The physiological properties and pharmacological regulation of mammalian ion channels

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Contents

Summary

This thesis describes experiments, carried out over the last forty years, which study the physiological properties and pharmacological regulation of mammalian ion channels and the importance of these channels in various disease states.

The first section concerns acetylcholine-activated nicotinic receptors in neurons and shows how the properties of these receptor/channels differ from the more widely studied (at the time) muscle nicotinic receptors. These channels show different conductance and kinetic properties compared to muscle nicotinic receptors and are characterised by strong inward rectification. The neuronal nicotinic receptors found in chromaffin cells of the adrenal medulla are shown to be regulated by the antihypertensive agent, clonidine.

The second section focusses on the indirect regulation of neuronal voltage-gated calcium (Ca^{2+}) and potassium (K^+) channels by activation of muscarinic acetylcholine receptors. The experiments distinguish two principal modulatory pathways that decreased the Ca^{2+} currents of sympathetic neurons. One pathway was fast, voltage dependent and pertussis toxin sensitive. It could explain presynaptic inhibition by M_4 muscarinic receptors and other G_i/G_o-coupled receptors in the nervous system. The other pathway was slow, blocked by intracellular Ca^{2+} chelators, not voltage dependent, and not pertussis toxin sensitive. It could explain Ca^{2+} channel inhibition by M_1 muscarinic receptors and other G_q-coupled receptors in the nervous system. This pathway also underlies the slow G_q-dependent modulation of the M-type (K^+) current by M_1 muscarinic receptors.

The third section describes a novel leak K^+ conductance in cerebellar granule neurons, regulated by activation of muscarinic receptors and by changes in external pH and termed IK_{SO} for "standing-outward" K^+ current. Inhibition of the current led to a depolarisation of the neurons thus regulating their excitability. The current bore all the hallmarks of members of the TASK family of two pore domain potassium (K_2P) channels.

Activation of M_3 muscarinic receptors inhibits both TASK3 channels and IK_{SO} through activation of the G protein G_q, because both effects are abolished by the selective G_q antagonist YM-254890. The cannabinoid methanandamide potently blocked TASK3 and TASK1 channels and both methanandamide and G_q mediated inhibition converged on the same intracellular gating pathway.

Like IK_{SO}, currents through TASK channels are inhibited by extracellular acidification. For TASK3, mutation of histidine (H)98 in the pore of the channel considerably reduced this effect. Zinc was found to be a selective blocker of TASK3 channels and IK_{SO}, is also sensitive to block by zinc. This suggests that TASK3 channels underlie a major component of IK_{SO}. Mutation of both E70 in the M1P1 loop and H98 of TASK3 abolished block, suggesting the two residues may contribute to a zinc binding site.

The cytokine TNFα enhanced TASK3 current. TNFα action occurs through the ASK1 pathway and is JNK- and p38-dependent. In combination, TNFα activation and TASK3 channel activity can promote cellular apoptosis.

The fourth section describes the characterisation of the functional properties and regulation of the related TREK family of K_2P channels.

TREK1 channels were inhibited by fluoxetine (Prozac) and its active metabolite, norfluoxetine. Additionally, the anticonvulsant agents, sipatrigine and lamotrigine inhibit TREK channels. The actions of these compounds on TREK channels may contribute to their current and potential use in the treatment of pain and depression. A splice variant of TREK1 (TREK1ΔEx4) expressed in the brain was identified, which encodes a heavily truncated TREK1 protein. TREK1ΔEx4 has no channel activity itself but reduced TREK1 whole cell current amplitude, thereby regulating channel function.

Fenamates such as flufenamic acid (FFA) and the related experimental drug BL-1249 enhance the activity of TREK1 currents, as does aristolochic acid which is used in traditional medicines to treat pain. A new, selective opener of TREK

channels, GI-530159 was described and characterised. Given the importance of TREK1 channels in pain, selective TREK channel openers like GI-530159 could aid in the development of novel analgesic agents.

G171F and A286 mutations in the pore of TREK1 substantially reduced current through the channel. This reduction in current could be reversed pharmacologically, by FFA. Pharmacologically reversible mutations of TREK channels can help to clarify the importance of these channels in pathophysiological conditions such as pain and depression and also suggest potential therapeutic benefits for reversing disease-causing channelopathies, pharmacologically.

The final section concerns the characterisation of a number of potassium channelopathies.

A mutation in TASK3 (G236R) was shown to be responsible for Birk Barel mental retardation syndrome (also named KCNK9 imprinting syndrome, KIS), a maternally transmitted developmental disorder. G236R mutated TASK3 channels give rise to a functional current, that is significantly smaller when compared with wild-type (WT) TASK3 channels. This reduction can be overcome, at least in part, by FFA. It is proposed that pharmacological enhancement of mutated TASK3 channel current during development may, therefore, provide a potentially useful therapeutic strategy in the treatment of Birk Barel syndrome.

In later experiments, the broader genetic and phenotypic variability for this disease was described, including the identification of an additional mutational hotspot (R131) which led to a paradoxical gain of channel function. This study demonstrated the complex etiology of this syndrome including gain and loss of channel function and consistent loss of channel regulation. This suggests that unique therapeutic strategies may be needed to address the specific functional consequences of mutation on channel function and regulation in different patients.

The TASK1 channel gene (KCNK3), expressed in pulmonary arterial smooth muscle cells, has been identified in heritable pulmonary arterial hypertension (PAH). Novel mutated TASK1 channels, seen in PAH patients, have a substantially reduced current compared to wild-type TASK1 channels but this could not be overcome pharmacologically by activators of TASK1 channels.

Numerous pathogenic variants in KCNB1, which encodes the voltage-gated potassium channel, $K_V2.1$, are linked to developmental and epileptic encephalopathies and associated with loss of function, regulation, and expression of the channel. A novel *de novo* variant (P17T) occurring in the $K_V2.1$ channel that results in a gain of channel function was described. It is clinically associated with neurodevelopmental disorders, but without the seizures seen in patients with loss of function channel mutations.

Abbreviations

5-HT	5-hydroxytryptamine
ACh	acetylcholine
ASK1	apoptosis signal regulating kinase 1
BAPTA	1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid
CFTR	cystic fibrosis transmembrane conductance regulator
CGN	cerebellar granule neuron
Cryo-EM	cryo-electron microscopy
DEE	developmental and epileptic encephalopathies
DMPP	1,1-dimethyl-4-phenylpiperazinium
DTT	dithiothreitol
FFA	flufenamic acid
GABA	gamma-aminobutyric acid
GIRK	G protein coupled inwardly rectifying potassium channel
GoF	gain of function
GPCR	G protein coupled receptor
GTP	guanosine triphosphate
IK_{SO}	standing outward potassium current
JNK	c-Jun N-terminal kinase
K_2P	two pore domain potassium channel
KIS	KCNK9 imprinting syndrome
LoF	loss of function
MTSET	2-(Trimethylammonium)ethyl methanethiosulfonate
NA	noradrenaline
nAChR	nicotinic acetylcholine receptor
Oxo-M	oxotremorine-M
PLC	phospholipase C
PAH	pulmonary arterial hypertension
PIP_2	phosphatidylinositol 4,5-bisphosphate
PKC	protein kinase C
TALK	TWIK-related alkaline pH-activated K^+ channel
TASK	TWIK-related acid-sensitive K^+ channel
THIK	tandem pore domain halothane-inhibited K^+ channel
TNFα	tumour necrosis factor α
TRAAK	TWIK-related arachidonic acid-stimulated K^+ channel
TREK	TWIK-related K^+ channel
TRESK	TWIK-related spinal cord K^+ channel
TWIK	tandem of pore domains in a weakly inward rectifying K^+ channel
VGCC	voltage gated calcium channel
WT	wild type

Introduction

This thesis describes experiments, carried out over the last forty years, which study the physiological properties and pharmacological regulation of mammalian ion channels and the importance of these channels in various disease states. The work is divided into five sections, roughly chronologically, covering 26 publications for which I was a principal author, either in terms of leading the work (corresponding author) or leading the data collection (1st or 2nd author) or both. Alongside these papers, I have published many other collaborative papers and reviews which have shaped my thinking and research over these decades, but these are not explicitly covered in this thesis.

For the first two sections of the thesis, I was the primary scientific researcher (publications 1-4) or joint scientific researcher (publications 5-7, with two others), carrying out all experiments and analysis and contributing to paper writing, as a postdoctoral researcher fellow. For the latter three sections of the thesis, I was head of the research laboratory and corresponding author (publications 8-17, 19-23, 25, 26) or joint corresponding author (publications 18 & 24) for all publications. For the publications in these sections, I led the research programmes, obtained funding for the work, made a major contribution to the conception and design of the experiments, contributed to the analysis and interpretation of the data and wrote the manuscripts, with appropriate input from colleagues.

By bringing together these 26 papers, a natural narrative suggests a plan and consistent direction of travel over these decades but, of course, this thesis has been written retrospectively and it was not my intention to compile this research as a single narrative from the outset. As such, I have tried to avoid stretching the connection between these papers too far. I believe that grouping the papers into five sections makes sense, both in terms of chronology and in terms of the objectives of the papers in each section. Whilst the thesis provides an overview of the papers, the papers themselves contain the detailed results and context at

the time of writing them and are provided as an appendix organised as they appear in the text below.

Section 1. Neuronal Nicotinic Acetylcholine Receptors

Summary

This section concerns acetylcholine-activated nicotinic receptors in mammalian neurons and shows how the properties of these receptor/channels differ from the more widely studied (at the time) muscle nicotinic receptors. These channels showed different conductance and kinetic properties compared to muscle receptors (Mathie et al 1987, Mathie et al 1991) and were characterised by strong inward rectification (Mathie et al 1990). The neuronal nicotinic receptors found in chromaffin cells of the adrenal medulla were shown to be regulated by the antihypertensive agent, clonidine (Cull-Candy et al 1998).

