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Allosteric mechanisms in receptor function and modulation: toward a new pharmacology

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The concept of allosteric interaction (1) was initially proposed to account for the inhibitory feedback mechanism mediated by bacterial regulatory enzymes. In contrast with the classical mechanism of competitive, steric, interaction between ligands for a common site, allosteric interactions take place between topographically distinct sites and are mediated by a discrete & reversible conformational change of the protein. The concept was soon extended to membrane receptors for neurotransmitters (2) and shown to apply to the signal tranduction process which, in the case of the acetylcholine nicotinic receptor (nAChR), links the ACh binding site to the ion channel (3). Pharmacological effectors, referred to as allosteric modulators, such as Ca^{++} ions and ivermectin, were discovered with nAChR that enhance the transduction process when they bind to allosteric mdulatory sites distinct from the orthosteric ACh site and the ion channel (3). The recent X-ray structures, at atomic resolution, of the resting & active conformations of several eukaryotic and prokaryotic homologs of the nAChR, in combination with atomistic molecular dynamics simulations (3) reveal two distinct quaternary transitions in the transduction process with tertiary changes which profoundly modify the boundaries between subunits. These interfaces host orthosteric and allosteric modulatory sites which structural organization changes in the course of the transition. These views apply, in addition to ligand- or voltage gated ion channels, to G protein-coupled receptors, nuclear hormone and tyrosine kinases receptors. All are allosteric proteins that carry multiple, spatially distinct, yet conformationally linked ligand-binding sites. Synthetic allosteric modulators are being discovered for these receptors, many of them currently used in the clinic, thus paving the way to a novel pharmacology.

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Exploring Class B GPCR signalling in sensory neurons

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Introduction. For people of all ages and ethnicities, chronic pain is an intolerable daily burden. It is a major factor in diseases including arthritis, migraine and chronic lower back pain. Currently only limited treatment options are available to sufferers. New medicines are required to address this condition. A major challenge in the development of new medicines targeting G protein-coupled receptors (GPCRs) is the ability to quantify drug action in physiologically relevant models. Primary cell models that closely resemble the clinically relevant in vivo site of drug action are important translational tools in drug development. However, pharmacological studies in these models are generally very limited due to the methodology used.
Aims. To determine the expression and functionality of class B GPCRs in primary sensory neurons derived from the trigeminal ganglia (TG), which plays a central role in craniofacial pain.
Methods. Receptor expression was determined using Taqman GPCR arrays and receptor pharmacology quantified using signalling assays in primary rat TG neurons and glia.
Results. In primary rat TG neurons GPCR arrays detected ~250 individual GPCRs. This included 13 of 15 class B GPCRs. Selected Class B GPCR agonists were screened in primary TG neurons and glia. Primary TG neurons displayed robust cAMP responses to PACAP38, αCGRP and amylin. PACAP-responsive receptors in rat TG neurons and glia were pharmacologically similar to transfected PAC_{1n} receptors, suggesting a PAC_1, receptor is present. However, distinct pharmacological profiles were also observed. In TG glia, PACAP38 and PACAP27 stimulate cAMP production, however, only PACAP38 activated ERK1/2 phosphorylation. Agonist and antagonist pharmacology in TG neurons also indicated the presence of CGRP and AMY_1 receptors. Interestingly, the 'CGRP receptor' antagonist, olcegepant, displayed signal-specific differences in antagonism of αCGRP responses, suggesting that the affinity of this antagonist can be dependent on the receptor and signalling pathway activated.
Discussion. The detection of ~250 GPCRs in TG neurons suggests that sensory neurons may express a large number of currently unexploited targets for the treatment of pain. The PAC_1 and AMY_1 receptors represent potential targets for treating pain. Understanding the complex pharmacology and signalling behaviours of these receptors in primary cells may help to more effectively direct the development of new treatments for chronic pain.

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Plasma membrane calcium channels in the regulation of hypoxia events in breast cancer cells

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Introduction. Calcium signalling plays a crucial role in a variety of cellular processes important in tumorigenesis including migration, invasion and proliferation. Calcium influx pathways in cancer cells are often mediated by plasma membrane calcium channels including Orai proteins and transient receptor potential (TRP) channels. Hypoxia is a hallmark of the tumour microenvironment and is associated with increased metastasis and poor patient survival. The association between hypoxia and Ca^{2+} influx in breast cancer cells has not been fully explored.
Aims. To assess the role of TRP and Orai-mediated calcium entry in hypoxia mediated cellular events in basal breast cancer cells.
Methods. Basal breast cancer cell lines assessed included MDA-MB-468, HCC1569 and MDA-MB-231. Pharmacological activators or inhibitors or siRNA were used to activate or block channel activity or expression. A Fluorescence Imaging Plate Reader ($FLIPR^{TETRA}$) was used to assess intracellular free Ca^{2+} ($[Ca^{2+}]_{CYT}$). RNA and protein levels were quantified using real-time RT-PCR and immunoblotting respectively. Real time RT-PCR and/or next-generation sequencing was used to identify targets that were sensitive to ion channel modulation during hypoxia.
Results. Specific TRP channels were identified as regulators of vimentin protein expression induced by hypoxia and epidermal growth factor (EGF) in basal breast cancer cells. Orai proteins were demonstrated to be isoform dependent regulators of cellular events associated with cell migration and inflammatory responses during hypoxia.
Discussion. The isoform specific roles of Orai and TRP proteins suggests that the hypoxic phenotype exploits different ion channels to achieve different cellular functions. These studies suggest that specific Orai and TRP isoforms may represent potential therapeutic targets for the control of processes associated with breast cancer progression.

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Integrating quantitative clinical pharmacology to expedite paediatric drug development

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Introduction. Paediatric drug development is challenging and is constrained due to obvious ethical considerations for conducting clinical trials in children. A promising solution is the application of mathematical models that quantitate disease kinetics, drug concentration and pharmacodynamic response in a suitable adult population. Once established, these models can be utilised to extrapolate dose recommendations that expedite paediatric drug development.
Aims. To describe how mechanistic models have assisted with developing new drugs in the treatment of respiratory virus infections in children.
Methods. Human challenge studies involve the artificial infection of healthy adults with respiratory viruses. Subjects are randomized by placebo or drug treatment several hours after the infection is established. Clinical measurements include the time course of viral load, drug concentration and symptom scores. Nonlinear mixed effects modelling was used to quantitatively describe viral kinetics, drug pharmacokinetics-pharmacodynamics, and changes in disease symptoms.
Results. Mechanistic target cell-limited models characterised the time course of influenza and Respiratory Syncytial Virus in placebo subjects. Substantial inter-individual variability was estimated for parameters of the viral life-cycle. Simple Emax models described the antiviral inhibition effect, with high effectiveness at the doses administered. The resulting models were then used for paediatric dose extrapolation by applying physiologically-relevant differences between adults and children.
Discussion. We have integrated mathematical modelling with human challenge studies to progress the development of several new drug candidates to treat respiratory virus infections. An additional benefit is the ability to estimate variability and explore covariate effects using biological principles. This quantitative pharmacological approach is useful for drug development in target populations where robust clinical study design and measurement is restricted.

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Using animal models to identify novel targets in respiratory disease

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Introduction. Lung diseases are highly prevalent worldwide, with asthma affecting ~10% of the population and chronic obstructive pulmonary disease (COPD) now the third leading causing of death. One of the common features of both of these diseases is small airway obstruction, caused in part by airway remodelling and increased inflammation. Current therapies aimed at reducing airway inflammation and reversing excessive airway contraction are relatively ineffective in opposing excessive contraction in small airways. In addition, respiratory viral infections in patients with asthma and COPD can also lead to exacerbations of their symptoms. Using mouse models of airway disease provides settings to assess changes in small airway reactivity and to identify potential novel therapeutic targets when current therapy is ineffective.
Aims: To assess airway reactivity and novel therapeutics in precision cut lung slices (PCLS) from mouse models of lung disease including ovalbumin, lipopolysaccharide, cigarette smoke (CS) exposure, and cigarette smoke with influenza A virus infection.
Results/Discussion: Exploration of small airway reactivity in PCLS from models that mimic key features of asthma and COPD has revealed key differences in the influence of inflammation, CS and/or infection on contraction and relaxation. CS exposure altered contractile responses to 5HT and not MCh, and led to downregulation of ryanodine receptors. Since ryanodine receptor isoform 3 is involved in agonist-induced calcium signaling and contraction, this may provide a novel therapeutic target to treat obstructive airway diseases. Furthermore, influenza A virus exposure either alone or in combination with CS decreased the bronchodilator potency and/or efficacy of the currently prescribed ß-adrenoceptor agonist, salbutamol. These models can be used to further assess mechanisms of impaired bronchodilator responsiveness and to assess novel therapeutic approaches to reverse small airway obstruction in asthma and COPD.

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Pharmacology and Functions of the Long Chain Fatty Acid Receptors FFA1/GPR40 and FFA4/GPR120

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Introduction The long chain fatty acid receptors FFA1/GPR40 and FFA4/GPR120 have attracted considerable interest in recent times as novel therapeutic targets for metabolic diseases including type II diabetes (Milligan et al., 2015, 2016) . However, expression patterns of these receptors extend beyond endocrine tissues and this suggests broader roles.
Aims I will discuss progress in the development of selective pharmacological tools and ligands for these receptors; consider roles beyond glycaemic control, including in chemotherapeutic resistance and lung function, and describe the generation of transgenic animal models expressing 'biased' receptors to explore roles of divergent signalling pathways.
Methods I will describe the identification of chemotypes of highly selective FFA4/GPR120 agonists and effects of these on glycaemic control (Azevedo et al., 2016), the development of fluorescent ligands to probe orthosteric versus allosteric binding sites (Christiansen et al., 2016), mapping of regulated sites of receptor phosphorylation (Prihandoko et al., 2016) and the use of such phosphorylation-resistant mutants alongside gene-edited cell lines that lack expression of G proteins or arrestins.
Results Whilst selective FFA1/GPR40 agonists have provided proof of concept as potential therapeutics to regulate blood glucose levels to date equivalently selective FFA4/GPR120 agonists have only been used in pre-clinical rodent models These, however, also show many beneficial effects. The expression of FFA4/GPR120 in each of splenocytes, macrophages and lung tissue and evidence suggesting key end points in white cells may be mediated by signals generated via non-canonical, G protein-independent pathways, led us to map sites of regulated phosphorylation in both human and mouse FFA4/GPR120, to demonstrate the loss of interaction with arrestins of variants of these receptors in which all agonist-regulated sites of phosphorylation were mutated, and the enhanced capacity of such mutants to regulate ERK1/2 mediated signalling. Generation of transgenic 'knock-in' mice expressing such a phosphorylation-deficient mutant provides the potential to define the contributions of different pathways to physiological functions, including an ability of FFA4/GPR120 to regulate airway contraction and resistance and to promote resistance to platinum-based chemotherapeutics.
Discussion Although now well established as therapeutic targets for metabolic conditions, the rapid development of pharmacological tool compounds for the long chain fatty acid receptors is opening up opportunities to explore roles of these receptors in a broader range of physiological and disease settings.
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Airway epithelial steroid insensitivity

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Synthetic glucocorticoids are among the most commonly used prescription medicines and have been remarkably successful in the treatment of chronic inflammatory diseases such as asthma. The therapeutic efficacy of glucocorticoids can be attributed to their broad spectrum of action, due to the pleiotropic effects of the glucocorticoid receptor (GR) on multiple signalling pathways. However, this pleiotropism also means that the clinical efficacy of glucocorticoids is accompanied by dose- and indication- limiting acute and chronic adverse effects. Intrinsic or acquired resistance to glucocorticoid actions also limits clinical efficacy, with subsets of patients with moderate to severe disease showing partial or complete resistance to the anti-inflammatory actions of glucocorticoids. The inflammation that occurs in other chronic airway diseases such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) and interstitial pulmonary fibrosis (IPF) is seemingly inherently glucocorticoid-resistant, although the reasons for this are not well understood. Airway epithelial cells are prominent targets for glucocorticoid action in treating respiratory disease such as asthma and COPD, as the epithelium is both the first site of contact for inflammatory and other stimuli from the physical environment, and is the site of deposition for inhaled glucocorticoids, and therefore exposed to much higher drug concentrations than any other cell type in the airway. Despite this, epithelial cells were only demonstrated to have functional glucocorticoid receptors (GR) in the late 1990s. The mechanisms of glucocorticoid resistance in this cell type are also underexplored. Our previous work has shown that transforming growth factor-beta (TGF-β) induces glucocorticoid insensitivity in bronchial epithelial cells through a signalling pathway distinct from Smad signalling, or MAP kinase pathways (1). Viral infection of the lower respiratory tract with respiratory syncytial virus (RSV) also results in a state of glucocorticoid insensitivity which we have shown to be mediated by TGF-β activity (2). Global inhibition of TGF-β signalling increases the risk of cancer and autoimmune disease. Therefore, selective targeting of discrete TGF-β signalling pathways presents as a novel approach to develop glucocorticoid-sensitising agents with the therapeutic potential to restore anti-inflammatory efficacy in glucocorticoid-resistant disease.

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Mast cells at the centre

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Mast cells are everywhere, are short-tempered and pack a punch. Whilst new work suggests these cells are just misunderstood and can perform a community service, 'locking up' mast cells is still seen as the safest bet in allergic disease. Mast cell presence in the airway smooth muscle is a defining feature of asthma. This location means that the many mediators that mast cells release are at precisely the right spot to exert maximum effects on airway calibre. But how can we control these miscreants? In this presentation, I will discuss our work on better understanding the interaction between mast cells and airway smooth muscle including some recent efforts in developing 3D spheroid models. I will also highlight our work identifying previously undescribed factors released from mast cells that may act as inflammatory mediators. Pathways prone to fire-off mast cells and inherent inhibitory pathways within these cells, that might be harnessed to calm them down, will be examined as possible therapeutic opportunities.

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Targeting oxidant-dependent mechanisms for the treatment of COPD and its co-morbidities

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Introduction. Reactive oxygen species (ROS) are a family of highly reactive molecules that are produced by a variety of cell types in the lung in response to chemical and physical agents in the environment. It is well known that ROS are critical in host defence as they kill invading pathogens, but that their excessive accumulation in the lung results in oxidative damage. Oxidative stress, which is defined as the persistent overproduction of ROS that overwhelms endogenous antioxidant defence systems, has been implicated in both acute (e.g respiratory virus infections, exacerbations of asthma and COPD) and chronic (e.g. COPD) lung diseases.
Aims & Methods. To determine whether inhibiting oxidative stress and ROS production may be a novel way to treat acute and chronic lung diseases using clinically relevant models of lung disease.
Results. We have shown that targeting oxidative stress with the Nox2 oxidase inhibitors and ROS scavengers, apocynin and ebselen can ameliorate influenza A virus (IAV)-induced lung inflammation and pathology, cigarette smoke-induced lung inflammation and acute exacerbations of COPD (AECOPD). In addition, we have found that treating mice with apocynin reduced cigarette smoke-induced skeletal muscle wasting in mice suggesting that this strategy can be useful in treating comorbidities associated with COPD.
Discussion. Targeting oxidative stress may be a novel strategy to treat both acute and chronic lung diseases.

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RGS Dysfunction in Asthma

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Introduction. Regulators of G protein Signaling (RGS) proteins act as multi-functional, GTPase-accelerating molecules that promote GTP hydrolysis by the alpha subunit of heterotrimeric G proteins and that inactivate G proteins. In asthma, airway smooth muscle (ASM) serves as the pivotal cell modulating bronchomotor tone that is dependent on excitation-contraction (E-C) signaling mediated by activation of G protein coupled receptors.
Aims. Our central hypotheses state that specific RGS proteins in airway smooth muscle and epithelial cells modulate E-C coupling and growth responses to evoke a phenotype of airway hyperresponsiveness (AHR) and remodeling in asthma or in models of allergen-induced AHR and airway inflammation in mice.
Methods. Using primary human ASM cells, precision cut lung slices, murine models of AHR and molecular gain-/loss of RGS protein, the role of RGS proteins in mediating agonist-induced calcium levels, phosphorylation of E-C coupling proteins and bronchconstriction were characterized. Immunocytochemical approaches of tissue derived from donors with varying asthma severity determined the differential RGS expression in asthma.
Results: RGS4 expression in human ASM increases with asthma severity. RGS4 is required for mitogen-induced human ASM cell proliferation in a PI3K dependent manner and mitogen-induced expression of RGS4 decreases agonist-induced cytosolic calcium mobilization and bronchoconstriction (1). Prolonged exposure to β2 agonists evokes enhanced agonist-induced calcium mobilization in human ASM cells by decreasing RGS5 expression (2); loss of RGS5 promotes baseline and allergen-induced AHR independent of airway inflammatory responses in mice (3). Additionally, loss of RGS2 that is abundantly expressed in airway epithelial cells and ASM promotes allergen-induced AHR in mice and is diminished in airways of asthma subjects (3). Further, SNP analyses of the RGS2 promoter showed significant differences between asthma and non-asthma subjects (5).
Discussion: Taken together, RGS 2, 4 and 5 play significant roles in modulating AHR and airway remodeling in asthma independent of airway inflammation. These molecules may serve as novel therapeutic targets in structural cells to promote bronchodilation and abrogate airway remodeling in asthma.
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Complexities of CYP mediated Kinase Inhibitors metabolism and interactions

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Targets of the small molecule tyrosine kinase inhibitors (TKI) are mainly the VEGF receptors. Their major advantage is the oral availability, and patients are being treated continuously. It is believed that increased exposure to TKIs will improve clinical outcomes. However, TKIs display a large inter-patient variability that is not at all understood so far. CYP3A amongst others (polymorphisms, drug transporters) seems to play an important role in the resulting variability of TKI exposure. Most TKIs are used in the target patient population in a dose which is the same in every patient (as recommended by SmPC). Taking into account that there will be numerous drug interactions especially with CYP3A but also drug transporters it is not surprising that there are patients with poor or no response, and patients with TKI related toxicities.
Because there are currently numerous TKIs available and they all seem to have complex pharmacokinetics and a range of important toxicities, therapeutic drug monitoring can be useful to optimise treatment. In addition, chronic underdosage with the risk of resistant clones and disease progression can be avoided as well as overdosage with the risk of dose-related toxicity. The key point is the knowledge of drug clearance and their underlying mechanisms. In combination with (to be) established therapeutic ranges, treatment with TKIs can be improved.

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Genetics of imatinib disposition

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Imatinib is a molecularly targeted therapy originally designed for the treatment of chronic myeloid leukaemia (CML), but also now indicated in other BCR-ABL-, c-KIT- and PDGFR-driven cancers. It is orally administered, predominantly hepatically cleared with a low hepatic extraction ratio, is ~95% bound to plasma proteins, and has an intracellular site of action. In the CML setting, treatment outcomes correlate with plasma imatinib concentrations, which show large interpatient variability. Treatment outcomes also correlate with markers of drug transporter variability thought to influence imatinib distribution into CML cells. Dose individualisation is therefore expected to improve imatinib treatment outcomes, and there is a potential role for a pharmacogenetic approach.
Imatinib is metabolised by CYPs 2C8 and 3A4 *in vitro*. Whilst there is no consistent evidence that *CYP3A4* genetics alter imatinib metabolism clinically, we have recently shown that *CYP2C8* genotype can significantly affect imatinib metabolism, and consequently imatinib exposure in CML patients. Imatinib is also a substrate for multiple uptake and efflux transporters. Some studies report effects of drug transporter genetics (e.g. *ABCB1*, *SLC22A1*, *SLCO1B3*, *ABCG2, SLC01A2*) on plasma imatinib concentrations and clearance, but results have been inconsistent. Correlations between drug transporter genetics and CML treatment outcomes have also been reported. However, due to study design limitations, it is unclear if drug transporter genetics influence treatment response via effects on plasma imatinib concentrations or intracellular distribution. Other potentially novel genetic factors influencing imatinib disposition, such as xenobiotic-responsive receptor gene polymorphisms, remain to be thoroughly investigated.
In summary, studies to date have shown a potential genetic influence on plasma imatinib exposure, however evidence is still lacking to support a pharmacogenetic approach to individualised dosing; whether such an approach might be complementary or redundant in the context of potential therapeutic drug monitoring/target concentration intervention for imatinib must also be considered. It is hoped through novel study designs incorporating both pharmacokinetics (especially unbound plasma and intracellular concentrations) and treatment outcomes, that we can gain a greater mechanistic understanding of factors governing imatinib intracellular distribution clinically, and move towards prospective trials of new tools to aid in CML treatment individualisation.

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Predictors of therapeutic and adverse outcomes with targeted anticancer drugs

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Introduction. Despite considerable advances in the treatment of cancer, the therapeutic and adverse effects of cancer medicines can differ considerably between patients.
Aims. To highlight the range of predictors and study data that may be of value in guiding treatment decisions for cancer medicines.
Methods. Study designs to be covered include the secondary analysis of pooled data from clinical trials of cancer medicines, experimental designs involving health participants, and observational studies of cancer patients.
Results. Results will be presented for a number of completed and ongoing studies demonstrating the range of different approaches (predictors, outcomes, study designs) that may be utilised to advance knowledge regarding predictors of therapeutic and adverse outcomes with targeted anticancer drugs. Some approaches have demonstrated great clinical utility for specific cancers and/or cancer medicines, but no single type of predictor has demonstrated consistent success in significantly improving treatment decisions across a wide range of cancer medicines and cancer types.
Discussion. There are a wide range of different study designs, outcomes and predictors relevant to improving the guidance of treatment decisions for cancer medicines. Experience indicates that different approaches are of variable value in guiding different treatment decisions. The value of a particular approach can also differ greatly between different types of cancer and types of cancer medicine. Therefore, evaluating a range of different prediction options for each given circumstance is likely to enhance the chances of identifying predictors of sufficient clinical utility to be translated into clinical practice.

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Bayesian Forecasting to Predict Drug Exposure

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Introduction. Achieving the optimal drug exposure for a given drug in a given patient is a cornerstone of pharmacotherapy. However, dosing strategies such as flat dosing or size-based dosing in a population of patients can leave some patients with sub-optimal drug exposure and outcomes. Using individual patient information (e.g. covariates such as genotype, lab values or biomarkers) or measured drug concentrations (e.g. Therapeutic Drug Monitoring, TDM) to modify dosing can help tune the exposure in a given patient. Bayesian forecasting is the most elegant method to date for integrating prior knowledge about a drug and individual patient information. Bayes forecasting methodology has been available for many years, but it has not widely adopted in clinical settings.
Aims. To introduce Bayes Theorem, the general principles of Bayes Forecasting in drug dosing and to illustrate potential benefits of Bayes Forecasting using the example of a mono-clonal antibody (MAb).
Methods. Bayes' theorem provides a mathematical rule for revising an estimate or forecast (“the posterior”) in light of experience (“the prior”) and observation (“the new data”). For Bayes Forecasting, the prior information is structured in the form of a population pharmacokinetic (or pharmacodynamic) model. The individual data comes from covariate values and measured drug concentrations in the individual patient. The Bayes method allows the parameters (and hence predictions) of a pharmacokinetic model to be weighted towards the prior in the absence of any individual data. As more individual data become available, the model predictions for an individual swing towards an individual prediction based on the amount of individual (posterior) data available. The revised model prediction can provide a dose that is most likely to achieve a target exposure given the available information.
Dosing strategies for an example MAb were assessed in silico. The aim was to keep exposure (via trough MAb concentrations) above a threshold value during a year of treatment, and label dosing was 5 mg/kg every 8 weeks.
Results. In silico assessment of the example MAb showed that Bayes based dosing resulted in substantially less patients being below the threshold Mab concentration than either label dosing or TDM based dosing.
Discussion. The adoption of Bayes methods will hopefully increase as: 1. Scenarios where it can have substantial benefit are identified. 2. New web-based applications, for making Bayes methods accessible for clinicians, become more widely used.

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Astrocytic calcium signaling in health and disease

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Introduction. Astrocytes ensheathe the synapses and blood vessels by their fine processes, which generate intracellular Ca^{2+} signals. The morphological features of astrocytes suggested that astrocytic Ca^{2+} signals may regulate synaptic functions and vascular contractions to control local cerebral blood flow. However, the roles of astrocytic Ca^{2+} signaling have not been fully understood.
Aims. To look into the roles of astrocytic Ca^{2+} signaling, we aimed at imaging Ca^{2+} signal in neurons, vascular smooth muscle cells, and astrocytes in living mice.
Methods. We generated tetracycline-regulated transgenic mice in which a genetically encoded Ca^{2+} indicator, YC-Nano50, is expressed in neurons, vascular smooth muscle cells, and astrocytes. Using a two-photon microscope, we compared the Ca^{2+} responses in these cells in the somatosensory cortex of living animals.
Results. Upon mechanical vibration of the hind paw, we observed an increase in Ca^{2+} concentration in neurons and astrocytes as well as a decrease in Ca^{2+} concentration in arterial smooth muscle cells. These Ca^{2+} signals may underlie the neuronal activity-dependent vasodilatation. Although the sensory stimulation-induced neuronal Ca^{2+} response preceded the vascular Ca^{2+} response, the astrocytic Ca^{2+} response seemed to lag the vascular response, apparently contradicting a potential role of astrocytic Ca^{2+} responses in mediating the neurovascular coupling. We also studied pathophysiological roles of astrocytic Ca^{2+} signaling. We found periodic increases in the astrocytic Ca^{2+} concentration in response to brain injury. Blockade of the injury-induced astrocytic Ca^{2+} responses resulted in reduced astrogliosis and increased neuronal cell death.
Discussion. These results indicate that astrocytic Ca^{2+} responses play important roles under pathological conditions, while their physiological roles require further clarification.

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Physiologically relevant opening of TRPV4 by signalling from G protein-coupled receptors

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Introduction. Endothelial cells react to blood hormones and blood flow to regulate vasodilatation, as well as regulating the flow of blood cells and metabolites into the interstitial space. We explored how endothelial cells sense forces and blood-borne signalling molecules regulate vessel function, looking for evidence of interaction between GPCRs and Transient Receptor Potential Vanilloid 4 (TRPV4) ion channels. Here, we 1) explored the effect of shear stress on vasodilatation caused by acetylcholine (ACh) and 2) investigated how 5-hydroxytryptamine (5-HT) receptors cause inflammatory plasma extravasation in visceral tissues in a mouse model of oedema through TRPV4. We went on to explore the potential for GPCRs subtypes to modulate the opening of the TRPV4 in HEK293 cells.
Aims. 1) To determine if TRPV4 plays an essential role in relaxation of rat cremaster arterioles by ACh. 2) To determine if 5-HT receptors recruit TRPV4 to cause vascular leak in a mouse model of plasma extravasation and 3) to determine potential signalling pathways between GPCRs and TRPV4 using HEK293 cells.
Methods. ACh-induced vasodilatation of rat cremaster arterioles was measured by pressure myography before or after exposure to shear stress (200 μl/min flow for 6 min). The effect of a TRPV4 antagonist GSK2193874 was investigated. The effect of TRPV4 agonist and TRPV4 and 5-HT receptor antagonists (i.v.) was studied on 5-HT-evoked Evans blue extravasation in C57Bl/6J mice. Cellular assays were performed in HEK293 TRex cells transfected with TRPV4 and GPCR cDNA and assayed by calcium imaging (FlexStation III).
Results. ACh-induced vasodilatation was not affected by exposure to shear forces. The dilator response to ACh was not affected by the TRPV4 antagonist (GSK2193874) in control arteries (pEC_{50} 7.24 ± 0.07 M) however, after shear, the ACh-response was significantly attenuated by the TRPV4 antagonist (pEC_{50} 6.26 ± 0.12 M, $p<0.05$) indicating that shear forces stimulate an interaction between TRPV4 and muscarinic receptors. Similarly, 5-HT- induced plasma extravasation was attenuated by GSK2193874 and in $TRPV4^{-/-}$ mice. In HEK293-TRPV4 cells, co-expression and agonist activation of several GPCRs caused TRPV4 to open.
Discussion. These studies provide evidence that TRPV4 opens in response to GPCR signalling and that this may be of relevance in regulation of vasodilatation and inflammation.

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TRPM2 channels as mediators of oxidative stress-induced liver damage

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Transient Receptor Potential Melastatine 2 (TRPM2) protein is a non-selective, Ca^{2+} permeable cation channel found in most tissues, including liver (Nagamine et al, 1998). The main known activators of TRPM2 are ADP-ribose (ADPR), nicotinamide adenine dinucleotide (NAD) and H_2O_2. Cytoplasmic Ca^{2+} plays a role of an important co-factor in channel activation, increasing TRPM2 sensitivity to ADPR (Perraud et al, 2003). Activation of TRPM2 channels by H_2O_2, other reactive oxygen species and ADPR may have significant implications in pathophysiological processes. Recently we have shown that TRPM2 channels are essential in the mechanism of paracetamol-induced liver damage (Kheradpezhouh et al, 2014). Paracetamol is the most commonly used analgesic and antipyretic drug. At the same time, paracetamol overdose is the most common cause of acute liver failure and the leading cause of liver failure requiring liver transplantation in developed countries. In hepatocytes, paracetamol overdose causes formation of ROS, deregulation of Ca^{2+} homeostasis, covalent modification and oxidation of proteins, lipid peroxidation, and DNA fragmentation. Using whole cell patch clamping, Ca^{2+} imaging and confocal microscopy we show that treatment of hepatocytes with paracetamol results in activation of Ca^{2+} entry and a cation current similar to that activated by H_2O_2 or the intracellular application of ADP-ribose. Furthermore, paracetamol-induced liver damage in TRPM2 knock-out mice, assessed by liver histology and the concentration of blood liver enzymes is significantly attenuated compared to wild-type mice. Presented results suggest that elevation in cytoplasmic Ca^{2+} induced in human liver by paracetamol overdose is mediated by TRPM2 channels and that blockade of TRPM2 may prove useful in treatment of paracetamol overdose and other liver diseases associated with oxidative damage.

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TRPC1 in the regulation of pathways involved in breast cancer metastasis

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Introduction. Transient receptor potential (TRP) ion channels are non-selective cation channels with diverse roles as versatile sensors to environmental cues. TRPV1 and TRPM8 are heat and cold responsive channels respectively, TRPM2 is an oxidative stress sensitive channel, and TRPC5 is important in neuronal development and is also associated with the acquisition of multidrug resistance in breast cancer cells. Although TRPC1 was the first TRP ion channel identified in mammals, its physiological function as a channel remains mysterious. The TRPC1 knockout animal has been described as having not "dramatic" phenotypes[1]. TRPC1 has been also described as a store-operated Ca^{2+} entry (SOCE) channel often in association with Orai and STIM proteins.
Aims. To assess the role of TRPC1 in hypoxia-mediated cellular events in PTEN-deficient breast cancer cells.
Methods. Cytosolic free Ca^{2+} ($[Ca^{2+}]_{CYT}$) levels were assessed using a Fluorescent Imaging Plate Reader (FLIPR). Quantitative real-time RT-PCR and immunoblotting were used to evaluate changes in mRNA and protein levels respectively. SiRNA-mediated silencing was used to block channel expression. Gene ontology analysis was performed to define genes and pathways associated with TRPC1 expression.
Results. This study identified TRPC1 as an integral player in the regulation of hypoxia-mediated events including specific regulation of the induction of specific epithelial to mesenchymal transition (EMT) markers, and activation of EGFR and STAT3 signalling. TRPC1 was also identified as a regulator of basal (constitutive) levels of HIF1α via an Akt-dependent pathway. Gene ontology analysis revealed a close association between TRPC1 and genes associated with EMT and metastasis in breast tumours. These studies represent TRPC1 as a potential therapeutic target for the control of breast cancer metastasis.

[1]Nilius B. et al (2014). Pharmacological Reviews, 66:676–814

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Positive allosteric modulators of muscarinic acetylcholine receptors for treatment of CNS disorders

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Previous clinical and preclinical studies suggest that selective activators of M1 and/or M4 subtypes of muscarinic acetylcholine receptors (mAChRs) could provide a novel approach to improving cognitive function and reducing psychotic symptoms in patients suffering from Alzheimer's disease and schizophrenia. Unfortunately, previous efforts to develop selective agonists of individual mAChR subtypes have not been successful and previous compounds have failed in development because of adverse effects due to activation of multiple mAChR subtypes. We have been highly successful in developing selective positive allosteric modulators (PAMs) of both M1 and M4 that have excellent properties for in vivo studies and potential development as drug candidates. Interestingly, selective M1 PAMs have robust efficacy in enhancing synaptic plasticity in the hippocampus and the medial prefrontal cortex (mPFC) in rodents. Furthermore, M1-selective PAMs induce robust improvements in specific domains of cognitive function in animal models that are dependent of hippocampal and mPFC function. Interestingly, our recent studies reveal that some M1 PAMs can display stimulus bias and potentiate coupling of M1 to activation of phospholipase C (PLC) and related signaling pathways, without potentiating M1 activation of phospholipase D (PLD). Furthermore, exciting new data suggest that activation of PLD is uniquely involved in a form of synaptic plasticity in the PFC that is thought to be important for the potential therapeutic effects of M1 PAMs. Thus, biased M1 PAMs that do not activate PLD signaling may not provide the efficacy observed with prototypical M1 PAMs. In contrast to M1 PAMs, highly selective M4 PAMs have robust antipsychotic-like effects in animal models. Recent studies suggest that the antipsychotic-like effects of M4 PAMs are mediated by actions of these compounds on a specific population of striatal neurons that receive dense dopaminergic inputs. M4 activation in these cells leads to release of an endocannabinoid that acts on CB2 receptors on presynaptic dopaminergic terminals to reduce dopamine release. Furthermore, M4 PAMs reduce transmission at cortico-striatal synapses and normalized pathological increases in cortico-striatal transmission in mice that bear mutations that give rise to Huntington's disease. Electrophysiology and genetic studies are providing important new insights into the mechanisms by which M1 and M4 PAMs act in specific cortical and midbrain circuits. These studies provide an exciting new approach to discovery of novel highly selective activators of individual mAChR subtypes and suggest that subtype specific mAChR PAMs may provide a novel approach for treatment of multiple CNS disorders.

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Dopamine in schizophrenia: where does it stand in the cascade of pathological events?

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Introduction. Molecular imaging with Positron Emission Tomography (PET) has been used to examine dopaminergic transmission in schizophrenia. Excess striatal presynaptic release and activity, as well as functional supersensitivity of striatal D2 receptors have been documented. The striatal dysfunction occurs early on in the course of the disease and predicts conversion to psychosis. We will present here an update including more recent studies examing extrastriatal release and their relationship to measures of brain connectivity, activation during cognitive tasks, as well as clinical symptoms.
Aims. Mapping of dopamine dysfunction in schizophrenia and its functional correlates.
Methods. PET imaging studies using D2/3 radiotracers combined with the amphetamine challenge in patients with schizophrenia and matched healthy controls to image striatal and extrastriatal capacity for dopamine release.
Results. Dopamine release is significantly reduced in patients with schizophrenia compared to controls matched for age, gender, ethnicity, smoking, parental socioeconomic status and catechol-O-methyltransferase (COMT) genotype in dorso-lateral prefrontal cortex (DLPFC) as well as in all cortical and in most extrastriatal regions. In particular the midbrain DA cell body region (i.e. substantia nigra and ventral tegmental area) showed a substantial blunting of release in drug-naïve and drug-free patients, compared to controls.
Discussion. The presence of opposing findings of striatal excess and extrastriatal deficit including midbrain deficit is puzzling as it suggests that striatal excess may not be a consequence of midbrain DA cells overactivity. A mechanistic understanding of the DA dysfunction is missing. Animal models may shed some light on the pathogenic mechanisms involved. In particular the D2 overexpressing (D2OE) mouse has shown that cortical dependent cognitive deficit and abnormal cortical DA signaling can be a consequence of developmental abnormalities in striatal D2 stimulation. Since many developmental factors, both genetic and environmental, have been shown to be at play in schizophrenia, and shown to affect dopaminergic indices, these combined lines of evidence suggest that DA dysfunction may be an early event leading to additional consequences on the rest of the circuitry and behavior.

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mGluR5 and its potential role in ASD

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The glutamatergic synapse is hypothesized to play a role in the pathogenesis of autism spectrum disorder (ASD). We have undertaken genetic, and postmortem mRNA and protein studies to investigate the potential role of mglur5 and the glutamatergic system in the pathogenesis of ASD.
In our cohort we find that GRM5 and *GRIA3 that mRNA and protein levels are statistically different amongst patients and neurotypicals*. In addition, within the DLPFC, levels of *GRM5* gene expression has a statistically significant inverse correlation with gene expression of the pro-inflammatory cytokine interleukin-4 (IL4) (p=0.02) interleukin-6 (IL6) (p=0.04), Nitric Oxide Synthase 2 (NOS2) (p=0.0003), Tumor Necrosis Factor-alpha (TNFα) (p=0.04) and Interferon Regulatory Factor 7 (IRF7) (p=0.0002).

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M_1-muscarinic allosteric modulators slow prion neurodegeneration and restore memory loss

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Introduction. The current frontline treatment for memory loss in Alzheimer's disease (AD) are acetylcholinesterase (AChE) inhibitors, which act to up-regulate cholinergic transmission by inhibiting the break down of acetylcholine. The non-selective mode of action of AChE inhibitors results in significant dose-related adverse effects that limit clinical usage. More selective approaches to restore cognitive function associated with a decline in cholinergic function have been focused on activation of M_1 muscarinic acetylcholine receptors (M_1 mAChRs). Recent studies have also implicated that targeting muscarinic receptors may not only be a strategy to improve cognitive decline but also modify disease progression by promoting non-amyloidogenic APP processing (Shirey et al., 2009; Davis et al., 2010). However, the potential that M_1 mAChR selective ligands might impact on disease progression and increase survival in terminal neurodegenerative disease has never been tested.
Aims. In this study, we aim to test the efficacy of M_1 mAChR positive allosteric modulators in alleviating symptoms of neurodegeneration as well as in modifying disease progression by employing mice with progressive, terminal neurodegenerative disease.
Methods. Prion infection of mice: Tg37 mice were inoculated with 1% brain homogenate of Rocky Mountain Laboratory (RML) prions aged 3–4 weeks (Mallucci *et al.*, 2003). Control mice received 1% normal brain homogenate (NBH). Fear conditioning: Mice were placed in the conditioning chamber and received 3 tone (30s, 2.8kHz, 85dB)/foot shock (2s, 0.4mA) pairings. The next day mice were placed back in the conditioning chamber and percent of time spent immobile (AnyMaze software) was recorded for 3min to assess context-dependent learning. Survival experiments: Control- or prion-infected Tg37 mice were injected daily with BQCA (15mg/kg; i.p.) from 7 weeks post inoculation (w.p.i.). Animals were culled when they developed clinical signs of scrapie; prion-infected mice were scored according to the appearance of recognised early indicator and confirmatory signs of prion disease. fMRI: Data was acquired using a Varian 9.4T 310mm internal bore MRI instrument. Mice were anaesthetised using isofluorane. Pre-drug recordings were obtained prior to administration of xanomeline (5mg/kg; i.p.) or BQCA (15mg/kg; i.p.). The post-drug fMRI recordings were acquired 30 minutes later.
Results. Prion-infected tg37 mice undergo significant neurodegeneration, with 50% loss of hippocampal pyramidal neurons by 10 w.p.i. and a reduction in levels of choline acetyltransferase. Despite this, we show that M_1 mAChR expression and G-protein coupling at 9- and 10 w.p.i. is maintained, as it is in human post-mortem tissue from AD patients. Furthermore, prion-diseased mice display deficits in hippocampal-dependent learning and memory by 9 w.p.i. Acute administration of xanomeline (5 mg/kg; i.p.), an M_1/M_4 mAChR agonist, results in a broad up-regulation of neuronal activity in fMRI and rescues the cognitive impairment displayed by prion-diseased mice. The M_1-selective *allosteric* agonist, BQCA (15mg/kg; i.p.), stimulated increases in neuronal activity within cortical regions of wild-type mice, restored fear conditioning responses in prion-diseased mice to levels displayed by control mice and, importantly, induced no adverse side-effects. This allowed us to investigate the impact of prolonged BQCA exposure on disease progression in prion-diseased mice. Daily treatment with BQCA (15 mg/kg; i.p.) from 7 w.p.i. significantly extended the life span of prion-infected mice, compared to vehicle treated controls (**Fig. 1**). Preliminary data suggests that BQCA may achieve these disease-modifying effects by down-regulating total prion-protein (both cellular and scrapie forms; PrP^c and PrP^{sc}), thereby limiting the amount of PrP^c which is converted to PrP^{sc} during the disease process.

