AuPS Meeting - Melbourne 2008

Symposium: Ion Channels as Therapeutic Targets for Multiple Diseases

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Chair: David Adams

Novel approaches for screening sodium channel function in drug discovery

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Sodium channels are vital constituents of excitatory cells and participate in a myriad of normal physiological functions. Voltage gated sodium channels are the molecular components that enable action potential generation in these cells and are comprised of a principal alpha subunit and accessory beta subunits. Ten alpha subunits have been identified each with a unique tissue and cellular distribution, and important differences in voltage dependent and kinetic properties. Congenital diseases such as long QT syndrome and epilepsy can be caused by mutations in voltage gated sodium channels and, therefore, they are validated "disease" genes. Modulation of sodium channel function by drugs is a strategy used to treat disease such as epilepsy, cardiac arrhythmia and chronic pain disorders as well as achieving local anaesthesia. As such they are attractive drug targets, yet many challenges remain. Because of the unique tissue distribution profile, achieving desired efficacy without adverse action demands subunit or functional selectivity. Current drug discovery efforts typically rely on high throughput low content screening based on fluorescence assays or on low throughput high content screening based on electrophysiological assays. An added complication is that *in vitro* efficacy and drug sensitivity do not necessarily predict desired *in vivo* efficacy and sensitivity. Here, we describe new approaches designed to address these issues and to provide a new screening method that should be able to meet the practical demands of the drug discovery cycle as well as increase the translation of drug effects from *in vitro* to *in vivo*.

Translational promise and physiological insights in Aquaporin drug discovery

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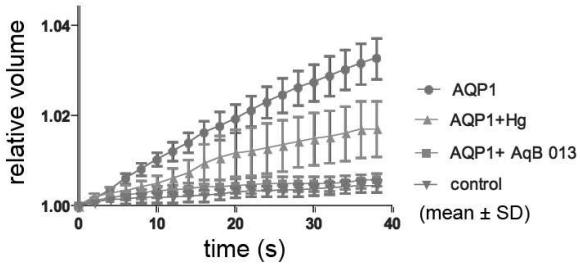
Aquaporins (AQPs) are expressed in tissues in which oedema and fluid imbalances are of major concern. In the mammalian brain, AQP4 water channels are localised in astroglial cells at the blood-brain-barrier interface; AQP1 channels are expressed in choroid plexus and serve in cerebral spinal fluid secretion. Potential roles in brain water homeostasis, oedema, angiogenesis, cell migration, development, neuropathological diseases, and cancer, suggest that AQPs are attractive drug targets. AQP1 is well known as a water channel and, under permissive conditions, also functions as a cGMP-gated cation channel; development of therapeutic strategies that involve differential targeting of AQP1 dual ion-and-water channel functions also is of interest. A need for pharmacological agents to dissect the roles of aquaporins in physiological and pathological processes is clear; however, to date little is known regarding the pharmacology of AQP channels. The classic AQP blocker mercury is toxic, and candidate agents such as tetraethylammonium and phloretin lack specificity and potency.

Our research has focused on the structure and function of AQPs, and on novel drug discovery. We have now identified a compound that has promise as a first-in-class lead compound (Migliati, DuBois, Meurice, Fang, Ritter, Flynn & Yool, 2008, submitted). An impressive array of crystal structure data is available for AQPs. From our structural modelling, two intracellular candidate binding sites on AQP4 were predicted from computational modelling. Synthetic derivatives were designed to enhance intracellular delivery and steric fit based on predicted binding in a homology model of AQP4. More than 40 novel derivative compounds were tested on AQP1 and -4 channels expressed in *Xenopus* oocytes, assayed by videomicroscopic analysis of swelling rates using a double-swell protocol in which each oocyte serves as its own control. One of the series of compounds, AqB013, proved to be an effective blocker of AQPs -1 and -4, with half maximal block (IC_{50}) of ~20 μM in the oocyte expression system (Figure). AqB013 is more effective in blocking AQP1 than is mercury at the same dose (50 μM). Altered efficacy of block after site-directed mutagenesis of amino acid residues in the candidate binding site supports the identification of the aquaporin channel as the direct molecular target of the blocking effect of AqB013. Collaborative studies using AqB013 *in vivo* and *in vitro* are in progress to assess effects of this compound in cell and systems models of pathophysiology. This novel class of pharmacological agents for aquaporins could be valuable as adjuncts in treating oedema and other conditions involving fluid imbalance in aquaporin-expressing tissues.