1) **Mathie A**, Cull-Candy SG, Colquhoun D (1987). Single-channel and whole-cell currents evoked by acetylcholine in dissociated sympathetic neurones of the rat. *Proc Roy Soc B* 232: 239-248. PMID: 2892208

2) **Mathie A**, Cull-Candy SG, Colquhoun D (1991). Conductance & kinetic properties of single nicotinic acetylcholine receptor-channels in rat sympathetic neurones. *J Physiol* 439: 717-750. PMID: 1716680

3) **Mathie A**, Colquhoun D, Cull-Candy SG (1990). Rectification of currents activated by nicotinic acetylcholine receptors in rat sympathetic ganglion neurones. *J Physiol* 427: 625-655. PMID: 1698982

4) Cull-Candy SG, **Mathie A***, Powis DA (1988). Nicotinic acetylcholine receptor-channels and their block by clonidine in cultured bovine chromaffin cells. *J Physiol* 402: 255-278. PMID: 2466982
*Journal listed authors in alphabetical order at this time.

See Appendix pages 57 - 155 for complete versions of these references.

This work was carried out between 1986 and 1989 whilst I was employed as a postdoctoral Research Fellow, funded by the MRC, in the laboratories of Professor David Colquhoun and Stuart Cull-Candy.

Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that play central roles in synaptic transmission, neuromodulation, and neural plasticity throughout the nervous system. They form part of the Cys-loop receptor superfamily, which also includes $GABA_A$, glycine, and $5\text{-}HT_3$ receptors. Each receptor is a pentamer surrounding a cation-permeable pore. The diversity of subunits, α2–α10 and β2–β4 in neurons, produces numerous receptor subtypes with distinct conductance, kinetics, and pharmacological properties (Dani & Bertrand 2007, Albuquerque et al 2009).

When acetylcholine or another agonist like nicotine binds to extracellular interfaces between α and β subunits, the receptor undergoes a rapid conformational change that opens its central pore, allowing and Ca^{2+} influx and K^+ efflux (Unwin 2005). In the case of neuronal nAChRs, this depolarises neurons and, often through Ca^{2+} entry, stimulates a variety of intracellular signalling pathways. The receptors are found at presynaptic, postsynaptic, and extra-synaptic sites. Presynaptic nAChRs enhance release of neurotransmitters such as glutamate, GABA, dopamine, and serotonin, impacting learning and reward pathways. Postsynaptic receptors mediate fast excitatory transmission in autonomic ganglia and specialized central neuron circuits. In addition, extra-synaptic neuronal nicotinic receptors modulate excitability and neurite growth (Gotti and Clementi 2004).

The subunit composition of nAChRs determines physiological function and regional expression. For example, the α4β2 subtype predominates in the mammalian brain and underlies nicotine reward via dopaminergic neurons in the ventral tegmental area, whereas the α7 homopentamer is highly Ca^{2+}-permeable and influences attention, cognition, and anti-inflammatory signalling. Many nAChRs desensitise rapidly. Sustained agonist exposure drives receptors into

long-lasting non-conductive states, explaining nicotine tolerance and the transient nature of cholinergic signalling (Gotti & Clementi 2004).

nAChRs have broad physiological and pathological significance. During development they guide synapse formation, while in adulthood they shape sensory processing, arousal, and reward. Dysregulation of receptor expression or signalling is implicated in Alzheimer's and Parkinson's diseases, schizophrenia, epilepsy, and anxiety (Changeux 2012).

Advances in structural biology, including cryo-electron microscopy, have revealed resting, activated, and desensitized conformations of these receptors, showing how ligand binding leads to channel gating (Unwin 2005). These structural insights, combined with mutagenesis and electrophysiology have been used to inform potential drug discovery.

At the time this work was undertaken, most mechanistic understanding of nicotinic receptors derived from studies of the neuromuscular junction. In contrast, neuronal nAChRs were less well characterised, particularly at the single-channel level (Colquhoun et al 1987). The studies described here addressed this gap by providing a detailed electrophysiological analysis of neuronal nAChRs and revealing several properties that distinguish them fundamentally from their muscle counterparts.

Results and Discussion

These four studies established a quantitative and mechanistic framework for neuronal nAChR function in sympathetic neurons and chromaffin cells. Key advances included the identification of structured burst kinetics, agonist-specific channel behaviour, and, most notably, the demonstration of strong inward rectification (whereby current passes more easily in an inward direction than in an outward direction).

Mathie et al (1987) provided the foundation by determining the unitary conductance and kinetic behaviour of sympathetic neuronal nAChRs. In 1 mM Ca^{2+} the conductance clustered around 37 pS, with patch-to-patch variability and

evidence of multiple closely spaced conductance states. Removal of divalent cations markedly increased conductance and revealed a clear ion selectivity sequence, $K^+ > Cs^+ > Na^+ > Li^+$, consistent with a pore accommodating monovalent ions in a size-dependent manner. The conductance increase seen when Ca^{2+} was absent, both reinforced the view that divalent ions exert a pore-blocking or screening effect and also suggested Ca^{2+} permeability of these receptors, a key property of neuronal nicotinic receptors described subsequently (Sands & Barish 1991).

A major conceptual advance was the identification of structured burst kinetics. Importantly, different agonists produced distinct intraburst behaviours. At low agonist concentrations, both ACh and the selective agonist DMPP produced bursts interrupted by brief gaps whose distributions required multiple exponential components. ACh produced many more short intraburst closures than DMPP, resulting in a shorter corrected mean open time (0.86 ms vs 2.3 ms), even though both agonists produced similar overall gating efficacy ($\beta/\alpha \approx 23–25$). This distinction (similar efficacy but different intraburst structure) helped separate the concepts of gating efficacy and open-time duration, influencing later models of agonist-specific receptor behaviour. The demonstration that both ACh and DMPP can also block the channel at high concentrations, with DMPP acting as a particularly potent open-channel blocker, provided one early mechanistic explanation for agonist-dependent desensitisation known to shape synaptic signalling.

Mathie et al (1990) described for the first time, one of the most striking properties of neuronal nAChRs: strong inward rectification. DMPP produced robust inward currents at negative potentials but virtually no outward current up to +70 mV. This held true regardless of the permeant ion, divalent cation presence, or pH buffer, implying that rectification is intrinsic to the receptor rather than a secondary effect of ionic composition. The possibility that intracellular Mg^{2+} acted as a voltage-dependent blocker was examined, but even Mg^{2+}-free recording conditions produced pronounced rectification, indicating that Mg^{2+} block alone could not account for the phenomenon.

A key physiological insight came from the observation that desensitisation was voltage-dependent. At positive potentials, receptors became nearly non-responsive despite agonist presence, but stepping the membrane potential negative triggered a large inward current with a rapid onset followed by desensitisation. This revealed that the receptor can progress into a non-conducting but activatable state at positive potentials, a subtle but important distinction from classical desensitisation models. The idea that rectification and voltage-dependent desensitisation arise from intrinsic gating transitions broadened the field's understanding of how ligand-gated channels are influenced by the membrane potential when adopting their functional state.

Parallel experiments on chromaffin cells (Cull-Candy et al 1998), used noise analysis to characterise nAChR behaviour under whole-cell voltage clamp. ACh generated a single-Lorentzian noise spectrum with a characteristic time constant (~11 ms), consistent with a dominant gating process shaping single channel current behaviour and very similar to the dominant burst length value seen for single channel activity both in chromaffin cells and in sympathetic neurons (see below). Clonidine was shown to be a potent modulator, substantially reducing ACh-evoked currents and splitting the noise spectrum into two Lorentzian components. Importantly, clonidine did not alter single-channel conductance, and its effect was neither reproduced by adrenaline nor evident when clonidine was applied intracellularly. This established clonidine as an extracellular, receptor-specific modulator with a complex blocking mechanism that did not conform to simple open-channel block. This work helped establish chromaffin nAChRs as pharmacologically distinct from other receptor types and demonstrated that noise analysis can detect subtle changes in channel kinetics not evident in conventional macroscopic recordings.

In the most comprehensive analysis of single channel behaviour of neuronal nAChRs at the time (Mathie et al 1991), we tied these themes together by directly comparing single-channel and whole-cell behaviours in the same neuronal preparation. Single channels displayed a conductance of ~35 pS in Ca^{2+} and ~51 pS without Ca^{2+}, in line with earlier results, but the striking feature was the unusually noisy open state which was five times more variable than at the

frog endplate. This implied rapid unresolved subconductance transitions or micro-fluctuations within the open state. Bursts again showed dual-component duration distributions, and the longer component matched synaptic EPSC decay kinetics (Derkach et al 1987), linking microscopic channel behaviour to macroscopic synaptic function.

Most intriguingly, the study confirmed a fundamental difference in that whole-cell currents showed strong inward rectification with no measurable outward macroscopic current, yet isolated outside-out patches clearly displayed outward single-channel openings of normal amplitude. This difference showed that rectification is not a property of the unitary conductance but, rather, is a result of channel gating and state occupancy within the intact cell environment regulated by, at the time, unknown factors, but subsequently attributed primarily to polyamine block of these channels (Haghighi & Cooper 1998).

These four papers established, for the first time, the kinetic and biophysical framework for neuronal nAChRs, distinguishing them from muscle-type nAChRs and highlighting receptor diversity long before subunit-specific cloning was widespread. Their detailed quantification of burst kinetics, intraburst closures, subconductance noise, and agonist-specific open-time differences influenced subsequent gating models, including multi-state Markov schemes. The discovery of strong inward rectification in combination with voltage-dependent desensitisation shaped later thinking about synaptic signalling and excitability within autonomic circuits.

The properties of the native channels closely resemble those of recombinant α3β4 receptors described by Ragozzino et al (1997) which are the most prevalent expressed in autonomic ganglia (Rust et al 1994).