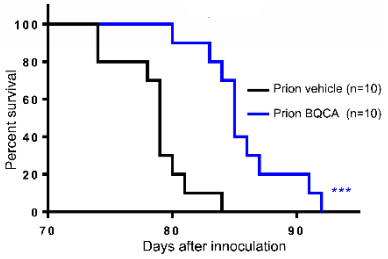

Figure 1 Kaplin-Meier survival plot for prion-infected mice treated daily with BQCA (15mg/kg) from 7 w.p.i. Gehan-Breslow-Wilcoxon test; ***P<0.001.

Discussion. M_1 mAChRs are considered to be an attractive therapeutic target in neurodegenerative diseases. However, drug development has been unsuccessful due to the problems of generating sufficiently selective orthosteric M_1 mAChR agonists. By employing prion-diseased mice with genuine neurodegenerative disease showing mechanistic, behavioural and neuroanatomical correlates to animal and human disorders, we show that M_1-selective positive allosteric modulators can restore memory loss associated with a decline in cholinergic function and prolong survival in terminal neurodegenerative disease.

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Extrapyramidal side effects of antipsychotic drugs are linked to their association kinetics at the dopamine D_2 receptor

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Introduction. Atypical antipsychotic drugs (APDs) have been hypothesized to show reduced extrapyramidal side effects (EPS) due to their rapid dissociation from the dopamine D_2 receptor (D_2R)[1]. However, support for this hypothesis is limited to a relatively small number of observations made across several decades and under a variety of different experimental conditions.
Aim. To measure the kinetic properties of a large number of APDs under the same experimental conditions in order to better correlate these properties with their EPS and prolactin-elevating liabilities at therapeutic doses.
Methods. Using a TR-FRET-based method[2] we monitored the association of fluorescent PPHT to terbium-labeled D_2R expressed in CHO cells in the presence of unlabeled APDs at 37°C. These data were fit to a competition association model to determine the kinetic parameters for the APDs. The resulting on- and off-rates were compared to side effect odds ratios from a comprehensive meta-analysis of clinical APD studies[3].
Results. The APDs displayed a wide range of on- and off-rates, with dissociation $t_{½}$ values ranging from 0.32 to 38.5 min. Remarkably, we found that association rates ($r = 0.68$, $P < 0.05$), but not dissociation rates ($r = -0.13$, $P = 0.68$), correlated with EPS. EPS was robustly predicted by a ligand rebinding model[4] that takes into account the microenvironment of postsynaptic D_2Rs and integrates association and dissociation rates to calculate the net rate of reversal of receptor blockade. In contrast, prolactin elevation was correlated with off-rate ($r = -0.82$, $P < 0.05$).
Discussion. We propose that rebinding of APDs in the D_2R synapse/apposition leads to prolonged receptor blockade, resulting in on-target EPS. In contrast, off-rate appears to govern hyperprolactinemia. Thus, optimizing both association and dissociation rates at the D_2R may lead to APDs with improved therapeutic profile.
(1) Kapur & Seeman (2001) Am J Psych 158:360-369; (2) Herenbrink et al (2016) Nat Commun 24:10842
(3) Leucht et al., (2013) Lancet. 382:951-962: (4) Vauquelin & Charlton (2010) Br J Pharmacol 161:488-508

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The use of three-dimensional cell culture models in the study of idiopathic pulmonary fibrosis

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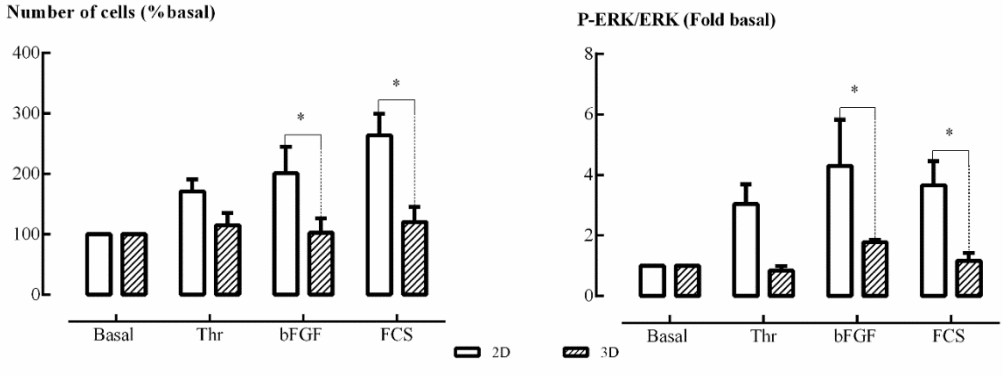
Introduction: idiopathic pulmonary fibrosis (IPF) is a chronic, irreversible, progressive and fatal fibrotic lung disease. It is characterized by, disorganized extracellular matrix (ECM) deposition and destruction of the lung architecture. The pathophysiology of the disease is poorly understood. Animal models of IPF have low clinical predictive value. Two-dimensional cell culture as model lacks the complexity of the *in vivo* conditions. Thus, the attrition rate of drugs for fibrosis is high.
Aim: three-dimensional (3D) cell culture models are better approaches to evaluate potential anti-fibrotic drugs *in vitro*.
Methods: 3D human pulmonary fibroblast (PFb) cell cultures were generated on ultra-low attachment plates or on polyhydroxyethylmethacrylate coated round-bottom 96-well plates. Coagulants and growth factor induced extracellular signal–regulated kinases (ERK) phosphorylation was analyzed by western blotting. The effect of transforming growth factor-β1 (TGF-β1) on expression of ECM and fibrogenic genes markers was measured using qRT-PCR. Cell count after 48 hours of incubation was done in a blinded fashion with a hemocytometer.
Results: Incubation of PFb cell cultures with known mitogens, such as basic fibroblast growth factor (bFGF), fetal calf serum (FCS) and coagulant thrombin (Thr) and factor Xa (FXa) increased ERK phosphorylation and cell proliferation in 2D but not 3D experiments. TGF-β induced mRNA expression of genes that codes for ECM protein was also reduced in 3D PFbs from non-IPF lungs. In contrast, the mRNA expression of αSMA, Col2 and SMAD2 in PFb cultures from IPF patients but not non-IPF culture was significantly increased only in 3D cultures.



*P<0.05; two-way ANOVA, Bonferroni's Post-hoc test

Discussion: In response to TGF-β exposure fibrotic markers expression in both IPF and non-IPF 2D cultures were increased. In 3D PFb cultures fibrotic markers expression was augmented only in fibroblasts from IPF patients.

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HTR_{2A} mediates 5-HT-induced oedema in the mouse trachea, oesophagus and bladder

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Introduction. Elevated serum 5-HT causes oedema in various body tissues by increasing microvascular permeability. Recent unpublished data from this laboratory implicate neurogenic inflammatory signalling from 5-HT receptors (HTRs) on primary afferent neurons as the cause of 5-HT-induced visceral oedema. The HTR subtype mediating this response is yet to be identified.
Aims. To determine the HTR subtype mediating oedema in the mouse trachea, oesophagus and urinary bladder.
Methods. Plasma extravasation of the albumin-binding dye Evans Blue (EB) in a C57Bl/6J mouse model of oedema was used to investigate the inhibition of 5-HT-induced oedema with selective HTR antagonists. Mice were anaesthetised with isoflurane and pre-treated with 100 µL i.v. bolus of antagonist for HTR subtype 1A (WAY-100635 80 µg/kg), 1B/D (GR 55562 300 µg/kg), 2A (Ketanserin 2mg/kg), 2B (RS-127445 300 µg/kg), 4 (GR 113808 30 µg/kg) or 7 (SB269973 300 µg/kg). EB (100 µL i.v. bolus, 5 mg/kg) was delivered 9 mins following antagonist treatment, and 5-HT (100 µL i.v. bolus, 100 µg/kg) was given 1 min later. Mice were culled 5 mins after 5-HT treatment and the circulation cleared of blood via left ventricular saline (0.9% NaCl) perfusion. Tissues were excised, incubated in formamide for dye extraction then oven-dried and weighed. EB concentration was determined at 620 nm using spectroscopy and expressed as mean ± S.E.M. ng/mg, EB/dried tissue.
Results. 5-HT-treated mice had significantly increased extravascular EB in the trachea, oesophagus and bladder compared with saline control. Oedema in these tissues was significantly inhibited by the HTR_{2A} antagonist. No other antagonist tested had a significant effect on vascular permeability.
Discussion. 5-HT is an agonist at thirteen G-protein coupled receptors. HTR_{2A} regulates affective and cognitive function in the CNS, is additionally expressed on primary afferents and is known to signal via $G_{q/11}$. Elevated serum 5-HT acts on sensory neurons to orchestrate disruption of interendothelial adherens junctions by release of inflammatory neuropeptides (McDonald, 1996). Elucidation of afferent intracellular signalling pathways downstream of HTR_{2A} offers an opportunity for pharmacological treatment of oedema in these tissues.
McDonald DM et al. (1996) Adv Exp Med Biol 410:453-62

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FGD2: A new modulator of mast cell activation

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Introduction: Mast cells are key contributors to allergic and pseudo-allergic reactions. Through IgE-dependent activation of FcεRI receptors, and via alternative pathways such as the GPCR MrgX2, mast cells are capable of releasing inflammatory mediators including histamine and a host of inflammatory cytokines. FGD2 is a Rho-family guanine nucleotide exchange factor thought to regulate numerous cellular functions through the small GTPase Cdc42.
Aims: This study investigated the role of FGD2 and Cdc42 in regulating mast cell activation.
Methods: Bone marrow from wildtype (WT) and $FGD2^{-/-}$ mice were cultured in IL-3 containing media to generate mature bone-marrow-derived mast cells (BMMCs). Mast cell phenotype was assessed by flow cytometry and histology. BMMCs, and the human mast cell line LAD2, were assayed for degranulation, inflammatory cytokine production, and changes in gene and protein expression upon stimulation with either antigen (IgE-dependent) or compound 48/80 (MrgX2-dependent). In some studies, the selective CDC42 inhibitor ML141 was used.
Results: $FGD2^{-/-}$ BMMCs showed a heightened degranulation response compared to WT cells. Cdc42 inhibition using ML141 produced a further enhancement of degranulation in both WT and $FGD2^{-/-}$ cells. FGD2 gene expression was strongly down-regulated by antigen stimulation. Moreover, Cdc42 expression at the protein level was markedly reduced by FGD2 deletion. In human LAD2 mast cells, Cdc42 inhibition with ML141 similarly produced a marked increase in degranulation following stimulation with compound 48/80.
Discussion: These results reveal FGD2 as a negative regulator of mast cell activation and also unexpectedly identify Cdc42 as also playing an important dampening role in mast cell activation. The consistent nature of Cdc42 inhibition on IgE and MrgX2 stimulated degranulation suggests that Cdc42 is acting at a common level in the two activation pathways. These findings suggest that harnessing the inhibitory actions of the FGD2/Cdc42 pathway might provide novel approaches to treating allergic and pseudo-allergic reactions.

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Respiratory syncytial virus-induced glucocorticoid insensitivity in bronchial epithelial cells: role of pathogen-associated molecular patterns (PAMPs)

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Introduction. Respiratory syncytial virus (RSV) is the prime cause of paediatric bronchiolitis and one of the leading triggers of asthma and COPD exacerbations. Despite the fact that RSV-induced pathogenesis is in part inflammatory, the beneficial effect of glucocorticoids (GC) in altering the course of the disease is still debatable. Infection with RSV has a detrimental effect on GC signalling through mechanisms that are not yet defined.
Aims. To ascertain whether Toll-like receptors (TLRs) contribute to RSV-mediated GC insensitivity in human bronchial epithelial cells (BEAS-2B).
Methods. The TLR ligands used in the current study included poly I:C; TLR3 ligand, LPS; TLR4 ligand, zymosan A; TLR2 ligand and imiquimod; TLR7 ligand. In addition, the RIG-I/MDA5 ligand; Poly I:C LyoVec, was also tested. Glucocorticoid response element (GRE) activity was assessed in BEAS-2B cells via transient transfection with a GRE-controlled secretory alkaline phosphatase (SEAP) reporter construct. GC-inducible gene expression was assessed by RT-qPCR. The selective ALK5 inhibitor, SB431542 (1μM) was used to identify the contribution of TGF-β. TLR3 was knocked down using small interfering RNA (siRNA).
Results. Among different PAMPs screened, only poly I:C and LPS significantly attenuated dexamethasone-induced GRE activation. Moreover, both PAMPs impaired the expression of epithelial sodium channel-α subunit (ENaCα) and glucocorticoid-inducible leucine zipper (GILZ), while upregulating plasminogen activator inhibitor-1 (PAI-1) expression, suggesting that these PAMPs initiated TGF-β expression. SB431542 (1μM) prevented the GC resistance. Transfected poly I:C failed to recapitulate the naked poly I:C detrimental effect on GC sensitivity. Moreover, TLR3 knockdown abolished poly I:C-induced GC responsiveness. In contrast, neither zymosan nor imiquimod had a detectable effect on dexamethasone induction of ENaC and GILZ expression or GRE activation.
Discussion. The current findings suggest that dsRNA/TLR3 and TLR4 pathways significantly contribute to RSV-mediated GC resistance. However, neither TLR2, TLR7 nor RLRs; RIG-I and MDA5, has a detectable effect on epithelial GC sensitivity. Moreover, ALK5 inhibition prevented both TLR3 and TLR4-mediated impairment of GC actions implicating TGF-β as a downstream mediator of RSV-induced GC resistance.

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GPR52 – A fine-tuner of striatal signalling in schizophrenia?

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Introduction. GPR52 is a $G\alpha_s$-coupled, orphan G protein-coupled receptor (GPCR) recently identified in literature as a novel target in schizophrenia due to expression in key areas of dopaminergic and glutamatergic dysregulation. GPR52 is highly expressed in the striatum, exclusively on dopamine D_2 receptor-expressing medium spiny neurons (MSNs) and predominantly on dopamine D_1 receptor-expressing cortical pyramidal neurons, in key neuronal populations that may regulate striatal hyper- and cortical hypodopaminergic states in schizophrenia.
Aims. To examine the role of GPR52 in striatal signalling, neurophysiology and striatal-dependent behaviours in order to gain insight into how this receptor may function as a novel target for schizophrenia.
Methods. We performed cAMP accumulation, Ca^{2+} mobilization and ERK1/2 phosphorylation assays in Chinese hamster ovary (CHO) cells stably transfected with human GPR52 (CHO GPR52) using selective synthetic agonist 3-[2-(3-Chloro-5-fluorobenzyl)-1-benzothiophen-7-yl]-N-(2-methoxyethyl)benzamide (3-BTBZ); cAMP assays were also performed in primary mouse embryonic striatal cultures. We used immunohistochemistry to investigate 3-BTBZ-induced phosphorylation of striatal signal integrator, 32kDa dopamine- and cAMP-regulated phosphoprotein (DARPP-32). Two key residues that modulate MSN excitability, threonine 34 (T34) and threonine 75 (T75), were examined in D_1- and D_2-expressing MSNs. In vivo activity of 3-BTBZ (1 - 30 mg/kg, IP) was assessed using amphetamine (3 mg/kg, IP)- and phencyclidine (3 mg/kg, IP)- induced hyperactivity in mice (n = 6-7 per group).
Results. 3-BTBZ induced cAMP production (pEC_{50} = 7.5 ± 0.2, n=4), but not Ca^{2+} mobilization or ERK1/2 phosphorylation, in CHO GPR52 cells and also induced cAMP production (pEC_{50} = 8.3 ± 0.2, n=4) in striatal neurons. 3-BTBZ increased T75 phosphorylation in D_1- but not D_2-expressing MSNs, with no effect on T34 phosphorylation in either population under these experimental conditions. Finally, 3-BTBZ showed efficacy in predictive animal models of psychotic symptoms of schizophrenia, inhibiting both amphetamine- and phencyclidine-induced hyperactivity at 30 mg/kg (IP) and 3-10mg/kg (IP), respectively.
Discussion. This data suggests that GPR52 activation, likely via regulation of cAMP signalling, modulates both DARPP-32 phosphorylation and behaviour in predictive models of schizophrenia.

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Do human amnion stem cell-derived exosomes improve stroke outcome?

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Introduction. Recent findings indicate that human amnion epithelial cells (hAECs) are neuroprotective following stroke. However, little is known about the factors these cells release to elicit neuroprotection. Interestingly, stem cells release extracellular vesicles called exosomes, which are thought to be the active component of stem cells and have an improved safety profile.
Aims. The study aimed to test whether hAEC-derived exosomes exhibit similar post-stroke neuroprotective effects to those of hAECs.
Methods. Male mice (8-12 weeks old) were anaesthestised with intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg) and subjected to 30 min middle cerebral artery occlusion or sham surgery (n=6). At 1 h following reperfusion, mice were injected intravenously with vehicle (saline; n=8), 10^6 hAECs (n=8) or 10 μg of hAEC-derived exosomes (n=6). After 24 h, functional outcomes and infarct volumes were assessed and immune cell infiltration was analysed via immunohistochemistry.
Results. Mice treated with either hAECs or exosomes were able to grip a wire for 63% and 45% longer compared to vehicle, respectively. Furthermore, the treated mice had a lower neurological deficit score compared to vehicle-treated mice. Infarct volume was reduced by 56 % ($p=0.05$) and 65 % ($p<0.05$) following hAEC and exosome administration, respectively, compared with vehicle treatment. Consistent with hAEC treatment, exosomes prevented the increase in neutrophils and T cells in the ischemic hemisphere. Finally, exosomes abolished, whereas hAECs tended to reduce, stroke-induced glial scar formation.
Discussion. These data indicate that exosomes provide similar neuroprotective benefits as hAECs following ischemic stroke.

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Magnesium Acetyltaurate provides Retinal Neuroprotection against NMDA-Induced Excitotoxicity

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Introduction. Glutamate excitotoxicity plays a major role in the loss of retinal ganglion cells (RGCs) in glaucoma. The toxic effects of glutamate on RGCs are mediated by the over stimulation of NMDA receptors. Accordingly, NMDA receptor antagonists have been suggested to inhibit excitotoxicity in RGCs and to delay progression and visual loss in glaucoma patients. From this point of view, magnesium (Mg) as physiological antagonist of NMDA receptors might be good therapeutic instrument in the treatment of NMDA-mediated ocular pathological conditions
Aims. The purpose of the present study was to examine the potential of neuroprotective effect of Mg acetyltaurate (MgAT) on RGC death induced by NMDA.
Methods. Rats were divided into 5 groups and were given intravitreal injections. Group 1 (PBS group) was injected with vehicle; group 2 (NMDA group) was injected with NMDA while groups 3 (pre-), 4 (co-) and 5 (post-) treatment were injected with MgAT, 24 hours before, in combination or 24 hours after NMDA injection. NMDA and MgAT were injected in PBS at doses 160 and 320 nmol, respectively. Seven days after injection, the histological changes in the retina and optic nerve were evaluated. The extent of apoptosis in retinal tissue was assessed by TUNEL assay and Caspase-3 immunohistochemistry staining. The estimation of neurotrophic factor, oxidative stress, pro/anti- apoptotic factors and caspase-3 activity in retina was done using ELISA technique.
Results. The retinal morphometry showed reduced thickness of ganglion cell layer (GCL) and reduction in the number of retinal cells in GCL in NMDA group compared to the MgAT treated groups. TUNEL and Caspase-3 staining also showed increased number of apoptotic cells in inner retina. The results were further corroborated by the estimation of neurotrophic factor, oxidative stress, pro/anti- apoptotic factors and caspase-3 activity in retina.
Discussion. Current study revealed that intravitreal MgAT prevents retinal and optic nerve damage induced by NMDA. Overall, our data demonstrated that the pre-treatment with MgAT was more effective than co- and post-treatment. This protective effect of MgAT against NMDA-induced retinal cell apoptosis could be attributed to reduction of retinal oxidative stress and activation of BDNF-related neuroprotective mechanisms.

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Deferoxamine can reduce brain injury and enhance functional recovery in a rat model of endothelin-1 induced focal stroke.

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Introduction. Stroke is a leading cause of death and disability and there are currently limited treatment options for this devastating neurological condition. Increasing expression of the transcription factor - hypoxia-inducible factor-1 (HIF-1) and its target genes, including erythropoietin and vascular endothelial growth factor can protect against brain injury. Under normoxic conditions HIF-1α protein is degraded due to HIF-1 prolyl hydroxylase enzymes (PHDs) which cause ubiquitination and proteosomal degradation and consequently, constitutive levels of HIF-1α protein are almost undetectable. Hypoxia and PHD inhibitors can cause accumulation of HIF-1 and increase target gene expression. Previously, we have shown that pretreatment with hypoxia and a PHD inhibitor (deferoxamine (DFX)) can reduce brain injury and this protection is largely due to HIF-1 and its target genes.
Aims. Here we have examined whether DFX can reduce brain injury and enhance functional recovery when administered after a stroke.
Methods. Adult male Sprague-Dawley rats (300-350g) were anaesthetised with isofluorane (1.5%, via inhalation in oxygen) and a cannula implanted just above the middle cerebral artery. Rats were allowed to recover for 7 days, and endothelin-1(120pmol) or saline injected into conscious rats to produce stroke or sham rats. A single, intraperitoneal (i.p.) injection of DFX (200mg/kg, i.p) or saline vehicle (i.p.) was performed 6h after stroke or sham treatment. Behavioural testing was performed prior to cannula implantation and 1, 7 and 14 days after stroke induction. On day 14, brains were removed for histological analysis to evaluate lesion volume.
Results. There was minimal tissue loss in both sham groups (n=3, per group; (vehicle) 4±1.mm^3, (DFX) 3±1.mm^3). Stroke induced an infarct size that was significantly larger than both sham groups (48±14mm^3; n=5). Stroke rats that received DFX showed a significant reduction in the infarct size (10±3mm^3; n=5) compared with vehicle treated stroke rats ($p<0.05$, ANOVA, Tukeys post-hoc test). Likewise, rotarod activity was improved at 14 days following stroke in the DFX, compared to vehicle-treated stroke rats.
Discussion. Our findings indicate that modulation of HIF-1 and its target genes after a stroke is an effective neuroprotective strategy that also appears to improve functional recovery.

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Cortisol inhibits selected actions of inhaled corticosteroids (ICS) in human airway epithelium

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Introduction. Cortisol is essential for normal growth and differentiation of bronchial epithelium. Alterations in epithelial differentiation and functions contribute to chronic inflammation in asthma. Anti-inflammatory actions of glucocorticoids (GC) in epithelium include transactivation of selected genes and inhibition of synthesis/release of pro-inflammatory cytokines. However, GC resistance remains a major obstacle to the successful treatment. Whether the physiological GC, cortisol, can affect actions of ICS and contribute to the resistance in asthma, remains to be established.
Aims. To investigate the effect of cortisol (hydrocortisone) on anti-inflammatory actions of ICS using human airway epithelial cell model.
Methods. BEAS-2B cells were exposed to 1-10μM hydrocortisone (HC) 30 minutes before fluticasone propionate (FP) (1-10nM) treatment for 4h and 24h, at which time expression of GC-inducible genes was measured by RT-qPCR and protein expression was assessed by western blot. Furthermore, following GC treatment, cells were challenged with 10ng/mL TNFα for 24h, and levels of pro-inflammatory cytokines were measured using ELISA. Results. FP induced PLZF, GILZ and MPK-1 gene expression in BEAS-2B cells in concentration-dependent manner. However, pre-treatment with 1μM HC significantly decreased the maximum response to FP, which was most prominent for PLZF gene expression (45±4% of the response to 1nM FP, n=4). The effect of HC was even greater in PLZF protein expression (31±4%, n=4). Although 10nM FP greatly inhibited TNFα-induced granulocyte macrophage colony-stimulating factor (GM-CSF) release from BEAS-2B, 1μM HC significantly limited the inhibitory effect ($p<0.05$, n=4).
Discussion. Cortisol limits maximum induction of selected genes and repression of GM-CSF by ICS in human airway epithelium. As some of the beneficial actions of GC are mediated through these pathways, cortisol may limit the efficacy of existing treatments and contribute to GC resistance in asthma, particularly when its levels are elevated by stress.

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Assessment of cytosolic free calcium levels in MDA-MB-231 breast cancer cells undergoing cell death following treatment with ceramide and staurosporine

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Introduction. Investigating the contribution of altered calcium signalling to key cancer hallmarks such as cell death and migration has been hindered by the poor suitability of small-molecule fluorescent dyes for long-term assessment of cytosolic free Ca^{2+} ($[Ca^{2+}]_{CYT}$). Genetically-encoded calcium indicators such as GCaMP6m could potentially allow continual long-term measurement of $[Ca^{2+}]_{CYT}$ in breast cancer cells, revealing the nature of alterations in the intracellular calcium signal in processes relevant to breast cancer progression.
Aims. To assess $[Ca^{2+}]_{CYT}$ levels in GCaMP6m expressing MDA-MB-231 breast cancer cells treated with inducers of cell death.
Methods. GCaMP6m expressing MDA-MB-231 cells were treated with either staurosporine (1 µM) or ceramide (100 µM) and imaged using an ImageXpress Micro (Molecular Devices) every 5 min for 6 h at 37°C in HEPES buffered Fluorobrite™ DMEM media containing propidium iodide (1 µg/mL), as an indicator of cell death.
Results. Addition of staurosporine was associated with an initial increase in $[Ca^{2+}]_{CYT}$ and subsequent changes in cell morphology, followed by sustained $[Ca^{2+}]_{CYT}$ oscillations over the 6 h measurement period. A significant increase in $[Ca^{2+}]_{CYT}$ was identified in ceramide treated cells, with clear variability between individual cells in the time to reach maximal $[Ca^{2+}]_{CYT}$ levels. Peak $[Ca^{2+}]_{CYT}$ increases were greater in the subset (~10%) of ceramide treated cells that underwent cell death and in all cases increases in $[Ca^{2+}]_{CYT}$ preceded cell death. siRNA-mediated silencing of the global $[Ca^{2+}]_{CYT}$ regulator, plasma-membrane calcium ATPase 1 (PMCA1), modestly augmented both peak $[Ca^{2+}]_{CYT}$ increases and cell death.
Discussion. Ceramide and staurosporine treatment resulted in differential changes in $[Ca^{2+}]_{CYT}$ in MDA-MB-231 over 6 h. This work has also identified a relationship between PMCA1 and the $[Ca^{2+}]_{CYT}$ accumulation associated with ceramide-induced cell death in MDA-MB-231 breast cancer cells. Future work aims to investigate the influence of regulators of store-operated calcium entry on staurosporine-induced cell death given the observed sustained $[Ca^{2+}]_{CYT}$ oscillations in MDA-MB-231 breast cancer cells.

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CCL18 and reactive oxygen species are potential mediators of the pro-fibrotic actions of M2 macrophages

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Introduction. M2 macrophages contribute to vascular fibrosis and stiffening in hypertension (Moore et al., 2015). Potential mediators of these actions include reactive oxygen species (ROS) and the macrophage-derived, pro-fibrotic chemokine CCL18. Thus whilst oxidative stress is associated with cardiovascular remodelling and fibrosis (Barnes and Gorin, 2011), the ROS-generating capacity of M2 macrophages has not been thoroughly investigated. Moreover, pro-fibrotic actions of CCL18 are evident in the lung yet its role in cardiovascular settings is unknown.
Aims. To investigate the ROS-generating and pro-fibrotic capacity of M2 macrophages.
Methods. PDBu (10µmol/L)-stimulated ROS generation from human M1 (5-20ng/ml IFNγ + 10-100ng/ml lipopolysaccharide, 24-72h) and M2 (25ng/ml IL-4, 24-72h) macrophages was detected via L012-enhanced chemiluminescence ($^{\cdot}O_2^-$), and amplex red (H_2O_2) fluorescence. CCL18 was measured via qRT-PCR and ELISA. Type 1 collagen was measured via western blotting in CCL18 (3-300ng/ml, 72h)-treated human cardiac fibroblasts.
Results. PDBu increased superoxide levels to a similar extent in M1 and M2 macrophages (1.5-2-fold, $P<0.05$, n=6-7); an effect that was negated by Nox2 siRNA. Hydrogen peroxide generation was greater in M2 versus M1 macrophages (1.5-fold, $P<0.05$, n=5). IL-4 caused concentration- and time-dependent increases in macrophage CCL18 mRNA (up to 1700-fold, $P<0.05$, n=5-7) and protein (up to 500-fold, $P<0.05$, n=4-7) expression, which was attenuated by STAT3 inhibition ($P<0.05$, n=7). CCL18 caused a concentration-dependent increase in type 1 pro-collagen (up to 4-fold, n=4) and increased mature type 1 collagen (1.5-fold, n=4) in cardiac fibroblasts.
Discussion. M2 macrophages generate substantial levels of ROS and CCL18. We provide the first evidence that CCL18 increases collagen synthesis in human cardiac fibroblasts. Therapeutic targeting of M2 macrophages, and their generation of ROS and CCL18, represents a novel approach for the treatment of fibrosis during hypertension.

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Pannexin-1 channels and P2X7 receptors in a human colonic mucosa model of colitis: potential roles in inflammatory bowel disease

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Introduction. The pannexin-1 (Panx1) channels are found on many cell types and ATP released from these channels can act on nearby cells to activate purinergic P2X7 receptors (P2X7R). This interaction was shown in some tissues to mediate inflammatory processes such as release of cytokines (Pelegrin & Surprenant, 2006). Inflammatory bowel disease (IBD) is a condition characterised by colonic tissue damage and alterations in cytokine levels. It would be of interest to study Panx1 and P2X7R in IBD to reveal their potential roles, as there are limited studies in this area.
Aims. The aim of the present study was to investigate the function of Panx1 and P2X7R in an adapted model of cytokine-induced colitis in the mucosa layer of human colon (Nicotra et al., 2013).
Methods. Mucosal strips (4 × 10 mm) were incubated in carbogenated RPMI 1640 media containing 1% foetal calf serum and 1% penicillin-streptomycin over 16 h at 37°C. Cytokines TNFα and IL-1β (10ng/ml for both) were used to induce colitis. Panx1 channel blocker ^{10}Panx1 (100 μM) and P2X7R antagonist A438079 (100 μM) were used to block function. Zonula occludens-1 (ZO-1) tight junctions were studied using immunofluorescence.
Results. The colitis model showed tissue damage in the cytokine-only group and not in the control group (*P<0.01*). Crypt damage showed a statistically significant decrease in groups co-treated with inhibitors compared to the cytokine-only group (^{10}Panx1, *P=0.02*; A438079, *P=0.02*; ^{10}Panx1 + A438079, *P=0.04*). Tight junction protein ZO-1 was abundant in all control mucosa and reduced in the cytokine-only group (*P<0.01*). This was attenuated in the presence of ^{10}Panx1 (*P=0.04*).
Discussion. Panx1 and P2X7R were found to have an influence on tissue integrity in a human colonic mucosa model of colitis. The blockage of Panx1 and P2X7R reduced the inflammatory cytokine induced crypt damage and loss of tight junctions. Thus, Panx1 and P2X7R may have roles in causing mucosal damage, which is a common feature seen in clinical IBD. This process may involve extracellular ATP signalling similar to that observed in other tissues.

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Proteome profiling reveals phospho-cofilin1 as a candidate mediator of TGF-β1-induced glucocorticoid resistance

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Introduction: Glucocorticoid (GC) resistance limits GC effectiveness in treating respiratory diseases, including severe asthma and chronic obstructive pulmonary disease (COPD). Transforming growth factor-β1 (TGF-β1) induces resistance to GCs in airway epithelium. However, the mechanisms remain unknown. We undertook a systems-based approach to identify candidate signal transducers of the GC resistance induced by TGF-β1.
Methods: The effects of TGF-β1 on GC activity were measured using a GC response element (GRE)-controlled reporter and GC-inducible gene expression. The TGF-β proteome of BEAS-2B cells was identified using differential in-gel electrophoresis (2D DIGE) and LC-MS/MS. The potential role of candidate mediators was validated using siRNA and small molecule kinase inhibitors. Data are presented as the mean ±SEM for *n* independent experiments.
Results: TGF-β1 and TGF-β3 impaired GC activity, whereas TGF-β2 did not. Inhibition of well-known non-canonical kinase pathways ((extracellular-signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), p38 mitogen-activated protein kinases (p38MAPK), phosphoinositide 3-kinase (PI3K)) of TGF-β1 did not prevent TGF-β1 impairment. Of the 748 (±35, n=5) protein changes induced by TGF-β1, only 24 were not inhibited by the cocktail of the above kinase inhibitors. Of these 24 candidates only 4 were common to TGF-β1 and TGF-β3, with phospho-cofilin being prioritised based on prior evidence of induction of GC insensitivity. TGF-β1-induced phospho-cofilin was inhibited by the LIM kinase (LIMK) inhibitor, as was GC insensitivity. LIMK2 and coflilin1 siRNA attenuated TGF-β1 suppression of GC activity. The association of phospho-cofilin1 with phospholipase D (PLD) activates support on use of the selective PLD inhibitor, which prevented TGF-β1-induced GC insensitivity.
Conclusions: Proteomic analysis identified phospho-cofilin1 as a potential transducer of TGF-β1-induced GC insensitivity, and as a determinant of GC sensitivity.

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The gut hormone INSL5 activates multiple signalling pathways in the human enterocyte cell line NCI-H716

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Introduction. Insulin-like peptide 5 (INSL5) is a peptide hormone secreted from enteroendocrine L-cells together with GLP-1. Its cognate receptor is the relaxin family peptide receptor 4 (RXFP4), a GPCR predominantly expressed in the colorectum (Grosse et al, 2014). Although recognised as an appetite stimulant (Grosse et al, 2014), a recent study suggested that INSL5 also stimulated GLP-1 release from GLUTag L-cells (Luo et al, 2015), suggesting a possible autocrine/paracrine feedback system.
Aim. To elucidate the biological roles of INSL5 in the GLP-1 secreting L-cell lines, NCI-H716 and GLUTag cells.
Method. NCI-H716 and GLUTag cells were grown in RPMI or DMEM with 5% FBS. Gene expression was studied using Taqman RT-qPCR assay. Cell signaling was performed using AlphaScreen and Western blotting. cAMP accumulation was measured using LANCE HTRF kit. GLP-1 secretion was measured using active GLP-1 kit.
Results. *RXFP4* and proglucagon (*GCG)* but not *INSL5* mRNA expression was identified in NCI-H716 cells. In these cells, INSL5 activated p-ERK1/2, p-AKT (p-Ser473 and p-Thr308) and p-S6RP with peak response observed at 5 min, 15 min and 30 min, respectively ($p<0.001$). In the same cells, INSL5 concentration-dependently inhibited forskolin-stimulated cAMP accumulation. Concentration-response curves revealed comparable pEC_{50} values for the pathways investigated (see table). However, INSL5 treatment did not affect GLP-1 secretion from NCI-H716 cells. In GLUTag cells little to no *Rxfp4* expression was detected in contrast to the previous study (Luo et al, 2015). Moreover GLUTag cells were unresponsive to INSL5 treatment in p-AKT and p-S6RP assays.
Discussion. NCI-H716 cells endogenously express RXFP4 and are responsive to INSL5 although the peptide does not appear to regulate GLP-1 secretion in these cells. This coupled with the lack of RXFP4 expression in GLUTag cells suggests that the roles of INSL5 in enterocytes have yet to be determined.

	p-ERK1/2 (5 min, n=5)	AKT p-Ser473 (15 min, n=5)	AKT p-Thr308 (15 min, n=5)	p-S6RP (30 min, n=5)	cAMP (30 min, n=5)
E_{max} (% basal)	154.3±7.86	259.2±5.6	162.4±6.0	115.2±1.98	51.16±5.4
pEC_{50}	8.4±0.45	7.9-±0.1	8.0±0.23	7.9±0.33	8.0±0.26

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GPR37L1: an orphan G protein-coupled receptor contributing to sex differences in blood pressure regulation

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Introduction. Over 100 mammalian G protein-coupled receptors (GPCRs) are yet to be matched with endogenous ligands; these so-called 'orphans' offer hope as novel drug targets in treatment of disease. GPR37L1 is one such orphan; abundant in the CNS and shown by our laboratory to be a novel protease-inactivated GPCR (Coleman et al, 2016). A previous study suggests GPR37L1 to regulate BP in mice (Min et al, 2010) yet this work lacked vital controls. We investigated GPR37L1 more thoroughly, hypothesising it is a CVS regulator with a role in BP control.
Aim. To investigate the effects of global GPR37L1 deletion on the CVS and to profile receptor expression.
Methods. Male/female GPR37L1 wild-type (WT) and knockout (KO) mice were subject to BP phenotyping (via invasive catheterisation under anaesthesia or conscious radiotelemetry) and cardiac hypertrophy assessment (via weighing of whole heart, left/right ventricle tissue). Receptor expression was investigated using immunohistochemistry of GPR37L1-lacZ tissue and immunoblotting of WT tissue.
Results. The previously described hypertensive phenotype of KO mice was limited to females, which had significantly elevated systolic (+11.6±3.9 mmHg, $n\geq13$, $p\leq0.05$) and diastolic BP relative to WT, with no difference in heart rate. Crucially, we have validated this phenotype using radiotelemetry in conscious mice. Contrasting previous reports of GPR37L1 mRNA in heart/kidney, we could not detect the GPR37L1-lacZ reporter *protein* in either organ. We observed an abundance of GPR37L1 in the brain, especially in astrocytes and Bergmann glia.
Discussion. The CNS expression pattern and female-specific phenotype of GPR37L1 suggests it to be a hitherto unknown contributor to both central CVS regulation and the sexual dimorphism of BP control.