The figure illustrates block of volume increase (swelling) by 50μM $HgCl_2$ or 50μM AqB013 in AQP1-expressing oocytes placed into 50% hypotonic saline at time 0. Data are mean and SD, for 10 oocytes per treatment. Control oocytes lack AQP.

The capsaicin receptor, TRPV1, as a target for chronic pain therapy

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Chronic pain is a debilitating and distressing condition which affects more than 300 million people worldwide. It is more prevalent in older populations and its incidence is increasing due to greater longevity. Current therapies are less than adequate and new therapies are needed. This presentation will review the basis of sensation of chronic pain and the potential of the transient receptor potential V1 (TRPV1) channel as a novel target for chronic pain therapy. I recently led a drug discovery program at Novartis Pharma R&D which developed antagonists of the heat-sensitive TRPV1. This group was the first to show the effectiveness of a TRPV1 antagonist (capsazepine) in reversing pain behaviors in animal models of chronic neuropathic and inflammatory pain (Walker *et al.*, 2003) and developed an orally active antagonist which was also successful in reversing pain behaviour in inflammatory and neuropathic models (Culshaw *et al.*, 2006). This and subsequent medicinal chemistry programs from other companies have generated TRPV1 antagonists which are effective in reversing pain behaviors in animal models of chronic pain and novel compounds from several pharmaceutical companies have entered clinical trials. Preliminary phase 1 studies are reporting interesting results. Thus, a phase 1b trial of the TRPV1 antagonist AMG-517 could not be completed because of a significant hyperthermia in all patients (Gavva *et al.*, 2008). Another TRPV1 antagonist, SB-705498 successfully reversed the hyperalgesia of a UV-induced burn and was not observed to cause hyperthermia (Chizh *et al.*, 2007). The different observations made regarding hyperthermia in these two trials could be due to off-target effects, differential exposure of the brain to the antagonists or different mechanisms of action via activity at qualitatively different forms of TRPV1. We have been investigating possible post-translational modifications of this ion channel which may have relevance to this observation. To this end, we have generated and partially characterized a series of Chinese hamster cell lines expressing a comprehensive array of rat TRPV1 variants with mutations of the major phosphorylation sites that have previously been reported for this receptor.

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The P2X7 receptor and its genetic variants; relation to mood disorders

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The P2X7 receptor is a ligand-gated cation channel which is activated by extracellular ATP and which is highly expressed in monocyte-macrophages as well as microglia of the central nervous system. P2X7 is a two-transmembrane receptor with intracellular amino and carboxyl termini and is present as a trimer in cell membranes. Prolonged exposure to ATP induces a larger permeability state (dilated channel or pore) which allows influx of the large fluorescent cation, ethidium (314 Da) and which is used to measure receptor function. The P2X7 receptor is regarded as a pro-inflammatory receptor since its activation initiates a cascade of downstream signalling events including processing and secretion of interleukin-1β and interleukin-18 from macrophages and microglia. The P2RX7 gene resides on human chromosome 12q24.31, a region which has been associated with bipolar and depressive disorders in genetic linkage studies. The gene is highly polymorphic with at least 12 non-synonymous single nucleotide polymorphisms (SNPs) which change the function of the receptor. Recently three independent case-control studies from Canada, Germany and the UK have identified a SNP at nucleotide 1405 A>G in the gene which is strongly associated with both bipolar and major depressive disorders. This SNP is present in about 15% of the Caucasian population and changes Glutamine-460 to Arginine in the long carboxyl tail of the receptor but the function of this polymorphic variant is not known. We have genotyped over 3000 subjects to examine the linkage disequilibrium of the 1405 SNP with other functional polymorphisms in the gene. Site directed mutagenesis was used to introduce mutations into a human P2X7 plasmid both in isolation and in combination to recreate the 1405 G haplotypes found in the population. In isolation the Gln-460 to Arg mutation reduces P2X7 function but in combination with certain other mutations a dramatic increase in ATP-induced ethidium uptake is seen, suggesting that the 1405 variant may confer gain-of-function on the P2X7 receptor and this predisposes to bipolar disorders.