At the time, several key questions remained unresolved. The molecular basis of rectification was not established until the later discovery of intracellular polyamine block (Haghighi & Cooper 1998). Similarly, the structural correlates of subconductance transitions awaited advances in structural biology (see Delgado-Velez et al 2021, Barrantes 2023). The identity of subunits composing

sympathetic nAChRs (later shown to be primarily α3 and β4, see Haghighi & Cooper 2000) had not yet been molecularly resolved. While clonidine's modulation was functionally described, the location of its allosteric site and its physiological importance remain speculative.

Another open question was how the biophysical complexity of single receptors scales to neural computation, how inward rectification and self-blocking shape excitability patterns in networks of sympathetic neurons. Inward rectification has been shown to provide an important mechanism to ensure that the receptors do not short circuit the action potential in the nerve terminal and reduce transmitter release (Haghighi & Cooper 2000).

Future work could address these gaps through structural and single-molecule approaches to resolve gating transitions, combined with subunit-specific pharmacology to define modulatory sites. Integrating detailed kinetic models into network-level analyses would also clarify how these complex channel properties influence neuronal signalling.

Collectively, these studies transformed the understanding of neuronal nAChRs. The demonstration of inward rectification provided early mechanistic insight into voltage-dependent modulation of receptor currents, while the identification of structured burst kinetics established that channel gating involves multiple discrete states rather than single opening events. Crucially, the finding that different agonists produce distinct patterns of channel activity revealed that ligand binding stabilises specific conformational states, anticipating later structural models of receptor function.

Section 2. G-Protein Coupled Receptor Regulation of Ion Channels

Summary

This section focusses on the indirect regulation of neuronal voltage-gated calcium and potassium channels by activation of G protein coupled receptors (GPCRs), primarily muscarinic acetylcholine receptors. The experiments distinguish two principal modulatory pathways that decreased the Ca^{2+} currents of sympathetic neurons (Beech et al 1991, Mathie et al 1992). One pathway was fast, voltage dependent and pertussis toxin sensitive. It could explain presynaptic inhibition by M_4 muscarinic receptors (Bernheim et al 1992) and other G_i/G_o-coupled receptors in the nervous system. The other pathway was slow, blocked by intracellular Ca^{2+} chelators, not voltage dependent, and not pertussis toxin sensitive. It could explain Ca channel inhibition by M_1 muscarinic receptors and other G_q-coupled receptors in the nervous system. This pathway also underlies the slow G_q-dependent modulation of the M (potassium) current by M_1 muscarinic receptors.

5) Beech DJ, Bernheim L, **Mathie A**, Hille B (1991). Intracellular Ca buffers disrupt muscarinic suppression of Ca-current and M-current in rat sympathetic neurons. *Proc Natl Acad Sci* 88: 652-656. PMID: 1846449

6) **Mathie A**, Bernheim L, Hille B (1992). Inhibition of both N- & L- type Ca channels by muscarinic receptor activation in rat sympathetic neurons. *Neuron* 8: 907-914. PMID: 1316767

7) Bernheim L, **Mathie A**, Hille B (1992). Characterization of muscarinic receptor subtypes inhibiting Ca current and M current in rat sympathetic neurons. *Proc Natl Acad Sci* 89: 9544-9548. PMID: 1329101

See Appendix pages 156 - 173 for full versions of these references.

This work was carried out between 1989 and 1991 whilst I was employed as a Fogarty International Research Fellow, in the laboratory of Professor Bertil

Hille. Together with David Beech and Laurent Bernheim and under the leadership of Bertil, we had an extremely exciting and productive 24 months addressing the mechanisms whereby ion channels in sympathetic neurons are regulated by G protein-coupled receptors.

Introduction

G protein-coupled receptors (GPCRs) modulate neuronal and cardiac excitability largely through their control of voltage-gated calcium (Ca^{2+}) and potassium (K^+) channels. When activated by neurotransmitters or hormones, GPCRs engage heterotrimeric G proteins (composed of α, β, and γ subunits) that directly or indirectly alter channel gating. This reversible coupling allows synaptic activity and autonomic input to finely tune activities as diverse as electrical signalling, transmitter release, and heart rhythm (Hille et al 1995, 2015, Dolphin 2016).

Voltage-gated Ca^{2+} channels (VGCCs)—particularly the N-type ($Ca_V2.2$) and P/Q-type ($Ca_V2.1$)—are classic targets of G protein regulation. Activation of $G_{i/o}$-coupled receptors such as $GABA_B$, α_2-adrenergic, or opioid receptors leads to dissociation of $G_{\beta\gamma}$ subunits, which bind directly to the channel's α_1 subunit at the I–II loop and β subunit. This interaction reduces channel open probability and slows activation, producing the hallmark "voltage-dependent inhibition". A brief depolarizing prepulse relieves inhibition by dislodging $G_{\beta\gamma}$, explaining the rapid, reversible control of transmitter release at presynaptic terminals. In addition to the direct pathway, slower "voltage-independent" inhibition can occur via second messengers: phosphorylation through protein kinase C (PKC) or depletion of membrane PIP_2 decreases channel availability. In neurons, such multilayered regulation limits Ca^{2+} entry during repetitive firing, curbing neurotransmitter release and preventing excitotoxicity (Wickman & Clapham 1995, Dascal 2001).

A parallel but opposite process governs several potassium channels that mediate membrane hyperpolarization. The best characterised are the G protein-activated inwardly rectifying K^+ channels (GIRKs, or $K_{IR}3.x$) (Yamada et al 1998).

Activation of $G\alpha_{i/o}$-coupled muscarinic M_2, adenosine A_1, or opioid receptors releases Gβγ, which directly gates the channels by binding to cytoplasmic domains on the Kir subunits. This produces fast inhibitory postsynaptic potentials that slow heart rate and suppress neuronal firing. GIRK currents require both Gβγ interaction and the presence of phosphatidylinositol (4,5)-bisphosphate, providing a lipid-dependent second level of control.

GPCRs also regulate voltage-gated (K_V) and leak (K_2P) potassium currents. Gq-coupled receptors activate phospholipase C, hydrolysing PIP_2 to IP_3 and DAG. The resulting Ca^{2+} elevation and PKC activation inhibit M-type K_V7 channels and certain K_2P channels, depolarising cells and enhancing excitability. Conversely, Gβγ released from Gi-coupled receptors can open GIRKs directly or modulate delayed-rectifier currents indirectly via kinases. These mechanisms operate in concert: calcium-inflow suppression limits excitation, while potassium-current activation stabilises the resting potential, giving G proteins powerful bidirectional control of cell firing (Hille et al 1995).

Functionally, such regulation coordinates synaptic strength, heart rate, vision, and pain perception, linking diverse receptors, including muscarinic, adrenergic, dopaminergic, and opiate receptors, to a common electrical output. Disruption of G protein control underlies several diseases: loss of voltage-dependent inhibition contributes to epilepsy, and aberrant GIRK activation causes bradycardia and atrioventricular block. As such, G protein modulation of Ca^{2+} and K^+ channels provides a rapid molecular bridge between receptor activation and electrical signalling, maintaining the delicate balance between excitation and inhibition in excitable tissues (Hille et al 2015, Dolphin 2016).

Results and Discussion

The first study (Beech et al 1991) revealed a striking paradox: muscarinic suppression of inward Ca^{2+} current (I_{Ca}) and M-type K^+ current (I_M) in isolated sympathetic neurons occurred without any detectable rise in intracellular

calcium concentration ($[Ca^{2+}]_i$) measured by fura-2, yet buffering intracellular calcium with the strong chelator BAPTA nearly abolished these inhibitory effects. Normally, acetylcholine acting on M_1 receptors suppresses the voltage-gated Ca^{2+} current and closes M-channels, producing excitatory depolarisation in sympathetic neurons. Here, suppression of both currents persisted when $[Ca^{2+}]_i$ increased only minimally, challenging the hypothesis that calcium elevation itself mediated the response.

Instead, blocking intracellular Ca^{2+} fluctuations prevented the receptor pathways from functioning, implying the need for a basal permissive level of Ca^{2+} for the coupling steps between receptor, G protein, and channel. This led to the concept that Ca^{2+} acts as a co-factor rather than as the trigger for this regulation. Moreover, some effects of BAPTA could not be fully explained by Ca^{2+} chelation, suggesting either direct interference with signalling proteins or sequestration of other divalent intermediates. This paper therefore showed that intracellular Ca^{2+} was not the messenger that carries muscarinic signals but, rather, acts as an enabling element within the signalling machinery itself, raising the question of what molecular reactions require such minimal $[Ca^{2+}]_i$ to proceed.

The second paper (Mathie et al 1992) advanced this discovery by quantifying how muscarinic (oxo-M) and noradrenergic (NA) stimulation differently regulated N- and L-type Ca^{2+} channels in the same cells. Using whole-cell clamp and single-channel patch recordings, we found that both agonists inhibit N-type Ca^{2+} channels, but only muscarinic stimulation via oxo-M suppresses L-type channels.

Two mechanistically distinct signalling pathways were identified:

- A membrane-delimited pathway, shared by oxo-M and NA, that acted rapidly through G-proteins tethered to the plasma membrane and targeted only N-type channels.

- A diffusible-messenger pathway, unique to muscarinic stimulation, that inhibited both N- and L-type channels and required intact cytoplasmic signalling components.

Intracellular BAPTA abolished the L-channel suppression but not the N-channel inhibition, proving that the diffusible pathway was Ca^{2+}-dependent while the membrane-delimited one was not. Single-channel measurements confirmed that oxo-M lowered the probability of channel opening rather than reducing unitary conductance providing evidence of gating modulation through second messengers.

This study thus produced an explicit demonstration that a single neurotransmitter receptor can operate through two biochemically distinct coupling modes: a fast, localised, G-protein-mediated mechanism and a slower, cytoplasmic, Ca^{2+}-dependent one. It reconciled varied results across tissues (why some muscarinic responses were fast and others delayed) by identifying the cellular context and signalling components determining channel specificity.