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Pickpocketing pharmacological neighbours reveals ligands for orphan receptors

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Introduction. Over 100 orphan G protein-coupled receptors (GPCRs) lack ligands to probe their function. Many synthetic ligands display polypharmacology but phylogenetic analyses do not always accurately predict their likely off-targets (Gloriam et al, 2009; Lin et al, 2013). Predicting this between known and orphan GPCRs is invaluable.
Aims. To predict pharmacological similarities between GPCRs through refined binding site sequence analysis accounting for residue:ligand interaction strength, and to predict ligands for orphan GPCRs.
Methods. We developed GPCR-Contact-Informed Neighbouring Pocket (CoINPocket): positional sequence similarity was weighted by the ligand:residue contact strengths observed in GPCR crystal structures. Relationships based on this metric were built for all GPCRs. Experimental validation was performed in reporter gene assays.
Results. The GPCR-CoINPocket arrangement of GPCRs recapitulated features of the ligand similarity organisation of GPCRs. In benchmarking studies, GPCR-CoINPocket showed better recognition of known GPCR ligand similarities (described in ChEMBL) compared to previous unweighted measures. Orphan receptor GPR37L1 was predicted to be pharmacologically similar to the bombesin, orexin and neuropeptide S receptors and not to the phylogenetically related endothelin receptors. Three ligands from these receptors displayed inverse agonism at GPR37L1 in the cAMP reporter assay (30% hit rate), demonstrating the utility of GPCR-CoINPocket.
Discussion. Predicted pharmacological similarity between known and orphan GPCRs can guide the search for potential ligands. These initial ligands act as a stepping-stone to explore the therapeutic potential of orphan receptors; the ligands can be used to refine homology models for virtual ligand screening and to facilitate parallel *in vitro* and *in vivo* studies of orphan GPCRs. GPCR-CoINPocket can be applied to any orphan GPCR of interest.

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The potential role of a novel endogenous allosteric modulator of muscarinic acetylcholine receptors

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Introduction. There is now emerging evidence that endogenous modulators can target G protein-coupled receptor (GPCR) allosteric sites (van der Westhuizen et al., 2015). Although the (patho)physiological roles of many endogenous GPCR allosteric modulators are poorly understood, major basic protein (MBP) has previously been identified as a putative negative allosteric modulator of neuronal auto-inhibitory M_2 muscarinic receptors (mAChRs) in the airways (Jacoby et al., 1993). We hypothesised that other highly basic endogenous proteins, such as the antimicrobial peptide, LL-37 that is involved in wound healing, chemotaxis, maturation of immune cells or, in some cases, cell death (Kahlenberg et al. 2013), can also interact allosterically with mAChRs.
Aims. To characterise the pharmacological properties of LL-37 at human mAChRs.
Methods. Using CHO cells stably expressing the M_1-M_3 mAChRs, we performed [^{3}H]NMS radioligand binding and G protein activation-mediated functional studies ([35S]GTPγS binding and IPone accumulation assay) to assess the putative allosteric effects of LL-37.
Results. LL-37 mediated a concentration-dependent but partial (saturable) inhibition of [^{3}H]NMS specific binding at all three M_1-M_3 mAChRs (pK_B=5.2±0.2, 4.9±0.3, and 5.3±0.1, respectively). Additionally, LL-37 negatively modulated ACh-mediated G protein activation at all M_1-M_3 mAChRs, completely abolishing responses to the endogenous orthosteric agonist.
Discussion. Our results suggest that LL-37 is an allosteric modulator of the M_1-M_3 mAChRs, negatively modulating ACh function. These mAChRs are expressed on various neuronal and non-neuronal cells, including immune cells, epithelial cells, and endothelial cells, and known to be involved in their survival outcome. The blockade of mAChR activity by LL-37 could therefore have unappreciated physiological and pathological consequences.

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Tacrine is a novel allosteric modulator of the β2-adrenoceptor

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Introduction. Tacrine is a small molecule that is a known allosteric modulator of the muscarinic acetylcholine receptors (mAChRs) and α_1-adrenoceptors. The only synthetic compound known to modulate the β_2-adrenoceptor (β_2AR) is THRX100361, which is also an antagonist at mAChRs. As the β_2AR shares homology with the mAChRs, we hypothesised that tacrine may be an allosteric modulator of the β_2AR.
Aims. To determine the allosteric effects of tacrine at the β_2AR, and characterise its binding site.
Methods. COS-1 membranes expressing the wildtype β_2AR were used to determine the affinity of tacrine and its effects on [^{3}H]dihydroalprenolol (DHA) dissociation and association kinetics. Functional assays using the cyclic AMP (cAMP) bioluminescent resonance energy transfer biosensor CAMYEL were used to further assess the allosteric effects of tacrine on agonist signalling. Tacrine was docked into the inactive and active state crystal structure of the β_2AR to identify potential interactions with an allosteric site. Based on these predictions, human β_2AR mutants were constructed and the modulatory effects of tacrine were characterised at these mutant receptors.
Results. Tacrine bound with low affinity to the β_2AR with a pK_{iApp} value of 5.0±0.2 (n = 3). At 10μM, tacrine significantly slowed the dissociation of [^{3}H]DHA (K_{obs}/K_{off} = 0.67±0.04, n = 3 $P<0.05$). cAMP accumulation was only affected by high concentrations of tacrine (300μM), which decreased the potency of isoprenaline by 2.5 fold (n = 6-7, $P<0.05$) and adrenaline by 5 fold (n = 8, $P<0.05$). In silico docking revealed interactions with multiple residues in the extracellular region of the β_2AR corresponding to residues that are involved in allosteric ligand binding at the mAChRs. Alanine substitution of these residues reduced the apparent affinity of tacrine for the β_2AR, decreased the ability of tacrine to modulate the binding of [^{3}H]DHA, and also reduced the ability to modulate isoprenaline induced activation of the receptor.
Discussion. We have shown that tacrine is a modulator of the β_2AR that interacts with the extracellular region of the β_2AR. The implicated residues of the β_2AR allosteric site are homologous with the $mAChR_{2/3}$ allosteric site, suggesting that a conserved allosteric site exists between these two receptors.

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Functional analysis of novel allosteric modulators at the M_1 muscarinic acetylcholine receptor

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Introduction. The M_1 muscarinic acetylcholine receptor (mAChR) is a G protein-coupled receptor (GPCR) expressed predominantly in the brain and its activation has been clinically proven to improve cognition in patients with Alzheimer's disease and Schizophrenia (Bodick et al., 1997; Carruthers et al., 2015; Foster et al., 2014). However, selective targeting of this receptor has remained suboptimal until the recent discovery of novel M_1 mAChR positive allosteric modulators (PAMs).
Aims. To synthesise and characterise novel M_1 mAChR allosteric modulators.
Methods. 52 novel compounds were synthesised and tested in recombinant FlpInCHO cells expressing the wild type and selected mutants of the M_1 mAChR using a cell-based functional assay of inositol phosphate 1 (IP1) accumulation. 5 lead compounds from the initial screening were selected for further characterisation on the Y82A, Y179A, F77I, and W400A M_1 mAChR mutants (Abdul-Ridha et al., 2014; Keov et al., 2014).
Results. Compounds #7, #22, #32, #34, #52 were representative compounds of the different scaffolds that had minimal allosteric agonist activity, but retained the ability to potentiate the actions of the endogenous agonist, acetylcholine (ACh). The novel PAMs potentiated ACh only on the wild type M_1 mAChR and the F77I mutant. No potentiation of the ACh-mediated IP1 response was observed in the Y82A, Y179A or W400A mutants.
Discussion. This study suggests that all the new PAMs bind to and regulate signalling of the M_1 mAChR via the classical allosteric binding site but are not bitopic in nature.

Abdul-Ridha A et al (2014) J Biol Chem 289:33701-33711
Bodick NC et al (1997) Arch Neurol 54:465-473
Carruthers SP et al (2015) Neurosci Biobehav Rev 55:393-402
Foster DJ et al (2014) Neuropsych Dis Treat 10:183-191
Keov P et al (2014) J Biol Chem 289:23817-23837

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Structure-Function Analysis of Orthosteric and Allosteric Ligand Binding at the Adenosine A_1 Receptor

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Introduction. The adenosine A_1 receptor (A_1AR) is an attractive therapeutic target for a range of cardiovascular and neuronal disorders (Jacobson *et al,* 2006). However, it remains sub-optimally targeted by both orthosteric and allosteric ligands. To facilitate the rational design of more selective and efficacious A_1AR therapeutics, greater structural knowledge of the orthosteric and allosteric binding sites is required. The current study has focussed on extracellular loop 2 (ECL2), as this region has previously been suggested to influence A_1AR pharmacology.
Aim. To establish the role of the A_1AR-ECL2 on orthosteric and allosteric ligand binding and function using a combination of mutagenesis, quantitative analytical pharmacology and molecular modeling
Methods. Mutant A_1ARs containing single alanine substitutions were expressed in FlpINCHO cells. Radioligand binding and cAMP accumulation assays were used to quantify orthosteric (DPCPX, NECA) and allosteric (PD81723, VCP171) ligand affinity, efficacy and cooperativity. Molecular modeling was performed using A_1AR 3D homology models based on inactive and partially active A_{2A}AR structures (PDB ID: 3EML and 3QAK).
Results. Mutations proximal to a conserved ECL2-TM3 disulphide bond selectively impacted orthosteric ligand affinity, whereas a cluster of five residues near the TM4-ECL2 juncture influenced orthosteric agonist efficacy. Substitution of $E172^{ECL2}$ for alanine reduced the affinity of both modulators. Positive cooperativity between PD81723 and NECA was reduced upon alanine substitution of a number of ECL2 residues, including $E170^{ECL2}$, and $K173^{ECL2}$, whereas mutation of $W146^{ECL2}$ and $W156^{ECL2}$ decreased VCP171 cooperativity with NECA. Molecular modeling localized a likely allosteric pocket to an extracellular vestibule that overlaps with a region utilized by orthosteric ligands as they transition into the canonical A_1AR orthosteric site within the transmembrane bundle.
Discussion. ECL2 contributes to the molecular mechanisms governing orthosteric and allosteric ligand pharmacology. $E172^{ECL2}$ is a key allosteric ligand-binding determinant, whereas hydrogen-bonding networks within the extracellular vestibule may facilitate the transmission of cooperativity between orthosteric and allosteric sites.

Jacobson K et al (2006) Nat Rev Drug Discov 5:247-264

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Characterising cellular uptake and distribution of a membrane-anchored Neurokinin 1 Receptor antagonist

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Introduction. Stimulation of the neurokinin 1 receptor (NK_1R) mediates receptor internalisation into endosomes and initiation of endosomal signalling profiles that are distinct from those at the cell surface. Endosomal signalling is associated with central pain transmission. Hence, antagonists that selectively target receptors in endosomes may be therapeutically advantageous. We have recently demonstrated that lipidated NK_1R antagonists inhibit endosomal signalling, yet it is unknown precisely how or where these ligand-receptor interactions occur.
Aims. Characterise cellular uptake and distribution of fluorescently labelled lipid-conjugated compounds.
Methods. Cyanine-5 fluorophore conjugated to spantide (Span-Cy5), cholestanol (Cy5-Chol), spantide and cholestanol (Cy5-Span-Chol), or a PEG-ethyl ester control (Cy5-EE) were synthesised in-house. Localised ligand concentrations in HBSS buffer preparations were accurately determined by fluorescence correlation spectroscopy (FCS). Live-cell confocal microscopy was performed in HEK293 cells and primary neurons.
Results. At 200um above the coverslip (nominal concentration = 10nM) Cy5-Chol and Cy5-EE were measured at 0.6±0.1 nM and 3.1±0.7 nM respectively. This increased to 34±5.9 nM and 67±28 nM, when measured 2um above the coverslip (nominal concentration = 5nM, n=3). Distributions were improved with BSA. Cellular uptake of Cy5-Span-Chol was greater than soluble Cy5-Span. The distribution of Cy5-Span-Chol was predominantly endosomal in HEKs and neurons, with significantly greater plasma membrane association observed in NK_1R-expressing cells. Discussion. FCS data provides valuable insights into actual drug concentrations. Despite strong deviations in expected concentrations of compound preparations, the lipophilicity enhanced cellular uptake and endosomal targeting of an otherwise soluble drug. The altered Cy5-Span-Chol intracellular distribution in NK_1R-positive cells confirms that drug-receptor interactions occur. We propose that lipidated antagonists inhibit signalling within endosomes or by preventing receptor internalisation.

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Molecular Determinants of Amylin Receptor Agonism

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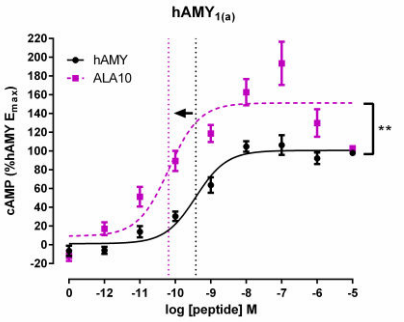
Introduction. Amylin is a glucoregulatory and satiety-inducing hormone produced in the pancreatic β-cells with clinical relevance towards diabetes and obesity. Amylin is a Family B G protein-coupled receptor peptide ligand proposed to activate its receptor(s) via a two-domain model whereby the peptide N-terminus is important for activation, particularly the N-terminal "activation loop". However, there is no data investigating the contribution of individual N-terminal residues of human amylin on receptor activation. In order to fully exploit the potential of the amylin system and further amylinmimetic drug development efforts, investigation into how the peptide structure translates to function is needed.



Aims. Synthesise single alanine or glycine analogues of the N-terminus of human amylin 1-17 and test pharmacological profiles *in vitro*.
Methods. Peptides were made using solid-phase peptide synthesis and tested in cultured Cos-7 cells transiently transfected with three amylin-responsive receptors for cAMP production using a LANCE assay to obtain pEC_{50} and E_{max} values. Binding assays were done for reduced-activity analogues by competing off radiolabelled I^{125}-hαCGRP to determine pIC_{50} affinity values.
Results. Several single-residue analogues displayed reduced activity, highlighting their importance in receptor binding and activation, however others unexpectedly presented with increased potency and E_{max} as shown in the above figure with the leftward shift in the concentration-response curve outlined with a black arrow.
Discussion. Residues beyond the activation loop were important for both activation and binding, highlighted by reduced pEC_{50} values. The increased-activity analogues outline the potential for single-residue replacements as a potential starting point for improved amylinmimetics.

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Ethanol-oxytocin interactions at homomeric glycine receptors expressed in Xenopus laevis oocytes

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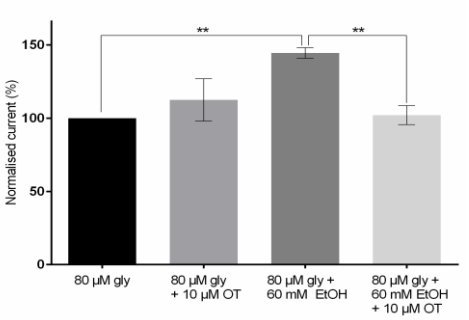
Introduction. Alcohol is one of the most widely used drugs, yet its targets in the brain have not been reliably established. Glycine (Gly) receptors have been implicated in ethanol's (EtOH) effects on motor coordination and reward pathways. While the neuropeptide oxytocin (OT) has been shown to attenuate EtOH-induced potentiation of extrasynaptic $GABA_A$ receptors in *Xenopus laevis* oocytes, and to counteract sedation and ataxia in severely intoxicated mice (Bowen *et al.,* 2015), its effects at Gly receptors have not yet been characterised.



Aims. To investigate the effects of ethanol and oxytocin at homomeric α1 and α2 glycine receptors.
Methods. Two-electrode voltage clamp recording from *Xenopus laevis* oocytes was used to evaluate the effects of EtOH and OT at human recombinant glycine receptors.
Results. At α2 Gly receptors, co-application of 60 mmol/L EtOH significantly potentiated 80 µmol/L Gly-gated current by 144%±4 (n = 5, P<0.002; One-way ANOVA with Sidak's multiple comparison test). Pre-treatment and co-application of 10 µmol/L OT abolished this potentiation, with Gly-gated currents significantly reduced by 42%±10 (n = 5, P<0.002; One-way ANOVA with Sidak's multiple comparison test). Individually, neither ethanol nor oxytocin activated the receptors, and oxytocin did not modulate currents generated by glycine in the absence of EtOH (Figure). Similar effects were observed with α1 glycine receptors.
Discussion. OT inhibited the effect of EtOH-induced enhancement of glycine currents, potentially contributing to the mechanism by which OT attenuates the intoxicating effects of EtOH in mice (Bowen *et al.,* 2015). The binding site for OT on Gly or $GABA_A$ receptors has not been identified. Future studies are required to identify this site.

Bowen MT et al (2015) Proc Natl Acad Sci U S A 112:3104-3109.

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Elucidating the mammary stem cell hierarchy: insights into breast cancer heterogeneity

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Introduction. The mammary gland undergoes cycles of growth, death and regeneration throughout reproductive life, a process that requires long-lived mammary stem cells. Whilst recent genetic fate-mapping studies, using promoters specific for either the luminal or basal epithelial cell lineages, have provided valuable insights into the mammary epithelial hierarchy, controversy still remains as to the true differentiation potential of adult mammary stem cells and whether there are distinct populations. As presumptive targets for transformation in breast cancer, the identity of adult mammary stem and progenitor cells, and the origin of luminal and basal cell lineages, have important implications for breast cancer and tumour heterogeneity.
Aims. To determine the capacity and differentiation potential of mammary stem cells during puberty and pregnancy.
Methods. We combined the use of a novel mouse model that enables genetic labelling of a single, random cell and all of its progeny *in situ* ($R26^{[CA]30}$ reporter mice) with tissue clearing and 3D confocal imaging. $R26^{[CA]30}$ mice have a dinucleotide repeat tract, $[CA]_{30}$, downstream of the translational start site of an out-of-frame reporter gene (EYFP or modified β-glucosidase) inserted in the constitutively expressed Rosa26 locus. The inherent instability of the microsatellite repeat resulted in rare, spontaneous frame-shift mutations during DNA replication, which could place the reporter gene in-frame, resulting in its expression. Tissue clearing and advanced 3D imaging enabled all of the progeny of a single labelled cell to be visualized, traced and analyzed using custom-built 3D imaging algorithms.
Results. Individual clones that arose from a single mammary stem cell could be visualized in their entirety, revealing that clonal progeny contributed exclusively to either the luminal or basal cell lineages and were distributed sporadically to branching ducts during puberty or alveoli during pregnancy. A quantitative analysis suggested that pools of proliferative unipotent stem cells contribute to adult mammary gland development.
Discussion. This study has demonstrated the capacity of a single, unipotent, adult mammary stem cell to contribute to normal breast development. A greater understanding of mammary stem cell traits will reveal novel strategies to target these cells for the treatment and/or prevention of breast cancer.

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Metabolism of the Australian medicinal plant compound polyandric acid A: in silico and in vitro approaches

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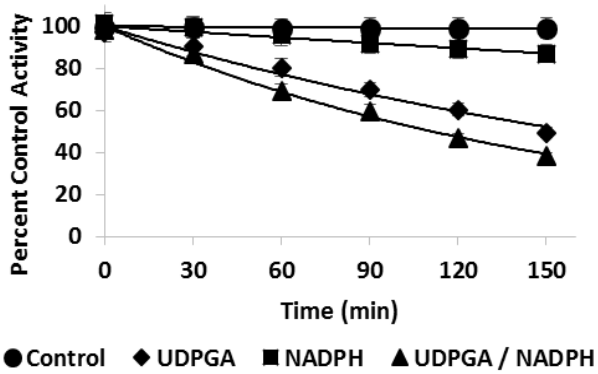
Introduction. Polyandric acid A (PAA) is an active anti-inflammatory component of the Australian medicinal plant *Dodonaea polyandra.*
Aims. Investigate through *in silico* and *in vitro* approaches, the enzymology of the metabolism of PAA and its de-esterified alcohol (PAAH).
Methods. Metabolism of PAA and PAAH were investigated *in vitro* by incubation with human liver microsomes (HLM), cytosol and recombinant enzymes in the presence of appropriate enzyme co-factors and selective inhibitors where applicable. Metabolism was assessed by the substrate depletion method. Structures of major glucuronide metabolites were determined by LC-MS/MS analysis. *In silico* methods included molecular overlays, protein docking and use of predictive models. Results are presented as Mean±SD.



Results. Hydrolysis of PAA occurred upon incubation with HLM ($t_{1/2}$, 67.0±7.86 min, n=3). Incubations of PAAH with HLM in the presence of UGT and CYP cofactors resulted in significant depletion, with UGT-mediated depletion as the major pathway ($CL_{int,UGT}$, 154±12.2 µL/min.mg, $CL_{int,CYP}$ 636±41.6 µL/min.mg, n=4). Reaction phenotyping revealed UGT 1A1 and 2B7 and CYP 2C9 and 3A4 as the major enzymes involved in the metabolism of PAAH, in agreement with predictions derived from *in silico* models. LC-MS/MS analysis identified glucuronide metabolites with a major acyl glucuronide metabolite, which was also in agreement with modelling.
Discussion. *In silico* predictions were found to be in good agreement with *in vitro* findings and the results represent the first systematic study of the metabolism of an active constituent of an Australian Aboriginal medicinal plant.

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Preclinical model to identify adverse geriatric outcomes from polypharmacy and Drug Burden Index

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Introduction. Polypharmacy (use of ≥ 5 drugs) is common in older people. Polypharmacy with increasing Drug Burden index (DBI; measure of patient's total exposure to anticholinergics and sedatives) is associated with impaired physical function, frailty, falls, hospitalization and mortality in older people. A preclinical model of polypharmacy would be a useful toxicological screening tool to evaluate drugs to guide clinical prescribing.
Aims. Establish a model to determine the effect of polypharmacy and increasing DBI on adverse geriatric outcomes.
Methods. Young (3 months) male C57BL/6 mice were treated with standard base diet for 2 weeks. This was followed by 4 weeks of control or treated feed/water containing therapeutic drug levels grouped into Zero DBI (simvastatin, metoprolol, omeprazole, paracetamol, irbesartan), Low DBI (simvastatin, metoprolol, omeprazole, paracetamol, citalopram), High DBI (simvastatin, metoprolol, oxybutynin, oxycodone, citalopram) and single drug groups: simvastatin, metoprolol, oxybutynin, oxycodone and citalopram (n=6/ group). A panel of functional tests was conducted at baseline and during drug treatment, and sera was collected when animals were euthanized.
Results. Animals tolerated the diet well and did not display overt signs of toxicity based on serum toxicity marker assessment. Based on food/ water intake, drug intake was within therapeutic doses (±30%). Increasing DBI significantly decreased locomotor activity (Distance, mobility, max speed and centre exploration time) but did not change grip strength (wire hang), muscle endurance (rotor rod) or frailty index score. Chronic treatment with each drug in the High DBI regimen individually did not cause any significant changes in function.
Discussion. We have established a preclinical model to determine the effects of polypharmacy and DBI. Increasing DBI causes significant declines in locomotor activity in young mice. Future research is required to determine whether serum drug levels associated with DBI correlates with increased adverse geriatric outcomes and confirm these findings in older mice with longer treatments to better replicate clinical drug usage. This will facilitate the development of an optimal preclinical polypharmacy and DBI model.

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Therapeutic Drug Monitoring (TDM) of Vancomycin

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Introduction. Vancomycin has an important role in treating infections caused by methicillin-resistant *Staphylococcus aureus*. Therapeutic drug monitoring (TDM) of vancomycin therapy is recommended due to its narrow therapeutic index. St Vincent's Hospital (SVH) guidelines suggest trough concentrations of 15-20 mg/L be targeted to maximise efficacy and minimise toxicity. This acts as a surrogate for the target $AUC_{0\text{-}24}$/MIC ≥ 400 h (Rybak et al, 2009).
Aims. To describe vancomycin prescribing patterns at SVH relative to dosing guidelines and evaluate the attainment of target $AUC_{0\text{-}24}$/MIC using the Bayesian-based computer software, DoseMe (DoseMe Pty Ltd, Brisbane QLD).
Methods. Dosing history, patient demographics, and confounding variables for vancomycin associated acute kidney injury (AKI) were collected prospectively over a 3 month period at SVH using both the electronic data capture system and paper chart records. Loading doses, collection of trough concentrations, and occurrences of AKI were assessed. Data was entered into DoseMe to generate $AUC_{0\text{-}24}$/MIC values. It was assumed that the MIC was 1 mg/L.
Results. Prescriptions of vancomycin (n=1207) and serum samples (n=461) were collected from 159 patients. Loading doses were not in accordance with guidelines in the majority (84.3%) of cases. Trough concentrations were rarely collected at the correct time (4.3% of cases that had TDM done). A proportion of patients (10.6%) experienced AKI, which was significantly associated with $AUC_{0\text{-}24}$/MIC ≥ 700 h ($P<0.05$).
Discussion. There was poor compliance to vancomycin dosing guidelines at SVH. The suggested trough concentrations correlated poorly with the calculated $AUC_{0\text{-}24}$/MIC ratios. It is suggested that the vancomycin guidelines recommend targeting $AUC_{0\text{-}24}$/MIC ≥ 400 h estimated using a Bayesian forecasting tool instead of direct measurements of trough concentrations.

Rybak M et al (2009) Am J Health Syst Pharm 66:82-98.

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Potential drug interactions with novel oral anticoagulants in elderly hospital inpatients

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Introduction. The novel oral anticoagulants (NOACs) are an attractive alternative to warfarin in selected patients because regular INR monitoring is not required. However, elderly patients often take multiple drugs with NOACs which increases the risks of therapeutic failure and bleeding. Currently, the extent of potential pharmacodynamic and pharmacokinetic drug-drug interactions (DDIs) with NOACs in clinical practice is not well understood.
Aim. To determine the prevalence of potential DDIs with NOACs in elderly hospital inpatients.
Methods. Inclusion criteria were: > 65 years of age, on apixaban, rivaroxaban or dabigatran, and admitted to the Repatriation General Hospital between April 2014 and July 2015. A list of clinically relevant DDIs with NOACs was compiled from product information, the Australian Medicines Handbook, the National Prescribing Service, and state guidelines. The prevalence and nature of potential DDIs with NOACs in the study population was then determined from inpatient medication charts.
Results. There were 122 patients in the study with a mean age of 86 years (48.4% male and 51.6% female). The mean creatinine clearance was 44.6 mL/min and the mean CHA_2DS_2-VASc score was 4.83. Most patients had atrial fibrillation and were taking NOACs to prevent thrombotic stroke (82.8%). Forty-nine patients (40%) were on rivaroxaban, 50 (41%) on apixaban and 23 (19%) on dabigatran. Overall, 45 patients (37%) had a total of 53 potential DDIs. Thirty five patients had potential pharmacodynamic DDIs with SSRIs/SNRIs, NSAIDs and antiplatelets (35/122, 29%). Seventeen patients had potential pharmacokinetic DDIs (17/122, 14%). Of these, 65 % (11/17) were taking medications that increase NOAC plasma concentrations (e.g., amiodarone, erythromycin, diltiazem, verapamil and cyclosporine), and 35% (6/17) were taking medications that decrease NOAC plasma concentrations (e.g., carbamazepine, primidone and phenytoin). There were no cases of patients on contraindicated interacting medications.
Discussion. Potential DDIs with NOACs in elderly hospital inpatients are relatively common (~1/3), particularly those that increase the risk of bleeding. Prescribers and pharmacists should consider carefully the risk:benefit of NOACs in elderly patients on polypharmacy.

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The pharmacokinetics and pharmacodynamics of febuxostat

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Introduction. Febuxostat is a hypouricaemic, xanthine oxidoreductase (XOR) inhibitor registered for the treatment of gout. The time to achieve full recovery of serum urate (SU) concentrations following a single dosage of febuxostat has not been reported.
Aims. To follow the concentration-time profiles of SU and plasma febuxostat over a period of ≥ 72 hours.
Methods. Two healthy male subjects were administered a single dose of febuxostat (80 mg) under fasting conditions. Plasma concentrations of febuxostat and SU were measured pre-dose and at 1, 1.5, 2, 4, 6, 9, 24, 30, 36, 48 and ≥ 72 h after dosing. Plasma concentrations of febuxostat were determined by HPLC assay with fluorescence detection.
Results. Initially, SU concentrations declined more slowly relative to plasma concentrations of febuxostat. SU then recovered gradually. In one subject, SU did not return to pre-treatment concentrations one week after febuxostat administration (Fig 1). The response of urate to febuxostat exhibited a negative hysteresis.

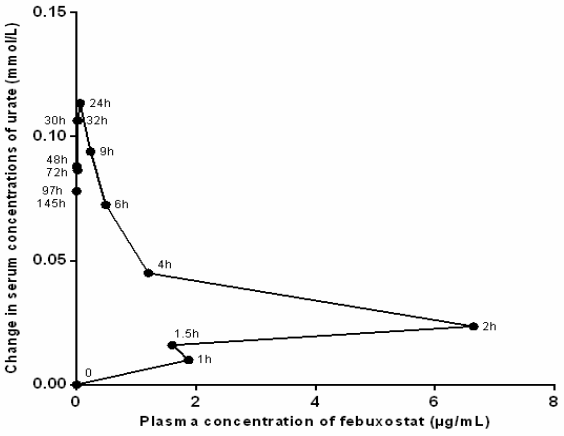

Fig 1. Change in concentrations of urate and febuxostat over 1 week after a single 80 mg dosage of febuxostat

Discussion. Febuxostat had a sustained hypouricaemic effect. The negative hysteresis is most likely due to the time to clear the urate already present. An additional factor may be the time taken for full recovery of XOR. The time course of inhibition and recovery of XOR activity *in vivo* with febuxostat requires further investigation.

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Extraction of Metformin During Haemodiafiltration

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Introduction. Type 2 diabetes mellitus patients (T2DM) with end-stage kidney disease (ESKD) who receive haemodiafiltration (HDF) may benefit greatly from the cardioprotective effects of the anti-hyperglycaemic agent metformin. However, a detailed understanding of metformin removal during HDF is required so that dosing may offset any risk of lactic acidosis.
Aims. To estimate the extraction of metformin during HDF in patients with T2DM.
Methods. Patients (n=4) received 500 mg of metformin (IR) after each HDF session (thrice weekly; 1500 mg/week) for 12 weeks. Patients were intensely sampled (paired blood samples; entering and exiting the haemodiafilter) during HDF sessions at 6 study visits. On 3 occasions, blood samples were also collected between HDF sessions to assess any endogenous clearance of metformin. Metformin concentrations were measured by HPLC.
Results. Mean extraction ratios and clearances of metformin from plasma for each patient ranged from 0.56-0.90, and 123-179 mL/min, respectively. Mean extraction ratios of metformin from erythrocytes for each patient ranged from -0.15-0.28. Metformin concentrations were generally constant between HDF sessions indicating minimal endogenous clearance.
Discussion. Metformin was extensively cleared from plasma, but not from red blood cells. This is in keeping with the physical properties of metformin (low molecular weight; lack of protein binding), and the very slow movement of metformin into and out of erythrocytes ($t_{1/2}$ ~ 20 hours). Minimal endogenous clearance of metformin was observed in these patients, which is consistent with metformin being totally cleared by the kidney. These important findings indicate that metformin dosing can be titrated to safe concentrations to match its extraction during HDF treatment in patients with T2DM and ESKD.

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Cannabinoid Interactions with Gabapentin in an Animal Neuropathic Pain Model

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Introduction. Clinical studies have suggested that the psychoactive ingredient of *Cannabis sativa*, D9-tetrahydrocannabinol (THC), may have efficacy in neuropathic pain states (Abrams et al, 2007). However, the efficacy and side-effect profile of THC is problematic. It has been proposed that THC might be used as an adjuvant.
Aims. We examined whether THC enhanced the actions of a major neuropathic pain medication, gabapentin, in a nerve injury induced model of chronic pain.
Methods. Ethics approval was from Royal North Shore Hospital Animal Ethics Committee. Adult male C57BL6 mice underwent chronic constriction of the sciatic nerve (CCI) under isoflurane anaesthesia (2% in saturated oxygen). Mechanical and cold allodynia was measured as the mechanical paw withdrawal threshold (PWT) and responses to acetone application to the operated hind paw. Motor incoordination, catalepsy and sedation were measured using the rotarod, bar test, and dark open field, respectively. The effect of acute drug administration was tested at 8 days post-CCI (volume of 0.01ml per g in saline with 10% dimethylsulphoxide and 5% Tween 80).
Results. Both THC and gabapentin produced a dose dependent reversal of mechanical and cold allodynia. THC, but not gabapentin produced dose dependent motor incoordination, catalepsy and sedation. In combination, THC and gabapentin reversed mechanical and cold allodynia with ED50s less than that predicted for an additive effect. In combination, THC and gabapentin produced side-effects with a similar profile to that of THC alone.
Discussion. This data indicates that THC and gabapentin act synergistically to reduce the allodynia associated with an animal model of neuropathic pain. By contrast, the side-effects of combination treatment are similar to those predicted for THC alone. Thus, THC represents a potential adjuvant in the treatment of neuropathic pain.

Abrams DI, Jay CA, Shade SB, Vizoso H, Reda H, Press S*, et al.* (2007). Cannabis in painful HIV-associated sensory neuropathy: a randomized placebo-controlled trial. Neurology 68**:** 515-521.

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Mechanism-based PK/PD Modelling Approach to Optimise Synergistic Combinations against Multidrug-resistant *Pseudomonas aeruginosa* (Pa) and *In-vivo* Evaluation in a Murine Thigh Infection Model

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Introduction. Carbapenem-resistant Pa are highly challenging clinically and effective early therapy is likely critical for treatment success.
Aims. We aimed to rationally optimize carbapenem + aminoglycoside (AGS) combination dosage regimens *via* mechanism-based modelling (MBM) and to prospectively evaluate them in a murine thigh infection model.
Methods. We performed MBM, based on data from *in vitro* static concentration time-kill studies, and Monte Carlo simulations to optimise combination dosage regimens against a double-resistant clinical Pa isolate (FADDI-PA088). Our optimised regimens were tested in a murine thigh infection model *via* a humanized dosing scheme.
Results. MBM indicated that AGS significantly enhanced the imipenem (IPM) target site concentrations, characterized by an up to 2.9-fold decrease in IPM concentrations required to achieve half-maximal bacterial load reduction. The optimized combination regimens (IPM continuous infusion at 4g/day or 5g/day + tobramycin (TOB) 7 mg/kg q24h as 0.5h infusion) were predicted to achieve >2 $\log_{10}$ killing and prevent regrowth at 24 and 48h in 81 to 90.3% of simulated critically-ill patients. The bacterial load in the mice immediately before the start of treatment (2h after inoculation) was 4.79±0.08 $\log_{10}$ CFU/thigh (mean±standard deviation) and was increased up to 8.11±0.11 $\log_{10}$ CFU/thigh at 24h in untreated control mice. IPM+TOB combinations (*i.e.* IPM 4g/day or 5g/day continuous infusion + TOB 7 mg/kg q24h, 0.5h infusion) provided a clear benefit with ≥2.51 $\log_{10}$ and ≥1.50 $\log_{10}$ CFU/thigh of bacterial load reduction compared to the most active monotherapy at 24h.
Discussion. MBM suggested that disruption of the outer membrane by an aminoglycoside contributed to the synergy for combinations of IPM plus an AGS. Monte Carlo simulations predicted 81 to 90.3% success rate to achieve ≥2 $\log_{10}$ killing at 24h and 48h for optimised combination dosage regimens. The IPM plus TOB combination regimens, which were rationally optimised *via* a translational modelling approach, demonstrated a synergistic effect *in vivo* against a double-resistant clinical Pa isolate and are therefore highly promising.

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FPR2 regulates chronic inflammation in Chronic Obstructive Pulmonary Disease (COPD)

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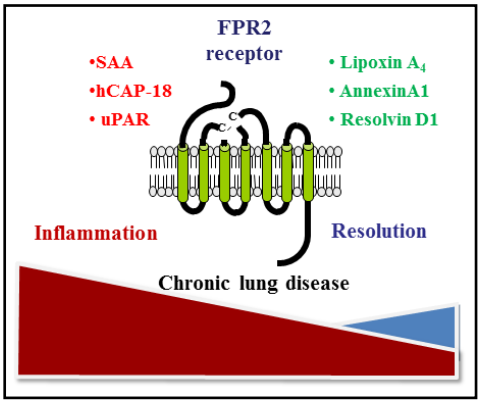
Introduction. Chronic Obstructive Pulmonary Disease (COPD) is an inflammatory lung condition that is associated airway remodelling and parenchymal destruction (emphysema), which manifests into persistent breathlessness. Chronic inflammation is driven by leukocyte mobilising cytokines stimulated by environmental triggers including cigarette smoke and lung infections.
Aims. We propose an alternative view on why inflammation persists in COPD. We have focused on the G-protein coupled receptor, formyl peptide receptor-2 (FPR2). FPR2 engages multiple agonists that can either promote inflammation (Serum Amyloid A; SAA) or initiate resolution of inflammation (Lipoxin A4). Agonist biased signalling may dictate whether inflammation or resolution pathways are initiated.



Methods. Expression of alternate FPR2 agonists was assessed in serum and lung tissue of COPD patients. An experimental mouse model was established to investigate the actions of alternate FPR2 agonists on lung inflammation.
Results. Circulating levels of SAA were disproportionally expressed relative to Lipoxin A4 in COPD patients during an acute exacerbation of COPD (AECOPD). SAA expression in lung resection tissue from patients with COPD correlated with tissue neutrophilia. *In vivo*, recombinant SAA potently stimulated recruitment of neutrophils via an IL-17A dependent mechanism in BALB/c mice.
Discussion. There is an imbalance in expression of alternative FPR2 agonists in COPD, which may favour the ongoing recruitment of leukocytes via IL-17A dependent mechanisms. Hence, the development of more stable pro-resolving FPR2 analogs provides therapeutic opportunities to counteract pathogenic signalling and promote resolution of inflammation in chronic lung diseases such as COPD.

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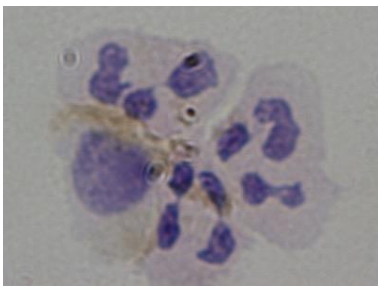
Do anti-viral neutrophil responses exacerbate lung inflammation in asthma?

Brian Oliver, David Van Ly, Fran Tang



Introduction. Respiratory viral infections precipitate exacerbations of respiratory diseases, characterised by tachyphylaxis to commonly used medications such as steroids and β2-agonists and neutrophilic inflammation. Surprisingly, the pathological role of neutrophils during exacerbations is not known.
Aims. To investigate the role of neutrophils in virus-induced exacerbations of asthma
Methods. In-vitro lung models using primary human cells were used to model virus-induced exacerbations.
Results and Discussion. Over the last ten years, our studies have discovered not only why β-agonist induced tachyphylaxis occurs during viral infections, we have also uncovered a unique immunomodulatory role of neutrophils during virus infection.

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Adenosine receptor biased agonism to treat ischaemic heart disease

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Introduction. Ischaemic heart disease and secondary heart failure, places an immense burden on society. Stimulation of adenosine G protein-coupled receptors can protect against cardiac ischemia-reperfusion injury (IRI) and subsequent cardiac remodeling. We recently described a rationally designed hybrid molecule, VCP746, a biased agonist with relatively high affinity for the adenosine A_1 receptor (A_1AR) and adenosine A_{2B} receptor (A_{2B}AR).
Aims. To investigate the potential anti-hypertrophic and anti-fibrotic effects of VCP746 in isolated neonatal rat cardiac myocytes (NVCMs) and fibroblasts (NCFs). Subsequently, to assess the influence of adenosine receptor biased agonism on cardiac remodelling *in vivo*.
Methods. The influence of VCP746 on hypertrophic and fibrotic signalling *in vitro* was investigated using [^{3}H]leucine and [^{3}H]proline incorporation and quantification of gene expression in NVCMs and NCFs. A rat 4-week IRI model was used to investigate the influence of VCP746 on cardiac remodelling *in vivo*.
Results. In NVCMs, VCP746 significantly inhibited IL-1β-, TNF-α- and Ang II-stimulated [^{3}H]leucine incorporation and ANP, β-MHC and α-SKA mRNA expression. The anti-hypertrophic effect of VCP746 was likely A_1AR mediated, and more potent than the prototypical A_1AR agonist, CPA. In NCFs, VCP746 decreased TGFβ- and angiotensin II- mediated [^{3}H]proline incorporation and Col I, CTGF and TGFβ mRNA expression. The anti-fibrotic signalling mediated by VCP746 in NCFs was selectively reversed in the presence of an A_{2B}AR antagonist. In the 4-week *in vivo* model of IRI, VCP746 improved cardiac function, while decreasing cardiac hypertrophy and fibrosis.
Discussion. Collectively, this study reveals that the previously characterized cardioprotective pharmacology of VCP746 now extends to include potent anti-hypertrophic and anti-fibrotic effects. That is, in addition to being a biased agonist that elicits A_1AR-mediated cardioprotection in the absence of adverse bradycardia and atrioventricular block, we have now demonstrated that VCP746 also decreases cardiac remodelling post IRI. As such we believe the development of compounds, such as VCP746, that stimulate potent A_1/A_{2B}AR biased agonism may represent a highly attractive therapeutic approach for modulating both myocardial fibrosis and hypertrophy in the treatment of heart failure.