The third paper (Bernheim et al 1992) applied subtype-selective muscarinic antagonists (pirenzepine and himbacine) and pertussis toxin to dissect the receptor and G-protein identities underlying each pathway. Building on earlier kinetic distinctions of "fast" vs. "slow" suppression, we showed:

- The fast, pertussis toxin-sensitive inhibition of Ca^{2+} current was mediated principally by M_4 receptors, using Gi/Go-type G-proteins.
- The slow, BAPTA-sensitive inhibition of Ca^{2+} current, and the corresponding suppression of M-type K^+ current, was mediated mainly by M_1 receptors, using Gq/11-type proteins and dependent on basal intracellular Ca^{2+}.

Both pathways required GTP binding, as GDP-β-S blocked their effects, confirming G protein mediation. Functionally, this established that sympathetic neurons co-express multiple receptor subtypes with distinct molecular coupling and that each receptor–G protein pair targets specific ion-channel populations.

The pharmacological discrimination between M_1 and M_4 receptors provided the first molecular separation of muscarinic actions in sympathetic neurons and became foundational for later cloning and expression studies of muscarinic receptor subtypes.

Considered together, these three studies constituted a comprehensive dissection of how muscarinic receptors modulate excitability in sympathetic neurons through Ca^{2+}-dependent and Ca^{2+}-independent G protein pathways. Major conceptual advances through this work included:

- A separation of receptor signalling modes. Rapid, membrane-delimited G_i/G_o pathways act locally on N-type channels, while slower, cytoplasmic $G_{q/11}$ pathways depend on intracellular calcium and a diffusible messenger to regulate L-type Ca^{2+} and M-type K^+ currents.
- A redefinition of the role of intracellular Ca^2 ions. Ca^2 serves not as the second messenger carrying muscarinic signals but as a cofactor enabling enzymatic interactions or maintaining messenger production.
- The discovery of subtype-specific receptor coupling. Individual receptor subtypes (M_1 vs M_4) employ different G proteins to drive these distinct pathways, accounting for diverse kinetics and pharmacology in sympathetic and central neurons.

By showing that receptor-channel coupling could be tuned at multiple biochemical levels, this body of work predicted many of the subsequent signalling pathways uncovered in central neurons, particularly the distinction between membrane-delimited inhibition of N-type channels by Gβγ subunits and Gq-coupled activation of phospholipase C leading to M-current suppression. The conceptual "dual-pathway" framework informed the interpretation of G protein modulation of calcium channels by numerous transmitters ($GABA_B$, opioid, and somatostatin receptors). The demonstration of subtype-specific coupling anticipated the explosion of receptor genomics in the early 1990s, when molecular cloning confirmed that M_1 receptors

preferentially couple to Gq/PLC pathways and M_4 to Gi/Go systems (Hille et al 2015).

This work raised several key questions:

- What is the nature of the diffusible messenger? The cytoplasmic intermediate responsible for Ca^{2+}-dependent suppression of L-type channels was left unidentified. Later hypotheses involved arachidonic acid derivatives and phosphoinositide metabolism, but the exact molecule remains debated.
- What are the target sites on channels? How G proteins or second messengers physically alter channel gating, through phosphorylation, membrane lipid changes, or direct binding, is unresolved.
- How are these proteins organised spatially? The structural relationship between receptor domains, G protein pools, and ion-channel clusters in sympathetic neurons is unknown.

Future research could extend these discoveries in several ways. Cryo-EM and proteomics can reveal how M_1- and M_4-associated G proteins interface with N- and L-type Ca^{2+} channels. Modern high-speed fluorescence or genetically encoded indicators could define the minimal $[Ca^{2+}]_i$ "set-point" required for slow muscarinic signalling. Lipidomics and targeted perturbation of PLC, diacylglycerol, or arachidonic pathways can test the molecular species mediating the BAPTA-sensitive effects. Modelling how co-activation of M_1 and M_4 receptors shapes firing patterns could bridge single cell mechanisms to autonomic reflex control. Finally, designing ligands that preferentially engage either the fast G_i/G_o or slow $G_{q/11}$ pathway could yield therapeutics that adjust neuronal excitability without broader off target side effects.

Collectively, this work shifted understanding of GPCR–ion channel coupling from a single, undefined inhibitory mechanism to a dual-pathway model in which distinct receptor subtypes engage separate G protein signalling pathways

to regulate channel gating. It established that Ca^{2+} is not the primary messenger in muscarinic inhibition, but a permissive cofactor, and demonstrated that signalling specificity arises from both receptor identity and the intracellular pathway activated. This framework provided a mechanistic foundation for current views of presynaptic inhibition, M-current regulation, and the broader integration of metabotropic signalling with neuronal excitability.

Sections 3-5 Potassium Channels

Introduction

The remaining three sections of this thesis describe work on two pore domain potassium (K_2P) channels carried out in my laboratory over the last 30 years. I was lead or joint corresponding author for all the selected papers which were carried out alongside other collaborative studies at University College London, Imperial College London and the University of Kent. I am indebted to the many colleagues who collaborated with me on this work. many of whom are co-authors on the contributory papers.

Over the past two decades, the family of K_2P channels has moved from a physiological curiosity described as a 'leak' current, to a major focus in neuroscience, pharmacology, and ion channel structural biology. Unlike classical voltage- or ligand-gated K^+ channels, K_2P channels provide constitutive "leak" currents which underlie background currents that stabilize the resting membrane potential of excitable and non-excitable cells (Enyedi & Czirjak 2010). There are 15 mammalian K_2P channels, which are grouped into six families based on their structural and functional properties (the TASK, TREK, TWIK, TRESK, THIK and TALK families, see Alexander et al 2025). When these background currents are altered, neurons can switch between quiescence and hyperexcitability, or muscles between contraction and relaxation, linking K_2P function to diverse disorders, from pain and depression to cardiac arrhythmia and pulmonary hypertension (Mathie et al 2021).

Section 3. TASK, Two Pore Domain Potassium Channels

Summary

This section describes a novel leak potassium conductance in cerebellar granule neurons (CGNs), regulated by activation of muscarinic receptors and by changes in external pH and termed IK_{SO} for "standing-outward" potassium current (Watkins & Mathie 1996, Millar et al 2000, Boyd et al 2000). Inhibition of the current led to a depolarisation of the neurons thus regulating their excitability. The current bore all the hallmarks of members of the TASK family of two pore domain potassium channels.

Activation of M_3 muscarinic receptors inhibits both TASK3 channels and IK_{SO} through activation of the G protein G_q, because both effects were abolished by the selective G_q antagonist YM-254890 (Veale et al 2007a). The cannabinoid methanandamide potently blocked TASK3 and TASK1 channels and both methanandamide and G_q mediated inhibition converged on the same intracellular gating pathway (Veale et al 2007b).

Like IK_{SO}, currents through TASK channels are inhibited by extracellular acidification. For TASK3, mutation of histidine (H)98 in the pore of the channel considerably reduced this effect. Zinc was found to be a selective blocker of TASK3 channels, and IK_{SO} is also sensitive to block by zinc (Clarke et al 2004, 2008). This suggests that TASK3 channels underlie a major component of IK_{SO}. Mutation of both the E70 in the M1P1 loop and the H98 of TASK3 abolished block, suggesting the two residues may contribute to a zinc binding site.

The cytokine TNFα enhanced TASK3 current (El Hachmane et al 2014). TNFα action occurs through the ASK1 pathway and is JNK- and p38-dependent. In combination, TNFα activation and TASK3 channel activity can promote cellular apoptosis.

8) Watkins CS, **Mathie A** (1996). A non-inactivating K^+ current sensitive to muscarinic receptor activation in rat cultured cerebellar granule neurons. *J Physiol* 491: 401-412. PMID: 8866863

9) Millar JA, Barratt L, Southan AP, Page KM, Fyffe REW, Robertson B, **Mathie A** (2000). A functional role for the twopore domain potassium channel, TASK-1, in cerebellar granule neurons. *Proc Natl Acad Sci* 97: 3614-3618.PMID: 10725353

10) Boyd DF, Millar JA, Watkins CS, **Mathie A** (2000). The role of Ca^{2+} stores in the muscarinic inhibition of the K^+ current, IK_{SO}, in neonatal rat cerebellar granule cells. *J Physiol* 529: 321-331. PMID: 11101643

11) Veale EL, Kennard LE, Sutton GL, MacKenzie G, Sandu C, **Mathie A** (2007a). $G\alpha_q$ mediated regulation of TASK3 two pore domain potassium channels: the role of protein kinase C. *Mol Pharmacol* 71: 1666-1675. PMID: 17374744

12) Veale EL, Buswell R, Clarke CE, **Mathie A** (2007b). Identification of a region in the TASK3 two pore domain potassium channel that is critical for its blockade by methanandamide. *Br J Pharmacol* 152: 778-786. PMID: 17828294

13) Clarke CE, Veale EL, Green PJ, Meadows HJ, **Mathie A** (2004). Selective block of the human 2-P domain potassium channel, TASK-3, and the native leak potassium current, IK_{SO}, by zinc. *J Physiol* 560: 51-62. PMID: 15284350

14) Clarke CE, Veale EL, Wyse K, Vandenberg JI, **Mathie A** (2008). The M1P1 loop of TASK3 K2P channels apposes the selectivity filter and influences channel function. *J Biol Chem* 283: 16985-16992. PMID: 18417474

15) El Hachmane MF, Rees KA, Veale EL, Sumbayev VV, **Mathie A** (2014). Enhancement of TWIK-related acid sensitive potassium channel 3 (TASK3) two

pore domain potassium channel activity by TNFα. *J Biol Chem* 289: 1388-1401. PMID: 24307172

See Appendix pages 174 - 254 for full versions of these references.

Introduction

In this section, the characteristics of the native leak current in cerebellar granule neurons (which we termed IK_{SO}, for standing-outward potassium current) are described and shown to correlate with the newly identified (at the time) TASK family of K2P channels, which are now known to underlie background leak K currents in many cells in the body (see Enyedi & Czirjak 2010, Mathie et al 2021). Subsequent work characterised the fundamental properties and regulation of recombinant TASK channels.

Results and Discussion

The earliest work (Watkins & Mathie 1996) identified a non-inactivating outward K^+ current, IK_{SO}, in rat cerebellar granule neurons. Using perforated-patch recordings, we documented that neurons cultured for seven days developed a large outwardly rectifying current that activated near rest, reversed around the K^+ equilibrium potential, and was insensitive to classical voltage-gated K^+ blockers such as 4-aminopyridine and tetraethylammonium. Muscarinic receptor activation with muscarine strongly suppressed IK_{SO} (EC_{50} ~ 0.17 μM), leading to membrane depolarization and enhanced excitability. The effect persisted despite pertussis-toxin pretreatment and in Ca^{2+}-free medium was diminished but not abolished, suggesting a Ca^{2+}-independent muscarinic receptor coupling mechanism, distinct from canonical G_i/o pathways. This characterization defined IK_{SO} as a novel "standing outward" leak conductance sustaining the hyperpolarized resting potential of CGNs. Its inhibition by muscarine implied that GPCRs can modulate resting leak channels to drive neuronal excitation, linking transmitter signalling to background conductances.

Subsequent molecular and physiological analyses pinned down the identity of this current (Millar et al 2000, Boyd et al 2000). Biophysical fingerprinting revealed voltage independence, rapid kinetics, Ba^{2+} and Zn^{2+} block, and sensitivity to extracellular acidification, all hallmarks of the newly cloned K_2P TASK channel family. Messenger RNA and immunostaining confirmed TASK1 expression in CGNs, and recombinant TASK1 currents in *Xenopus* oocytes were inhibited by M_3 muscarinic receptor activation, replicating IK_{SO} modulation.

These results firmly established that IK_{SO} was a TASK-like current, marking one of the first identification of a K_2P channel's physiological correlate in the mammalian CNS (Millar et al 2000). Functionally, this defined a known molecular entity to the setting of neuronal resting potential and muscarinic cholinergic control of excitability, both long-standing physiological areas of interest.

Further experiments revealed that muscarine's effects required M_3 receptors but were only loosely coupled to Ca^{2+} signalling (Boyd et al 2000). CGNs lacked sizeable endoplasmic reticulum Ca^{2+} stores and only depolarisation primed them to show a detectable Ca^{2+} transient after M_3 activation. Blocking phospholipase C or chelating Ca^{2+} prevented the Ca^{2+} rise yet scarcely affected IK_{SO} inhibition. The key inference from these experiments was that M_3 receptor activation suppresses IK_{SO} through a PLC-dependent but Ca^{2+}-independent pathway, while also mobilising residual Ca^{2+} stores through a parallel branch. This separation of signalling modes suggested that GPCR control of TASK currents occurs largely by Gαq interactions but not using Ca^{2+} as the second-messenger (Boyd et al 2000).

Once TASK channels were established as underlying IK_{SO}, our attention broadened to characterisation of TASK channel regulation. Recombinant TASK3 currents were strongly inhibited by protein kinase C, blocked by the PKC antagonists bisindolylmaleimide 1 and Gö6976, and abolished after silencing PKCα (Veale et al 2007a). TASK3 and CGN IK_{SO} were inhibited by M_3 receptor activation through pathways abolished by the $G\alpha_{(q)}$ antagonist

YM-254890, confirming the role of Gαq. PKC functioned through direct phosphorylation at position T341 in the C terminus, reducing channel current. Interestingly, activation of M_3 receptors also inhibited TASK3 via Gαq, but the inhibition persisted in PKC-resistant mutant, demonstrating that muscarinic suppression does not require PKC phosphorylation. Instead, Gαq interacts with TASK3 by a separate mechanism, while PKC serves as a feedback regulator that dampens prolonged inhibition (Veale et al 2007a).

Methanandamide, a non-hydrolysable analogue of anandamide, potently and irreversibly blocked TASK1 and TASK3 channels (Veale et al 2007b). The inhibition targeted an intracellular region bridging the final transmembrane segment and C terminus (the VLRFLT motif, now known to contribute to the X-gate of these channels, Rodstrom et al 2020). Strikingly, both methanandamide and GPCR activation (via Gαq) converged on this same site, implying a shared intracellular gating pathway. This provided one of the earliest molecular descriptions for receptor-independent cannabinoid effects on neuronal excitability, linking the cannabinoids to the K_2P channel family.

Human and mouse TASK3 channels were shown to be selectively enhanced by tumor necrosis factor-α (TNF-α), acting through the ASK1, JNK, and p38 MAP-kinase pathways (El Hachmane et al 2014). This modification increased channel open probability without altering surface expression. TNF-α therefore functions as a positive modulator of a leak conductance with pro-apoptotic consequences in neurons, suggesting that inflammation can tune membrane potential and viability via TASK3 channels.

Detailed mutagenesis studies demonstrated that at physiological pH 7.4, TASK3 channels are potently blocked by Zn^{2+} ($IC_{50} \approx 20\ \mu M$), whereas TASK1 and TASK2 are nearly insensitive (Clarke et al 2004). Two positions, E70 in the extracellular M1P1 loop and H98 in the pore, constitute a cooperative binding site for Zn^{2+} and mediate acid sensitivity. Swapping these residues between TASK1 and TASK3 exchanged their Zn sensitivity, directly proving structural causation (Clarke et al 2008). Since native CGN IK_{SO} is Zn-sensitive and zinc is synaptically released from inhibitory interneurons, this work suggested

a physiological synaptic mechanism that transiently depolarises granule neurons by inhibiting leak channels.

Follow-up biophysical analyses confirmed spatial proximity between E70 and H98 by introducing paired cysteines that formed disulfide bridges reversible by DTT and susceptible to Cd^{2+} block, strong chemical evidence that the M1P1 loop lies close to the selectivity filter, dynamically regulating TASK3 gating and extracellular modulation (Clarke et al 2008).

While Zn^{2+} engaged an outer vestibule site, kinases and cytokines acted via cytoplasmic domains; this bidirectional control exemplified the versatility of K_2P channel gating. Together, these studies established TASK channels as versatile integrators of neurotransmitter, kinase, and cytokine signals, modulating excitability over time scales from seconds to hours.

This work helped to establish two-pore-domain K^+ channels as the molecular substrates of neuronal leak currents and confirmed that TASK channels set the resting membrane potential in cerebellar granule neurons. Before this, inward rectifiers or delayed rectifiers were thought to dominate resting conductance (see Enyedi & Czirjak 2010).

The mutational mapping of extracellular and intracellular binding sites guided later high-resolution crystallographic and cryo-EM studies of K_2P channels, many of which confirmed the residue interactions proposed here (see Mathie et al 2021).

Despite major progress, several key uncertainties remained:

- Although residues critical for blockers and activators were identified, how these chemical interactions translate into concerted pore and helix movement remains incompletely defined. More recent advances in this area have advanced the field considerably for TASK channel structure and function (e.g. Rodstrom et al 2020, Hall et al 2025, Jouen-Tachoire et al 2026).

- The net contributions of TASK1 versus TASK3 vary among brain regions; many neurons co-express multiple K_2P subtypes (see Crowther et al 2025 for our more recent work in this area).
- The cross-regulation between PKC phosphorylation, Gαq binding, and lipid modulation is intricate. Whether these act sequentially or competitively remains unclear.
- TNF-α-induced enhancement suggests potential pro-apoptotic outcomes, yet K_2P activation is neuroprotective in ischemia. This needs further work to understand and reconcile these observations perhaps through in vivo models.

Building upon these foundational studies, several future directions emerge for this research area:

- Cryo-EM now enables atomic capture of open, closed, and modulated states. Determining structures of TASK channels complexed with muscarinic receptor peptides, Gαq, PKC, or small-molecule activators and inhibitors will be crucial to visualize gating transitions proposed here and considerable recent progress has been made in this area (see, for example, Rodstrom et al 2020, Hall et al 2025).
- Combining patch-clamp with voltage-sensitive fluorophores or genetically encoded K^+ indicators could map spatiotemporal dynamics of leak-current modulation during synaptic transmission or glial signalling.
- Mathematical modelling incorporating G-protein kinetics, Ca^{2+} feedback, and PKC regulation could predict how combined neuromodulatory inputs summate to control membrane potential.
- Structure-guided drug discovery could target extracellular sensor regions (M1P1 loop, pore helix) and intracellular gating motifs to produce selective, state-dependent modulators.

Collectively, this work shifted understanding of TASK channels and the native leak conductance, IK_{SO}, from passive leak conductances to multistate, highly regulated proteins. Rather than serving as static background currents, TASK channels operate as dynamic effectors that couple GPCR activation, pH sensing, lipid signalling, and inflammatory pathways to changes in resting membrane potential.

Section 4. TREK, Two Pore Domain Potassium Channels

Summary

The fourth section describes the characterisation of the functional properties and regulation of the related TREK family of K_2P channels.

TREK1 channels were inhibited by fluoxetine (Prozac) and its active metabolite, norfluoxetine (Kennard et al 2005). Additionally, the anticonvulsant agents, sipatrigine and lamotrigine inhibited TREK channels (Walsh et al 2021). The actions of these compounds on TREK channels may contribute to their current and potential use in the treatment of pain and depression.

A splice variant of TREK1 (TREK1ΔEx4) expressed in the brain was identified, which encodes a heavily truncated TREK1 protein (Veale et al 2010). TREK1ΔEx4 has no channel activity itself but reduced TREK1 whole cell current amplitude, thereby regulating channel function.

Fenamates such as flufenamic acid (FFA) and the related experimental drug BL-1249 enhances the activity of TREK1 currents (Veale et al 2014a), as did aristolochic acid which is used in traditional medicines to treat pain (Veale & Mathie 2016). A new, selective opener of TREK channels, GI-530159 was described and characterised (Loucif et al 2018). Given the importance of TREK1 channels in pain, selective TREK channel openers like GI-530159 could aid in the development of novel analgesic agents.