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Anti-oxidant effects of hydrogen sulfide in the cardiovascular system

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Introduction. Hydrogen sulfide (H_2S) is an endogenous mediator with the unique properties of a gasotransmitter and many and varied physiological effects. Many reports indicate that H_2S acts as an anti-oxidant. The mechanism of this activity has been reported to be at several levels; as a scavenger of reactive oxygen species (ROS) (Wedmann 2014), an inhibitor of ROS production (Muzaffar 2008) and to turn on and/or boost endogenous cellular anti-oxidant defenses (Kimura 2010). In cardiovascular diseases elevated oxidative stress is an early and common pathological event. Increased oxidative stress in the vasculature is associated with endothelial dysfunction, reduction of $NO^{\bullet}$ bioactivity and further progression of vascular disease (Brandes 2010).
Aims. These studies investigated the role of H_2S as antioxidant and vasoprotectant in oxidative stress-induced vascular dysfunction.
Methods. *In vitro* and *in vivo* models of oxidative stress, hypertension, atherosclerosis and diabetes were used to test the hypothesis that H_2S can act as an antioxidant and vasoprotectant.
Results. The data show that H_2S scavenges superoxide ($O_2^{\bullet -}$) and inhibits vascular NADPH oxidase activity. H_2S donor treatment *in vivo* restores endothelial function and $NO^{\bullet}$ bioactivity, in models of hypertension, atherosclerosis and diabetes.
Discussion. These data confirm that H_2S donors can act as an anti-oxidant that is useful in protecting endothelial function *in vivo* in these models of vascular disease. The mechanism of this effect is partly through their ability to scavenge ROS, and partly due to inhibitory effects on the activity of vascular NADPH oxidase, but not through reduction of NOX2 protein expression nor by induction of eNOS protein expression. Further studies to determine the molecular mechanism of these effects are needed for the development of H_2S donors as potential therapeutic agents.

Wedmann R et al. (2014). Nitric Oxide 41: 85-96.
Muzaffar S et al. (2008). J Vasc Res 45(6): 521-528.
Kimura Y et al. (2010). Antioxid Redox Signal 12(1): 1-13.
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Specific issues for geriatric patients in clinical trials

Ingrid Hopper[1]. School of Public Health and Preventive Medicine, Monash University[1], Melbourne, VIC.

Patients aged 65 and over are the primary drug users in many diseases, yet this group is underrepresented in all phases of clinical trials. Studies in non-geriatric populations cannot predict all of the potential differences in pharmacokinetics, pharmacodynamics, drug-drug interactions, drug-disease interactions, and clinical responses in geriatric populations. The elderly are often arbitrarily excluded from clinical trials, and few trials are specific to elderly patients. Drivers of exclusion include increased risk of adverse drug effects, multi-morbidity, reduced life expectancy, difficulties with transportation and ethical challenges around informed consent especially in the presence of cognitive impairment. Industry guidance recommends that participants in clinical trials should be reasonably representative of the population to be treated by the drug, have no arbitrary upper age limit, and sufficient geriatric participants to compare the drug response to younger patients. Some of Henry Krum's clinical trial work in elderly populations will be reviewed.

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Clinical Trials for The Real World

Danny Liew. Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, VIC.

Clinical trials provide 'gold standard' evidence of the *efficacy* of drug therapy, but not necessarily of *effectiveness* nor *cost-effectiveness*. Indeed, there is often a large gap between efficacy and effectiveness, and again between effectiveness and cost-effectiveness. Furthermore, many drug trials are designed for the purposes of obtaining drug registration, which further limits their real-world relevance. In this presentation, Professor Liew will discuss a ways in which clinical trial results are translated for real-world applicability using *comparative effectiveness research* and *health technology assessment*. He will also touch on clinical trial designs that allow for more direct application and translation of their results.

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The Legacy of Henry Krum –making clinical trials fit practice: Where are the children?

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Children make up approximately one third of the world population, however, when it comes to the use of medicines in children, the information needed is often lacking. This has resulted in children (along with the elderly and pregnant) being classed as therapeutic orphans. Past reviews of therapeutic labelling in Australia and internationally have identified that up to 80% of medicines lack prescribing information for children, with neonates having the least information. Because of this, much of the prescribing in children is off-label or with unlicensed formulations. This increases the risk of incorrect dosing, reduced efficacy, and an increased risk of adverse events in an already vulnerable population.
Paediatric Clinical Pharmacology has been an important advocate for prescribing in children through the development of guidelines and dosing information as well as promoting and conducting clinical studies with new and established medicines. The World Health Organisation established the Essential Medicines List for Children and produced a supporting Children's Formulary in 2009. There have been legislative changed in Europe and the USA to encourage the appropriate study of new medicines in children which are now starting to bear fruit. However, many medicines still lack the information needed for appropriate treatment of children.

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Fitting the evidence to drug practice

Prof Jennifer Martin, The University of Newcastle

Fitting the evidence to drug practice is a key difficulty for clinical trials which, for new medicines have undergone a transformation over the last 15 years. Specifically, recruitment strategies may now be based on highly selected clinical groups of one racial background, a single comorbidity and middle age. Genetic subgroups may also now be used for recruitment, rather than the traditional functional class. The studies, particularly the larger cardiovascular or oncology studies have also involved cross over from placebo arm during the study, use surrogate and/or composite outcomes, and have often been studied use in more advanced settings or in less comorbid populations. Whilst much of this change has been necessary due to expense in developing drugs and clinical trials, increasing numbers of subgroups in which to study therapy, and increasing numbers of subjects required to show benefit mean that a number of clinical issues have arisen on their use in the subsequent clinical setting. These include uncertainties over how to extrapolate actual comparative benefit in the clinical trial, and how to subsequently translate clinical trial data to clinical practice, particularly in terms of dosing and frequency of drug administration. Lastly, data on actual benefit in clinical practice is not often collected, thus ensuring the actual benefit of some therapies in some population groups is never known. This presentation focuses on techniques adapted from clinical pharmacology to attempt to ensure therapeutics developed and tested in clinical trials are used in ways more commensurate with individuals' variable pharmacology and phenotypic variables. It discusses the need for post marketing research including pharmacoepidemiology and measurements of drug exposure to provide informative data to refine optimum drug use in the clinical setting.

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Demonstration of knowledge and understanding using visualization

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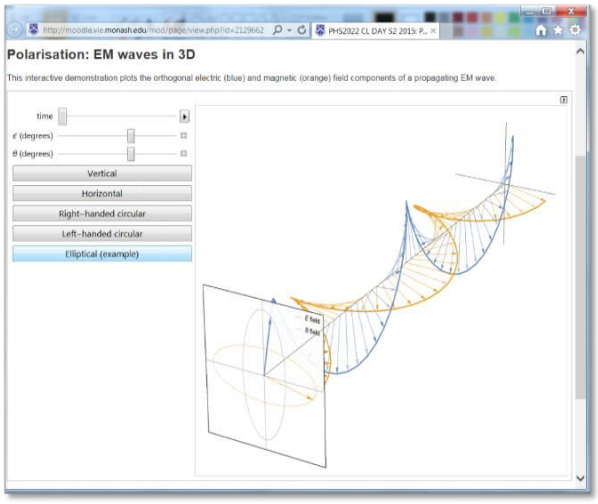

Introduction. Visualisation is essential to coming to grips with abstract concepts. This is especially true in physics, where visualisation is key to seeing common threads between abstract mathematics and the physical universe. I'll discuss innovative approaches to embedding pervasive visualisation in the undergraduate physics curriculum, which are transferable to other disciplines.

Aims. I seek to show students that equations are merely machines with dials, and that by literally turning those dials they can better understand the physical world. Like Keanu Reeves' character in the popular sci-fi trilogy, I want my students to 'see the Matrix', instead of just seeing the matrix.

Methods. I demonstrate scientific programming live in lectures, and complement this with original interactive visualisations. The visualisations are embedded into the learning management system (Moodle); an example is shown (right) as viewed in a browser. The interactive visualisations are tightly wed to concepts introduced in lectures and laboratories. Students can use them to check their answers to particular assigned problems, in a way that demands deep understanding, not akin to looking in the 'back of the book' for answers.

Results & Discussion. Moodle analytics revealed the above measures significantly enhanced student engagement. The power of visualisation has underpinned the introduction of scientific computing in the restructure of undergraduate physics units at Monash in 2016. This transformation has been widely welcomed by students, and we intend to market computational problem solving as a key graduate attribute.

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Comparison of visual modalities to enhance student learning

Anna-Marie Babey. Pharmacy Discipline, School of Science & Technology, University of New England, Armidale, NSW

Introduction. Moving students from fact-based knowledge to a deeper understanding of concepts that facilitates creativity and problem-solving skills can be difficult from the point of view of both teaching and assessment. The impetus to reject the traditional didactic approach in favour of students' personal discovery through the use of visualisation modalities has been growing since Novak developed one of the first concept mapping tools in 1972. While inclusion of enhancements beyond paper-based resources is potentially valuable, there is evidence that it does not necessarily enhance learning (Horton, et al., 1993); however, the primary issue might well be that novel learning and teaching approaches are often married to more traditional assessment formats, creating a mismatch as the student endeavours to put into words that which they have learned visually (McGrath and Brown, 2005; Ruiz-Primo and Shavelson, 1996). Further, it has been proposed that instead of relying solely on a single visualisation modality for all content, the choice of tool should be based on best fit to the different facets of the content (Eppler, 2006).
Aims. To provide an overview of the strengths and weakness of the more common visualisation tools to better inform decisions about the inclusion of these approaches in pharmacology teaching.
Discussion. The value of visualisation tools such as concept mapping, mind mapping, conceptual diagrams, word clouds (which has given rise to molecule clouds), conceptual diagrams and visual metaphors to enhance student learning is predicated on a clear understanding of the content to which they are best applied rather than choosing a single modality with which to engage. Providing students with a clear understanding of the advantages and disadvantages of each tool allows them to choose the one that best serves their needs, and recognises that not all approaches will work for all students, regardless of the content at issue.

Horton PB *et al*. (1993) Science Education 77(1):95-111
McGrath MB; Brown JR (2005) IEEE Computer Graphics and Applications. 25(5):56-63
Ruiz-Primo MA; Shavelson RJ (1996) Journal of Research in Science Teaching 33(6):569-600
Eppler MJ (2006) Information Visualization 5:202-210

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Linking drug-target interaction to response: Filling the gap

Janet K Coller[1]. Discipline of Pharmacology, The University of Adelaide[1], Adelaide, SA.

Introduction. There is substantial evidence that team-based learning (TBL) that incorporates the 4 S's framework (Significant problem, Same problem, Specific choice and Simultaneous report) improves student learning of complex concepts (Michaelsen et al, 2008).
Aims. To design and implement a team-based learning tutorial session to facilitate engaged learning of key pharmacodynamic concepts, namely drug-target interactions to cause a cellular response, for first year Bachelor of Nursing students.
Methods. Students who do not have an extensive background in pharmacology participated in the tutorial session in groups of 5 (total class size of 150) and needed to complete three activities that supported different learning styles with group discussion at the end of the session. Student were asked to provide feedback about their learning experience during the session from a three item paper survey at the end of the session.
Results. Student engagement with, and understanding of the content during the session improved significantly from a prior non-TBL session. Student feedback was positive and exam performance indicated understanding of the concepts.
Discussion. To teach advanced concepts to students with wide background knowledge of pharmacology, TBL sessions provide an ideal environment to reinforce lecture content and provide an engaging environment for learning.

Michaelsen et al (2008) New Directions for Teaching and Learning 116:1-104

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3D Molecular Visualisation in Pharmacology Teaching

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The interaction of drugs with receptors, enzymes and transporters is central to pharmacology. However, the concepts of ligand receptor interactions are often over-simplified with cartoon shapes. Even when two dimensional representation of three dimensional (3D) structures are used, these can be confusing, especially to students with little biochemistry or chemistry background 3D molecular visualization may help overcome these problems, and contribute of a better understanding of drug-protein interactions. 3D visualizations can be physical models, 3D simulations (eg JMOL) or even virtual reality immersive simulations. The kinds of simulation used will depend on the stage and size of the class, and the educational objectives. A VR simulator would not be appropriate for a 200 plus class of health professionals, but would be useful for a drug discovery major. Mere presentation of 3D structures is not sufficient, these structures need to be tied into appropriate assessment to reinforce the knowledge the 3D visualizations are being used to present.

Some example 3D visualizations will be demonstrated, and their appropriateness and level of expertise to utilize them discussed.

Barnea N and Dori YJ (1996) J. Chem. Inf. Comput. Sci., 36: 629–636
Satyanarayanajois SD (2010) Am J Pharm Educ.;74: 147-156.
Berry C and Baker MD (2010) Biochem Mol Biol Educ. 38:425-9.

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Therapeutic targeting of cardiac fibrosis

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Fibrosis is a key component of pathologic remodeling in multiple tissues, yet there are no effective, approved therapies that specifically target maladaptive fibrosis. Exploring similarities in the mechanism(s) of fibrotic remodeling in diverse tissues may hold substantial therapeutic promise. Heart failure (HF), the final manifestation of many cardiovascular pathologies, is a devastating disease with poor prognosis. Fibroblasts play a key role in tissue fibrosis, including decreased compliance and dysfunction. Pathologically activated fibroblasts transition to myofibroblasts, releasing pro-hypertrophic/fibrotic mediators that exacerbate tissue remodeling. Although ECM deposition may provide early mechanical support, sustained myofibroblast activity/proliferation underlies tissue stiffness and progressive fibrosis. The ECM protein fibronectin (FN) plays a key role in pathologic remodeling of the ECM; fibroblasts are the major source of cellular FN in most tissues. Production and polymerization of FN are both elevated in clinical and experimental fibrosis; these processes are essential for maladaptive MF transition and progression of pathologic fibrosis. FN polymerization tightly regulates the assembly of different ECM proteins; it also promotes cell adhesion, growth, migration and contractility. We recently described a peptide, pUR4, which binds to FN and inhibits its cell-mediated polymerization; it also attenuates pathologic myofibroblast transition. Our data further suggest that 7 days of pUR4 post-I/R reduces FN deposition, collagen accumulation and myocardial fibrosis and significantly improves cardiac function for up to 4 weeks post-I/R. Inhibition of FN polymerization may be a new therapeutic approach for treating pathologic fibrotic remodeling in the heart and possibly in other tissues.

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GPCRs beyond the bench: lessons from the CaSR signalling pathway

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The extracellular calcium (Ca_o^{2+})-sensing receptor (CaSR), a G-protein-coupled receptor (GPCR), regulates Ca_o^{2+} homeostasis by detecting alterations in Ca_o^{2+} concentrations and activating G-protein mediated signalling cascades, which modulate parathyroid hormone (PTH) secretion and urinary calcium excretion. Much has been learnt about the role of the CaSR in calcium homeostasis by studying human disorders. Thus, CaSR mutations resulting in loss-of-function lead to familial hypocalciuric hypercalcemia (FHH), a lifelong disorder associated with mild-to-moderate elevations of serum calcium concentrations, normal or elevated PTH concentrations, and inappropriately low urinary calcium excretion. CaSR mutations are detected in ~65% of FHH patients, referred to as FHH type 1 (FHH1), and genetic studies in other FHH kindreds have revealed genetic heterogeneity and defined two additional types, FHH2 and FHH3. FHH2 is due to mutations of G-protein subunit α_{11} ($G\alpha_{11}$), encoded by *GNA11*, and *in vitro* expression of FHH2-associated *GNA11* mutations were found to diminish the sensitivity of CaSR-expressing cells to Ca_o^{2+}, consistent with a loss-of-function. FHH3 is due to loss-of function mutations affecting adaptor protein-2 sigma subunit (AP2σ), encoded by *AP2S1*. AP2, a hetrotetrameric complex, is involved in clathrin-mediated endocytosis and AP2σ mutations, which all affect the Arg15 residue that interacts with the dileucine motif of cargo proteins and comprise Arg15Cys, Arg15His and Arg15Leu, result in increased CaSR cell surface expression likely due to decreased CaSR internalisation. Such AP2σ mutations are found in >20% of FHH patients who do not have CaSR or $G\alpha_{11}$ mutations. These studies have provided new insights into CaSR signaling and trafficking, and advanced therapeutic options, such as CaSR allosteric modulators, for disorders of calcium metabolism.

1. Howles SA et al (2016) New England Journal of Medicine 374:1396-1398
2. Nesbit MA et al (2013) New England Journal of Medicine 368: 476-86
3. Nesbit MA et al (2013) Nature Genetics 45:93-97
4. Babinsky VA et al (2016) Journal of Biological Chemistry 291:10876-10885
5. Hannan FM et al (2016) Journal of Molecular Endocrinology 57:R127-R142

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The role of MRGPRs in Itch and inflammation

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Despite of the clinical importance, cell surface receptors mediating non-histaminergic itch are largely unknown. We identified a large family of G protein-coupled receptors in mice called Mrgprs. Many of these receptors are exclusively expressed in distinct subsets of small-diameter dorsal root ganglion (DRG) neurons. We found that MrgprA3 functions as a receptor for chloroquine (an anti-malaria drug) and is required for chloroquine-induced itch. Besides chloroquine, Mrgprs also respond to several itch-inducing compounds such as BAM8-22, SLIGRL, and beta-alanine suggesting that Mrgprs are novel itch receptors by directly sensing these compounds. Our data have shown the involvement of Mrgprs in mouse chronic itch models such as dry skin, contact dermatitis, and allergic itch. Importantly, some of the results have been confirmed in human psychophysical studies. Besides the sensory neuron specific Mrgprs, we discovered another member of the gene family, MrgprB2, is exclusively expressed in mast cells, a type of innate immune cells, which secret many pro-inflammatory mediators like histamine upon activation. We found that MrgprB2 contributes pain and itch. Therefore, we believe that targeting Mrgprs may lead to novel treatment of chronic itch in the future.

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The inner workings of a GPCR: Molecular basis for biased G protein activation and beta-arrestin recruitment

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G protein coupled receptors (GPCRs) are pharmacologically important membrane proteins involved in the transmission of signals into the cell. While they can be activated by a diverse set of ligands including small molecules, hormones, neurotransmitters or photons, GPCRs signal through only 16 G proteins and 2 β-arrestins. Insights into how and why GPCRs select specific G proteins opens up new opportunities for drug design and the possibility of drugs with fewer side effects.
Using alanine scanning mutagenesis on a GPCR, coupled with pluridimensional signalling profiling, we determined a molecular map of residues involved in the activation of G proteins from different subfamilies and recruitment of β-arrestins at single amino acid resolution. While some residues were important for both G protein activation and beta-arrestin recruitment, others specifically affected either of the signalling pathways. Clustering of the residues involved in those signalling pathways allowed us to connect ligand binding pocket and G protein/arrestin binding region by several distinct allosteric paths[1,2], which were specific for the activation of G proteins, G protein subtypes and the recruitment of β-arrestins. Our data gives us insights into the molecular basis of G protein activation, selection of G protein subtype and allows us to better understand the conformational changes needed for β-arrestin recruitment, desensitization and internalization of receptors.

[1] Isogai, S. *et al*. Backbone NMR reveals allosteric signal transduction networks in the β1-adrenergic receptor. Nature 530, 237-241 (2016).
[2] Venkatakrishnan, A. J. *et al.* Diverse activation pathways in class A GPCRs converge near the G-protein-coupling region. Nature 536, 484–487 (2016)

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VCP746, a biased adenosine A_1/A_{2B} receptor agonist, promotes divergent cardioprotective signalling from that of NECA, a prototypical adenosine receptor agonist

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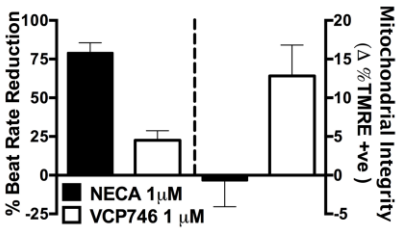
Introduction. Adenosine A_1 receptor (A_1AR) stimulates powerful cardioprotection; however, therapeutic targeting has been largely unsuccessful due to on-target adverse hemodynamic effects. Recently, the biased agonist, VCP746, was shown to protect against ischaemia in neonatal ventricular cardiomyocytes (NVCM), but did not slow isolated atrial beating.

Aims. To compare the cardioprotection and haemodynamic effects stimulated by VCP746 and NECA *in vivo*. Further, to investigate *in vitro* the mechanism underlying the atypical signalling profile of VCP746.



Methods. VCP746 and NECA were assessed in an acute myocardial ischemia-reperfusion (IR) model and their signalling profile for pathways implicated in cardioprotection and bradycardia was characterized in rat NVCMs.

Results. NECA and VCP746 mediated a significant decrease in infarct size *in vivo* (n=6-9; P<0.05; One-way ANOVA, Dunnetts post-hoc). However, in contrast to NECA, VCP746 had no significant effect on heart rate or blood pressure. In rat NVCMs, A_1AR activation decreased NVCM beat rate frequency via GIRK channels; VCP746 had reduced potency relative to NECA. Both agonists stimulated canonical $G_{i/o}$ protein signaling ($[^{35}S]GTP\gamma S$ binding and inhibition of cAMP accumulation; n=4-12) and reperfusion injury salvage kinase pathways (ERK1/2, AKT and S6 ribosomal protein phosphorylation; n=4-12). Under simulated IR conditions, both NECA and VCP746 mediated a significant decrease in lactate dehydrogenase release (n=6; P<0.05; One-way ANOVA, Dunnets post-hoc), however, only VCP746 significantly enhanced mitochondrial integrity (n=4; P<0.05; one-sample t-test).

Discussion. In contrast to the prototypical agonist, NECA, the biased agonist VCP746 promoted cardioprotection in the absence of bradycardia. In isolated rat NVCMs, only VCP746 enhanced mitochondrial integrity after simulated IR at concentrations that had minimal effect on GIRK channel function. These findings suggest divergence between NECA and VCP746 for pathways implicated in cardioprotection relative to those associated with bradycardia.

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Receptor residence time determines bias towards internalisation of the μ opioid receptor

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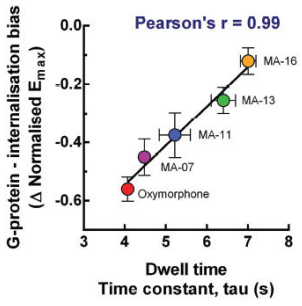
Introduction. μ-Opioid receptor (MOPr) agonists are analgesic via G-protein signalling however additional pathways such as ß-arrestin dependent signalling & regulation contribute to their clinical effects. Signalling bias between these pathways has been extensively demonstrated but the mechanisms remain unclear. Agonist residence at the receptor is required to maintain the ß-arrestin bound receptor state and thus residence time at the receptor may contribute to G-protein/ß-arrestin-2 bias.

Aims. To investigate the relationship of receptor residence time to G-protein/ß-arrestin 2 bias at the MOPr.



Methods. We used a series of oxymorphone analogues with extended aliphatic substitutions at the 6-position to systematically investigate this relationship. Studies were conducted on AtT20 cells stably transfected with mouse MOPr. MOPr-mediated potassium channel (GIRK) activation and rate of channel deactivation were assessed using patch clamp electrophysiology. A resonance transfer assay was used to determine degree of ß-arrestin 2 recruitment to the MOPr and rate of ß-arrestin 2 dissociation. MOPr internalisation was quantified with immunocytochemistry. Direct agonist affinity was quantified by $[^3H]$ DAMGO binding displacement.

Results. Increasing substituent length in the analogue series had little effect on agonist binding affinity or GIRK assay potency but systematically slowed both decay of MOPr-mediated channel activation and ß-arrestin 2 dissociation indicating increased agonist MOPr residence time. Extension of aliphatic chain led to unchanged efficacy for G-protein activation but increased efficacy for ß-arrestin 2 recruitment and MOPr internalisation.

Discussion. The increase in ß-arrestin 2 bias was highly correlated with the increase in receptor residence time. Receptor residence time is therefore a factor determining G-protein/ß-arrestin 2 bias at the MOPr.

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Functional consequences of different conformational states of GPCR–β-arrestin complexes

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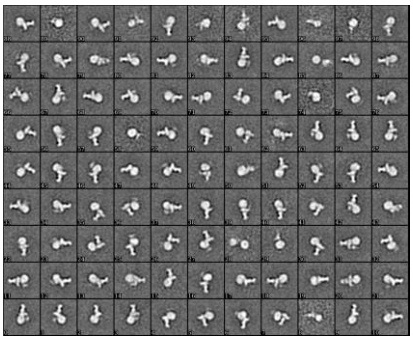

Introduction. Recent data suggests that GPCR–β-arrestin (βarr) interactions occur through a 'biphasic' mechanism, in which the βarr initially interacts with the phosphorylated carboxy terminus of the GPCR (tail conformation) before rearranging to more fully engage the transmembrane core (core conformation) of the receptor via an interaction with the fingerloop region (FLR) of βarr.

Aims. Investigate whether GPCR–βarr interactions occur through a 'biphasic' mechanism by employing novel biophysical and structural techniques, and to explore whether a new paradigm, whereby conformational isomers of GPCR–βarr exist, which might then mediate distinct functional outcomes.

Methods. We have developed a method for screening purified GPCR–βarr complexes for biochemical, biophysical, and structural features. To investigate the GPCR–βarr complex, mutations were introduced into the amino acids of the βarr FLR. Upon forming GPCR–mutant–βarr complexes, transmission electron microscopy (EM) was employed; single particle analyses and reconstructions provided structural data. Multiple assays were performed on relevant mutants to assess how structural differences impacted functionality.

Results. By introducing different mutations or deletions within the βarr FLR we were able to significantly alter which type of conformation (tail or core) between the receptor and βarr was present. For instance, wild type GPCR–βarr forms complexes, which always exist in the ratio of ~70% tail conformation and ~30% core conformation as assessed by EM. However, altering the FLR led to a 3-fold decrease in the core conformation.

Discussion. The core and tail GPCR–βarr complexes do indeed represent unique conformations, which are significantly altered through changes to the βarr FLR, and that have a direct impact on functionality.

Shulka A et al (2014) Nature 512:218-222

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β-Arrestin-biased agonists of the GLP-1 receptor from backbone-modification of GLP-1

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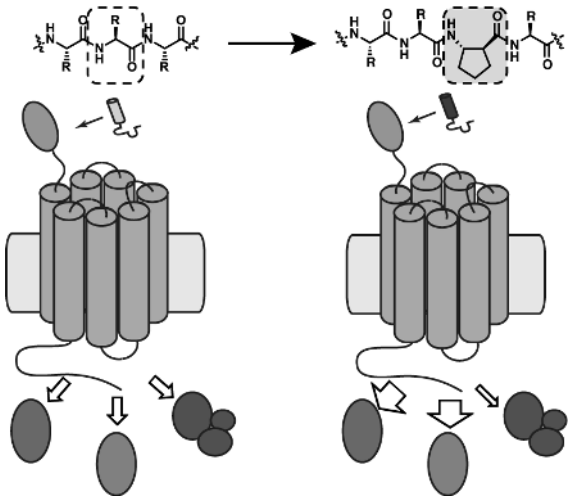
Introduction. The glucagon-like peptide-1 receptor (GLP-1R) is a major therapeutic target for the treatment of diabetes. The GLP-1R can signal through several intracellular pathways upon activation by GLP-1. Biased agonists of the GLP-1R have the potential to maximize beneficial aspects of GLP-1R activation while minimizing side effects that might arise from augmentation of some GLP-1R-initiated signaling.



Aims. To identify biased agonists of the GLP-1R by incorporating backbone-modified amino acids into GLP-1.

Methods. Backbone-modified GLP-1 analogues were synthesized *via* solid phase peptide synthesis. The activity of each analogue in activating the GLP-1R to cAMP production or β-arrestin recruitment was assessed in HEK293-derived cells. Ligand bias was determined by fitting concentration-response curves for each analogue to an operational model of agonism and comparing extracted transduction coefficients for each analogue to that of GLP-1 in each pathway.

Results. We found that backbone-modification of GLP-1 affects cAMP production more strongly than β-arrestin recruitment, leading to β-arrestin-biased agonists of the GLP-1R.

Discussion. We identify the central portion of GLP-1 as critical in promoting β-arrestin recruitment over cAMP production. In addition, our results suggest that backbone-modified GPCR ligands may have general utility as a novel source of biased agonists.

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Is the differential reversal of opioid tolerance by ethanol a consequence of ligand bias at the μ opioid receptor?

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Introduction. In mice tolerance develops to the respiratory depressant effect of morphine but at a slower rate than tolerance to antinociception (Hill et al 2016). On prolonged treatment tolerance also develops to the respiratory depressant effect of oxycodone and methadone and pseudo tolerance develops to buprenorphine. Tolerance induced by morphine or oxycodone can be reversed by acute administration of a low dose of ethanol as well as by PKC inhibitors (calphostin C and tamoxifen) whereas the tolerance induced by methadone or buprenorphine is not reversed by these drugs. The effects of ethanol and calphostin C were non-additive.

Previously we have observed that in rat locus coeruleus neurones μ opioid receptor (MOPr) desensitization and cellular tolerance to morphine, a low efficacy MOPr agonist, are mediated by PKC (Bailey et al 2009) and can be reversed by ethanol (Llorente et al 2013) whereas desensitisation induced by the high efficacy agonist DAMGO is mediated by G-protein-coupled receptor kinase (GRK) and is insensitive to ethanol (Lowe et al 2016). Morphine and oxycodone have low efficacy in recruiting arrestin to the MOPr whereas methadone and DAMGO have high efficacy (McPherson et al 2010).

Discussion. Selective reversal of tolerance induced by morphine and oxycodone would suggest that ethanol acts to reduce the PKC component but not the GRK/arrestin component of opioid tolerance. Reversal of tolerance to the respiratory depressant effects of morphine (a metabolite of heroin) and oxycodone may contribute to overdose deaths in opioid abusers.

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Hill R et al (2016) Neuropsychopharmacol.41:762-773
Llorente J et al (2013) Mol Pharmacol. 84:252-260
Lowe J et al (2016) Mol Pharmacol. 88:347-356
McPherson J et al (2010) Mol Pharmacol. 78:756-766

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The GABA-B receptor and psychiatric disorders

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As γ-aminobutyric acid (GABA) is the major inhibitor neurotransmitter in brain, GABAergic transmission is associated with virtually all central nervous system functions. This proposition is supported by findings with human brain autopsy material indicating significant and regionally selective changes in GABA synthesis and receptor binding and activity associated with a host of neurodegenerative and psychiatric disorders, including Alzheimer's, Huntington's and Parkinson's diseases, and major depression. Brain areas found to display changes in GABA synthesis, receptor binding or function include the frontal cortex, globus pallidus, putamen, and hippocampus. Because modifications in cognition are among the symptoms thought to be associated with alterations in GABA activity, efforts have been made to define the role of this transmitter in learning and memory. These have included laboratory animal studies of $GABA_A$ and $GABA_B$ receptor subtype-selective, full and partial agonists, antagonists and inverse agonists. Whereas conventional benzodiazepines, which are positive allosteric modulators at $GABA_A$ receptors, compromise cognition, $GABA_A\alpha_5$-selective partial inverse agonists, which are negative allosteric modulators, reverse deficits in working and spatial memory and improve executive function. Similarly, $GABA_B$ receptor agonists suppress, and $GABA_B$ receptor antagonists enhance, cognition. These findings indicate that GABAergic drugs might be of value in reversing or moderating cognitive deficits associated with psychiatric and neurological disorders. Challenges faced in exploiting these findings are the need to develop receptor subtype-selective agents with appropriate human pharmacokinetic and safety profiles, and the identification of patient populations in which the necessary GABA circuitry is sufficiently intact to sustain the desired response.

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Pharmacology of Chaperones in the CNS: Re-emergence of Sigma-1 receptors for Neuropsychiatric and Neurological Disorders

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Introduction: The evolution of our understanding of the sigma (σ) receptor has been a fascinating journey. The σ receptor was originally proposed by Martin in the 1970s as one of four opioid receptor subtypes, designated as μ, δ, κ and σ. While the first three of these receptor subtypes were closely related to one another, the σ receptor was subsequently determined to be unique in that the effects of the putative σ receptor agonist, SKF-10,047 (±)-N-allylnormetazocine) were not blocked by the opioid receptor antagonists naloxone or naltrexone. Further, because SKF-10,047 produced dysphoria and psychotomimetic effects, it was thought that the σ receptor was a phencyclidine (PCP-like) receptor located within the NMDA glutamate receptor complex. The resolution that the σ receptor was neither an opioid receptor nor a PCP-like receptor was resolved when it was recognized that the σ sites have a higher affinity for (+)-enantiomers while opioid receptors bind to (-)-isomers of the racemic benzomorphans such as (±)-N-allylnormetazocine. Recent research focusing on Sigma-1 (σ_1) receptor antagonists has indicated that these compounds have significant potential for the treatment of a wide variety of neuropsychiatric and neurological disorders, including pain. σ_1 receptors are widely distributed in the central and peripheral nervous system. In the CNS, immunostaining revealed a uniform localization along the plasma membrane with intense staining associated with the membrane thickenings facing the synaptic contacts, suggesting a possible role in the modulation of synaptic neurotransmission. When cells are stimulated by ligands or undergo stress, σ_1 receptors translocate from the mitochondrial associated membrane to the ER reticular network and plasma membrane where they interact with and regulate a variety of proteins that include ion channels, receptors and kinases. These findings support pharmacological data indicating an important role for σ receptors in modulating synaptic neurotransmission. The finding that the σ_1 receptor is involved in affecting several neurotransmitter systems (referred to as a 'pluripotent modulator') has been suggested by a number of studies. The lengthy trajectory of research on the σ_1 receptor has resulted in the current view that the σ_1 receptor is an 'enigmatic' endoplasmic reticulum-resident transmembrane chaperone protein that has been implicated in a wide variety of disorders. The crystal structure of σ_1, characterized as an 'evolutionary isolate with no discernible similarity to any other human protein' has been published very recently documenting the molecular structure of the σ_1 receptor as a single-pass transmembrane that helps to explain the diverse functions of this promising pharmacological target, thereby enabling much greater insight to the biochemical and biophysical features of this intriguing target for drug action.
Methods and Discussion: This talk will review recent findings with σ_1 receptor antagonists, particularly in the area of neurological disorders, including pain, where they have been clearly implicated both genetically and pharmacologically. The role of σ_1 receptors in analgesia is particularly relevant in light of the social issues surrounding opioid abuse

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Addiction and the brain: The role of the immune system

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The neuronal understanding of the cortico-mesolimboc dopamine reward pathway and the neuroadaptations that underpin drug addiction have been complimented recently by an understanding of the impact that neuroimmune signalling has on normal and maladaptive brain function. This work originally stemmed from explorations of the illness response to infection and how hedonic and anhedonic behavioural responses were integrated and processed in the brain, driven by classical immune signals. A paradigm shift occurred when it was discovered that drugs of dependence triggered neuroimmune cell responses directly via innate immune receptor systems, and that these neurokine signals and cellular responses were critical for the presentation of classical behavioural drug responses. In this presentation data will be shared demonstrating how this neuroimmune mechanism has relevance across multiple drug classes and where novel neuroimmune targeted clinical interventions have a future in the management of drug addictions.

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Macrophage mineralocorticoid receptor signalling regulates cardiovascular remodelling

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Introduction. Sustained mineralocorticoid receptor (MR) signalling promotes cardiac inflammation and fibrosis, which ultimately leads to cardiac failure. While the clinical use of MR antagonists are protective in cardiac disease, hyperkaelemia and other off target effects have limited their use.
Aims/Methods In order to identify key MR-dependent mechanisms of heart disease progression that are distinct to normal renal electrolyte control, we have developed a series of tissue selective MR null animal models and determined their responses to the DOC/salt model and other models of cardiac remodelling.
Results. We have shown that the MR plays a critical and selective role in the macrophage, cardiomyocyte and in endothelial cells in the progression of cardiac inflammation and fibrosis. Macrophage MR signalling drives the proinflammatory macrophage responses to tissue injury and is central to the onset of fibrosis. The MR in cardiomyocytes has important roles in early chemoattractant signals and determines the hearts response to ischemia reperfusion. Regulation of endothelial cell function by the MR is dependent upon the vascular bed and, but the MR has a central role in macrophage recruitment in these cells.
Discussion. Together, recent data from our lab and elsewhere support a broad but carefully orchestrated role for MR signalling in the cardiovascular system in cardiac pathology and also in the physiological setting. Defining the key mechanistic whereby MR signalling pathways in macrophages and other non-epithelial cells in the cardiovascular system is an essential first step towards identification of therapeutic targets that may preserve potassium homeostasis, especially those cell types in which the MR is most likely acting as a cortisol receptor.

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Targeting adenosine receptors for cardiac remodelling

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Introduction. Ischaemic heart disease and secondary heart failure, places an immense burden on society. Stimulation of adenosine G protein-coupled receptors can protect against cardiac ischemia-reperfusion injury (IRI) and subsequent cardiac remodeling. We recently described a rationally designed hybrid molecule, VCP746, a biased agonist with relatively high affinity for the adenosine A_1 receptor (A_1AR) and adenosine A_{2B} receptor (A_{2B}AR).
Aims. To investigate the potential anti-hypertrophic and anti-fibrotic effects of VCP746 in isolated neonatal rat cardiac myocytes (NVCMs) and fibroblasts (NCFs). Subsequently, to assess the influence of adenosine receptor biased agonism on cardiac remodelling *in vivo*.
Methods. The influence of VCP746 on hypertrophic and fibrotic signalling *in vitro* was investigated using [^{3}H]leucine and [^{3}H]proline incorporation and quantification of gene expression in NVCMs and NCFs. A rat 4-week IRI model was used to investigate the influence of VCP746 on cardiac remodelling *in vivo*.
Results. In NVCMs, VCP746 significantly inhibited IL-1β-, TNF-α- and Ang II-stimulated [^{3}H]leucine incorporation and ANP, β-MHC and α-SKA mRNA expression. The anti-hypertrophic effect of VCP746 was likely A_1AR mediated, and more potent than the prototypical A_1AR agonist, CPA. In NCFs, VCP746 decreased TGFβ- and angiotensin II- mediated [^{3}H]proline incorporation and Col I, CTGF and TGFβ mRNA expression. The anti-fibrotic signalling mediated by VCP746 in NCFs was selectively reversed in the presence of an A_{2B}AR antagonist. In the 4-week *in vivo* model of IRI, VCP746 improved cardiac function, while decreasing cardiac hypertrophy and fibrosis.
Discussion. Collectively, this study reveals that the previously characterized cardioprotective pharmacology of VCP746 now extends to include potent anti-hypertrophic and anti-fibrotic effects. That is, in addition to being a biased agonist that elicits A_1AR-mediated cardioprotection in the absence of adverse bradycardia and atrioventricular block, we have now demonstrated that VCP746 also decreases cardiac remodelling post IRI. As such we believe the development of compounds, such as VCP746, that stimulate potent A_1/A_{2B}AR biased agonism may represent a highly attractive therapeutic approach for modulating both myocardial fibrosis and hypertrophy in the treatment of heart failure.