G171F and A286 mutations in the pore of TREK1 substantially reduced current through the channel (Al-Moubarak et al 2019). This reduction in current could be reversed pharmacologically, by FFA. Pharmacologically reversible mutations of TREK channels can help to clarify the importance of these channels in pathophysiological conditions such as pain and depression and also suggest potential therapeutic benefits for reversing disease-causing channelopathies, pharmacologically (see section 5).

16) Kennard LE, Chumbley JR, Ranatunga KM, Armstrong SJ, Veale EL, **Mathie A** (2005). Inhibition of the human two-pore domain potassium channel, TREK-1, by fluoxetine and its metabolite norfluoxetine. *Br J Pharmacol* 144: 821−829. PMID: 15685212

17) Walsh Y, Leach M, Veale EL, **Mathie A** (2021) Block of TREK and TRESK K2P channels by lamotrigine and two derivatives, sipatragine and CEN-092. *Biochem Biophys Rep* 26: 101021. PMID: 34041373

18) Veale EL, Rees KA, **Mathie A***, Trapp S (2010). Dominant negative effects of a non-functional TREK1 splice variant expressed in brain. *J Biol Chem* 285: 29295-29304. PMID: 20605797 *Joint corresponding author

19) Veale EL, Al-Moubarak E, Bajaria N, Omoto K, Cao L, Tucker SJ, Stevens EB, **Mathie A** (2014a). Influence of the N-terminus on the Biophysical Properties and Pharmacology of TREK1 Potassium Channels. *Mol Pharmacol* 85: 671-681. PMID: 24509840

20) Veale EL, **Mathie A** (2016) Aristolochic acid, a plant extract used in the treatment of pain and linked to Balkan Endemic Nephropathy, is a regulator of K2P channels. *Br. J. Pharmacol* 173: 1639-1652. PMID: 26914156

21) Loucif A, Saintot P-P, Liu J, Antonio BM, Zellmer SG, Yoger K, Veale EL, Wilbrey A, Omoto K, Cao L, Gutteridge A, Castle NA, Stevens EB, **Mathie A** (2018). GI-530159, a novel, selective, mechano-sensitive two-pore-domain potassium (K2P) channel opener, reduces rat dorsal root ganglion (DRG) neuron excitability. *Br J Pharmacol* 175: 2272-2283. PMID: 29150838

22) Al-Moubarak E, Veale EL, **Mathie A** (2019). Pharmacologically reversible, loss of function mutations in the inner pore helices of TREK-1 K2P channels. *Scientific Reports* 9: 12394. PMID: 31455781

See Appendix pages 255 - 329 for full versions of these references.

Introduction

TREK K_2P channels are mechanosensitive channels which contribute to background conductances in many neurons and other cells (Enyedi & Czirjak 2010, Feliciangeli et al 2015, Djillani et al 2019). Their activity is now known to be regulated by a number of different physical and chemical stimuli which include membrane stretch, membrane depolarisation, heat, arachidonic acid, and other polyunsaturated fatty acids (see Honore 2007, Mathie & Veale 2015). TREK1 and TREK2 channels show bidirectional modulation. They are inhibited by $G\alpha_q$ and $G\alpha_s$, but enhanced by $G\alpha_i$ pathways such as those activated by certain opioids or $GABA_B$ receptors (Mathie 2007, Feliciangeli et al 2015). TREK channels have emerging roles in a number of physiological responses and pathophysiological conditions including depression, ischemia, nociception, migraine and general anaesthesia (Devilliers et al 2103, Mathie et al 2021) and so pharmacological regulation of their activity may be of therapeutic benefit.

Results and Discussion

TREK1, was shown to be potently inhibited by fluoxetine (IC_{50} ~ 19 μM) and its metabolite norfluoxetine (~ 9 μM) (Kennard et al 2005). Block was voltage-independent and only partial at related channels (TASK3 ~31 % inhibition). Structural mapping found that deletions in the C-terminus reduced fluoxetine sensitivity, implicating regions near E306. This blocking action of fluoxetine and norfluoxetine has subsequently proven to be critical for structural studies of TREK channels allowing both the identification of the binding site for these compounds on the channel and a deeper understanding both of how these channels gate and how these compounds interfere with channel gating (Dong et al 2015, Proks et al 2021). This effect of fluoxetine also provided supporting evidence to suggest a role of TREK1 channels in depression given the observation that TREK1 channel deletion results in a depression-resistant phenotype in mice (Heurteaux et al 2006).

Extending this pharmacological regulation by known CNS-active drugs, the *in vitro* profiling of the anticonvulsants lamotrigine and sipatrigine, and the

novel compound CEN-092 showed that these triazine compounds inhibit TREK1, TREK2 and TRESK channels with mid-micromolar potency (Walsh et al 2021). Mutational analyses pinpointed leucine (L289A and L320A) sites shared with the fluoxetine pocket (Dong et al 2015), while analogous pore-helix mutations (F145A and F352A) altered TRESK sensitivity. These results suggested overlapping but distinct binding cavities and might explain differences in clinical efficacy and tolerability among these compounds.

A novel TREK1ΔEx4 splice variant, encoding only one transmembrane segment, was discovered as an intracellularly retained dominant-negative regulator that diminished surface expression and current of full-length TREK1 (Veale et al 2010). Its selective effect on long versus short TREK1 isoforms identified the N-terminus and first transmembrane region as essential for dimerisation and trafficking. This finding introduced post-transcriptional control of leak K conductance via non-functional subunits as a novel layer of K_2P regulation.

Alongside the inhibitory pharmacology, several studies (Veale et al 2014a, Veale and Mathie 2016, Loucif et al 2018) uncovered small-molecule activators of TREK channels. Fenamates (flufenamic, mefenamic, niflumic acids, diclofenac) and BL-1249 markedly enhanced TREK1 currents (Veale et al 2014a). Comparison of long and short TREK1 isoforms showed both forms could be activated, but gating mutations that prevent channel opening abolished fenamate potentiation. Thus, fenamates act by stabilising the open state of TREK channels. Notably, in the short isoform of TREK1, they restore strong K^+ selectivity.

Aristolochic acid (Veale & Mathie 2016) selectively enhanced TREK1 and TREK2 but inhibited TRESK channels through interaction with inner-pore phenylalanine residues. This dual action could underlie both analgesic and nephrotoxic properties, highlighting how natural compounds can differentially modulate K2P subfamilies. GI-530159 also proved to be a potent and selective opener of TREK1 and TREK2 channels but inactive at TRAAK or some

non-K_2P channels (Loucif et al 2018). In dorsal root ganglion neurons, 1 μM GI-530159 hyperpolarised the membrane potential and reduced firing frequency, offering an important proof of concept for TREK channel activation as an analgesic strategy.

Further structure-function analysis showed that flufenamic acid (FFA) requires conformational freedom between the TM2 and TM4 helices of the channel. Gain-of-function mutations (G137I, Y284A) or targeted cross-linking of residues (A286C + MTSET) altered or prevented its activation, demonstrating that FFA opens TREK1 by biasing helical motion in the inner bundle (Al-Moubarak et al 2019). This study described the first pharmacologically reversible mutation in TREK channels and paved the way for future studies developing pharmacological reversal of inhibitory mutation by activators as a valid therapeutic approach.

The identification of antidepressants, anticonvulsants and anti-inflammatory agents as K_2P channel modulators, both clarified off-target actions of established drugs and expanded the pipeline for future analgesic development. TREK channel openers emerged as prototypes for pain therapeutics. Conversely, TREK channel inhibition was implicated in antidepressant efficacy (Mathie et al 2021).

Although a number of pharmacological tools for K_2P channels were discovered, few are truly selective among K_2P channel members. For example, sipatrigine, fenamates, and aristolochic acid all affect multiple channels. Achieving subtype selectivity without off-target toxicity is a continuing goal. Nevertheless, the more selective activators (GI-530159) and inhibitors (sipatrigine derivatives) should be tested in animal models of pain, depression, and epilepsy to confirm therapeutic relevance.

Section 5. Potassium Channelopathies

Summary

The final section concerns the characterisation of a number of potassium channelopathies.

A mutation in TASK3 (G236R) was shown to be responsible for Birk Barel mental retardation syndrome (also named KCNK9 imprinting syndrome, KIS), a maternally transmitted developmental disorder (Veale et al 2014b). G236R mutated TASK3 channels give rise to a functional current that is significantly smaller when compared with wild-type (WT) TASK3 channels. This reduction could be overcome, at least in part, by FFA. It is proposed that pharmacological enhancement of mutated TASK3 channel current during development may, therefore, provide a potentially useful therapeutic strategy in the treatment of Birk Barel syndrome.

In later experiments (Cousin et al 2022), the broader genetic and phenotypic variability for this disease was described, including the identification of an additional mutational hotspot (R131) which led to a paradoxical gain of channel function. This study demonstrated the complex etiology of this syndrome including both gain and loss of channel function and the more consistent loss of channel regulation by GPCRs and pH. This suggests that unique therapeutic strategies may be needed to address the specific functional consequences of mutation on channel function and regulation in different patients.

The TASK1 channel gene (KCNK3) has been identified in heritable pulmonary arterial hypertension (PAH). Novel mutated TASK1 channels, seen in PAH patients, had a substantially reduced current compared to wild-type TASK1 channels but this could not be overcome pharmacologically by activators of TASK1 channels (Cunningham et al 2019).

Numerous pathogenic variants in KCNB1, which encodes the voltage-gated potassium channel, $K_V2.1$, are linked to developmental and epileptic

encephalopathies and associated with loss of function, regulation, and expression of the channel. A novel *de novo* variant (P17T) occurring in the $K_V2.1$ channel that results in a gain of channel function was described. It is clinically associated with neurodevelopmental disorders, but without the seizures seen in patients with loss of function channel mutations (Veale et al 2022).

23) Veale EL, Hassan M, Walsh Y, Al Moubarak E, **Mathie A** (2014b). Recovery of current through mutated TASK3 potassium channels underlying Birk Barel syndrome. *Mol Pharmacol* 85: 397-407. PMID: 24342771

24) Cousin MA, Veale EL, [....], Zimmermann MT, **Mathie A***, Klee EW (2022). Gain and loss of TASK3 channel function and its regulation by novel variation cause KCNK9 imprinting syndrome. *Genome Medicine* 14: 62. PMID: 35698242 *Joint corresponding author

25) Cunningham KP, Holden RG, Escribano-Subias P, Cogolludo A, Veale EL, **Mathie A** (2019). Characterisation and regulation of wild type and mutant TASK-1 two pore domain potassium channels indicated in pulmonary arterial hypertension. *J Physiol* 597: 1087-1101. PMID: 30365877

26) Veale EL, Golluscio A, Grand K, Graham JM, **Mathie A** (2022). A KCNB1 gain of function variant causes developmental delay and speech apraxia but not seizures. *Frontiers in Pharmacology* 13: 1093313. PMID: 36618935

See Appendix pages 330 - 390 for full versions of these references.

Introduction

Potassium (K^+) channels are prominent gatekeepers of neuronal excitability, cardiac rhythm, and vascular tone. They control resting membrane potential, repolarisation, and cell signalling. Mutations in K^+ channels disturb these processes and increasingly appear among the growing catalogue of channelopathies, disorders rooted in altered channel function or regulation.

The four studies summarised here explore disease-causing variants in three distinct K^+ channel genes:

- KCNK9 which codes for the K_2P background channel, TASK3. TASK3 channels are critical for setting neuronal resting potential and implicated in neurodevelopmental disorders such as Birk Barel syndrome / KCNK9 imprinting syndrome (KIS) (Veale et al 2014b, Cousin et al 2022).
- KCNK3 which codes for the K_2P channel TASK1. TASK1 channels are central to pulmonary vascular tone, and KCNK3 is now recognised as a gene whose mutations cause heritable pulmonary arterial hypertension (PAH) (Cunningham et al 2019).
- KCNB1 which codes for the voltage-gated K^+ channel $K_V2.1$. $K_V2.1$ channels are widely expressed in cortex and hippocampus whose variants underlie developmental and epileptic encephalopathies (DEEs) (Veale et al 2022).

Together, these studies broaden understanding of how K^+ channel mutations translate molecular dysfunction into clinical phenotypes, revealing a spectrum that ranges from loss of function and aberrant gating in TASK channels to gain of function in $K_V2.1$ and, in some cases, TASK3 channels. Collectively these studies advance electrophysiological, structural, and therapeutic insight into potassium channelopathies.

Results and Discussion

Birk Barel mental retardation dysmorphism syndrome, the first recognized KCNK9 (TASK3) imprinting disorder, is maternally inherited, arising from monoallelic expression of the maternal copy in brain tissue. Our study of the G236R mutant offered the first detailed electrophysiological explanation for the disease (Veale et al 2014b).

Whole-cell recordings showed that mutant TASK3 channels still produce measurable current, but the amplitude of outward K^+ current is markedly reduced and the current-voltage relationship displays inward rectification, the opposite of the outwardly rectifying behaviour of wild-type channels. This rectification and smaller outward current imply that at depolarised potentials, neurons will fail to maintain proper leak conductance, thereby depolarising too easily. In developing neurons, such instability could disrupt migration and network formation, consistent with the neurodevelopmental phenotype observed clinically.

G236R channels also exhibit abnormal regulation. They responded differently to extracellular acidification, zinc, and M_3 muscarinic receptor activation. Hence, the mutation perturbs both the pore's biophysical properties and the allosteric coupling to extracellular modulators. A striking therapeutic insight came from the observation that gain of function mutation A237T, immediately adjacent to G236R, or treatment with the non-steroidal anti-inflammatory drug flufenamic acid (FFA) restored outward current toward normal and enhanced conductance far more in mutant channels than in wild type. FFA, a known TREK/TASK modulator, appeared to "rescue" channel opening. We proposed that pharmacological potentiation of mutant TASK-3 channels during development may ameliorate Birk Barel symptoms, establishing, for the first time, a precision-medicine concept for a K2P-related neurodevelopmental disorder.

A subsequent large-scale genomic and clinical investigation (Cousin et al 2022) transformed the single-variant view of KIS into a syndromic spectrum. By identifying 15 novel KCNK9 alterations in a cohort of 47 individuals, we broadened both the genotypic diversity and phenotypic range of the condition. Computer-assisted facial recognition confirmed shared dysmorphic traits and developmental hallmarks: motor and speech delay, hypotonia, behavioural issues, and feeding difficulties, while stressing that no single feature was a specific indicator of the syndrome. Molecular diagnosis therefore remains essential.

Electrophysiological characterisation and modelling revealed both gain- and loss- of function (GoF/LoF) variants within TASK3. Importantly, most mutations converged on disrupted regulation, rather than simple current amplitude changes. Some mutants lost responsiveness to pH or G-protein modulation; others showed aberrant gating kinetics. These findings overturned the earlier assumption that KIS stems solely from a LoF mutation and instead pointed to a spectrum of TASK3 dysregulation, from over-active to under-active channels.

This diversity implies that therapies must be mutation-specific. Potentiators like FFA or gain-of-function substitutions that aid G236R could aggravate disease for GoF variants. Mapping each variant's functional class is therefore critical before intervention. The study's integration of computational modelling, electrophysiology, and clinical data established a template for functional interpretation of rare variants in ion-channel genes.

While TASK3 mutations cause neuronal disorders, loss of function TASK1 (KCNK3) mutations disrupt pulmonary vascular homeostasis. The third study (Cunningham et al 2019) characterised two newly discovered PAH-linked mutations (G106R and L214R) and evaluated whether pharmacological activators can restore channel current.

Whole-cell recordings in transfected tsA201 cells showed that both mutants are properly trafficked to the plasma membrane but produce markedly reduced K^+ currents, confirming true loss of function at the pore level. Several established TASK1 activators, extracellular alkalisation (pH 8.4) and the phospholipase-A_2 inhibitor ONO-RS-082, failed to rescue activity. Likewise, riociguat, a guanylate-cyclase stimulator already approved for PAH treatment, enhanced current through wild-type but not mutant channels. This ruled out defective localisation or signalling deficiency and pinpointed intrinsic gating impairment resistant to available ligands.

Loss of K^+ efflux in pulmonary arterial smooth muscle cells would depolarise

the membrane, open voltage-dependent Ca^{2+} channels, elevate intracellular Ca^{2+}, and promote vasoconstriction and proliferation, canonical hallmarks of PAH. The location of G106 and L214 near the selectivity filter suggests altered conduction rather than trafficking. Riociguat's partial effectiveness on WT TASK1 implies its pulmonary benefits may stem at least in part from enhancing residual TASK1 currents in non-mutant cells. However, the failure to rescue mutant channels highlights the need for mutation-specific pharmacology.

The fourth study (Veale et al 2022) shifted focus from K_2P leak channels to the Shab-related voltage-gated channel $K_V2.1$, encoded by KCNB1. Many prior KCNB1 mutations produce loss-of-function and manifest clinically as severe developmental and epileptic encephalopathies. In sharp contrast, the newly discovered P17T variant, identified de novo by exome sequencing, caused a gain of function (GoF) phenotype associated with developmental delay and hypotonia but not epilepsy.

Whole-cell patch-clamp analysis revealed larger macroscopic currents and a right-shifted steady-state inactivation curve, meaning that channels remain open over a broader voltage range. The mutant also showed reduced sensitivity to guanxitoxin-1E, a selective $K_V2.1$ inhibitory peptide. Functionally, this would hyperpolarise neurons or shorten action potentials, potentially reducing excitability and explaining the absence of seizures despite cognitive impairment.

This is the first demonstration that GoF in $K_V2.1$ yields a neurodevelopmental disorder distinct from epileptic encephalopathies caused by LoF. It underscores how directionally opposite channel defects, deficient versus excessive current, can produce divergent clinical outcomes, requiring functional assays for every variant found by sequencing. The study also validates precision-phenotyping across channelopathies, even within a single gene given that biophysically opposite effects (gain vs. loss of function) determines the presence or absence of seizures.

Together, these four papers exemplify a growing transition from genetic discovery to mechanistic channel physiology, providing model cases for how

inherited or de novo K^+ channel mutations drive human disease (see also Mathie et al 2024). They consolidated K_2P (TASK1/TASK3) and voltage-gated ($K_V2.1$) channels as clinically relevant targets. Each study demonstrated that understanding the functional properties of mutated channels through electrophysiological characterisation is integral to variant interpretation. In both KCNK9 and KCNK3 syndromes, sequence variation alone offered limited predictive value. Some mutations were benign, others pathogenic, and some produced opposing effects. Functional assays defined the true pathogenic mechanism and informed tailored therapy design. Similar reasoning applied to KCNB1 as clinical phenotype could not be predicted until the P17T GoF nature was revealed.

The TASK3 G236R rescue by FFA provided one of the first concrete demonstrations that channel potentiation can correct a developmental K2P defect, analogous to CFTR correctors in cystic fibrosis. Parallel implications emerge for PAH, where restoring TASK1 activity could normalise pulmonary tone, and for the $K_V2.1$ GoF mutation, where mild inhibitors might prevent excessive neuronal repolarisation.

Despite the major advances made in these studies, key uncertainties remain:

- For KCNK9, it is unclear when during neurogenesis decreased TASK3 current exerts the greatest impact, and how imprinting restricts the phenotype to maternal inheritance.
- The structural basis for gating failure remains unresolved; high-resolution structures capturing G106R or L214R conformations are needed.
- Our studies highlight that altered regulation (pH, G proteins, Zn^{2+}) rather than pure conductance loss can dominate pathology, yet the downstream signalling perturbations are undefined.
- Most functional analyses relied on heterologous tsA201 cells. Native neurons, pulmonary smooth-muscle cells, or patient-derived induced

pluripotent-stem-cell (iPSC) models could reveal coupling to endogenous partners and give drug responses more accurately.