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Mechanisms downstream of IGF-1R for targeting diabetes-induced cardiac remodelling

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Diabetes imposes a significant burden on society, and with its incidence predicted to affect 600 million adults globally by 2035, it represents a major threat to human health. More than 1 million Australians have diabetes, with this number growing exponentially. Many of these patients will die from cardiovascular disease such as heart failure, coronary heart disease, atherosclerosis, with the incidence of heart failure increasing 6 fold in 45-65 year old diabetics. Essentially, diabetes-induced cardiac disease (termed diabetic cardiomyopathy) is characterised by cardiac fibrosis, cardiomyocyte hypertrophy, inflammation, LV diastolic dysfunction, and generation of cardiac reactive oxygen species (ROS), resulting in impairment of cardiac relaxation and contractility. Diabetic cardiomyopathy is also associated with an increased and sustained activation of the hexosamine biosynthesis pathway (HBP), which is considered to be detrimental to cardiac function over the longer term, and ultimately leading to heart failure. Currently, there is no specific therapy for diabetes-induced heart failure.

This presentation will summarise findings of current treatment strategies that our laboratory is focussed on for targeting diabetes-induced cardiac remodelling and function. This will include investigation into the role of the IGF-1-PI3K pathway in amelioration of LV remodelling including fibrosis, ROS generation and pathological hypertrophy, as well as the involvement of HBP signalling (O-GlcNAcylation), in both type 1 and type 2 diabetic mouse models. In these models of diabetic cardiomyopathy, we have investigated transgenic over-expression of cardiac-specific IGF1R (which has been shown to be cardioprotective), the use of adeno-associated viral (AAV) gene therapy with PI3K(p110α) to increase the cardioprotective PI3K gene, and separately the OGA gene to assist with the removal of diabetes-induced O-GlcNacylation of SERCA2a, as treatments to ameliorate the cardiomyopathies associated with diabetes.

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Targeting inflammation and fibrosis in early life to protect against life-long respiratory and cardiovascular complications

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It is well established that early life exposures to inflammatory stimuli have consequences for life-long lung health. In premature infants, the combined insults of in utero and neonatal inflammation with therapeutic mechanical ventilation and oxygen therapy may lead to bronchopulmonary dysplasia (BPD), which compromises respiratory function and heightens risk of asthma and pulmonary arterial hypertension. More broadly, exposure to allergens, environmental challenges such as cigarette smoke, and to viral and bacterial infections, can lead to changes in airway responsiveness (Donovan et al, 2016; FitzPatrick et al 2016), childhood asthma, and COPD in adulthood.

New treatment options are required to target the common features of these respiratory diseases, whereby chronic inflammation is associated with airway and/or vascular remodelling, including fibrosis. While anti-inflammatory glucocorticoids are widely used in asthma and COPD exacerbations, their use in early life has potential adverse impacts and they do not reverse fibrosis. In a mouse model of BPD, the interleukin-1 receptor antagonist (IL-1Ra, anakinra) opposes the lung histopathology caused by inflammation and hyperoxia, but not airway hyperresponsiveness (AHR) (Royce et al, 2016), suggesting that antagonising IL-1β alone is insufficient to target additional factors influencing contraction. Targetting established fibrosis has proven difficult, but the antifibrotic hormone relaxin reverses both airway remodeling and AHR in a mouse model mimicking key features of asthma (Royce et al, 2009). These preclinical studies have provided key mechanistic insights to inform clinical development of novel therapies to oppose life-long impairments in lung function associated with inflammation and fibrosis.

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FitzPatrick M, et al (2016) *Sci Rep.* 4;6:22751.
Royce SG, et al (2016) *Am J Respir Cell Mol Biol* doi: 10.1165/rcmb.2016-0031OC.
Royce SG, et al (2009) *Endocrinology* 150(6):2692-9.

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Targeting epigenetic mechanisms to limit pulmonary fibrosis in COPD

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Introduction. COPD is currently the 3rd commonest cause of morbidity and mortality globally. It is an inflammatory and fibrotic progressive lung disease and there are no treatments that halt the progression or reverse disease features. Epigenetic changes affect DNA transcription without its sequence, and include alterations in acetylation and methylation of histones and other proteins.
Aims. To assess the roles and potential for therapeutic targeting of acetylation in inflammation and fibrosis in experimental and human COPD.
Methods. Mice were exposed to cigarette smoke for 8-12 weeks, and changes in acetylation activity and enzymes were assessed. Samples from human COPD patients were also assessed.
Results. Acetylation activity was increased in lung tissues and airway biopsies from mince with experimental COPD and human COPD patients. This was due to increases in the activity of acetylating and a reduction in deacteylating enzymes. Prophylactic and therapeutic use of an inhibitor of acetylation suppressed the development of COPD pathogenesis including inflammation and airway fibrosis.
Discussion. Targeting epigenetic changes such as acetylation may be a novel therapeutic approach for the suppression of airway fibrosis and other features of COPD.

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What clinical and surrogate outcomes should be used to evaluate medicines in older adults?

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Introduction. Outcome measures used to evaluate the safety and effectiveness of medicines are traditionally based on disease diagnosis. There may be outcomes related to geriatric syndromes that are relevant to best evaluate medicines in some groups of older individuals. In addition patient reported outcomes are gaining traction as part of the safety and efficacy evaluation.
Aims. To compare and contrast how selected example medicines/therapeutic indications may be evaluated using disease diagnosis based outcomes, geriatric syndrome based outcomes, and patient reported outcomes for older patients.
Methods/Results/Discussion. Two therapeutic areas, osteoporosis and type 2 diabetes mellitus, will be examined. Current outcome measures using disease diagnosis are vertebral/axial skeleton fracture and haemoglobin A_{1c}. The concept of surrogate outcome will be defined, and its relevance to these two diseases outlined. Geriatric syndromes that often associate with these two diagnoses, falls, urinary incontinence, frailty, and functional decline may be put in the context of secondary clinical trial outcomes for medicine evaluation. This may provide increased understanding of the risk/benefit relationship that a clinical trial characterizes. Patient reported outcomes that may represent meaningful outcome measures such as pain, fatigue, social participation, and sense of well-being may also provide increased understanding of the effectiveness of a drug therapy. This is an area of research opportunity that will potentially improve the understanding of effectivenss of specific drug therapy interventions in older individuals. For this to be fully realized reproducible and validated measures that characterize geriatric syndromes and patient reported outcomes must be available and able to be efficiently administered in the medication clinical trial setting.

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Polypharmacy intervention trials

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Polypharmacy represents a major burden for older adults and is associated with adverse outcomes such as decreased quality of life, falls, morbidity and increased mortality (1). Reduction of polypharmacy, usually defined as more than 5 different drugs per day, seems to be an attractive goal. However, according to recent systematic reviews, most studies failed to demonstrate a direct impact on the above mentioned outcomes (2-4). What are the limitations of the studies conducted until now, how can they be improved?

The setting of the studies seems to be an important factor: studies conducted in (geriatric) hospitals appear to be very effective, however, the duration of the effect usually vanishes after weeks or months. Trials in community-dwelling seniors are difficult, since primary care physicians have to participate during their daily routine. Changes in prescribing practices are difficult to achieve. Due to disappointing results, pharmacists are frequently involved in these studies and care for the appropriateness of medications, although they are in many countries not involved in routine daily care. Interventions in nursing homes tackle a particularly vulnerable population in a setting, where specialised care is needed. Here, team interventions appear to be promising.

The tools to reduce inappropriate polypharmacy include brown bag reviews, PIM-lists, START/STOPP and other criteria. A high potential for improvement offer studies focussing on a few drugs and combinations only.

The major criticism is indeed the lack of convincing effects on patient-related endpoints, which are thought to be related with polypharmacy. There is doubt that the most frequently applied MAI score and other medication quality measurements are valid surrogate endpoints for intervention studies in older adults with polypharmacy.

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2. Alldred et al (2016) Cochrane Database of Systematic Reviews, Issue 2, CD009095
3. Smith et al (2016) Cochrane Database of Systematic Reviews, Issue 3, CD006560
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Evidence for deprescribing in older people

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Polypharmacy in older people is associated with harms including increased risk of mortality, hospitalization, institutionalization, falls and confusion. Therefore there has been increased interest in whether ceasing medications is useful in the setting of polypharmacy. Deprescribing has been defined as the process of withdrawal of an inappropriate medication, supervised by a health care professional with the goal of managing polypharmacy and improving outcomes. There is a growing evidence based supported by clinical trials that deprescribing supervised by a clinician can be associated with improved outcomes including mortality, falls and behaviour. This evidence base applies mostly to deprescribing of individual classes of medications, although there are now trials that have or are investigating outcomes following reduction of polypharmacy.

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Implementation of Drug Burden Index as a clinical risk assessment tool to guide management of polypharmacy

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Introduction. Drug Burden Index (DBI) is a pharmacological measure of a person's total exposure to medicines with anticholinergic and sedative effects. Higher DBI is associated with adverse outcomes in older people internationally. DBI may have a role as a clinical risk assessment tool.
Aims. To consider whether and how to implement DBI as a clinical risk assessment tool in different practice settings.
Methods. Summarise the design and outcomes of clinical studies using DBI as a clinical risk assessment tool, and to discuss implications for implementation.
Results. While there is a lot of observational data on the association of DBI with adverse outcomes in older people, and several feasibility studies have been conducted on its implementation into practice, there is limited data from randomised clinical studies using DBI as an intervention. DBI has been assessed as a clinical risk assessment tool in older people living in the community, retirement villages, residential aged care facilities and in older hospital inpatients. Methods have included participant reports and computerised clinical decision support systems, usually conducted by the researcher or a clinical pharmacist, who then provides feedback to the prescriber. Challenges in implementation have been quick, accurate calculation of the DBI in the clinical setting, and generation of a clinically relevant report that the prescriber can interpret and act on.
Discussion. DBI is currently being evaluated as a clinical risk assessment tool. It will be important to evaluate the effectiveness of this risk assessment tool in terms of both prescribing and clinical outcomes in practice.

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Exploiting GPCR Bias at Formyl Peptide Receptors to Protect Against Myocardial Injury

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Myocardial infarction (MI) and the resultant heart failure remain a major cause of death across the globe, despite clinical advances such as thrombolysis and percutaneous revascularisation interventions. These treatments have focused on restoring blood flow to the ischaemic tissue to prevent tissue necrosis in the very short-term (hours). Myocardial injury however continues to evolve over days and weeks post MI, with adverse cardiac remodelling and cardiac contractile dysfunction ultimately progressing to heart failure. This presentation explores the potential for targeting the formyl peptide receptor GPCR family for cardioprotection, beyond their early anti-inflammatory effects to the prevention of late cardiac remodelling and dysfunction up to several weeks after myocardial ischaemic insults in mice *in vivo*. By obtaining signalling fingerprints across pERK1/2, $pAkt^{T308}$, $pAkt^{S473}$, Ca^{2+}-mobilization and cAMP inhibition of two small-molecule FPR agonists *in vitro* in concert with multiple myocardial injury responses across several different timepoints following coronary artery occlusion in mice *in vivo*, we have now demonstrated ligand-selective cardioprotection at FPRs. We have revealed for the first time evidence of bias away, from intracellular Ca^{2+}-mobilization (a trigger of inflammation and of cardiomyocyte death) in parallel with preserved signalling at cell survival kinases. This FPR bias was associated with cardioprotective actions in primary cardiomyocytes and cardiac fibroblasts *in vitro*, together with reductions in each of infarct size, cardiac inflammation, cardiac apoptosis and cardiac dysfunction *in vivo.* These findings demonstrate ligand-selective cardioprotection at FPRs. This breakthrough observation is the first to demonstrate GPCR-agonist bias in the context of cardioprotection *in vivo*, suggesting a new approach for development of small-molecule FPR-pharmacotherapies for treating myocardial ischaemic injury.

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Type 1 cholecystokinin receptors in metabolic disease. Target of endogenous and therapeutic allosteric modulation

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Obesity has become a major public health problem, with increasing prevalence around the world. Lifestyle modification and existing drugs can effectively induce acute loss of weight, however, this has not been durable. Also, while bariatric surgery can be effective in morbidly obese patients, it is not scalable to meet the current need. Insights from gastrointestinal handling of and response to nutrients can provide clues to new therapeutic strategies. A key gut hormone for regulation of satiety is cholecystokinin (CCK), acting through the type 1 CCK receptor (CCK1R) on vagal afferent neurons. Full agonists of this receptor have been developed, but have not achieved approval for clinical use, due to limits in efficacy relative to lifestyle modification, side effects correlating with potency, and the possibility of toxicity. Another potential strategy involves the use of positive allosteric modulators (PAMs) without intrinsic agonist activity. Such an agent would have the advantage of only being active for a finite period of time after meal ingestion. We will review the molecular basis of docking the orthosteric agonist and allosteric small molecule ligands. Strategies for the development of drugs targeting the receptor allosteric pocket, yet devoid of intrinsic activity, in attempt to prepare non-caloric satiety agents to reduce meal size and frequency will be discussed. Lateral allosteric regulation of this receptor by elevated cholesterol in the bilayer, such as has been described in metabolic syndrome, is also prominent. This interferes with CCK stimulus-activity coupling, with high affinity ligand binding and low potency of biological responses observed. This provides a need for new types of drugs and an opportunity for corrective PAMs. An effort to define what clinical groups may exhibit reduced sensitivity to CCK at the CCK1R is in progress, and will be used to determine who might need and benefit from this new type of chemical agent.

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Identification of positive allosteric modulators of the Glucagon-like peptide-1 receptor

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Introduction. Agonism of the glucagon-like peptide-1 receptor (GLP-1R) is a clinically validated therapeutic intervention for type 2 diabetes mellitus. Activation of the GLP-1R induces glucose-dependent insulin secretion and improves energy balance in diabetic patients. Currently available GLP-1R agonists require parenteral administration and a key therapeutic goal is the discovery of orally available small molecules. One approach to activate the GLP-1R is to potentiate circulating GLP-1R ligands such as oxyntomodulin and the inactive GLP-1 metabolite GLP-1(9-36).
Aims. The identification and in vitro and in vivo pharmacological characterization of small molecules that potentiate signal transduction of the GLP-1 inactive metabolite GLP-1(9-36) at the GLP-1R.
Methods. We identified compounds as weak potentiators of GLP-1(9-36) by quantifying cAMP accumulation in HEK-293 cells expressing the GLP-1R. We performed iterative optimization of in vitro pharmacology (cAMP, β-arrestin), pharmacokinetics, and pharmacodynamics to generate tool compounds with in vivo activity in hyperglycemic rodent models.
Results. We identified compounds that selectively potentiate the affinity and efficacy of GLP-1(9-36) at the GLP-1R. Characterization by functional assays indicated a high degree of probe dependence for orthosteric ligand with ligands potentiating GLP-1(9-36) and GLP-1(7-36) but not oxyntomodulin. Moreover, compounds strongly potentiated the Gαs-cAMP signal transduction pathway with limited effects on β-arrestin signal transduction.
Discussion. We have identified allosteric GLP-1R ligands with robust in vitro activity and glucose-lowering efficacy in preclinical models.

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Role of cyclic AMP in BRL37344 and isoprenaline mediated glucose uptake by β2-adrenoceptors

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Introduction: Cyclic AMP (cAMP) is a key second messenger involved in β_2-AR mediated glucose uptake in skeletal muscle but in vivo may causes adverse effects including altered vasoreactivity and cardiac hypertrophy. It has been suggested that BRL37344 (BRL), a dual β_2/β_3-AR agonist originally developed for the treatment of obesity, increases skeletal muscle glucose uptake via β_2-ARs independently of cAMP, but the mechanism has not been rigorously examined (Nevzorova et al 2002). This study examines the signalling mechanisms activated by isoprenaline (Iso) and BRL to promote glucose uptake in CHO-K1 cells stably expressing the β_2-AR and myc-tagged GLUT4 (CHOβ_2GLUT4myc) and L6 skeletal muscle cells.
Methods. We measured glucose uptake with [^{3}H]-2-deoxy-glucose, the production of cAMP was determined by LANCE cAMP assay and Förster resonance energy transfer (FRET; pmEpac2, cytoEpac2), receptor internalisation was measured by Bioluminescence resonance energy transfer (BRET; β-arrestin, Kras).
Results. In L6 cells, BRL increased glucose uptake with a similar potency and efficacy to Iso (pEC_{50} 7.41±0.2 and 7.45±0.3; Emax 168.1±4.6 and 186.8±7.9 respectively, n=6), but was a partial agonist when measuring global cAMP levels (Iso pEC_{50} 8.44±0.1 Emax 15.5±0.5pmol cAMP/well; BRL pEC_{50} 6.57±0.1 Emax 6.9±0.3pmol cAMP/well; n=4-6). However glucose uptake to both Iso and BRL was partialy inhibited by PKI, a competitive inhibitor of PKA by 74.5% and 54.3% respectively (n=5). FRET studies in CHOβ_2GLUT4myc cells showed that BRL and Iso activated the plasma membrane cAMP sensor pmEpac2 to the same degree but that BRL caused significantly less activation of the cytoplasmic cAMP sensor cytoEpac2. BRET studies showed that Iso, but not BRL, caused interaction between the β_2-AR and β-arrestins, internalization and desensitization.
Discussion. Our results show that BRL has equivalent effects to Iso on glucose uptake, preferentially increases cAMP at the plasma membrane and does not produce desensitization or internalization of the receptor.

Nevzorova, J., et al. (2002) Br J Pharmacol, 137:9-18.

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Engineering ultra-stable α1-adrenoceptor subtypes allows the interrogation of ligand selectivity and fragment screening using ligand-observed NMR

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α_{1A}- and α_{1B}-adrenoceptors (α_{1A}-AR and α_{1B}-AR) are closely related G protein-coupled receptor (GPCRs) that modulate the cardiovascular and nervous systems in response to binding epinephrine and norepinephrine. Using transgenic mouse models these receptors have been shown to mediate opposing responses to adrenaline and noradrenaline release, especially in relation to: cardioprotection during heart failure; epileptic seizures; and neuroprotection in neurodegenerative diseases (1). Elucidation and clinical targeting of the individual roles of α_{1A}-AR and α_{1B}-AR has been hindered however, due to the lack of subtype selective ligands and antibodies. The GPCR gene super-family (>800 genes) is made up of numerous sub-families that, like α_{1A}-AR and α_{1B}-AR, are activated by the same endogenous agonists but may modulate different physiological processes. Thus a major challenge in GPCR drug discovery is understanding how compounds interact with receptors at the molecular level in order to optimize the efficacy and selectivity of drug leads. Ligand-observed NMR methods such as saturation transfer difference NMR (STD NMR), water-ligand observed via gradient spectroscopy (WaterLOGSY); and transferred-NOE spectroscopy (NOESY) are powerful methods for characterizing ligand-protein interactions, epitope mapping and fragment screening (2). Such techniques have not been widely employed to study ligand binding at GPCRs due to the challenges associated with purifying GPCR proteins and maintaining them in solution. To overcome this, we evolved ultra-stable α_{1A}-AR and α_{1B}-AR variants using Cellular High-throughput Encapsulation, Solubilization and Screening (CHESS) (3). The long-term stability of these receptors in detergent solutions enabled the analysis of ligand binding using STD NMR, WaterLOGSY, and NOESY, which revealed the different binding modes selective ligands use at each subtype. Furthermore, an NMR based fragment screen was conducted using both ultra-stable receptors in parallel, identifying several hits that were subsequently shown to include both antagonists and agonists at the wild-type receptors in cell-based assays. Because GPCRs are the largest, yet underexploited class of drug targets, this work has broad implications for studying GPCR-ligand interactions and for drug discovery and optimization.

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Identification of novel irritant sensing mechanisms in the bladder

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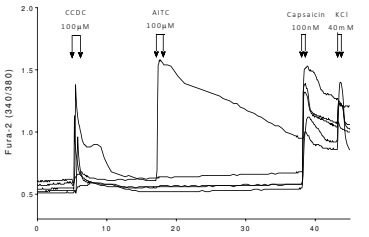
Introduction: Studies suggest that the symptoms associated with overactive bladder syndrome (OAB) and interstitial cystitis (IC) may be due to a disrupted mucosal barrier. Access of the urine to underlying sensory structures is hypothesised to induce bladder irritation which, in the absence of an ability to 'scratch', manifests in the symptoms of urinary urgency and frequency. The TGR5 receptor has been implicated in mediating non-histaminergic itch responses in the skin and is responsible for bile acid induced pruritis (itch). Bile acids are secreted in high concentrations in the urine and may be responsible for the sensory abnormalities seen in OAB and IC.

Aims: To determine the expression and function of TGR5 in bladder afferent neurons.



Methods: Retrogradely traced bladder DRG neurons from mice were isolated and dissociated for single cell RT-PCR, calcium imaging and whole cell patch clamp recordings. *Ex-vivo* bladder afferent recordings were used to identify the role of TGR5 in bladder mechanosensation.

Results: Single cell PCR data identified TGR5 mRNA expression in bladder afferent neurons. CCDC (100µM) was able to induce significant calcium transients in 60% of neurons. Application of CCDC (10µM) was able to induce a significant hyperexcitability of bladder DRG as indicated by an increase in the number of action potentials at 2X rheobase (1.1±0.14 vs 3.0±0.48, n=6, P≤0.01) and a decrease in rheobase compared to control (100±10 vs 88±11, n=6, P≤0.001). CCDC (300µM) also induced significant mechanical hypersensitivity to bladder distension versus control (8.0±0.96 vs 18.6±2.4, n=5, P≤0.001).

Discussion: This data shows for the first time that TGR5 is functionally present on bladder DRG neurons and its activation is able to induce calcium transients, neuronal hyperexcitability and mechanical hypersensitivity. This suggests that histamine-independent itch mechanisms are not exclusive to the skin, but are present and functional in the viscera and may hold the key to bladder hypersensitivity in various pathologies and syndromes.

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Nav1.8 is required for mechanosensation in hypersensitive colonic afferents and Na_v-induced colonic pain *in vivo*

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Introduction. Voltage-gated sodium (Na_v) channels regulate action potential generation and cell membrane excitability in sensory neurons, and are implicated in several pain phenotypes. In this study, we examined the contribution of $Na_v1.8$ in colonic pain signaling in a mouse model of chronic visceral hypersensitivity (CVH).

Aims. Identify which Na_v channels are expressed in sensory neurons that innervate the colon, and assess if modulation of identified channels can have an analgesic effect in a model of CVH.

Methods. Gene expression profiles were obtained by quantitative RT-PCR in dorsal root ganglia (DRG) at spinal levels T10-S1 from healthy and CVH mice. *In vitro* electrophysiological recordings were performed on colonic nociceptors from healthy and CVH mice in the presence and absence of a $Na_v1.8$-selective antagonist A-803467. *In vivo* pain behavior studies were performed in mice with an intra-colonically administered pan-Na_v channel activator in the presence or absence of A-803467.

Results. Na_v channel isoforms $Na_v1.1$, $Na_v1.2$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$, $Na_v1.9$, and β-subunits β1-β4 were expressed in neurons from all spinal levels (T10-S1). $Na_v1.8$ gene expression was consistently higher than all other Na_v channels and β-subunits across all levels. *In vitro* electrophysiology recordings showed that A-803467 administration caused a significant reduction in CVH colonic nociceptor mechanosensitivity ($p<0.05$, n = 8), whereas nociceptors from healthy mice were not significantly affected by $Na_v1.8$ inhibition. *In vivo* intra-colonic administration of a pan-Na_v channel activator significantly increased pain behaviors, which were fully reversed by co-administration with A-803467 ($p<0.05$, N = 6-12 animals/group).

Discussion. Inhibition of $Na_v1.8$ in DRG neurons had a pronounced effect on nerves from CVH animals, suggesting that $Na_v1.8$ may contribute to CVH colonic nociceptor hypersensitivity. In conjunction with *in vivo* studies, our findings indicate that targeting $Na_v1.8$ could be beneficial in the treatment of chronic colonic pain syndromes.

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Pharmacology of the human β_3-adrenoceptor agonist mirabegron at the mouse β_3-adrenoceptor

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Introduction. The β_3-adrenoceptor (AR) is a pharmacological target for overactive bladder syndrome (Bhide et al., 2012) and has a postulated role for the treatment of obesity (Hainer, 2016). The human β_3-AR agonist mirabegron has recently been approved for use in Australia although there is little information on the selectivity or potency of mirabegron at either the human or mouse β_3-AR.
Aim. To examine the pharmacological profile and selectivity of mirabegron at the mouse β_3-AR
Method. Mouse primary brown, white and brite adipocytes from FVB mice, CHO-K1 cells expressing the human or mouse β_3-AR, or bladder detrusor muscle from FVB and β_3-AR knockout mice were used. β_3-AR function (cAMP production, [^{3}H]-2-deoxyglucose uptake, glycolysis, oxygen consumption, relaxation of carbachol and KCl pre-contracted detrusor muscle) was assessed in response to mirabegron. P was evaluated using 2-way ANOVA or Student's t-tests.
Results. In CHO-K1 cells expressing either the mouse or human β_3-AR, mirabegron increased cAMP levels with a similar Emax to isoprenaline, but with a greater potency at the human receptor. In mouse brown and brite (but not white) adipocytes, mirabegron increased cAMP levels, glucose uptake, glycolysis and oxygen consumption rates. In mouse detrusor muscle, mirabegron concentration-dependently relaxed muscle pre-contracted with either KCl or carbachol in both FVB and β_3-AR knockout mice.
Discussion. While mirabegron shows agonist properties at both the human and mouse β_3-AR, questions remain about its selectivity since relaxation responses in mouse detrusor muscle were intact in β_3-AR knockout animals.

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Hainer V (2016). Expert Opin Pharmacother, DOI: 10.1080/14656566.2016.1233177

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Evidence for mu and delta opioid receptor coexpression and functional interactions in the mouse colon

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Introduction. Opiates acting through the mu opioid receptor (MOR) are effective analgesics and anti-diarrheals. However, their use can be severely limited by on-target side-effects including intractable constipation, which is mediated via the enteric nervous system (ENS). Recent studies have demonstrated that MOR and the related delta opioid receptor (DOR) can functionally interact, leading to altered pharmacological and signaling profiles of these receptors. Whether MOR and DOR are coexpressed in the ENS and can functionally interact is unknown.
Aims. To investigate the co-expression of MOR and DOR in the mouse colon and to determine whether interaction between MOR and DOR occurs.
Methods. MOR and DOR co-expression was detected using MORmCherry/ DOReGFP knockin mice. Neuronal phenotypes were determined using established markers. The effects of the putative MOR-DOR heteromer agonist CYM51010 on neurogenic contractions of the mouse distal colon were examined.
Results. Approximately 25% of all HuC/D-immunoreactive myenteric neurons of the colon were positive for both MORmCherry and DOReGFP. The majority of these neurons were cholinergic excitatory neurons, identified by ChAT and calretinin labelling. CYM51010 inhibited neurogenic contractions of the distal colon in a concentration-dependent manner (pEC50= 7.20±0.14, n=11). The addition of the MOR selective antagonist CTOP (1μM; pEC50= 6.79±0.18, n=6) or the DOR-selective antagonist naltrindole (100 nM; pEC50= 6.81±4.41, n=6) individually did not significantly alter the response to CYM51010. In contrast, the simultaneous addition of both naltrindole and CTOP produced a 36-fold shift in the CYM51010 concentration-response curve (pEC50= 5.64±0.28, n=11, P<0.001).
Discussion. In summary, our data demonstrate extensive coexpression of MOR and DOR in myenteric neurons of the mouse distal colon. Furthermore, we provide evidence to support functional interaction between these two opioid receptor subtypes in the ENS. This study has potential implications for the therapeutic targeting of MOR and DOR in the gut, and for the development of opiate analgesics with reduced constipatory side-effects.

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The tachykinin NK_2 receptor antagonist, ibodutant, displays gender- and agonist-dependence in the human colon

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Introduction. Upon release from enteric neurons, neurokinin A (NKA) contracts intestinal smooth muscle via the tachykinin NK_2 receptor (NK_2R). In clinical trials, the NK_2R antagonist, ibodutant, produced a better treatment outcome in female patients with diarrhoea-prominent irritable bowel syndrome (IBS-D), compared to males (Menarini Group, 2015). On the other hand, we have previously reported that [^{125}I]NKA binding sites on colonic smooth muscle were more abundant in males than in females (Burcher et al, 2008).
Aims. We investigated the gender-related differences surrounding the NK_2R in the human colon.
Methods. Fresh human colonic circular muscle strips were mounted in organ baths and contractile responses to NKA and the selective NK_2R agonist, [Lys^5,MeLeu^9,Nle^{10}]NKA(4-10) were recorded. Strips were pre-incubated either with ibodutant (0.01, 0.1 and 1 µM) to determine its antagonist potency, or the rho kinase inhibitor, Y27632, and the PLC inhibitor, U73122 hydrate (3, 30, 300 µM) to investigate the second messenger signalling pathways involved in NK_2R-mediated contractions.
Results. Contractile responses induced by NK_2R agonists were more prominent in females than in males. Interestingly, ibodutant displayed a significantly higher degree of competitive antagonism against NKA in females, pA_2 = 8.38 (95%CI 7.75-10.35, n = 9), compared to males, pA_2 = 7.80 (7.26-9.45, n = 8, $P<0.05$). However, the gender discrepancy for ibodutant did not exist when against [Lys^5,MeLeu^9,Nle^{10}]NKA(4-10). Both Y27632 and U73122 significantly reduced NK_2R-mediated colonic smooth muscle contractions.
Discussion. Ibodutant appeared agonist-dependent in females, suggesting that ligand-dependent conformations of the NK_2R may underlie the gender discrepancies of ibodutant in the treatment of IBS-D. NK_2R-mediated human colonic smooth muscle contractions may involve both Gαq-PLC-DAG-PKC and rho kinase signalling pathways.

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Burcher E et al (2008) J Pharmacol Exp Ther 324:170-8

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Legumain is a novel biomarker and therapeutic target in inflammatory bowel disease

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Introduction. Inflammatory bowel disease (IBD), encompassing ulcerative colitis and Crohn's Disease, is a major health problem worldwide. While the etiology of IBD remains incompletely understood, it is becoming increasingly clear that proteases play critical disease-promoting roles. Among these are cysteine cathepsins and matrix metalloproteases, both of which can be regulated by another cysteine protease called legumain. Legumain is upregulated in inflammation associated with cancer, atherosclerosis, and pancreatitis, but it has not yet been studied in the gut. We therefore hypothesized that legumain might be a key driver of gut inflammation.
Aims. We aimed to measure the activation of legumain during colitis and whether this activity contributes to pathogenesis of IBD.
Methods. In the DSS mouse model of colitis compared to healthy controls, legumain activation was measured with a fluorescent activity-based probe, LE28, by ex vivo IVIS imaging, SDS-PAGE, and confocal microscopy. A legumain-specific inhibitor, LI-1, was used to determine the contribution of legumain activity to disease symptoms.
Results. Legumain activity was upregulated in the proximal colon of DSS-treated mice compared to healthy controls. Legumain was predominantly found within CD68$^+$ macrophages of the mucosal layers of the colon, with little activity in the muscle. Active legumain was also detected in luminal fluid and feces of mice with colitis, but not healthy mice. LI-1-treated mice exhibited delayed symptoms of colitis compared to vehicle-treated controls, including diarrhea and fecal blood. At end point, LI-1-treated mice exhibited less weight loss and less shortening of the bowel. Biochemical characterization of the colons revealed that legumain and cathepsin L activation was impaired in mice treated with the legumain inhibitor, indicating that legumain may promote the symptoms of colitis directly or through modulation of downstream proteases. Production of several pro-inflammatory cytokines was also reduced upon legumain inhibition.
Discussion. Our data demonstrate that legumain is activated in the inflamed mouse colon and promotes inflammation. The detection of activated legumain in colon, luminal fluid and feces of mice with colitis indicates that legumain may be a disease biomarker. Legumain inhibitors may represent a novel therapy for inflammation.

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Identification of Novel sites of TRPV4 Expression in the Mouse Colon

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Introduction. Transient Receptor Potential Vanilloid 4 (TRPV4) is a non-selective cation channel activated by mechanical stimuli, lipid mediators and GPCR-mediated signalling cascades. TRPV4 is implicated in the aetiology of visceral hyperalgesia, inflammatory bowel disease and irritable bowel syndrome. Although TRPV4 plays important roles in colonic function, very little is known about where TRPV4 is expressed in the gut.
Aims. To characterize the functional expression of TRPV4 in the mouse colon by defined cell types.
Methods. TRPV4 activity was detected by Ca^{2+} imaging of colonic myenteric and submucosal wholemounts. Cellular identity was confirmed by immunofluorescence using established markers. Functional expression was characterised using TRPV4-selective agonists and antagonists in relevant cultured primary cells and cell lines.
Results. Increased intracellular Ca^{2+} was detected in enteric glial cells, macrophages, vascular and lymphatic endothelial cells in response to the selective TRPV4 agonist GSK1016790A (100nM). Glial responses were characterized by transient oscillatory Ca^{2+} waves within a subset of glia that were attenuated in frequency by the gap junction inhibitors carbenoxolone and $^{43}GAP^{26}$. Tissue-resident macrophages exhibited marked morphological changes in response to TRPV4 agonist. All responses to GSK1016790A were abolished by pre-treatment with the TRPV4 antagonist HC-067047 (10μM) and in $trpv4^{-/-}$ tissues, confirming specificity. Identity of cells was confirmed by post-staining with GFAP/S100B, CD68/F4/80/CD169, CD31 and LYVE1/VEGFR3, respectively. Direct activation was confirmed in isolated glial cells, macrophages (RAW264.7, resident, peritoneal and bone-marrow derived macrophages) and primary human dermal lymphatic endothelial cells.
Discussion. We have defined novel and previously unappreciated sites of functional TRPV4 expression in the colon. These findings differ from those reported using immunofluorescent detection and demonstrate TRPV4 function in cells potentially involved in colonic motility (macrophages, glia) and inflammation (vascular and lymphatic endothelia, macrophages). Thus, these findings have major implications for our current understanding of the proposed neurogenic role of TRPV4 in colonic inflammation and in intestinal motility.

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Biological effects on rat prostatic smooth muscle and chemical fractionation of *Costus speciosus* rhizome

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Introduction. *Costus speciosus* (Crêpe ginger or Spiral ginger) has been traditionally used by Sarawak natives to treat urological disorders.
Aims. To assess the biological effects of *C. speciosus* on prostate contractility and isolate bioactive components.
Methods. *C. speciosus* rhizome and roots were harvested from Sarawak. Extracts of dried and ground plant materials were obtained using water at different temperatures (i.e. 100°C, ~70°C, and ~21°C). Activity of these extracts was evaluated pharmacologically by assessing their effects on contractions of isolated rat prostate gland. Nerve mediated contractions were evoked electrically (0.1-20 Hz, 0.5 ms pulse duration, 60 V) while direct muscle stimulation was achieved by application of the exogenously administered agonists: noradrenaline, acetylcholine or ATP. Various pharmacological tools were used to identify mechanisms of action.
Results. *C. speciosus* rhizome and root decoction (100°C) extract (2.0 mg/mL) inhibited electrical field stimulation (EFS) induced contractions of rat prostatic smooth muscle by 64 ± 9.8% and 60 ± 11.5% at frequencies of 1.0 Hz and 2.0 Hz, respectively (p = 0.0161, n=4); whereas cold water (~21°C) extract (2.0 mg/mL) inhibited contractions by 73 ± 3.4% and 76 ± 2.2% at 1.0 Hz and 2.0 Hz, respectively (p < 0.0034, n=4). Contractions mediated by exogenous administration of noradrenaline (n=6), acetylcholine (n=6) or ATP (n=4) were not inhibited by rhizome extract (2.0 mg/mL). EFS induced contractions were still attenuated by the extract (2.0 mg/mL) in the presence of all inhibitors tested. In addition, extract (2.0 mg/mL) caused a concentration-dependent transient tonic contraction (p = 0.0092, Mean = 1.31 ± 0.32 g, n = 6) of the unstimulated prostatic tissue. The magnitude of the tonic contraction produced was also different in the presence of different pharmacology tools.
Discussion. The *C. speciosus* rhizome and root extract inhibits nerve mediated prostate smooth muscle contraction presumably by inhibition of neurotransmitter release by an unidentified pre-junctional mechanism. Tonic prostatic smooth muscle contraction induced by addition of extract indicates the existence of bioactive compounds in the rhizome extract that act via a different mechanism.

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A pilot feasibility study to assess *CYP2B6*6* genotype frequency and adverse effects to efavirenz in HIV/AIDs patients in Papua New Guinea

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Introduction. Efavirenz is a major antiretroviral drug for HIV infection therapy. CYP2B6 is the major enzyme in its metabolism and people with the **6* variant allele (516G>T) have substantially higher blood concentrations and the risk of severe CNS adverse effects, including psychiatric effects such as suicidal ideation, depression and aggression. Mehlotra et al. (2006) reported a **6* frequency of 65% (Caucasian ~20%) in 172 East Sepik (PNG) Province HIV/AIDs subjects but found a significant loss of heterozygosity.
Aims. To determine the feasibility of collecting, transporting and analysing saliva samples from HIV/AIDs patients in PNG maintained long term on 600 mg efavirenz daily and to collect data on CNS adverse effects.
Methods. Following ethics approval, HIV/AIDs participants were to sign an informed consent form and provide a saliva sample using Oragene DNA Saliva Kits (DNA Genotek Inc. Canada) to be transported unrefrigerated to Adelaide for DNA isolation and genotyping. Demographic data collection included age, sex, ethnicity (village, district, province), language, comorbidities and comedications. Adverse effects data included CNS and psychiatric.
Results. Fifty-two participants' samples and data were obtained within 3 weeks. The saliva samples were discoloured due to betel nut chewing thus requiring an extra DNA purification step, after which 51/52 samples met assay requirements for DNA purity and quantity. The T variant frequency was 52% and genotype frequencies were 25% GG, 45% GT and 29% TT (Hardy-Weinberg P=0.5). There was no association between carriers of the **6* variant and CNS (P>0.053, drowsiness), or psychiatric (P>0.4) adverse effects.
Discussion. The main aim was to determine the feasibility of conducting pharmacogenetic studies in PNG, which was successful. The *CYP2B6*6* genotyping data were similar to a previous study but with no loss of heterozygosity. The lack of association between **6* carriers and adverse effects to efavirenz requires further studies including blood concentration analysis and assessment of efavirenz adherence to dosing.

Mehlotra RK et al (2006) Eur J Clin Pharmacol 62:267-275.

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Relationship between whole blood and intra-graft tacrolimus concentrations in renal transplant recipients

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Introduction. Whilst cyclosporine and tacrolimus have significantly improved graft survival, their long-term use is limited by nephrotoxicity. Both are substrates for cytochrome P450 3A (CYP3A) and P-glycoprotein (P-gp). P-gp expression determines chronic tubulointerstitial damage in transplanted kidneys (Naesens et al, 2009), and intra-renal cyclosporine concentrations (Sallustio et al, 2012). Although tacrolimus has largely replaced cyclosporine, little is known regarding the factors that determine its intra-renal exposure, and hence potential nephrotoxicity.
Aims. To investigate the relationship between trough blood (C_B) and intra-renal (C_R) tacrolimus concentrations, and the role of *CYP3A5* genetic polymorphisms or P-gp expression in determining graft tacrolimus exposure.
Methods. This was a retrospective study in 134 transplant recipients from whom 239 matching blood and renal biopsy samples had been collected between 2-2490 days post-transplantation. C_B and C_R were measured by LC-MS/MS. P-gp expression was assessed by immunohistochemistry in paraffin-embedded biopsy samples. Donor *CYP3A5* genotypes (**1/*1*, **1/*3*, **3/*3*) were determined by TaqMan SNP Genotyping (ThermoFisher Scientific).
Results. C_B ranged from 2.6-52.3 μg/L and C_R from 33-828 pg/mg tissue (n=239). There was a weak but significant correlation between C_B and C_R (Spearman r = 0.44, P<0.0001), and within individuals, changes in C_R largely mirrored those in C_B. C_R/C_B (an estimate of 'net' renal uptake) was inversely correlated with time post-transplant (Spearman r = -0.16, P=0.03) and C_B (Spearman r = -0.40, P<0.0001). In the first month post-transplantation there was no effect of either graft P-gp expression (r = 0.01) or donor CYP3A5 expressor phenotype (P=0.81) on C_R/C_B.
Discussion. Blood tacrolimus concentrations only predict 20% of variability in intra-renal concentrations. Net renal uptake of tacrolimus may be saturable, and may be greater in the early compared to later post-transplant period. Renal cellular clearance pathways (P-gp or CYP3A5) do not significantly affect graft tacrolimus exposure.
Naesens M et al (2009) J Am Soc Nephrol 20:2468-2480
Sallustio BC et al (2012) ASCEPT-APSA Proc (P155), 52

334

Statin induced myalgia, is there a genetic link?

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Introduction. Statins are drugs that reduce the risk of cardiovascular disease. A proportion (up to 25%) of patients prescribed statins report a spectrum of muscle aches and pains ranging from myalgia to myopathy, and rarely, rhabdomyolysis.
Aims. Statin-associated muscle pain is largely dose-dependent, some patients experience muscle aches and pains at relatively low doses and across various types of statins. Therefore, excluding dose as a factor, genetic variants, particularly in the *SLCO1B1* gene, have been associated with an increased risk of statin-induced muscle ADRs. However, we suspect that there may be additional and/or different genes that pre-dispose a patient to be statin-intolerant.
Methods. To assess this, we collected blood samples from a group of patients who have trialled various statins, but have suffered muscle myalgia on at least two re-challenges (these patients were defined as statin-intolerant (n=8)). We then conducted exome sequencing to seek genetic variants that predispose to statin-intolerance. Genetic variation identified was analysed using standard bioinformatics to identify rare variants i.e. variants that are reported to occur in less than 1% of the population, as well as common variants, in curated genetic variant databases and user-created candidate gene lists. As this is an exploratory study in eight patients, statistics were not conducted.
Results. Statin intolerance was associated with variants in the *CYP2C19*, *CYP2D6*, *NOX4,* and *SLCO1B1* genes.
Discussion. Common genetic variants (expressed in >5% of the population) that affect the PK of statins, may explain statin-induced myalgia in some patients, however, rare variants will require further large scale studies and variant specific functional assays to determine the association with statin-intolerance. Together, this approach will support our goal to characterise genetic variants that may predispose to statin-induced myalgia, and to then apply this information clinically to identify those patients who may benefit from alternative medication.

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A pharmacological exploration of the *in vitro* neurotoxicity of king cobra (*Ophiophagus hannah*) venom

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Introduction. Despite recent proteomic studies indicating the presence of neurotoxins in king cobra (*Ophiophagus hannah*) venom (Petras et. al., 2015; Tan et. al., 2015), little is known about these toxins or the neurotoxicity of whole venom.
Aim. To study the *in vitro* neurotoxicity of king cobra venom, and to isolate and characterise the major neurotoxins.
Methods. A series of chromatographic steps was used to fractionate *O. hannah* venom. *In vitro* neurotoxicity was determined using chick-biventer cervicis nerve-muscle (CBCNM) preparation. Isolated fractions were analysed by gel electrophoresis (GE), matrix-assisted laser desorption/ionisation (MALDI-TOF) mass-spectrometry and liquid chromatography mass spectrometry (LC-MS). The *in vitro* efficacy of Thai Red Cross King cobra antivenom (TKCAV) was assessed by either addition before adding venom or after the twitches were inhibited by 90%.
Results. *O. hannah* venom caused concentration-dependent inhibition of indirect twitches in the CBCNM (1-10 µg/ml; n=5). A neurotoxic fraction constituting 3.9% of the venom was isolated, which inhibited indirect twitches (0.225-2.25 µg/ml; n=4) and responses to exogenous acetylcholine and carbachol (n=4, $P<0.05$). TKCAV prevented the neurotoxic effects of venom and the neurotoxic fraction. TKCAV, as well as repeated washing of the preparation, partially reversed the neurotoxicity of the venom and the fraction. However, GE and MALDI-TOF mass spectrometry analysis indicated that the fraction contained two toxins with intact masses of 7941.5Da and 7521.7Da, which were identified by LC-MS as LNTX-2 and Haditoxin.
Discussion. This study demonstrated that *O. hannah* venom and its major neurotoxic fraction are post-synaptically neurotoxic *in vitro* and can be neutralised and partially reversed by TKCAV. The neurotoxic fraction contains LNTX-2, which requires further purification.

Petras D et al (2015) J Proteome, 14, 2539-2556
Tan C et al (2015) BMC Genomics, 16:687

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Combining Adverse Outcome Pathways with multitask machine learning to simultaneously improve in silico rodent carcinogenicity prediction performance and enable mechanistic interpretation

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Introduction. Adverse Outcome Pathways (AOP) are frameworks that integrate the results of high throughput *in vitro* and omics assays with existing knowledge derived from *in vivo* bioassays. This approach enables mechanistic interpretation of assay results in order to improve understanding for regulatory decision making. The computational adaption of AOPs with toxicogenomics and multiple *in silico* models of toxicological assays could potentially identify mechanisms which are not well-characterised in existing models. Multitask machine learning paradigms complement *in silico* AOPs by aiming to elucidate a single model that could assess multiple toxicological endpoints.
Aims. This project aims to investigate the performance of multitask machine learning approaches compared to multiple single-task models in modelling chemical datasets with rodent carcinogenicity and in vitro genotoxicity assay outcomes. Additionally, this project aims to compose an AOP from *in silico* assay model predictions and additional toxicogenomics findings.
Methods. Single-task and multitask machine learning algorithms were used to model chemicals to their carcinogenicity or genotoxicity outcomes by relating physicochemical features to binary toxicity results. Gene expression data from the Comparative Toxicogenomics Database was analysed to determine non-genotoxic carcinogenicity outcomes that cannot be detected by *in vitro* assays. AOPs were to be composed in the end by grouping experimental findings into blocks or clusters.
Results. A single-task toxicogenomics machine learning model featured 75.09% accuracy with 10 times 10-fold Cross Validation (10x10CV), indicating the gene expression data is capable of being modelled. While this project is still ongoing, initial single-task models all feature above 80% accuracy (10X10CV) in predicting genotoxicity, however, the size of the genotoxicity assay dataset is not indicative of in silico to in vivo extrapolation performance.
Discussion. Despite good performance, there are barriers to implementing toxicogenomics data as it is hard to generalise findings to unprofiled chemicals which include large numbers of chemicals used for in silico modelling. Limitations in the assay design may account for marginal improvement with multitask machine learning paradigms.

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The changing face of snakebite in Australia: 10 years of the Australian Snakebite Project (ASP).

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Introduction. Snakebite is rare but medically important in Australia. There have been no large epidemiological studies of Australian snakebite. Aims. We aimed to define the epidemiology of snakebite and envenoming, including changes in treatment over a 10y period, using data collected by the Australian snakebite project (ASP).
Methods. ASP recruits suspected and confirmed snakebite throughout Australia. Data is collected prospectively including demographics, bite circumstances, clinical and laboratory effects, treatment and complications. Blood is collected to measure venom concentrations. The national coronial information system (NCIS) was searched for deaths. Results. 1549 cases were recruited (2005-2015); median age 38y (6m-92y); 1135 (73%) were male, 168 (11%) were snake handlers, 54 (3.5%) were intoxicated. Systemic envenoming occurred in 833 (54%) patients with a median length of stay of 41h (interquartile range:25-68h), including brown snake envenoming: 281 (34%), red-bellied black snake: 117 (14%), tiger snake: 113 (14%), rough-scaled snake: 61 (7.1%), taipan: 32 (3.8%), mulga snake: 30 (3.6%), *Hoplocephalus* spp.: 26 (3.1%), and death adder: 21 (2.5%). Brown snakes (24h) and red-bellied black snakes (22h) had the shortest median length of stay, taipans (71h) and mulga snakes (65h) had the longest. Envenoming syndromes included venom-induced consumptive coagulopathy (73%), myotoxicity (17%) and acute kidney injury (11.5%). There were 25 deaths, including 16 from NCIS; 16 brown snake, 4 tiger snake and 5 unknown. Seqirus snake venom detection kits were used in 694 (83%) envenomed patients and 366 (51%) non-envenomed patients. Antivenom was given to 756 patients, 194 (26%) had an adverse reaction. Over ten years the median total antivenom dose decreased for brown snake (5.5 to 1 vial), tiger snake (4 to 1 vial), mulga snake (3 to 1 vial), and taipan (3.5 to 1 vial).
Discussion. Australian snakebite carries significant morbidity and mortality, despite being relatively rare. Antivenom dosing has changed over the last decade, possibly due to research highlighting that one vial is sufficient for neutralisation but some envenoming syndromes are not reversed by antivenom.

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Utilization of multiple reaction monitoring scan mode for the simultaneous determination of amlodipine and lercanidipine in human plasma

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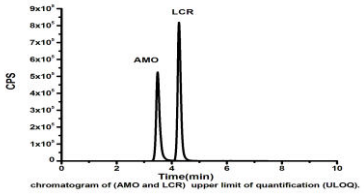

chromatogram of (AMO and LCR) upper limit of quantification (ULOQ).

Introduction: The calcium channel blockers Amlodipine (AMO) and Lercandipine (LER) are used in the treatment of chronic stable angina and in management of mild-to-moderate essential hypertension. They inhibit the entry of calcium into vascular and cardiac muscle. There are many reported methods for determination of AMO and/or LER in biological and pharmaceutical dosage forms but these require complex sample preparation that is time consuming.

Aim: To develop and validate a simple, rapid method for the simultaneous quantitation of AMO and LER to evaluate poisoning in single and mixed ingestions with other vasodilators.

Method: Chromatographic separation was achieved on a Luna 5 µm C18 (2) (Phenomenex) 50×2.0 mm column using gradient flow of a 5:95 mixture of 0.1% aqueous formic acid and acetonitrile. The eluates were detected by coupling the liquid chromatography (LC) with ESI-tandem mass spectrometer.

Results. The method was validated according to FDA guidelines with accuracy and precision within acceptable ranges (<15%). The method was effectively applied to clinical samples from patients under treatment with concentrations ranging from (13.9-148) and (8.5-88) ng/ml for AMO and LER respectively.

Discussion: The use of multiple reaction monitoring (MRM) decreases the interference of the matrix. Simple protein precipitations was used successfully to extract analytes from patients' plasma from dihydropyridine toxicity project. The developed method separated AMO, LER (Figure) and prednisolone (internal standard) in 5 min run time.

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Gelofusine ameliorates colistin-induced nephrotoxicity

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The decline in the discovery of novel antibiotics and the emergence of bacterial superbugs present a significant global medical challenge. Currently, polymyxin B and colistin are being used as the last-line defence for life-threatening Gram-negative infections. Polymyxins were approved for clinical use in the late 1950s, however due to concerns regarding their safety they were replaced by 'less' toxic antibiotics. As a result of low usage rates, polymyxin resistance among multi-drug resistant (MDR) Gram-negative organisms remains low. Acute kidney injury (AKI) attributed to treatment with colistin severely limits their clinical applications. The nephrotoxicity rate in patients receiving currently recommended dosage regimens is high. Colistin nephrotoxicity is dose-dependent but usually reversible upon discontinuation of therapy. Gelofusine a blood volume substitute is routinely used in the clinics and very recently has been shown to be nephroprotective against radiolabeled somatostatin analogs. Using a murine model coupled with histopathological evaluation and a pharmacokinetic study was used to examine the nephroprotective effect of gelofusine against colistin. Mice (n=12) were treated subcutaneously injected with colistin (14mgS/kg x 6 doses every 2 hours = accumulated dose 84mgS/kg) and simultaneously injected in the intraperitoneal region with either a treatment of gelofusine (600mg/kg x 6 = 3600mg/kg) or saline (same volume) for control. At twenty hours after the last dose, the mice were euthanized and severity of renal alteration was examined histologically and difference in colistin concentrations between the control group and the gelofusine group were examined in the plasma and the kidney homogenate. Significant histological abnormalities were detected in the kidneys of the saline-colistin group. The Gelofusine-colistin group were completely void of any renal damage. The pharmacokinetic profile of colistin did not change when co-administered with gelofusine. This finding has great potential to increase the therapeutic index of colistin for clinical use as gelofusine is a well-known and generally used blood volume substitute that can be applied safely without the induction of toxicity.

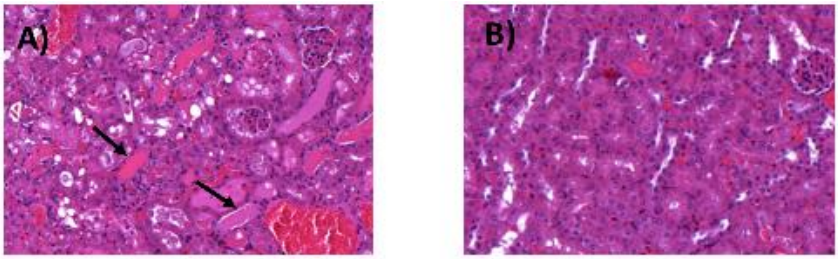

Figure 1. Representative histological images of murine kidney sections collected 20 h following treatment with colistin co-administered with saline (A) or Gelofusine (B). Tubular casts, indicative of kidney damage are highlighted with arrows.

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Fracture risk with zopiclone: Application of a varying-coefficient Cox model to examine influence of age as a risk factor for fractures

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Introduction. Z-hypnotic drugs are increasingly being used to treat insomnia in older people due to their perceived safety relative to benzodiazepines. Recent epidemiologic studies have reported increased risk of fractures in patients taking zolpidem, however the results have been inconsistent.
Aims. To evaluate the risk of fracture with zopiclone in a population-based cohort of older people in New Zealand (NZ) in a real world setting.
Methods. Prescription records (2005-2014) of zopiclone were sourced from NZ Pharmaceutical Collections (Pharms). The first-time coded diagnosis of fracture was extracted from National Minimal Datasets (NMDS). Datasets were linked by a unique patient identifier to set up case-crossover designs. Relative risks (RR) of fracture associated with zopiclone was calculated using conditional logistic regression. A varying-coefficient Cox model was employed to examine the influence of age on risk of fractures. Sensitivity analyses were performed with shortened windows for case and control periods.
Results. The risk of fracture associated with zopiclone is higher (Adjusted RR 7.54, 95% CI: 4.10 - 13.85), compared to non-users. The increased risk of fracture associated with zopiclone (ARR 2.47, 95% CI: 0.90 - 6.75) remained significant after adjusting for concomitant use of alpha blockers, antipsychotics, beta blockers, benzodiazepines and tricyclic antidepressants. The risk of fracture increased disproportionately with the age.
Discussion. The results support that the magnitude of the risk of fracture is higher with zopiclone compared with non-users. Confounding by indication and time varying confounders such as comorbidities could bias the risk of fractures. Marginal structural models could provide unbiased estimates of the risk of zopiclone exposures on risk of fracture.

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Do medications and prescribing patterns increase the risk of hospitalisations from aged care facilities? A systematic review

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Introduction. Residents of aged care facilities are at a high risk of hospitalisation with medications being a potentially modifiable risk factor.
Aims. To conduct a systematic review on medications and prescribing patterns as factors associated with all-cause and cause-specific hospitalisations from aged care facilities.
Methods. EMBASE, MEDLINE, IPA and CINAHL were searched to identify published, peer-reviewed, original research articles in the English language. Included studies reported the longitudinal association between prescribing patterns, medication classes or specific medications with hospitalisations. Quality and risk of bias assessment was performed using the Joanna Briggs Institute Critical Appraisal Checklist Tools.
Results. Of the 2673 articles identified, 19 were included and assessed to be of high quality. Cohort studies measured outcome/s as time to first hospitalisation (n=4), number of admissions (n=11), length of stay (n=1) and hospitalisation at 6-months or 12-months (n=1). Three case-control studies and one case-time-control study were also included. All articles on polypharmacy (n=3) and potentially inappropriate medication use (n=4) reported an association with all-cause hospitalisation. Five of the 10 articles exploring antipsychotic medications showed increased risks of all-cause or cause-specific hospitalisation for at least one antipsychotic medication/class. Evidence for non-steroidal anti-inflammatory drugs (NSAIDs) was mixed, with some NSAIDs being associated with gastrointestinal-related hospitalisations (relative risks ranging from 1.95 to 6.03) and some not (n=1). One study reported an association between warfarin and bleeding-related hospitalisations (odds ratio 1.26, 95% confidence interval 1.11-1.43). No significant associations were found between statins and hospitalisation (n=1).
Conclusion. Polypharmacy, potentially inappropriate medication use, warfarin and NSAIDs were associated with an increased risk of hospitalisation. Further studies are required to substantiate the evidence and better understand the associations for other classes of medications.

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Investigation of Hospital Opioid Prescribing among Opioid Naïve Surgical Patients

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Introduction. Opioids are commonly used to treat post-operative pain following acute hospitalisation. However, prescribing of opioids can lead to a range of short and long-term adverse effects, and at present it is unclear how post-operative opioid usage contributes to the wider patterns of opioid use, particularly among opioid naïve patients.
Aims. To investigate the utilisation of opioids after hospital discharge amongst opioid naïve surgical patients, and the association between opioid use, pain management and opioid related difficulties experienced in this setting.
Methods. A prospective observational study of opioid naïve patients undergoing elective surgery admitted to the Pre-Admission Clinic at a tertiary hospital in Sydney is being conducted. Data on socio-demographics, medical history, and pain levels using the Brief Pain Inventory (BPI) are obtained at recruitment before their operation. Medications prescribed at discharge are recorded from patients' discharge summaries. Patients are being contacted at 1-week, 1, 3 and 6-months after discharge to assess patterns of opioid use, pain levels and patient-reported problems and concerns using the Prescribed Opioid Difficulties Scale (PODS).
Results. At present, 127 patients have been recruited; 42.5% (n=54) were female with a mean age of 61.7±16.1 years. On preliminary analysis, 40.0% (n=32) of patients have been discharged with an opioid, either oxycodone when required (90.6%, n=29), a fixed combination of oxycodone/naloxone (43.8%, n=14), or both (34.4%, n=11). The baseline mean BPI pain severity was 1.5±2.3 (range: 0-10) and mean pain BPI interference score was 1.5±2.4 (range: 0-9.7). Of those who have completed the 1-week follow up (n=41), 39.0% (n=16) of patients had taken opioids within 1-week, and of 14 followed up within 1-month, 21.4% (n=3), reported using an opioid. A total of 37.5% (n=6) of patients experienced a high level of opioid related difficulties and concerns after 1-week.
Discussion. Our preliminary finding suggests that opioid naive patients generally experience very little pain preoperatively, but are prescribed opioids frequently after surgery. Use of newly prescribed opioids within 1-week and 1-month following hospital discharge is common among surgical patients. Future studies need to evaluate factors associated with opioid prescribing in this setting.

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"Real world" patients are different to trial participants - Application of direct oral anticoagulant clinical trial eligibility criteria to a hospitalised cohort

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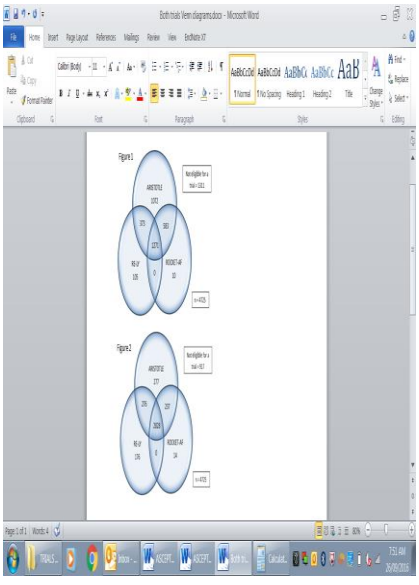
Introduction: Trial results inform clinical decision-making, but extrapolation of trial data to individual patients challenges prescribers.
Aims: Our objective was to determine what proportion of hospitalised patients with atrial fibrillation (AF) would have been eligible for inclusion in the three pivotal trials of the direct oral anticoagulants (DOACs) rivaroxaban, dabigatran and apixaban.
Methods: We conducted a cross-sectional study of 4,725 patients with a diagnosis of AF discharged from six hospitals in metropolitan Melbourne between 2012 and 2015. Comorbidity data and prescription data were linked for analysis. Inclusion and exclusion criteria from the three pivotal DOAC trials were applied to our patient population.
Results: When both the inclusion and exclusion criteria were applied to the sample population, 27% (n=1271) of patients would have been eligible for all three trials and 28% (n=1311) of patients would not have been eligible for any trial (Figure 1). On application of only the exclusion criteria, 60% (n=2828) would have been eligible for all three trials and 19% (n=917) would not have been eligible for any trial (Figure 2).
Discussion: Up to one-third of hospitalised patients with AF do not fit the profile of participants of all the pivotal trials for DOACs. The current approach to providing evidence still leaves a large gap between trial populations and patients, that needs to be addressed.



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N-of-1 trials for assessing the effects of deprescribing medications on short-term clinical outcomes in older adults: a systematic review

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Introduction. Deprescribing research, the investigation of the effects of supervised discontinuation of treatments, is a growing field. Most studies have been randomised controlled trials (RCTs), however methods more applicable to clinical practice that can provide rigorous data on causation and reversibility have been recommended. The N-of-1 methodology may allow this and provide evidence on individual responses to medications – and inform patient-centred care.
Aims. To determine the feasibility of using the N-of-1 method for deprescribing trials in older adults.
Methods. A search was conducted between May 31st, 2016 and September 23rd, 2016 in Embase, PubMed, Informit, Scopus, International Pharmaceutical Abstracts, PsychINFO, Cochrane Central Register of Controlled Trials (CCTR) and CINAHL for studies conducted in older adults (≥ 50 years), deprescribing any long-term treatment conducted over less than a year using the N-of-1 trial method. Two authors independently reviewed all articles for eligibility and extracted data. The review was conducted according to PRISMA guidelines.
Results. Six studies of deprescribing any treatment using the N-of-1 method in older adults were found (e.g. theophylline, digoxin, pacemaker use). These trials all investigated the efficacy of treatments for treating diseases including cardiovascular disease, asthma, chronic airflow limitation and skeletal muscle cramps. Four trials resulted in a significant number of patients discontinuing their medication due to non-significant benefits of treatment (44-64%). Two studies determined that the respective treatment was effective, and the majority of patients continued their treatment. Preliminary quality assessment of trials has been carried out using the PEDro scale.
Discussion. The ability of the N-of-1 method to effectively determine the individual efficacy of long-term treatments was powerful, resulting in strong patient-specific outcomes impacting on care of adults. However, use of the N-of-1 method has rarely been reported in deprescribing trials, although it has been used in other field. Further research using the N-of-1 method is required in the field of deprescribing, with the methodology demonstrated to be feasible.

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Polypharmacy and the incidence of falls, fractures and weight loss in residential aged care facilities

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Background: Polypharmacy has been associated with a range of adverse drug events in residential aged care facilities (RACFs) however its association with falls, fractures and weight loss is unclear in this setting.
Methods: We conducted a longitudinal study of 201 Australian RACFs providing 13.7 million bed days of care between 2007 and 2013. Data were collected quarterly at the RACF level. Polypharmacy rate in each RACF was recorded as the number of residents using ≥9 regular medications divided by the number of occupied bed days per quarter. The numbers of falls, fractures and residents with unplanned weight loss of ≥3 kg in each quarter were also recorded. Generalized Estimating Equations with Poisson distribution were used to compute incidence rate ratios (IRRs) with 95% confidence intervals (CIs) for the effect of polypharmacy rate on the incidence of falls, fractures and weight loss.
Results: There was no overall association between polypharmacy rate and the incidence of falls (IRR 1.003, 95%CI 0.982-1.024) or fractures (IRR 1.045, 95%CI 0.992-1.101) in adjusted analyses. In sub-analyses, each 1-unit increase in polypharmacy rate was associated with a 7.4% increase in fractures in RACFs providing high-level care (IRR 1.074, 95%CI 1.008-1.144). There was no association between polypharmacy rate and weight loss (IRR 1.007, 95%CI 0.988-1.025).
Conclusions: Facilities with higher rates of polypharmacy do not necessarily have higher rates of falls, fractures or weight loss. However, polypharmacy rate was associated with a small increase in the risk of fractures in facilities or wards that provide high-level care.

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Medication review in Australian residential aged care facilities: a systematic review

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Introduction. People living in residential aged care facilities (RACFs) are susceptible to medication errors and adverse drug events (ADEs). Medication review in this setting has been remunerated since 1997 in the form of residential medication management reviews (RMMRs).
Aims. To systematically review literature on medication review for people living in RACFs in Australia.
Methods. This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses checklist. Studies reporting medication review or reconciliation interventions in Australian RACFs and published between 1995 and 2016 were included. Keywords for medication review, medication reconciliation, and nursing homes were used to search MEDLINE, EMBASE, CINAHL, PubMed, and Informit Health. Grey literature and local journals were manually searched.
Results. Twelve studies were included. Eight were retrospective studies. The range of mean resident ages was 82.0 to 86.4 years. The mean number of regular medications per resident ranged from 3.9 to 11.4. Medication-related problems (MRPs) were reported in eight studies and categorised using four different classification systems. Two studies reported MRPs without using recognised classification systems. Medication reviews identified between 2.7 and 3.9 MRPs per resident. Two studies reported a significant association between increasing numbers of regular medications and higher number of MRPs. The most common recommendation of medication reviews was laboratory monitoring. Implementation of recommendations ranged between 30% and 83.8%. No studies have reported whether RMMRs improve quality of life or reduce hospitalisations and mortality.
Discussion. Collectively, data suggest RMMR is a useful strategy to identify MRPs. RMMRs may reduce number of inappropriate medications and prescribing of sedative and anticholinergic medications. It was unclear whether RMMRs were targeted to residents at high risk of medication errors and ADEs.

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Evaluation of the National Minimum Dataset for neurological conditions in older adults

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Introduction. The National Minimum Dataset (NMDS) is a collection of coded clinical data for hospital discharge information and the International Resident Assessment Instrument (interRAI) is a validated source for capturing important patient-specific clinical and diagnostic information including neurological conditions. To evaluate health related outcomes, it is important to establish the accuracy of the diagnostic information coded in the NMDS.
Aims. To evaluate the degree of agreement between the administrative and clinical data in capturing a diagnosis of Alzheimer's and other dementias (dementia) or Parkinson's disease (PD) in older individuals aged ≥ 65 years.
Methods. De-identified NMDS data from 1st September 2012 to 30th June 2014 was matched with the interRAI Home Care (HC) assessment records. A diagnosis of dementia or PD was compared for each individual present in both the clinical and administrative records in the 90 days preceding and subsequent to the date of diagnosis in the interRAI – HC. Diagnostic consistency was measured through sensitivity, specificity, positive predictive value (PPV), negative predictive value (NVP), weighted kappa analyses and McNemar's test.
Results. In the two large study samples of older individuals aged ≥ 65 years (NMDS: n = 59328 and interRAI: n = 21784) the NMDS demonstrated moderate and significant agreement in capturing a diagnosis of dementia or Parkinson's when compared to the interRAI within three months. For a diagnosis of dementia there was 60.20% sensitivity, 96.03% specificity with 72.80% PPV and 93.19% NVP. For PD there was 62.75% sensitivity, 99.64% specificity with 84.0% PPV and 98.88% NVP. Weighted kappa coefficient ($\kappa = 0.61$ for dementia and $\kappa = 0.71$ for PD) and the McNemar's test was significant at $P<0.000$. InterRAI assessments captured a larger number of diagnoses per population size compared to the NMDS.
Discussion. Substantial agreement between reliable sources of data, enables linking of data collections, assists in understanding the epidemiology of neurological conditions and how to provide appropriate support for older adults. Routinely collected administrative datasets such as the NMDS can be a valuable source for research to impact evaluation and inform care planning, resource allocation decisions and in predicting adverse health outcomes.

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Allosteric Targeting of Metabotropic Glutamate Receptor 5: New Insights and Opportunities

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Introduction. Dysfunctional glutamatergic neurotransmission is implicated in the pathophysiology of numerous CNS disorders. Allosteric modulation of metabotropic glutamate receptor 5 ($mGlu_5$) represents an attractive therapeutic strategy to restore glutamatergic neurotransmission in multiple disorders including depression, schizophrenia and anxiety. However, select allosteric $mGlu_5$ ligands are associated with adverse effects. We hypothesise that potential on-target adverse effects of certain allosteric ligands are related to unappreciated stimulus-bias profiles, encompassing context-dependence, biased agonism and biased modulation.
Aims. To rigorously investigate the molecular pharmacology of $mGlu_5$ allosteric ligands in both recombinant and native systems. By using primary cultures from different brain regions we aim to understand how context influences allosteric modulator activity.
Methods. We probed multiple measures of $mGlu_5$ activation and regulation by diverse allosteric modulators to gain a better understanding of the functional consequences of $mGlu_5$ allosteric modulation in recombinant and native systems. Allosteric ligands were chosen that represented distinct chemotypes and pharmacological activity as classified using glutamate stimulated $mGlu_5$ intracellular Ca^{2+} (iCa^{2+}) mobilisation (i.e. both negative and positive modulators). Operational models of agonism and allosterism were employed to rigorously quantify $mGlu_5$ allosteric ligand cooperativity, affinity and efficacy. Biased allosteric agonism between pathways was determined relative to the surrogate orthosteric agonist DHPG.
Results. Multiple allosteric agonists were biased toward inositol phosphate (IP_1) accumulation and/or ERK1/2 phosphorylation over iCa^{2+} mobilisation. Distinct bias profiles were linked to $mGlu_5$ allosteric ligands with therapeutic versus adverse effects. Allosteric ligand bias profiles were different in cortical versus striatal cortical neuron preparations.
Discussion. Our studies will help to elucidate mechanisms of on-target $mGlu_5$ modulator adverse versus therapeutic effects. Further, establishing a "biased modulation fingerprint" has the potential to provide a framework for future novel biased modulator discovery.

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Exploring conformational landscapes of muscarinic acetylcholine receptors

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Introduction. Muscarinic acetylcholine receptors (mAChRs) are an important family of Class A GPCRs widely expressed throughout the central and peripheral nervous systems, where they mediate many of the actions of the neurotransmitter, acetylcholine. Alterations in mAChR levels and activity, particularly with respect to the M1 and M4 mAChR subtypes, have been implicated in the pathophysiology of major neurological and psychiatric diseases, including Alzheimer's disease, Parkinson's disease, mood disorders and schizophrenia. As such, mAChRs have remained important targets for drug discovery, despite the difficulty associated with designing molecules to selectively target the highly conserved orthosteric binding site. We have recently determined the crystal structures of the M1 and M4 receptors bound to the inverse agonist tiotropium, and alongside the previously determined M2 and M3 receptor structures we now have a near complete view of the inactive state of this important subfamily. However, the relatively small number of ligand-bound structures that exist across all five mAChR subtypes can still preclude structure- and/or diversity-based drug discovery efforts.
Aims. To determine co-crystal structures of the M4 receptor bound to different ligands.
Methods. Crystal structures of the M4 receptor were determined using lipidic cubic phase X-ray crystallography and characterized using biophysical and pharmacological assays.
Results. We have now solved the structure of the M4 receptor bound to the antagonist, N-methylscopolamine.
Discussion. We will report on new insights gained by comparison of this structure to the previously determined mAChR structures. Furthermore, we have been working towards solving a structure of the M4 receptor bound to a computationally designed negative allosteric modulator 4P-C7 (1), and will report our recent progress towards this novel co-structure.

(1) Dror, R., et al. (2013) Nature **503**, 295-299

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From surrogate ligands to phenotype: exploring the function of orphan GPCR, GPR37L1

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Introduction. Orphan G protein-coupled receptors (GPCRs), a subset of receptors that are yet to be paired with their endogenous ligand, hold much hope as a source of novel therapeutic targets. Our work focuses on the cardiovascular role of the cerebellar receptor, GPR37L1, using *in silico*, *in vitro* and *in vivo* techniques.
Aims. To understand the pharmacology and physiology of the orphan GPCR, GPR37L1, in the context of cardiovascular disease.
Methods. We developed a computational approach, GPCR-CoINPocket, to predict nearest pharmacological neighbours in order to identify surrogate ligands for GPR37L1. In parallel, we have established high throughput signalling assays to screen both surrogates and novel chemical entities via a cAMP response element-luciferase reporter gene. Finally, we have generated GPR37L1 reporter and gene deletion mouse lines to investigate the cardiovascular function of GPR37L1.
Results. GPR37L1 is a constitutively active receptor that signals through Gαs to increase cAMP, both in HEK293 cells and murine cerebellar slice cultures. Receptor activity is abolished by N-terminal proteolysis by metalloproteases both *in vitro* and *ex vivo*, with the inactive species of the receptor predominating in the cerebellum. Surrogate ligands have been identified by GPCR-CoINPocket and *in silico* screening has commenced for future GPR37L1 studies. In our animal model, 10-12 week old GPR37L1$^{-/-}$ female, but not male, mice have elevated blood pressure at baseline but do not display cardiac hypertrophy. After cardiovascular challenge (AngII osmotic mini-pump), GPR37L1$^{-/-}$ males die suddenly from apparent aortic rupture and have exacerbated left ventricular hypertrophy and fibrosis, while their female counterparts are no different to wild type mice.
Discussion. GPR37L1 is required for cardiovascular homeostasis, with effects on blood pressure and left ventricular hypertrophy that are gender-specific. We have also identified a novel signal transduction mechanism of the receptor and a series of surrogate ligands that can be applied to our animal models of cardiovascular disease, furthering our understanding of the physiology and pathophysiology of GPR37L1.

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The role of central beta-3 adrenergic receptors in the control of metabolism and glucose homeostasis

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Introduction. The G protein-coupled adrenergic β3 receptors are ubiquitously distributed including in the small intestine, adipose tissue, both white and brown and on the vascular endothelium.
Aims. To determine if β3 receptors in the brain have a role in regulating metabolic homeostasis.
Methods. Both lean and Diet Induced obese (DIO) mice and measured numerous metablisc phenotypes.
Results and discussion: We have discovered that in mouse, β3 mRNA is also present in hypothalamic nuclei in the brain. We have demonstrated that in both lean and diet induced obese (DIO) mice that peripheral selective β3 agonism results in lower plasma glucose concentration at baseline. During glucose tolerance testing, pre treatment with a β3 agonist improved glucose response throughout. Peripheral treatment of β3 in both lean and DIO mice acutely reduced food intake and increased energy expenditure resulting in reduced body weight. Investigation into the actions of β3 receptors in the brain unveiled that central β3 treatment in both lean and DIO mice resulted in reduced basal blood glucose, a trend we have explored further using Data Science International continuous glucose telemetry recorders, in which we can demonstrate a complete reversal of blood glucose decline following central β3 antagonist administration. Interestingly while central β3 administration improves glucose tolerance in lean mice when challenged with glucose, this did not occur in DIO mice. Using chronic glucose telemetry recorders we can demonstrate that when challenged with glucose central β3 administration into DIO mice cannot improve glucose tolerance. Insulin secretion is impacted. Performing euglycemic clamp experiments in freely moving lean and DIO mice, following central β3 administration has unveiled that β3 impacts glucose uptake in numerous peripheral tissues. Acute Central β3 administration caused a decrease in food intake, an increase in brown adipose tissue temperature resulting in decreased body weight. Further investigation into the neuronal populations responsible for these physiological actions has demonstrated that neurons in several hypothalamic nuclei are activated by β3 administration including the POMC neurons of the arcuate nucleus of the hypothalamus, which is being investigated further.

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Diverse activation pathways in class A GPCRs converge near the G-protein-coupling region

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Class A G-protein-coupled receptors (GPCRs) are a large family of membrane proteins that mediate a wide variety of physiological functions, including vision, neurotransmission and immune responses. They are the targets of nearly one-third of all prescribed medicinal drugs such as beta blockers and antipsychotics. GPCR activation is facilitated by extracellular ligands and leads to the recruitment of intracellular G proteins. Structural rearrangements of residue and water-mediated interactions in the transmembrane domain serve as 'activation pathways' that connect the ligand-binding pocket to the G-protein-coupling region within the receptor. We aimed to understand how similar the activation pathways are across class A GPCRs. We represented crystal structures and simulations as networks of interactions mediated by residues and waters. Based on comparisons of these networks, we showed thatDe despite the diversity in activation pathways between receptors, the pathways converge near the G-protein-coupling region. This convergence is mediated by a highly conserved structural rearrangement of residue- and water-mediated interactions between transmembrane helices 3, 5, 6 and 7 that releases G-protein-contacting residues. The convergence of activation pathways may explain how the activation steps initiated by diverse ligands enable GPCRs to bind a common repertoire of G proteins.

Venkatakrishnan et al., Nature. 2016 Aug 25;536(7617):484-7.

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Enhancing Endogenous Opioid Signalling in the Amygdala through Positive Allosteric Modulation of DOR

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Introduction. Fear-associative learning, a cognitive process that relies heavily on amygdala activity, is modulated by endogenous opioids and is disrupted in anxiety disorders. The intercalated cells (ITCs) gate amygdala output and express both enkephalin and μ-opioid receptors (MORs), whilst the basolateral amygdala (BLA) receives amygdala input and expresses δ-opioid receptors (DORs). Selective MOR and DOR agonists, induce opposing behavioural outcomes, MOR is anxiogenic whilst DOR is anxiolytic. However, the cellular basis underlying endogenous opioid signalling is poorly understood.
Aims. To characterise exogenous and endogenous opioid signalling at BLA-ITC synapses.
Methods. Whole-cell recordings from ITCs and changes in excitatory postsynaptic currents (EPSCs) were measured following application of opioid receptor agonists/antagonists, inhibitors of known endogenous opioid peptidases and the recently developed DOR positive allosteric modulator (PAM). EPSCs were evoked using either a low (2 pulses, 50ms interval) or moderate (5 pulses, 150Hz, followed by 1 pulse, 500ms interval) stimulus delivered to the BLA.
Results. MOR (DAMGO, 1μM) and DOR (Deltorphin, 300nM) agonists reduced EPSCs whilst naloxone (10μM), a pan opioid receptor antagonist, increased EPSCs under moderate but not low stimulus conditions. This was potentiated by peptidase inhibitors and mimicked with a DOR antagonist (ICI174864, 1μM). Further, when peptidase activity was inhibited, naloxone increased EPSCs under low stimulus conditions and this was enhanced by the DOR PAM BMS-986187 (1μM; n=5, $p<0.01$).
Discussion. Here we show endogenous opioids are released by low stimuli but a moderate stimulus is required for sufficient release to overcome peptidase breakdown. We find endogenous opioids preferentially signalled at DORs at the BLA-ITC synapse and we show for the first time that a DOR PAM can enhance this signalling.

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GPCRs and ACRs (Arrestin-coupled receptors): A Tale of Two Transducers

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Understanding of the function and regulation of G-protein coupled receptors (GPCRs) has evolved very significantly over the past decade. In particular, the two component system of G-protein coupled receptor kinases (GRKs) and β-arrestins, which were originally discovered in the context of their desensitizing G-protein signaling, are now known to carry out a variety of other receptor functions such as endocytosis and signaling. Moreover, ligands which can selectively activate signaling through either G-proteins or β-arrestins, so-called biased agonists, represent a potentially novel form of more selective therapeutic with fewer side effects. My laboratory is currently focusing on trying to understand in structural terms, how β-arrestin mediated and β-arrestin biased signaling are mediated. Single particle averages of negative stain EM images have revealed the architecture of receptor-β-arrestin complexes, and demonstrated two different conformational arrangements. We have characterized the specific functional capabilities of each conformation. Moreover, we have demonstrated how one of these conformations of the complex can further associate with heterotrimeric G-proteins to form a “megaplex” which may explain the recently discovered ability of several GPCRs to signal to adenylate cyclase from internalized endosomes. In another approach, we are using a variety of affinity based selection techniques to isolate allosteric receptor modulators. We have identified both negative and positive allosteric modulators, from libraries of nanobodies, RNA aptamers, and DNA encoded small molecules. These novel allosteric modulators allow us to capture, and then characterize specific conformations of the receptors involved in discrete receptor functions.

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Thomsen, A.R.B., Plouffe, B., Cahill III, T.J., et al (2016) Cell 166: 1-13

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What I wish I knew: An intern's prescribing journey

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Introduction. This presentation aims to provide a reflection of an intern's prescribing journey, while briefly exploring the research surrounding medical education and junior doctors' prescribing experiences.
Methods. Personal experiences pertaining to the transition from academic medicine to 'independent' prescribing in the hospital setting are identified and discussed in further detail. This presentation incorporates informal feedback provided by interns and nursing staff at a tertiary hospital in Australia in order to comment on prescribing-related expectations, challenges, and preparation through medical school. A literature search was performed in order to review contemporary articles focusing on medical education and junior doctors' preparedness for prescribing.
Discussion. Similar challenges were identified through the literature and personal feedback provided by interns. These included: uncertainty when prescribing medications with open-ended clinical guidelines, limited exposure to prescribing during medical emergencies, and variability in the awareness of potential drug-interactions. Strategies to improve education for future prescribers include practical sessions through clinically-relevant scenarios, and greater familiarity with the use of online or print resources such as MIMS or the AMH.
Conclusion. This presentation briefly summarises research pertaining to interns' prescribing preparedness and outlines potential strategies to enhance confidence when prescribing.

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Prescribing curriculum and competency

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Introduction. Safe and effective prescribing requires a strong knowledge of clinical pharmacology and therapeutics as well as specific practical prescribing skills.
Aims. To describe and compare strategies used nationally and internationally to ensure that medical students can prescribe safely and effectively on graduation.
Methods. Published literature and national and international initiatives will be reviewed, with a focus on the role of clinical pharmacologists and addressing the complex needs of the ageing population.
Results. Curriculum and delivery of teaching in clinical pharmacology, therapeutics and prescribing skills is highly variable between and even within medical schools. This knowledge and skill base is particularly important for the care of our ageing population, with an increase in polypharmacy and in vulnerability to adverse drug events. Assessment of prescribing competency by all medical graduates is advocated to reduce prescribing errors by junior medical officers and improve patient safety.
Discussion. There is an important role for clinical pharmacologists to ensure that testing of competencies covers not only the practicalities of good communication and writing clear, legal prescriptions, but also the clinical pharmacology and therapeutics knowledge base that underpins complex clinical decision making.

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Prescribing skills assessment (PSA) – Is it the solution?

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Introduction: Safe use of medicines is a key priority for medical schools, patients and healthcare systems.
Medical students and junior doctors report under-confidence in their ability to prescribe safely. Limited opportunities for pre-graduation practice may contribute to prescribing errors by junior doctors. The PSA is an online examination, allowing medical students to practice prescribing and to assess competence pre-graduation.
Aim: To determine the relevance and utility of the PSA to drive learning in pre-registration medical students.
Methods: Final year medical students at Monash University were invited to take the PSA exam, to complete pre- and post-PSA surveys and then to access an online educational package (4 practice PSA papers and resources from NPS MedicineWise). Institutional ethics approval was obtained in advance.
Results: 231 (45.6%) students from a cohort of 507 completed the pre-PSA survey. Of these, 82 students (35.5%) recommended 'more rigorous assessment' and 118 (51.1%), "compulsory online modules" to improve performance in prescribing. 225 students (44.4%) chose to sit the PSA. Of these, 222 (98.7%) completed the post-PSA survey in which 200 (90.1%) agreed or strongly agreed that the PSA was 'an appropriate test of prescribing skills'. Overall, 193 (86.9%) agreed or strongly agreed that 'the online interface was easy to use' and 192 (86.5 %) agreed or strongly agreed that 'the questions were clear and unambiguous'. Data collection is in progress to measure activity in the educational package, to quantify the effect of participation in the PSA to drive subsequent learning activities.
Discussion: The PSA is likely to be part of the solution to guide students and graduates towards safe prescribing. It gives relevant, practical experience in tasks that will be encountered upon graduation, is acceptable to participants and preliminary results indicate that it engages learners to do more work in this area.

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ePrescribing – the devil is in the detail

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Introduction. Prescribers spend their early years of prescribing in hospitals in a team environment. Hospitals in Australasia are rapidly moving from paper to electronic prescribing systems. For training we have the same prescribers, same drugs and same principles but different tools.
Aims. To describe how changes in the prescribing environment have affected prescribing and prescriber training.
Methods. A review of published literature and report of experiences from an academic health service.
Results. Lack of student access to the hospital electronic prescribing systems is a barrier to training in some health services. Clinical decision support can provide context for therapeutic decisions. The WHO/IUPHAR/CIOMS model curricula and ASCEPT standards remain fit for purpose. Junior doctors adapted rapidly to new systems but senior doctors required additional training support to maintain senior oversight of pharmacotherapy.
Discussion. Clinical pharmacology and therapeutics underpin prescribing and should remain the focus of student learning. The prescribing environment provides a context and access to real world electronic prescribing systems are essential to training. Electronic prescribing systems reduce some medication errors but introduce new errors. In migrating to electronic systems the training needs of senior prescribers need to be considered to maintain prescribing quality.

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IUPHAR: Its organization and mission for world health

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The International Union of Basic and Clinical Pharmacology (IUHAR) has 68 member societies representing approximately 36,000 pharmacologists worldwide. It mission is to foster education, training and research in the discipline internationally. Organizationally, these efforts are overseen by seven Sections, one Division and one Standing Committee. Current initiatives include the Integrative Organ System Pharmacology (IOSP) project, and joint programs with the American, Chinese, Japanese and Hungarian pharmacology societies to establish the Pharmacology Education Program (PEP;www.pharmacologyeducation.org) and with the British Pharmacological Society to create and maintain the Guide to Pharmacology (GtP;guidetopharmacology.org) site. The GtP combines information from the IUPHAR receptor database with the British Pharmacological Society Guide to Receptors and Channels (GRAC). Because of these efforts, and various others undertaken by IUPHAR Sections and the Clinical Division, there is a renewed appreciation for pharmacology among organizations and individuals engaged in medical education, drug discovery and development. This not only enhances career opportunities in the field, but helps ensure the future of the discipline.

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IUPHAR: Pharmacological variables and therapeutic targeting

Michael Spedding[1,2]. Secretary General, IUPHAR[1]; Spedding Research Solutions, Le Vesinet, France[2]

Therapeutic targeting and precision medicine requires detailed knowledge of pathophysiology and the molecular targets of drugs. The pharmaceutical industry is finding it difficult to find any new drugs for diseases such as Alzheimer's, and at the same time there is a switch to antibody-based therapy where the epitope is relatively specific. However, the complexity of deconvoluting all the pharmacological variables in drug: receptor interactions, particularly at a tissue level, is such that expert analysis is needed rather than computerized data-trawling, hence the creation of 90 expert subcommittees (700 scientists) by NC-IUPHAR to review the data in IUPHAR's database of drug targets and ligands (IUPHAR/BPS guidetopharmacology.org). In many cases, drug discovery programs massively underestimate sources of complexity and the number of experimental variables, which are difficult to transpose from lab to lab. Furthermore, human spontaneous mutations in disease are very high. Fortunately, some of these variables converge on key nodes where epigenetics, ageing, metabolism and inflammation may switch protein phenotype in cells by splicing, and switch cellular signaling, so these may become key targets, but where expert advice is needed.

The production of one gene/one protein is evidently not true - particularly for humans, where gene number is low. Recent knowledge changes old concepts which had been (and are still being) used for drug discovery. For example, both in real and virtual screening, gene sequences are used to encode real and virtual protein structures: one to one. Yet the pathophysiological interest is in variability and multiplicity – complex but crucial.

IUPHAR is therefore prioritizing the new variables and translational pharmacology:

a. Alternative splicing of proteins: Bonner TI et al (2014) Br J Pharmacol: 171, 1231-40
b. Receptor polymorphisms.
c. Epigenetic drug targets: Tough DF et al, Br J Pharmacol: 171, 4981-510,
d. Allosteric modulation of these targets: Christopoulos A et al (2014) Pharm Rev: 66, 918-47,
e. Immunological/inflammatory targets: Tiligada E et al (2015) Br J Pharmacol: 172, 4217-4227.

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Synergies and challenges for academic/pharma drug targeting

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Introduction: The pharmaceutical industry has undergone tremendous consolidation over the past decade or so. In addition to these changes, some of the major pharmaceutical companies have abandoned certain therapeutic areas, such as neuroscience, because they are too risky, have poor predictive or translational models, are too costly, have a relatively low probability of success, and the development time is lengthy. There has been growing recognition that academic institutions need to play a more significant role in filling the gaps in areas that have been abandoned by pharma. Traditionally, a major strength of academic research has resided in the pursuit of basic research and, indeed, many of the discoveries in academic research laboratories have let to novel therapeutics. The path from academia to drug discovery to commercialization has included the identification of new mechanisms and pathways, characterization and validation of new targets and the exploration and development of new model systems from whole animal assays to organs-on-a-chip. Academic drug discovery centers have proliferated in recent years as they are seen more frequently as a means of addressing the innovation gap, particularly in the earliest phases of drug discovery. There are several challenges and constraints on these initiatives, one of which is the cost associated with drug discovery and secondly, the technologies involved to even minimally embark on these paths. Yet another challenge is the fact that many academicians have little understanding of what is required to advance a compound through even preclinical phases to position it as a drug candidate. Some of these challenges are mitigated by the 'reverse flow' of scientists from the pharmaceutical sector back to academic research centers into which they bring their experience and knowledge. These changes have had an unequivocal effect on academic structures and culture which will likely have a lasting impact.

Methods and Discussion: This presentation will cover these issues, will highlight some of the developments that have emerged, including academic – industry partnerships, and initiatives stemming from the federal government in the U.S. and elsewhere to initiate drug discovery and development, particularly in areas of unmet medical need such as in rare or neglected ("orphan") diseases.

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The specific challenges in India

Dr Bhagirath Patel, General Secretary, Indian Pharmacological Society, India

With development to agro based Indian economy there is addition of service oriented economy changing face of India to be a developed economy. Partly this is owned to the growing healthcare industry within the country that attracts heavy investment particularly in terms of capital investment and provision of private healthcare. We can expect Indian healthcare industry number to rise up to a staggering $280 billion dollars. However, there are numerous challenges faced by this industry both modifiable and non-modifiable. The non modifiable include our geographical location which is responsible for Tropical climate and thus its associated disease. We are world's greatest democracy. As a consequence of which for many policies quantity rules quality. We are a country with numerable dialects which change at short distances making the health counseling expensive and region specific. Our religious perspective also influences our belief systems which influences our healthcare more so in the low socioeconomic strata.

Pharmacology and clinical pharmacy can serve as intercepts to modify these risk associations. Improving upon our specializations in health hygiene, community pharmacy, drug addiction, rational drug therapy, patient counselling, ADR, drug interactions, Pharmacoeconomics, Pharmacovigilance and the list stands endless can intercept health care by create awareness and expertise and specializations in various concepts of Drugs list, Formulations, Kinetic profiles, Therapeutic profiles, Safety profiles etc. serving as a link in basic concepts of healthcare Education including life style modification, Documentation, analysis, developing Indian guidelines , implementing Prophylactic measures and Cost effective treatments.

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Critical Role of Clinical Pharmacology in Therapeutic Targeting

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Introduction. Translation of mechanistically defined therapeutic targets for specific disease indications has a high failure rate. Precision medicine approaches to identify molecular subgroups of a disease is being effectively applied with the hypothesis that this will decrease the failure rate in drug development and offer more effective therapies in relevant disease-patient subgroups.

Aims. Examples of the utility of molecular characterization of diseases and the application of this knowledge to define subgroups of patients that are more likely to respond to a targeted therapy are most numerous in oncology drug development. Effective clinical pharmacology evaluation in proof of concept phase 2 studies is key to furthering this approach. Extension of these approaches to non-cancer indications has been much more limited, and careful clinical pharmacology evaluation, including development of appropriate disease and drug biomarkers, will be critical to advancing precision medicine outside of oncology.

Methods/Results. Examples of drug development programs that utilized effective clinical pharmacology input to efficiently move a drug development program forward will be presented. Limitations of precision medicine of targeted therapies and their development will be discussed

Discussion. The current enthusiasm for developing specific treatments for specific molecular subgroups of patients is starting to be realized, however there remain many challenges. A key part of addressing these challenges is the utilization of effective clinical proof of concept programs that implement rigorous clinical pharmacology trial designs.

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TRPA1 channels and serotonin release from enterochromaffin cells

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The transient receptor potential (TRP) family of cation channels act as transducers of environmental stimuli and form a common transduction pathway for a large number of G-protein coupled receptors. The TRPA family is unique in that there is only a single member, the TRPA1 channel. TRPA1 are often expressed in nociceptive afferent neurons where they are associated with pain, itch and neurogenic inflammation.TRPA1 can be opened by mechanical forces, environmental irritants, noxious cold, or pungent natural compounds. One such compound is cinnamaldehyde which is the flavour and odour of cinnamon. It can act on TRPA1 channels as a full, albeit weak, agonist which covalently binds to the channel in a time-dependent manner via cysteine residues.

Work in the gastrointestinal tract has focused on TRPA1 mediated changes to motility. TRPA1 agonists evoke contractions in the ileum and colon, but in the stomach delay emptying. Immunohistochemical studies have shown TRPA1 on a variety of enteric neurons in the colon, but not ileum. Of interest is the finding that TRPA1 channels are richly coexpressed with serotonin in the enterochromaffin cells in the mouse ileum but not stomach or colon.

Our recent work has shown that TRPA1 is the main target of cinnamaldehyde to induce serotonin release from enterochromaffin cells. We used carbon fibre amperometry to record 5-HT release in real time from *in vitro* tissues. We have found that cinnamaldehyde potentiated the mechanosensitive release of serotonin from enterochromaffin cells in the stomach, and small and large intestine of guinea pigs. Similar results were seen in rat ileum where cinnamaldehyde significantly potentiated peak 5-HT release and basal 5-HT levels. This increase was abolished in the presence of the specific TRPA1 channel blocker HC030031. The antagonist alone had no effect on 5-HT release. Similar experiments in tissues from TRPA1 -/- mice showed no effect of cinnamaldehyde but surprisingly weak effects of cinnamaldehyde were observed in TRPA1 +/+ mice.

These data suggest that the effect of cinnamaldehyde on 5-HT release is mediated through TRPA1 channels. In addition, the lack of effect of the TRPA1 blocker on control 5-HT responses suggests that these channels do not participate in mechanosensitive 5-HT release and are not endogenously active in the intestine. However, there are unanswered questions such as whether the antagonist is equally effective in blocking mechanical stimuli as chemical activation of TRPA1, and why the observed effects of cinnamaldehyde were apparently weak in mouse tissue.

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Ion channels and visceral afferents in the gut and bladder

Ion channels and visceral afferents in the gut and bladder

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There is increasing pre-clinical and clinical evidence that infection and inflammation are key risk factors for the development of some subtypes of Irritable Bowel Syndrome (IBS)[1]. Extrinsic sensory afferents are at the start of the pain-processing pathway and are therefore key targets for treating chronic visceral pain (CVP) associated with IBS. This seminar will highlight the fundamental properties of extrinsic sensory afferent nerves innervating the gut and bladder, and the ion channels (specifically TRP and Na_V[2]) that underlie their function. In particular it will focus on the latest evidence for altered sensory signaling from colonic and bladder afferents in models of IBS. This talk will highlight how inflammation can trigger long-term neuroplasticity, through distinct interactions between inflammatory/immune cells and neurons. Such interactions trigger altered neuronal ion channel and receptor expression and ultimately aberrant neuronal function and gastrointestinal and bladder symptoms. Finally, this talk with highlight recent evidence that has identified several novel receptors ($GABA_B$[3] and oxytocin[4]) that hold promise for future selected pharmacotherapy for inhibiting colonic afferents in the treatment of CVP in IBS.

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[3] Castro et al., (2016). Gut. 2016 Feb 17. pii: gutjnl-2015-310971. doi: 10.1136/gutjnl-2015-310971.
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412

Local and regional regulation of endothelial function in diabetes

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Introduction. Endothelial dysfunction is a risk factor for the vascular complications of diabetes mellitus. The effects of diabetes on endothelial function exhibit regional heterogeneity. We investigated the local mechanisms regulating endothelial vasodilator function in mesenteric and hindlimb beds in a model of Type I diabetes mellitus.
Methods. Eight weeks after *iv* injection (diabetes, streptozotocin (60mg/kg *iv*); control, citrate buffer) vascular function was studied in male Wistar rats using *in vitro* techniques including intracellular microelectrode recordings of endothelial and smooth muscle cell membrane potential, wire myography, real time PCR and superoxide production using lucigenin-enhanced chemiluminescence; and *in vivo* measurement of local blood flow using transit-time ultrasound flow probes.
Results. Following 8 weeks of diabetes endothelial vasodilator dysfunction was present in the mesenteric vasculature and the distal, but absent in the proximal, branches of the hindlimb bed. Endothelial cell hyperpolarization was halved in mesenteric arteries and unchanged in proximal branches of the femoral bed. The functional roles of intermediate- and small-conductance calcium-activated potassium (K^+) channels were reduced in mesenteric endothelial cells and accounted for the impaired smooth muscle relaxation attributed to endothelium-derived hyperpolarization (EDH), while myoendothelial gap junction (MEGJ) function remained intact. Superoxide production was markedly increased in mesenteric arteries of diabetic rats and unchanged in proximal femoral arteries. Correspondingly, l-NAME-sensitive endothelium-dependent relaxation was impaired in mesenteric arteries and was unaffected by diabetes in proximal femoral arteries, respectively. In these arteries both nitric oxide (NO) and its one electron reduced and protonated form, nitroxyl (HNO), contribute to l-NAME-sensitive endothelium-dependent relaxation. The contributions of HNO in both arteries were preserved in diabetes.
Discussion. Despite the wide-reaching effects of diabetes across the body, there are regional differences in the effects of this disease on vascular function. These heterogeneous effects on the vasculature are underpinned by differences in local factors including K^+ channel activity, superoxide production, NO and HNO bioavailability and MEGJ function.

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TRPV3 control of uterine blood vessels

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Introduction. Transient receptor potential vanilloid proteins (TRPV) represent a class of voltage-insensitive, non-selective cation channels with emerging roles in vascular biology. The hypothesis of this study was that changes in the expression of TRPV3 contributed to pregnancy-induced functional changes in rat uterine radial arteries.
Aims. This study investigated the expression and function of TRPV3 in uterine radial arteries isolated from non-pregnant and twenty-day pregnant rats.
Methods. TRPV3 mRNA and protein expression was assayed through quantitative and analytical PCR, immunofluorescence (IF) and Western blot analysis. Functional studies in isolated vessels were performed using pressure myography. Data were analysed utilizing GraphPad Prism 6.0 ().
Results. IF studies suggested TRPV3 is primarily localized to the smooth muscle in uterine radial arteries from both pregnant and non-pregnant rats. Pregnancy increased TRPV3 mRNA expression in the arteries but protein assays yielded conflicting results suggesting post-translational modification. The TRPV3 activator carvacrol caused a concentration-dependent dilation of arteries which was not affected by pregnancy (pEC_{50} Non-preg 4.03 ± 0.08, n = 11; Preg 4.07 ± 0.1, n = 10). Inhibition of nitric oxide synthase or cGMP-dependent protein kinase (PKG) enhanced responses to carvacrol in arteries from non-pregnant rats only. In arteries from both non-pregnant and pregnant rats responses to carvacrol were prevented by TRAM-34 (1 µM), a blocker of the K^+ channel $K_{Ca}3.1$.
Discussion. Smooth muscle TRPV3 mediate dilation of rat uterine radial arteries through activation of $K_{Ca}3.1$, the intermediate-conductance Ca^{2+}-sensitive K^+ channel. Pregnancy increased expression of TRPV3 in the arteries, but this did not alter responses to the activator, carvacrol. TRPV3 activity in these vessels is inhibited by NO-cGMP-PKG, but this effect is lost in pregnancy (Murphy *et al.*, 2016).

Murphy TV et al. (2016) Vasc Pharmacol 83:66-77

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Molecular Mechanisms of Pain Control by Opioid Receptors

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Introduction. Opioids remain the gold standard to treat severe pain. However, opioids also cause detrimental side effects including analgesic tolerance and opioid-induced hyperalgesia (OIH). Tolerance and OIH counteract opioid analgesia, and drive dose escalation. Whether the mechanisms underlying opioid analgesia are distinct and dissociable from those promoting deleterious side effects is currently unclear. The cell-types and receptors on which opioids act to initiate maladaptive processes remain disputed, preventing the
Aims. We aim to identify the cell type and receptor responsible for morphine analgesia and side effects, to develop therapeutic strategies to maximize and sustain opioid analgesic efficacy.
Methods. We identified mu opioid receptor (MOR)-expressing cell-types using immunostaining, in situ hybridization, RNA-sequencing and transgenic reporter mice. We then selectively deleted MOR from nociceptors (MOR cKO mice) and evaluated the effects on tolerance, OIH, and maladaptive synaptic long term potentiation (LTP) induced by morphine. We also co-administered a peripherally restricted MOR antagonist with morphine.
Results. We confirm selective loss of MOR in dorsal root ganglion, with intact expression in spinal and brain neurons in MOR cKO mice. Knockout of MOR in nociceptors was sufficient to eliminate tolerance and OIH, as well as maladaptive LTP. Importantly, MOR cKO mice display intact analgesia following systemic morphine, suggesting that MOR in nociceptors is dispensable for analgesia. Finally, we demonstrate that administration of a peripherally restricted MOR antagonist prevents morphine tolerance and OIH without diminishing analgesia.
Discussion. We conclude that MOR expressed on nociceptors initiates maladaptive processes that promote the development of tolerance and OIH. Collectively, our data support the development of strategies interfering with MOR function in peripheral nociceptors to stabilize morphine's antinociceptive efficacy during chronic treatment.

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Location-dependent signaling of metabotropic glutamate receptor, mGlu5

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Introduction. GPCRs are primarily known for converting extracellular signals into intracellular responses. However, the metabotropic glutamate receptor, mGlu5, is also localized on intracellular membranes where it mediates both overlapping and unique signaling effects. Because mGlu5 receptors play important roles in both normal development and disease, a large number of drugs are being developed to allosterically target this receptor. Thus, it is critical to understand how such drugs might affect mGlu5 function on different membranes and in different brain regions.
Aims. The goals of these experiments were to determine the role of intracellular mGlu5 in striatal cultures and slices and to determine whether various negative allosteric modulators (NAMs) differentially affected these signalling pathways.
Methods. We used cellular, pharmacological and electrophysiological methods to test endogenous intracellular mGlu5 function.
Results. Using a pharmacological approach to isolate different pools of mGlu5, both intracellular and cell surface receptors activated the PI3K/AKT pathway whereas only intracellular mGlu5 induced ERK1/2, Arc, protein synthesis, and both electrically induced and chemically induced long-term depression. Known NAMs exhibited differential IC50's depending upon which neuronal background endogenous mGlu5 was expressed in.
Discussion. These data suggest a physiologically relevant and important role for intracellular mGlu5 in striatal synaptic plasticity. Thus, besides "ligand bias," whereby a receptor's signaling modality can shift from G protein dependence to independence, canonical mGlu5 receptor signaling can also be influenced by "location bias" (i.e., the particular membrane from which it signals). Cell type bias also exists leading to differential allosteric effects on receptor function. Given that mGlu5 NAMs are being developed for various disorders, further elucidation of the site(s) of action of these drugs may determine which signaling pathways mediate therapeutic efficacy.

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Orphans and veterans: GPCR-mediated regulation of striatal function

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The striatum is a nexus in the brain, integrating input from both midbrain dopaminergic nuclei and cortical regions and regulating reward, cognition and motor control. As result the region is a key target in diseases such as schizophrenia, addiction, ADHD, Parkinson's and Huntington's disease. A number of G protein-coupled receptors (GPCRs) are localised in the striatum, including orphans such as GPR88 and GPR52. Notably, GPR88 (inhibitory) and GPR52 (stimulatory) differentially regulate cAMP signalling. GPR52 is highly expressed in the striatum on dopamine D_2 receptor-expressing medium spiny neurons (MSNs) and in the cortex on dopamine D_1 receptor-expressing pyramidal neurons, whereas GPR88 is largely restricted to the striatum and expressed in both dopamine D_1 and D_2 MSN populations. As such, both orphan receptors are well placed to modulate synaptic dysregulation in diseases such as schizophrenia. We have extended our studies to examine activation of GPR52 on G protein-dependent and independent signalling, regulation of the key striatal phosphoprotein DARPP-32 and neurobehavioural models relevant to cognition and schizophrenia. Using the synthetic agonist, 3-BTBZ (Setoh et al., 2014), we show that GPR52 stimulates cAMP, but not calcium mobilization. Nonetheless, 3-BTBZ differentially regulates DARPP-32 phosphorylation at Thr34 and Thr75 in striatal MSNs and reverses psycho-stimulant induced hyperactivity in mice, indicative of potential antipsychotic activity. Further studies are ongoing to determine the mechanism of action and effects on cognition of GPR52 agonists; these studies will help determine the future utility of GPR52-targeted therapeutics in schizophrenia.

Setoh M (2014) *J Med Chem* **57**:5226-37

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Controlling efficacy at G protein coupled receptors; ligand-dependent modulation of G protein conformation and activity at the calcitonin receptor

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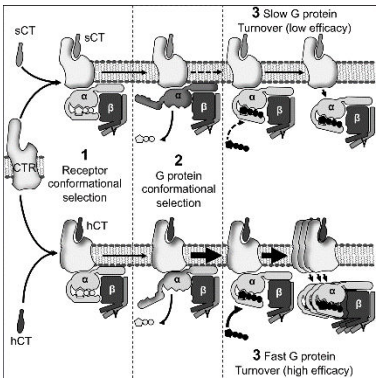

Introduction. Different ligands acting at the same G protein-coupled receptor (GPCR) can illicit divergent cellular responses. This phenomenon is known as differential efficacy and it underlies agonist dependent signal bias. The ability of ligands to illicit divergent responses from the same receptor is thought to principally arise from stabilisation of different receptor conformations.

Aims. We sought to understand the molecular basis for differential efficacy at the calcitonin receptor, a prototypical family B GPCR.

Methods. We used a variety of biochemical techniques, including native polyacrylamide gel electrophoresis and resonance energy transfer to examine whether receptor conformational changes were coupled to G protein conformational changes. We then used cell biological techniques including total internal reflection microscopy and real-time cyclic-adenosine monophosphate production to link the observed G protein conformational differences with the cellular response.

Results. We show that there are ligand dependent differences in the conformation of the G protein in the ternary complex. This results in different on-rates for guanosine triphosphate binding and therefore different G protein exchange rates at the active receptor and underlies differential efficacy for these ligands.

Discussion. We show that efficacy extends beyond the conformation of the receptor to encompass the G protein. This provides a new insight into the underlying molecular basis of efficacy and has important implications for understanding the basis of biased agonism.

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Cooperativity as the molecular basis of allosteric modulator receptor subtype-selectivity at muscarinic receptors

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Introduction. The muscarinic acetylcholine receptors (mAChRs) are prototypical members of the G protein-coupled receptor (GPCR) superfamily that regulate numerous fundamental processes in both the central and peripheral nervous system. Unfortunately, this family of receptor remains suboptimally targeted due to the very high degree of conservation of the orthosteric binding site across all 5 receptor subtypes. Fortunately, it is now well acknowledged that these, and other GPCRs, possess spatially distinct allosteric sites that provide greater selectivity in modulating receptor function (Christopoulos et al., 2014). This novel type of selectivity begs two questions: (i) where do allosteric modulators bind, and (ii) what is the molecular basis governing their selective actions?
Aim. To assess the pharmacological properties of two mAChR positive allosteric modulators (PAMs), BQZ-12, previously characterized as an M_1 mAChR-selective PAM (Abdul-Ridha et al., 2014) and LY-2119620, previously characterized as an M_4/M_2 mAChR-selective PAM (Kruse et al., 2013) at M_1-M_4 mAChRs.
Method. Radioligand binding assays in CHO cells expressing M_1-M_4 mAChRs were used to determine the affinity and cooperativity of BQZ-12 or LY-2119620 against orthosteric ligands. Functional interactions between PAMs at the M_1 and M_4 mAChRs were investigated in receptor-mediated ERK1/2 phosphorylation assays.
Results. In radioligand binding assays, BQZ-12 and LY-2119620 bound to M_1-M_4 mAChRs with similar affinity across subtypes, but with distinctly different degrees of cooperativity with orthosteric ligands. In functional studies, interaction of one allosteric modulator with another was consistent with a competitive mechanism.
Discussion. Our study suggests that the mAChR modulators interact with a 'common' allosteric domain, and that observed subtype selectivity of mAChR PAMs is thus not driven by differential affinity for structurally distinct allosteric sites, but rather subtype-selective cooperativity mediated between the allosteric and orthosteric sites.

Christopoulos (2014) Mol Pharmacol 86: 463.
Abdul-Ridha et al (2014) J. Biol. Chem. 2869:6067.
Kruse et al (2013) Nature 504: 101.

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Diagnosing and recording adverse drug reactions in general medical patients, a cross-sectional study

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Background: Adverse drug reactions (ADRs) are a significant cause of patient morbidity and mortality. In practice, a patient's ADR history is assimilated via several sources, of variable quality, on multiple occasions. The absence of a standard diagnostic process for ADRs, and a single record, creates uncertainty for clinicians. Recording ADRs has been demonstrated to be substandard, but the validity of ADR documentation in New Zealand is unknown.
Aim: To determine the validity of ADR documentation for patients admitted to the general medical service at Canterbury District Health Board (CDHB).
Methods**:** Consecutive patients admitted to general medicine at CDHB were recruited over a six week period in 2014. A reference list of ADRs for each patient was established by patient interview and review of records and assessed using the Naranjo Score by a study doctor. The ADRs recorded in each source document were entered into a database and compared with the reference ADR list. Comparisons between sources were made using Chi-squared tests.
Results: Sixty-one of the first 100 patients had a history of definite or probable ADRs, with a mean (sd) of 2.5 (1.7) per patient. The GP electronic record had a true positive rate (TPR) of 79%, with 93% true negative rate (TNR). The resident doctor review (on admission) had a TPR and TNR of 70% and 98%, respectively. The pharmacist review had a TPR of 87%, with a TNR 95%. Pharmacist review was superior to either of the other sources when examined by pairwise comparison ($\chi^2(1) > 4.3$, $P < 0.037$). However, the ADR documentation in the patient discharge summary had a significantly lower TPR of 76% than pharmacist review ($\chi^2(1) > 7.9$, $P < 0.005$). The drugs most commonly associated with ADRs were antibiotics (45%).
Conclusion: ADR documentation is inaccurate but improves following pharmacist review. However, the benefit of this review is not reflected in the discharge summary for the patient. Assessing ADRs retrospectively is difficult because there is usually insufficient information to make a diagnosis. Processes to diagnose and record ADRs at the time of the original event are needed. A single valid ADR list is needed to inform prescribing decisions.

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Effectiveness of interventions to deprescribe inappropriate proton pump inhibitors in older adults: a systematic review

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Introduction. Proton pump inhibitors (PPIs) are often inappropriately prescribed in older adults. There is also increasing evidence that PPI use is associated with an increased risk of adverse clinical outcomes in this group.
Aims. We assessed the effect of PPI deprescribing interventions in older adults.
Methods. We searched EMBASE, MEDLINE, Pubmed and the Cochrane Library (from inception to 19 July 2016) for studies including participants with a mean or median age of >/= 65 years and taking a PPI inappropriately. We included randomised controlled trials (RCTs) and cohort studies published in English. Our primary outcome of interest was the effect of the intervention on inappropriate PPI use (details of the protocol are registered on PROSPERO, http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016046356).
Results. After removing duplicates, 2,829 articles were screened, 189 articles were reviewed in full-text, and 16 were included in the final analysis. Conducting a meta-analysis was not possible because of significant differences in interventions and assessed outcomes between studies. There were 4 RCTs: 1 was specific to PPIs, and 3 were on general deprescribing with a PPI subgroup. There were 10 non-randomised studies, only 1 of which was controlled. Risk of bias was variable. No single study convincingly demonstrated a superior method to effectively deprescribe inappropriate PPIs.
Discussion. An optimal method to deprescribe inappropriate PPIs in older adults is yet to be identified.

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The uptake of TPMT pharmacogenomic testing across specialty units at a tertiary hospital

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Introduction. Thiopurine methyltransferase (TPMT) activity is an important predictor of the risk of cytopenias following azathioprine administration, and can be partially predicted by pharmacogenomic testing for low activity polymorphisms. This testing has been readily available for over ten years, however a recent critical episode at our hospital suggested that it may not be universally used. One of the difficulties in pharmacogenomic testing for TPMT is the delay in laboratory results returning, which means that unless testing is performed anticipatorily then results may not be available before drug commencement, especially if this is unexpected, as occurred in the index case.
Aims. To determine the rates and patterns of the use of TPMT testing in patients prescribed azathioprine, and in patients who have a reasonable likelihood of being prescribed azathioprine in the future.
Methods. We performed a retrospective chart review of patients who had electronic outpatient prescriptions for either azathioprine and mycophenolate (an indicator of potential future azathioprine use), issued between January-June 2016 inclusive. Liver transplant patients, patients commenced on the relevant drug before July 2013, patients with azathioprine introduced outside of the hospital, and patients previously prescribed thiopurines were excluded.
Results. Two investigators reviewed 161 medical records together. Of the 55 suitable patients prescribed azathioprine, 5 had not had TPMT tested, and only the index case did not have intensive cytopenia monitoring as a substitute precaution. Fewer patients with TPMT tested developed a cytopenia with six months of commencement (40% vs 2%, $p<0.02$). Of the 9 suitable patients prescribed mycophenolate, 6 had not had TPMT tested. Two of these patients could reasonably have started azathioprine in the future. Both patients are currently suffering from mycophenolate side effects. The patients at risk from both groups were cared for by a broad range of specialty units.
Discussion. TPMT testing was frequently performed for patients commencing azathioprine but was infrequently performed anticipatorily. The wide number of specialties involved and the turnover of medical staff means that improved education alone is unlikely to be sufficient to change practice. Implementation of an electronic medical record pharmacogenomic decision support tool to improve this usage has begun.

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Restarting psychoactive medications post intentional overdose: a move towards a consensus

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Introduction. There is an ongoing concern with regards to the optimal timing for restarting psychoactive medications post intentional overdose. No current guidelines exist to aid this important decision frequently faced by clinicians. Aims. We attempt to formulate an evidence-based guideline to support this decision-making skill and to make recommendations towards improvement in utilising therapeutic drug monitoring in psychoactive medications.
Methods. Review of pre-existing, available literature involving pharmacokinetics (including toxicokinetics) of commonly ingested psychoactive medications in overdose, and with reference to recommended timing of psychoactive medication reintroduction. Drug-drug interactions and effects of tobacco smoking in the metabolism of psychoactive medications were also explored. A list of commonly prescribed and ingested psychoactive medications was reviewed.
Results. No published data regarding recommended timing of reinstitution of psychoactive medication post overdose was found. Toxicokinetics for a number of psychoactive medications that we focused on were not well defined. Some psychoactive medications have saturable metabolism in overdose which makes the estimation of expected duration of toxicity and timing of medication recommencement challenging, unlike first-order kinetic metabolism. This encourages the use of serial serum concentrations as a form of best evidence-based guidance in the clinical setting.
Discussion. Our recommendations for safe reintroduction of psychoactive medications following overdose are based primarily on the pharmacological behaviour of the drugs when taken in excessive amount. We suggest the use of serial serum concentrations to help investigate the toxicokinetics of drug(s) ingested, in particular first- or zero-order kinetics and to help predict toxicity and offer the best guide to further management in both the acute phase of overdose and in the consideration of safe timing for recommencement of therapeutic psychoactive medication.

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Meropenem stability in 24 hour continuous infusion; a simulation study

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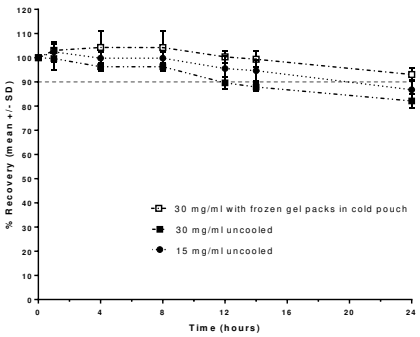
Introduction. Meropenem is a time-dependent antibiotic and continuous infusion administration is advocated to improve time above mean inhibitory concentration (T>MIC%). However, issues with stability, especially at higher temperatures, has limited its use, especially in an ambulatory setting where the infusion fluid is likely to approach body temperature. To improve stability, cooling has been investigated but the stability of high concentrations of meropenem over 24 hours in clinical settings is limited.



Aims. To assess the stability of meropenem in an infusion bag using ice packs in a cold pouch over 24 hours.
Methods. We performed an observational simulation study of the stability of 15mg/ml and 30mg/ml meropenem in a polyvinyl chloride infusion bag of 0.9% normal saline over 24 hours. In addition, stability at 30mg/ml was assessed when cooled. Meropenem concentrations were determined by liquid chromatography-mass spectroscopy (LC-MS) and t90; time at which the concentration of meropenem equals 90% of the initial concentration, was determined.
Results. Meropenem 30 mg/ml when cooled was stable for greater than 24 hours. However, uncooled meropenem 30mg/ml was stable for only 12 hours. Uncooled meropenem 15 mg/ml was stable for 20 hours.
Discussion. Meropenem at high concentrations is unsuitable to be given as a 24 hour infusion unless it is cooled. However, at high ambient temperatures, cooling may be inadequate to ensure stability up to 24 hours and shorter infusion times would be recommended.

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Challenges of an Electronic Medication Management System, The Evolving Role of a Clinical Pharmacologist in Interpreting Current Evidence and Implementing This Into an eMM System

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Introduction. An Electronic Medication Management (eMM) system changes the way we prescribe and also changes how a Clinical Pharmacologist can impact on prescribing habits. The current eMM project in Queensland Health involving the Princess Alexandra Hospital, as the pilot site, is the largest undertaken in Australia to date.
Aims. To explore some of the key challenges in implementing a state-wide eMM solution and to discuss the role and experience of a Clinical Pharmacologist in customising this solution.
Methods. Reviewing current literature to guide design decisions around allergies, interactions, dose-range checking and alert fatigue. First-hand experience in developing an eMM system to apply this evidence will be discussed.
Results. PubMed was searched for sulphonamide allergy and cross-reactivity (321 articles), QT-interval prolongation alerts in eMM systems (190 articles), dose range alerts (17 articles) and alert fatigue (34 articles).
Discussion. There is a lack of quality evidence around many decisions. The balance of evidence suggests that sulphonamide allergy cross-reactivity is not clinically significant (1), but the question of whether this evidence is strong enough to suppress such alerts is more difficult. The list of potential QT-prolonging drugs grows with time, yet the clinical significance of such interactions is difficult to assess and the existing evidence suggests alerts about these are largely ignored (2). It is well recognised that alert fatigue can compromise patient safety and alerts should be rationalised. The role of a Clinical Pharmacologist must evolve to fit in with these technological advances in order to aid rational and safe prescribing and to promote effective drug utilisation. We will present our experience and the challenges faced during the development of this eMM system.

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Participation of clinical pharmacology trainees in TGL expert groups

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Introduction. The first version of the Australian Antibiotic Guidelines (ABG) was published in 1978. Over the following 18 years several organisations took responsibility for publishing revisions of ABG, and, commencing with the Analgesic Guidelines in 1988, also for publishing groups of topics in other therapeutic areas. To consolidate these activities, Therapeutic Guidelines Ltd (TGL) was established as a not-for-profit company in 1996. TGL now publishes prescribing guidelines on groups of topics in most therapeutic areas which are widely used in all medical and pharmacy schools in Australia, as well as all public hospitals and many private practices and healthcare facilities. TGL aims to revise each group of topics in a particular therapeutic area approximately every 4 or 5 years. Revisions are undertaken by an “expert group” of about 14 people, half of whom are specialists in the relevant area; the others include general practitioners, pharmacists, and other relevant health professionals. In 2010, it was suggested to TGL that inclusion of a clinical pharmacology trainee on each group might be useful, first so they could obtain firsthand experience in developing prescribing guidelines, and second so they could bring to the group the perspective of junior hospital doctors and trainees, who are an important target audience for TGL’s guidelines.
Methods. Since 2010, during the planning and scoping phase of the revision of each version of Therapeutic Guidelines topics, all clinical pharmacology trainees have been invited to submit an expression of interest in being a member of the expert group, and one is selected by a member of the RACP specialty training committee in clinical pharmacology.
Results. A clinical pharmacology trainee has been a member of the expert groups that revised the following topic groups: Analgesic (2012), Cardiovascular (2012), Toxicology and Wilderness (2012), Psychotropic (2013), Endocrinology (2014), Antibiotic (2014), Respiratory (2015), Dermatology (2015), Gastrointestinal (2016) Palliative Care (2016), Rheumatology (in progress), Neurology (in progress), and Cardiovascular (in progress).
Discussion. From TGL’s perspective, the clinical pharmacology trainees have been useful members of our expert groups. We plan to more formally evaluate the program in the near future.

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Substantial impact of altered PK in critically ill (ICU) patients on the antibacterial effects of meropenem evaluated via mechanism-based modelling (MBM) and the hollow-fibre infection model (HFIM)

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Introduction. Severely altered PK due to pathophysiological changes, including in renal function, is often observed in ICU patients and can lead to widely varying antibiotic exposures. The potential impacts on bacterial killing by meropenem, frequently administered to ICU patients with serious infections, have never been characterized.
Aims. To evaluate the impact of altered PK on bacterial killing and resistance of meropenem in the HFIM.
Methods. A *Pseudomonas aeruginosa* isolate was studied in the HFIM (initial inoculum ~$10^{7.5}$ colony forming units (CFU)/mL). PK profiles of 3 meropenem dosing regimens (0.5, 1 and 2g q8h, 0.5h iv infusion) were simulated for augmented renal clearance (ARC), normal or impaired renal function (creatinine CL 250, 120 or 30 mL/min) based on a published population PK model. Viable counts of total and less-susceptible (5× and 10× minimum inhibitory concentrations (MIC)) bacteria were determined over 10 days. A MBM was developed to co-model the PK and PD.
Results. For all regimens with ARC, initial bacterial killing of ~2.5 $\log_{10}$ cfu/mL at 7 h was followed by regrowth to control values at 72 h; less-susceptible bacteria increased ~500,000-fold compared to controls. With normal renal function the 0.5g and 1g regimens provided ~3.4 - 4.0 $\log_{10}$ bacterial killing at 24 h followed by rapid regrowth and a large increase in less-susceptible bacteria. The 2g regimen at normal renal function and all regimens at impaired renal function suppressed regrowth over 10 days. The MBM including 3 bacterial subpopulations with different susceptibilities to meropenem successfully characterised bacterial killing and regrowth for all profiles.
Discussion. For ARC, extensive bacterial regrowth with resistance occurred with even the highest approved meropenem dose. Individualized dosing regimens accounting for altered PK in ICU patients and aiming for higher than standard antibiotic exposures are necessary to maximise bacterial killing and suppress emergence of resistance. Optimised dosing regimens supported by MBM and Monte Carlo simulations, *e.g.* including extended or continuous infusions, should be evaluated in the context of ARC.

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Cardiac-selective bone morphogenetic protein 7 (BMP7) gene therapy to target cardiac fibrosis in a mouse model of diabetic cardiomyopathy

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Introduction. Increasing evidence indicates that targeting BMP signalling reduces fibrosis, particularly in idiopathic pulmonary fibrosis and diabetic kidney disease. However, its potential role in the diabetic heart is unclear.
Aims. To study the impact of recombinant adeno-associated virus (rAAV)6-BMP7 administration on diabetic cardiomyopathy.
Methods. Following 8 weeks of untreated STZ-induced diabetes (55mg/kg/d i.p., 5 days) and the confirmation of diastolic dysfunction by echocardiography, male FVB/N mice received a single tail vein injection of rAAV6-BMP7 ($2x10^{12}$vg or null vector), incorporating a cytomegalovirus promoter to achieve cardiac selectivity. Mice were followed for a further 8 weeks prior to detailed *in vivo/ex vivo* analyses (n=8-15/group).
Results. Cardiac BMP7 levels in both nondiabetic (ND) and diabetic (STZ) mice were elevated following BMP7 gene therapy compared to null vector (RT-PCR: ND-Null 1.0±0.2 vs ND-BMP7 5.1±1.2, P=0.01; STZ-Null 1.1±0.2 vs STZ-BMP7 2.6±0.5, P=0.03). BMP7 treatment had no effect on diabetes-induced increases in blood glucose and HbA1c (STZ-Null 9.8±0.6% vs STZ-BMP7 10.9±0.2%, P=NS), and did not impact the diabetes-induced decrease in heart weight and increase in kidney weight. Interestingly, BMP7 treatment improved STZ-induced diastolic dysfunction, as assessed by echocardiography (E/A ratio: STZ-Null 1.4±0.04 vs STZ-BMP7 1.9±0.12, P=0.01). Moreover, improved cardiac function in BMP7-treated mice was associated with a preferential reduction in interstitial fibrosis (STZ-Null 4.8±0.5% vs STZ-BMP7 2.4±0.3%, P<0.001) and modulation of extracellular matrix-associated gene expression, including fibronectin (STZ-Null 2.4±0.7 vs STZ-BMP7 0.7±0.1, P=0.01) and MMP-2 (STZ-Null 1.0±0.3 vs STZ-BMP7 4.2±1.2, P<0.02).
Discussion. Cardiac-selective BMP7 gene therapy attenuates diabetic cardiomyopathy in mice, via preferential regulation of the extracellular matrix, suggesting specific targeting of BMP signalling may represent a novel therapeutic strategy for heart failure in diabetes.

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Clinical experience with pharmacological conditioning of donor hearts with glyceryl trinitrate (GTN) and erythropoietin (EPO) after declaration of circulatory death (DCD)

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Background: Hearts from DCD donors have not been considered for transplant due to injury sustained during warm ischaemia after withdrawal of life support. In a porcine transplant model we showed viable donor heart recovery is possible, given 3 pre-conditions: i) warm ischaemic time <30 min [1]; ii) hearts flushed with with preservation solution containing 0.1mg/ml GTN + 5U/ml EPO (pharmacological conditioning) [1]; iii) cold static storage replaced by ex vivo normothermic perfusion (Transmedics Organ Care System; OCS®) [2] . We now present results of the first 10 human heart transplants using hearts donated after declaration circulatory death performed at St Vincent's Hospital.
Methods: Recipients received hearts donated after circulatory death (Maastricht category III) from donors <40 years of age, with warm ischaemic times <30 min. All hearts were retrieved after a protective myocardial flush with GTN/EPO supplemented St Thomas' Solution (1L). Hearts were then instrumented and transferred to an OCS® circuit for preservation, resuscitation & continual assessment during transportation to St Vincent's Hospital.
Results: Recipients (6M, 4F; ages 22-65y) received hearts from donors (8M, 2F), ages 21-38y. Mean donor heart warm ischaemic time was 24±4 min, with OCS® perfusion times from 206-409 min. Three patients needed temporary mechanical support for 2-5 days. Length of hospital stay for the group was 7-38 days. All recipients regained normal cardiac function after 1 week. One year follow-up showed i) no mortality; ii) mean ejection fraction of 65%; iii) all recipients were NYHA class 1 (asymptomatic).
Conclusions: Careful donor selection, minimisation of warm ischaemic myocardial damage, and use of portable ex-vivo organ perfusion allows successful transplantation of DCD hearts.

References:
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Interleukin-18 is crucial for the development of renal inflammation and elevated blood pressure in a mouse model of hypertension

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Introduction. Clinical studies have shown that levels of pro-inflammatory, T_H1-polarising cytokine, IL-18, are elevated in hypertensive patients. However, whether IL-18 plays a causal role in hypertension is unknown.
Aims. To determine (1) whether IL-18 and/or its signalling system is upregulated in the kidneys of hypertensive mice and (2) if IL-18 deficiency is protective against the development of hypertension and renal inflammation.
Methods. Hypertension was induced in male wild-type (WT) and IL-$18^{-/-}$ mice by uninephrectomy and treatment with deoxycorticosterone acetate (2.4 mg/d, *s.c.*) and saline (0.9%) drinking water (1K/DOCA/salt). Control mice received uninephrectomy, a placebo pellet and normal drinking water (1K/placebo). Blood pressure (BP) was measured via tail cuff. After 21 days, kidneys were harvested to assess: renal hypertrophy; expression of IL-18 and IL-18 receptor (IL-18R; qRT-PCR); and immune cell numbers and cellular localisation of IL-18R (flow cytometry).
Results. 1K/DOCA/salt mice displayed elevated BP and kidney weights (140±3 mmHg; 390±8 mg) compared to 1K/placebo mice (118±2 mmHg; 291±7 mg; n≥17, P<0.05). 1K/DOCA/salt treatment also caused renal inflammation indicated by a 2-fold increase in CD45+ leukocytes. IL-18 expression was elevated by 40% in the kidneys of 1K/DOCA/salt versus 1K/placebo mice (n≥7; P<0.01). IL-18R expression was also elevated by 3-fold (n≥6; P<0.0001) with CD4+ T cells representing the major cell type expressing this receptor. Importantly, IL-$18^{-/-}$ mice were profoundly protected from the hypertensive actions of 1K/DOCA/salt compared to WT mice (119±6 vs 145±6 mmHg; n=5, P<0.05). Renal expression of the classical T_H1 cytokine interferon-γ also appeared to be reduced by 90% in IL-$18^{-/-}$ vs WT mice (n=2).
Discussion. 1K/DOCA/salt-induced hypertension is associated with increased inflammation and upregulation of components of the IL-18 signalling system in the kidneys. IL-18 deficiency protects against the development of high BP and renal inflammation in 1K/DOCA/salt-induced hypertension, highlighting the IL-18 system as a potential target for future anti-hypertensive therapies.

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Functional and inflammatory effects of thrombotic cortical stroke in males and females

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Introduction. Sex differences in stroke outcome are common in clinical and experimental studies; younger females being typically less functionally impaired than age-matched males. Estrogen is known to exert neuroprotective effects after stroke, and can modulate inflammation. Sex-dependent outcomes have not been investigated in experimental models of cortical thrombotic stroke.
Aims. To characterise the time-course of infarct development, functional impairment and brain inflammation over 7 d following photothrombosis-induced stroke in 8-12 week old male (n = 32) and female (n = 34) C57BL6 mice.
Methods. Under isoflurane inhalation (2-2.5% in O_2), thrombosis was induced in the left primary motor cortex using Rose Bengal (2 mg i.p) followed by direct illumination with cold light for 15 min. At 1 and 7 d post-stroke, infarct volume, edema, brain inflammation and lung infection were assessed. Motor function and estrous cycle stages were evaluated on d 0, 1, 3 and 7.
Results. At d 1, infarcts were ~20 % smaller in females than males (n = 8-9). By d 7, infarct volume was reduced, and no sex differences were evident. Females in the proestrus stage, where 17β-estradiol levels are shown to be the highest, at the time of stroke (n = 5) were ~50 % less lateralised to their contralateral forelimb than males (n = 17) and females in the estrus (n = 4) and metestrus (n = 1) stages at d 7. Numbers of macrophages/microglia and T cells were increased by 40-50 % from d 1 in both males and females, in the non-infarct region of the ipsilateral hemisphere at d 7 (n = 8-9, $P<0.05$). Neutrophil numbers were unchanged in males over the 7 d but were increased ~2-fold in females (n = 8-9, $P<0.05$). Stroke had no detectable effect on levels of bacteria in lungs (n = 9-10).
Discussion. This is the first study to characterise early functional and inflammatory changes following experimental thrombotic cortical stroke. The findings suggest that the estrogen level at the time of stroke in females is important in determining stroke severity. The role of estrogen and its effects on the post-stroke inflammatory response need to be further investigated using this model.

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Shear stress mediates cell surface expression and interaction of TRPV4 with other mechanoreceptors

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Introduction. Endothelial responses to haemodynamic forces associated with blood flow play a pivotal role in the acute regulation of vascular tone. Transient receptor potential vanilloid 4 (TRPV4), a non-selective cation channel, is a candidate mechanoreceptor that is widely expressed in the endothelium. Ca^{2+} influx through TRPV4 is an important source of local Ca^{2+} increase in the endothelium, and it has been shown that the cooperative opening of as few as three clustered TRPV4 channels per endothelial cell, causes maximal vasodilation through the activation of intermediate- and small- conductance, Ca^{2+} sensitive potassium channels (Sonkusare et al., 2014).
Aims. To study the effect of shear stress on plasma membrane translocation of TRPV4 channels and understand the signalling pathway(s) that control the trafficking of TRPV4 to the plasma membrane.
Methods. Here, we used a combination of microfluidic and Ca^{2+} imaging using to measure changes in intracellular Ca^{2+} levels. Cell surface biotinylation assay and super-resolution microscopy were used to study the localisation and the interaction of TRPV4 with other mechanoreceptors at the cell membrane.
Results. We found that TRPV4 channels form small clusters with components of adherens junctions, such as VE-cadherin and β-catenin, at the endothelial cell membrane. Shear stress stimulation increased the density of TRPV4 channels at the cell membrane and also increased the density and size of TRPV4 clusters. Shear stress stimulation resulted in trafficking of TRPV4 channels from the basolateral membrane to the basal membrane and reduced the density of TRPV4 channels in complexes with β-catenin while increased complexation of TRPV4 with β1 integrin. TRPV4 trafficking to the cell membrane was regulated by AKT, β1 integrin and Rac1 signalling pathways but was independent of PI3K.
Discussion. In mesenteric arteries, Ca^{2+} influx through TRPV4 ion channels regulates relaxation of smooth muscle cells, and such relaxation is impaired during hypertension. Such changes in TRPV4 membrane expression and interaction with other mechanoreceptors are likely to be relevant to diseases where mechanical signalling is altered and it may be possible to target this process for therapeutic benefit.

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Influence of ACE inhibitors on frailty and cardiac function in middle-aged female C57BL/6 mice

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Introduction. Frailty is the accumulation of health deficits over the lifespan. Frailty results in increased risk of adverse outcomes including poor cardiovascular outcomes. Clinical interventional studies aimed at attenuating frailty are limited and inconclusive. Angiotensin converting enzyme (ACE) inhibitors improve exercise capacity in older adults without cardiovascular disease (Sumukadas et al, 2007), and could be a potential frailty intervention.
Aims. To investigate whether chronic treatment with, the ACE inhibitor, enalapril would attenuate frailty in mice, and whether this occurs through changes to cardiac function.
Methods. Female C57BL/6 mice (12 months) were fed enalapril containing chow (40 mg/kg/day; n=10) or control feed (n=10) for 3 months. Frailty was quantified with the mouse clinical frailty index (Whitehead et al, 2014). Blood pressure (BP) was measured with a tail-cuff and in vivo cardiac function was measured using echocardiography. Cardiomyocytes were isolated for field-stimulation and voltage clamp experiments (2 Hz).
Results. Frailty index scores were significantly lower in the enalapril group when compared to control mice (0.14 ± 0.01 vs 0.21 ± 0.03, $p<0.05$) after 3 months. BP, heart structure and contractile function were not significantly different between the enalapril and control groups. Field stimulation experiments showed that enalapril treatment increased cell shortening (1.6 ± 0.2 vs 3.0 ± 0.5 %, $p<0.001$), velocity-to-peak contraction (0.068 ± 0.005 vs 0.133 ± 0.016 µm/ms, $p<0.001$) and ½ relaxation (0.044 ± 0.005 vs 0.100 ± 0.016 µm/ms, $p<0.001$), with no change in underlying calcium transients. Under voltage clamp conditions both calcium transients (37.6 ± 3.2 vs 49.0 ± 3.9 nM, $p<0.05$) and contractions (5.7 ± 0.7 vs 8.9 ± 0.9 %, $p<0.05$) were increased by enalapril treatment. Calcium current and sarcoplasmic reticulum (SR) calcium content were unchanged.
Discussion. Enalapril treatment attenuated frailty in middle-aged female mice. Mechanisms contributing to this attenuation may include improved cardiomyocyte contraction and increased calcium release from the SR.

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CCL18 as a mediator of the pro-fibrotic actions of M2 macrophages in the vessel wall during hypertension

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Introduction. M2 macrophages contribute to vascular fibrosis and stiffening in hypertension (Moore et al., 2015). Given that M2 macrophages can generate the pro-fibrotic chemokine, CCL18, we hypothesised that CCL18 contributes to hypertension-associated fibrosis via its cognate receptor, CCR8.
Aims. To determine if angiotensin II promotes CCL18 generation from human primary macrophages, and to identify cellular targets of CCL18.
Methods. Human primary macrophages were treated with either the M2-polarising stimuli, IL-4 and IL-13 (5-50 ng/ml) or with angiotensin II (100 pM-1µM), for 6-72 h and CCL18 expression was measured (qRT-PCR, ELISA). Male C57BL6/J mice were treated with saline or angiotensin II (0.7 mg/kg/d, 28 d; *s.c.*). Localisation and expression of CCR8 and CCL8 in the thoracic aorta were measured by immunohistochemistry and qRT-PCR, respectively.
Results. IL-4 and IL-13 evoked time-dependent increases in CCL18 mRNA (1500-fold at 72 h, $p<0.05$, n=5-7) and protein (500-fold at 72 h, $p<0.05$, n=4-7) expression in human primary macrophages. Angiotensin II alone did not increase CCL18 expression (n=3-5). However, in M2-polarised macrophages (0.5 ng/ml IL-4), angiotensin II (100 pM; 48 h) appeared to increase CCL18 protein levels by a further 70 % (n=2-3). In aortas from hypertensive mice, mRNA expression of CCL8 (murine homologue of human CCL18), but not CCR8, was elevated 3-fold ($p<0.05$, n=4-8). In the aortic wall, CCL8 and CCR8 were co-localised with macrophages and endothelial cells, respectively. CCR8 expression was also evident in the adventitia.
Discussion. Angiotensin II increases CCL18 generation from M2 macrophages, which may target CCR8-expressing endothelial and/or adventitial cells to promote fibrosis. CCL18 and its receptor CCR8, may represent targets for the treatment of fibrosis during hypertension.

Moore JP et al. (2015) *Am J Physiol Heart Circ Physiol*, 309 (5): H906-H917.

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Plasma cell differentiation is not essential for the pro-hypertensive actions of B cells

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Introduction. Mice deficient in mature B cells are protected from the hypertensive actions of angiotensin (Ang) II. However, it is unknown whether this is due to the ability of B cells to differentiate into antibody-secreting plasma cells or related to other known B cell functions such as antigen-presentation and cytokine production.
Aims. We tested (1) whether Ang II-induced hypertension in mice is associated with differentiation of B cells into antibody-producing plasmablasts/plasma cells and (2) if blocking plasmablast/plasma cell differentiation by genetic ablation of the transcription factor B-lymphocyte-induced mature protein-1 (Blimp-1) protects against hypertension.
Methods. Male wild-type (WT) and B cell-specific Blimp-$1^{-/-}$ mice were anaesthetized with isoflurane (0.4 L/min, 2.5%) and implanted with mini-pumps to deliver Ang II (0.7 mg/kg/d, *s.c.*) or vehicle (saline) for 5, 14 or 28 days. BP was measured by tail cuff. Flow cytometry was used to measure B cell, plasmablast and plasma cell numbers in the spleen. Luminex immunoassays were used to measure the concentration of IgG in the serum.
Results. BP was elevated by 25 mmHg (n=7; $P<0.05$) in Ang II- vs vehicle-treated WT mice at day 5 and remained at similar levels at days 14 and 28 (n≥8; $P<0.05$). At day 5 there were no differences in splenic B cell, plasmablast or plasma cell numbers between groups. However, at day 14, both plasmablasts (101±10 $x10^3$ vs 65±8 $x10^3$; n=8-9; $P<0.05$) and plasma cells (68±10 $x10^3$ vs 39±6 $x10^3$; n=8-9; $P<0.05$) were elevated in hypertensive vs normotensive WT mice. By day 28, only plasma cells were elevated in spleens of Ang II-treated WT mice (67±12 $x10^3$ vs 40±4 $x10^3$; n=11-15; $P<0.05$). Despite evidence of B cell differentiation into plasmablasts and plasma cells in hypertensive WT mice, no changes in serum IgG levels were observed at any time-point. Moreover, although mice with B cell-specific Blimp-1 deficiency had 65% fewer splenic plasma cells, they were not protected from the hypertensive effects of Ang II (ΔBP 32±7 vs 42±6 mmHg in WT mice; n=5-6).
Discussion. Chronic Ang II infusion results in B cell differentiation into plasmablasts and plasma cells, but these processes do not appear to be crucial to the development of hypertension. Hence, other B cell functions, such as cytokine production or antigen presentation, may explain the pro-hypertensive actions of these lymphocyte.

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Peer feedback and scaffolding student learning: developing an authentic assessment

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Introduction. Performing and responding to peer review is the basis of scientific publication and an important professional skill for students to learn. Furthermore research has suggested that formative feedback from peers increases student self-assessment skills and the immediate application of feedback improves learning outcomes[1].
Aims. To design a scientific writing assignment for second year Introductory Pharmacology and Toxicology students that develops critical thinking, scientific writing and peer review skills.
Methods. We developed an authentic assessment to mirror the process of a scientist goes through to publish a scientific paper i.e. submit an article for peer review, receive feedback, respond to feedback to produce the published article. In the same manner the commentary assignment is scaffolded with students having the following tasks (i) identify 3-4 key ideas from the scientific article provided and search for extra supporting sources that provide information in the context of the field, and submit their notes; (ii) write and submit a commentary article; (iii) two classmates peer review the commentary and provide feedback anonymously via Moodle 'workshop' activity; (iv) students revise the articles according to the feedback received and write a response which outlines how the feedback has been addressed; (v) the final version of the commentary article is submitted for grading. Prior to the students undertaking each task, it is practised and reviewed in tutorial classes.
Results. The scaffolded nature of the assignment and the need to respond and act upon feedback and thereby closing the feedback loop has resulted in improved student writing. We have observed that the majority of students provide constructive feedback and that the reviewees incorporate this feedback into the final version of their commentary, improving the overall quality. Furthermore, students report in course surveys that they like the scaffolded nature of the assessment task.
Discussion. This assignment cultivates the students' abilities in identifying key ideas and places them in the context of the broader research field. Furthermore, it develops students' professional peer review skills, in both providing and responding to feedback.

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Evaluating student perceptions of current laboratory report feedback

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Introduction. Practical classes are considered an integral part of undergraduate pharmacology curricula. Individual students' written practical reports thus form a primary method of not only assessing their understanding of theory, but also allow them to develop and enhance skills in written communication, data analysis and presentation. Although we aim to provide constructive feedback on these reports, our suggestions for improvement are often not taken up. This observation suggests that we are not sufficiently engaging students in the feedback process.
Aim. The current study aimed to evaluate the perceptions of students on our current feedback practice for laboratory reports, in order to assess what factors might influence their uptake of, and receptiveness to this feedback.
Methods. Respondents were undergraduate students (n=79) undertaking a third year pharmacology unit in semester 1, 2016. This unit included three assessed written practical reports, collectively contributing 15% to the final unit mark. At the conclusion of the unit's practical teaching program, students were invited to complete an anonymous survey that asked them to define the concept of feedback and rate their perceptions of the feedback they received on the reports in this unit.
Results. Students consistently stated that feedback in general must be constructive, timely and should include suggestions for improvement of future tasks. The majority (57%) of students indicated that they always reviewed the feedback provided to them on their practical reports, irrespective of the mark. Furthermore, students overwhelmingly agreed (>90%) that the feedback was useful for improving their future reports, and could be applied to other units of study (78%). Several did however, indicate that feedback needed to be more prompt to allow enough time to incorporate suggestions for improvement into subsequent reports. Interestingly, although nearly 50% of students disagreed that they pay less attention to written feedback than when it is provided in person, several still indicated that they would value the opportunity to follow up on their practical report feedback with their demonstrator face-to-face.
Discussion. Students appeared to respond favourably to the feedback provided in this pharmacology unit, yet individual comments suggest that students would value more personalised feedback. We are currently undertaking an evaluation of audio visual feedback approaches to investigate their potential for such.

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Surviving bioscience and pharmacology; an eBook for accelerated nursing students

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Introduction. In Australia, diploma, and international and domestic graduate students are given academic credit to accelerate their entry into the Bachelor of Nursing at second year level. These students struggle with the biosciences and pharmacology. At QUT, we have shown that the diploma-entry students have high attrition rates, but that this can be overcome by using a face-to-face/community website strategy including a formative activity of key concepts as "Getting started" and resource lectures as "Highlights of first year" (Doggrell & Schaffer, 2016).
Aims. The aim was to turn this successful strategy into an eBook to support the accelerated nursing students.
Methods. For the eBook, the original strategies of "Getting Started" and "Highlights from first year" were revised and rewritten, in plain English, in the form of eChapters. This included creating simplified diagrams in Powerpoint to illustrate and complement the text. All diagrams were copyrighted with Creative Commons so that the eBook could be made freely available to students and academics. The eBook was evaluated by the students.
Results. The "Getting Started" section of the eBook comprises eight eChapters covering medical and anatomical terminology, some fundamental concepts relating to the organisation of the human body e.g. homeostasis, molecules of life, cells and tissues, and an introduction to the principles of pharmacology, including binding sites and pharmacokinetics. "Highlights from first year" comprises six eChapters covering fundamental anatomy and physiology of the major systems of the human body (nervous, endocrine, cardiovascular, digestive and urinary), as well as an introduction to basic microbiology concepts. The eBook was evaluated positive by both bioscience and pharmacology students with most strongly agreeing or agreeing that both the "Getting Started" and. "Highlights from first year" were presented in a clear and organised manner.
Discussion. The creation of a simplified, customised, eBook covering the fundamental concepts of bioscience and pharmacology has allowed diploma-entry and graduate students to review some pre-requisite concepts prior to and during their pharmacology studies and this may fill their transitional learning gaps.

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Developing student awareness of potential career options in the pharmaceutical industry

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Introduction. Students undertaking courses without a defined vocational outcome (e.g. B.Sc, B.Biomed. Sci) are not always aware of potential career pathways, which can affect their motivation and engagement. Indeed a lack of knowledge of career pathways may contribute to the passive approach many students adopt in their final year of study.
Aim. The current study aimed to heighten the engagement and career awareness of pharmacology students via the development, implementation and evaluation of a career portal with a focus on the pharmaceutical industry.
Methods. Pharmaceutical industry roles were identified, a synopsis for each role written and a series of interview questions for Monash graduates working in the pharmaceutical industry compiled. Monash Graduates were contacted via LinkedIn or email and invited to participate in an on-line survey or video interview, focused on the nature of their job, career journey and skills and attributes they attained during their tertiary studies. A careers portal was established outlining career pathways in the pharmaceutical industry with links to profiles and video interviews of Monash graduates. Undergraduate students (n=35) enrolled in a third year pharmacology unit in semester 2, 2016, were invited to complete anonymous surveys pre- and post-implementation of the careers portal. Surveys were designed to assess students' knowledge and understanding of job opportunities in the pharmaceutical industry.
Results. Of the Monash graduates surveyed the majority indicated that communication skills, attention to detail and teamwork, were important attributes in their roles in the pharmaceutical industry. Four of these participants completed video interviews which were incorporated into the careers portal. Prior to access to the careers portal, students identified research (49%) and sales representatives (37%) as roles within the pharmaceutical industry of which they were aware. In addition, the majority of students (63%) indicated that they would use Google to obtain further information relating to career prospects. Interestingly, 51% of students identified communication as a skill they believed was needed to undertake a career in the pharmaceutical industry. Survey data post implementation of the careers portal is pending.
Discussion. Undergraduate students have limited knowledge of career opportunities within the pharmaceutical industry. The implementation of a careers portal will highlight potential career pathways for pharmacology students and provide context for the development of employability skills.

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Allostery in GPCR signalling

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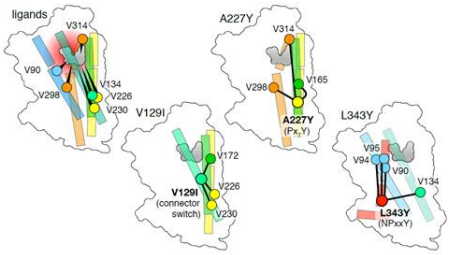

The signaling events in G protein coupled receptors (GPCRs) propagate through protein by concerted local conformational changes, forming allosteric pathways. Using a combination of NMR and mutagenesis as well as structural bioinformatics, we identified several independent allosteric activation pathways of the β1-adrenergic receptor that connect the ligand binding pocket with the activity of the receptor. The network analysis of active and inactive structures of GPCRs suggested that the activation pathways converge near the G protein binding site, consistent with NMR data. In order to identify the allosteric networks in the G protein, we generated comprehensive single amino acid resolution maps of the residues stabilising the human Gαi1 subunit in nucleotide- and receptor-bound states. We generated these maps by measuring the effects of alanine mutations on the stability of Gαi1 and of the rhodopsin Gαi1 complex. We identified stabilization clusters in the GTPase and helical domains responsible for structural integrity of the protein, the conformational changes associated with activation, as well as changes in the dynamics of individual amino acids. The proposed methods can be readily applied to identify and study allosteric pathways in other proteins.

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Novel insights into Class B GPCR activation and signaling

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Class B G protein coupled receptors (GPCRs) are activated by large peptide hormones and are important drug targets in many human diseases including metabolic, neurodegenerative and cardiovascular diseases. Like most GPCRs, these receptors are pleiotropically coupled with the physiological impacts of receptor activation dependent on the spectrum of signalling and regulatory events initiated by ligand binding. Peptide hormones interact with Class B GPCRs via a common two-domain binding mechanism, but to date, the molecular details for these interactions at any Class B GPCR and how this results in receptor activation is not fully understood. In addition, due to their pleiotropic coupling, Class B GPCRs are subject to biased agonism, a phenomenon that describes the ability of distinct ligands binding to the same receptor to promote diverse signalling profiles. The principal driver for this differential efficacy is thought to arise due to different ligands stabilising distinct receptor conformations. While biased agonism holds great promise for developing better and safer therapeutics, exploitation of this for therapeutic development requires an understanding of the mechanistic/structural basis of how Class B GPCRs are activated by peptide ligands and how this differs for distinct ligands. In this talk I will cover some of our work with Class B GPCRs that are enabling us to begin to uncover molecular mechanisms of Class B GPCR activation and those linked to biased behaviours of individual ligands. Collectively, this work is starting to provide novel insights into Class B receptor structure and function that might be exploited for the development of novel therapeutics.

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Structural mechanism of ligand activation in class C GPCRs

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Introduction. Class C G-protein coupled receptors (GPCRs) mediate a number of key biological phenomena including excitatory and inhibitory neurotransmission, calcium homeostasis, taste and smell[1]. These receptors are characterized by a large ligand-binding extracellular domain in addition to the canonical seven-helix transmembrane domain. A unique feature of the class C receptors is that they require dimerization for function[1,2].

Aims. We are investigating the structure and function of two class C GPCRs, human $GABA_B$ receptor and calcium-sensing receptor (CaSR). Our goal is to understand the ligand-dependent activation mechanism of these receptors.

Methods. We use protein x-ray crystallography to determine the extracellular-domain structures of $GABA_B$ receptor and CaSR in multiple functional states.

Results. Human $GABA_B$ receptor mediates inhibitory neurotransmission in the brain[3]. It functions as an obligatory heterodimer of GBR1 and GBR2 subunits. Here we present the first crystal structures of a heterodimeric complex between the extracellular domains of GBR1 and GBR2 in the apo, agonist-bound, and antagonist-bound forms. These structures reveal the molecular basis of ligand recognition by $GABA_B$ receptor. The apo and antagonist-bound structures represent the resting state of the receptor; the agonist-bound complex corresponds to the active state. Both subunits adopt an open conformation at rest, and only GBR1 closes upon agonist-induced receptor activation. $GABA_B$ receptor activation involves the formation of a novel heterodimer interface between subunits.

Human CaSR maintains extracellular Ca^{2+} homeostasis through the regulation of parathyroid hormone secretion[4]. Here we present the crystal structures of the entire extracellular domain of CaSR in the resting and active conformations. We provide direct evidence that L-amino acids are agonists of the receptor. In the active structure, L-Trp occupies the orthosteric agonist-binding site at the interdomain cleft, and is primarily responsible for inducing extracellular domain closure to initiate receptor activation. Our structures reveal multiple binding sites for Ca^{2+} and PO_4^{3-} ions. While Ca^{2+} ions stabilize the active state, PO_4^{3-} ions reinforce the inactive conformation.

Discussion. Our data reveals a universal activation mechanism for class C GPCRS that involves extracellular domain closure and association of membrane-proximal domains.

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Chemokines and their receptors: structural insights into binding, activation, specificity, and antagonism

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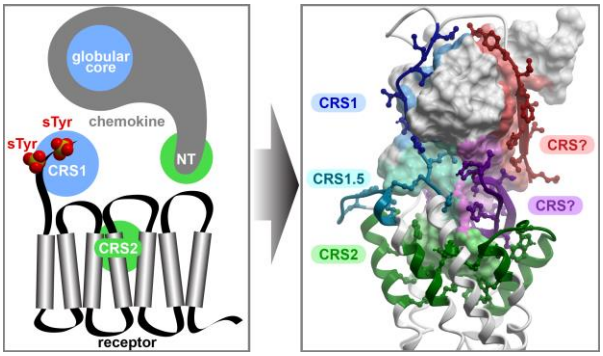

Introduction. Interaction of chemokines with 7TM membrane receptors drives cell migration in development, immunity, inflammation, and cancer, making them attractive therapeutic targets.

Aims. Understanding the structural basis of receptor interactions with chemokines and small molecules, as well as receptor activation, specificity, and antagonism, to facilitate the discovery of therapeutics targeting the chemokine receptor axis.

Methods. Molecular modelling, crystallography, biophysical and functional experiments.

Results. Crystallography and experiment-guided modelling of receptor:chemokine complexes support the canonical two-site interaction hypothesis but reveal unanticipated interaction epitopes important for function and specificity (*1, 2*). A contiguous signalling pathway between the chemokine and G protein has been defined (*3*). Structures with small molecules reveal novel mechanisms for antagonism and allostery (*4*).

Discussion. Findings significantly expand the understanding of receptor:chemokine recognition, and suggest new avenues for pharmacological modulation of chemokine receptors.

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Structural studies of purinergic receptors $P2Y_1R$ and $P2Y_{12}R$

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In response to adenosine 5'-diphosphate, purinergic receptors $P2Y_1R$ and $P2Y_{12}R$ regulate platelet activation and thrombus formation, and thus serves as important antithrombotic drug targets. Here we report the crystal structures of human $P2Y_{12}R$ in complex with an antagonist AZD1283, with a full agonist 2-methylthio-adenosine-5'-diphosphate (2MeSADP), and with the corresponding ATP derivative 2-methylthio-adenosine-5'-triphosphate (2MeSATP). As a first example of a GPCR where agonist access to the binding pocket requires large scale rearrangements in the highly malleable extracellular region, the structural studies therefore will provide invaluable insight into the pharmacology and mechanisms of action of agonists. We also report the crystal structures of human $P2Y_1R$ in complex with a nucleotide antagonist MRS2500, and with a non-nucleotide antagonist BPTU. The $P2Y_1R$ structures reveal two distinct ligand binding sites, providing atomic details of $P2Y_1R$'s unique ligand binding modes. MRS2500 recognizes a binding site within the seven transmembrane bundle of $P2Y_1R$, which, however, is different in shape and location from the nucleotide binding site in the $P2Y_{12}R$ structure. BPTU binds to an allosteric pocket on the external receptor interface with the lipid bilayer, making it the first structurally characterized selective G protein-coupled receptor (GPCR) ligand located entirely outside of the helical bundle. These high-resolution insights into $P2Y_1R$ should enable discovery of new orthosteric and allosteric antithrombotic drugs with reduced adverse effects.

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Structural insights into the dynamic process of G protein coupled receptor signaling

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G protein coupled receptors (GPCRs) conduct the majority of transmembrane responses to hormones and neurotransmitters, and mediate the senses of sight, smell and taste. The β_2 adrenergic receptor (β_2AR), the M2 muscarinic receptor and the mu-opioid receptor are prototypical Family A GPCRs. We have obtained three-dimensional structures of these receptors in inactive and active conformations, as well as a structure of the β_2AR in complex with the G protein Gs. Comparison of these structures provides insights into common mechanisms for propagation of conformational changes from the agonist binding pocket to the G protein coupling interface. We have also used fluorescence, EPR and NMR spectroscopy to study the dynamic properties of the β_2AR. I will discuss what we these studies have taught us about allosteric regulation of GPCR structure by G proteins and ligands.