- While FFA rescues G236R TASK-3 current in vitro, its safety and blood–brain barrier penetration in foetuses or children are unknown. Equivalent mutation-specific modulators for TASK1 or K_V2.1 P17T remain to be found.
- The same magnitude of channel dysfunction may produce divergent clinical severity, indicating epigenetic, transcriptional, or environmental modifiers that are yet to be identified.

High resolution cryo-EM structures of KCNK9 and KCNK3 (in wild-type and mutant forms, e.g. Hall et al 2025) will enable rational design of channel-specific potentiators or inhibitors. For G236R TASK3, mapping the altered pore configuration could help identify molecules that replicate the restorative effect of FFA but with safer pharmacology with less side effects. Similar structural efforts for TASK1 mutants may pinpoint allosteric sites capable of reopening closed channels (see Ma et al 2013).

Because KCNK9 variants display mixed gain and loss of function, mutation-tailored screening pipelines are required. Automated electrophysiology or optogenetic assays in iPSC-derived neurons could test diverse compounds rapidly, supporting individualised therapy analogous to oncology mutation panels.

Generating knock-in mice, C Elegans worms or zebrafish carrying specific mutations (e.g. KCNK9 G236R or A237T) will help clarify how leak-current defects translate to altered neuronal migration and behaviour. For PAH-related TASK1 mutations, smooth-muscle-specific models could reveal vascular remodelling pathways.

Understanding the epigenetic regulation of the KCNK9 locus, such as its methylation dynamics during embryogenesis, could uncover why paternal

alleles remain silent and how to transiently reactivate the healthy copy as a therapeutic strategy.

Future work should combine electrophysiology, proteomics, and computational modelling to trace how mutated channels perturb global cellular signalling. For example, does reduced TASK current alter Ca^{2+} homeostasis via membrane potential shifts, or modify neurotransmitter release probability? For $K_V2.1$ P17T, simulations of synaptic integration under GoF conditions could predict circuit-level consequences.

Clinically, genotype–phenotype correlations from these papers emphasise that molecular diagnosis must precede therapy. Developing clinical scoring systems informed by specific biophysical fingerprints (e.g. rectification for KCNK9 variants, reduced activation for KCNK3 mutants, shifted inactivation for KCNB1 GoF variant) will improve identification of atypical channelopathies.

Across these four studies, molecular insight from potassium channel research has led to translational foresight. The KCNK9 G236R description illuminated how subtle structural distortion in a background leak channel can disrupt neuronal development and introduced pharmacological rescue as a tangible objective. The extensive KCNK9 variant study reframed that single-gene disorder into a functionally heterogeneous imprinting syndrome, requiring variant-specific analysis. The KCNK3 G106R and L214R work contributed to defining TASK1 loss of function as a mechanistic driver of pulmonary arterial hypertension and demonstrated the limitations of existing activators. Finally, characterisation of the KCNB1 P17T GoF variant provided further evidence that even the same channel family can produce radically different clinical manifestations depending on whether conductance is reduced or increased.

Together these papers consolidate several themes central to the understanding of monogenic channelopathies associated with potassium channels. A central conceptual advance is that clinical phenotype depends on the direction, magnitude, and regulatory context of channel dysfunction**,** not gene identity

alone. Both LoF and GoF mutations of the same channel can be pathogenic. Many variants affect channel modulation and regulation, not just baseline conductance. Therapeutic interventions must be matched to biophysical defect(s), not merely to gene identity.

Consolidation

This thesis provides a comprehensive account of research I conducted over four decades on the physiological, molecular, and pharmacological properties of mammalian ion channels, with a particular emphasis on their roles in health and disease. The work is organised into five thematic sections encompassing 26 core publications, spanning early studies of neuronal nicotinic acetylcholine receptors through to detailed investigations of G protein signalling, Ca^{2+} channel modulation, and K_2P channels, culminating in the study of K^+ channelopathies.

Across these studies, a clear progression emerges from descriptive electrophysiology to mechanistic and structural insight. Early work established fundamental properties of ion channel function in neurons, while later studies elucidated how these channels are dynamically regulated by neurotransmitters, G proteins, kinases, lipids, and cytokines. In particular, the identification of background "leak" K^+ currents as mediated by K_2P channels represented a conceptual shift in the field, redefining these currents from passive conductances to highly regulated determinants of cellular excitability.

A major contribution of this body of work lies in defining the physiological roles and regulatory mechanisms of K_2P channels, especially the TASK and TREK families. These studies demonstrated that TASK channels underlie resting membrane conductances in neurons and are modulated by GPCR signalling pathways, while TREK channels act as polymodal sensors integrating mechanical, thermal, and chemical stimuli. The discovery of pharmacological modulators, including both inhibitors and activators, further established K_2P channels as tractable therapeutic targets in disorders such as pain and depression.

The final section extends these mechanistic insights into the area of human disease. Studies of mutations in KCNK9 (TASK3), KCNK3 (TASK1), and KCNB1 ($K_V2.1$) illustrate how alterations in potassium channel function give rise to diverse clinical phenotypes, including neurodevelopmental disorders and pulmonary arterial hypertension. Importantly, these works demonstrate that channelopathies cannot be understood solely in terms of gene identity; rather,

the specific biophysical consequences of each mutation, gain or loss of function, altered gating, or disrupted regulation, determine disease manifestation.

A key translational advance emerging from this research is the demonstration that defective ion channel function can, in some cases, be pharmacologically rescued. The partial restoration of TASK3 G236R channel activity by flufenamic acid provides a proof-of-principle for mutation-specific therapeutic strategies in K_2P-related disorders. More broadly, these findings highlight the importance of integrating electrophysiological characterisation with genetic diagnosis to enable precision medicine approaches.

Looking forward, advances in cryo-electron microscopy, live-cell imaging, and computational modelling are expected to further refine our understanding of ion channel structure–function relationships. These approaches will facilitate the development of selective, state-dependent modulators capable of targeting specific channel conformations or disease-associated variants. In parallel, the use of patient-derived cellular models and in vivo systems will be essential for linking molecular dysfunction to physiological and behavioural outcomes.

Taken together, the work presented in this thesis shows the evolution of ion channel research from foundational biophysical observations to clinically relevant mechanistic insight. It underscores the central role of K^+ channels in regulating cellular excitability and demonstrates how detailed understanding of their function can inform the development of targeted therapeutic strategies. The convergence of structural biology, electrophysiology, and human genetics now offers a realistic pathway toward precision treatments for a wide range of neurological and cardiovascular channelopathies.

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References (additional to the 26 core papers)

Albuquerque EX et al. (2009). Mammalian nicotinic acetylcholine receptors: from structure to function. Physiol Rev. 89(1):73-120.

Alexander SPH et al. (2025). The Concise Guide to Pharmacology 2025/26: Ion channels
Br J Pharmacol 182 Suppl 1: S152-S241

Barrantes FJ (2023). Structure and function meet at the nicotinic acetylcholine receptor-lipid interface. Pharmacol Res. 190:106729.

Changeux JP (2012). The nicotinic acetylcholine receptor: an allosteric protein prototype of ligand-gated ion channels. Nat Rev Neurosci. 13(6):419-431.

Colquhoun D et al (1987). Nicotinic acetylcholine receptors of nerve and muscle: functional aspects. Trends in Pharmacol Sci 8(12): 465-472

Crowther KM et al. (2025). Equivalent gain-of-function variants in KCNK3 and KCNK9 and their contribution to distinct TASK K2P channelopathies. medRxiv. 2025.12.15.25342159.

Dani JA, Bertrand D (2007). Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol. 47:699-729.

Dascal N (2001) Ion-channel regulation
by G proteins. Annu Rev Physiol. 63:89-118.

Delgado-Vélez M et al. (2021). Pursuing high-resolution structures of nicotinic acetylcholine receptors: lessons learned from five decades. Molecules. 26(19):5753.

Derkach V.A. et al. (1987). Single channels activated by acetylcholine in rat superior cervical ganglion. Journal of Physiology 388, 141-151.

Devilliers M et al. (2013). Activation of TREK-1 by morphine results in analgesia without adverse side effects. Nature Commun 4:2941.

Dolphin AC (2016). Calcium channel regulation by G proteins and associated proteins. Cold Spring Harb Perspect Biol. 8: a028142.

Dong YY et al. (2015), K2P channel gating mechanisms revealed by structures of TREK-2 and a complex with Prozac. Science 347:1256-1259

Enyedi P, Czirjak G (2010) Molecular background of leak K+ currents: two-pore domain potassium channels. Physiol Rev. 90(2):559-605.

Feliciangeli S et al. (2015) The family of K2P channels: salient structural and functional properties. J Physiol. 593(12): 587-603

Gotti C, Clementi F. (2004). Neuronal nicotinic receptors: from structure to pathology. Prog Neurobiol. 74(6): 363-396.

Haghighi AP, Cooper E (1998) Neuronal nicotinic acetylcholine receptors are blocked by intracellular spermine in a voltage-dependent manner. J Neurosci 18(11): 4050-4062

Haghighi AP, Cooper E (2000) A molecular link between inward rectification and calcium permeability of neuronal nicotinic acetylcholine alpha3beta4 and alpha4beta2 receptors. J Neurosci 20(2): 529-541.

Hall PR et al. (2025). Structures of TASK-1 and TASK-3 K2P channels provide insight into their gating and dysfunction in disease. Structure. 33(1): 115-122.

Heurteaux C et al. (2006). Deletion of the background potassium channel TREK-1 results in a depression-resistant phenotype. Nat Neurosci 8 1134-1141.

Hille B et al. (1995). Multiple G-protein-coupled pathways inhibit N-type Ca channels of neurons. Life Sci 56: 989-992.

Hille B et al. (2015) Phosphoinositides regulate ion channels Biochim Biophys Acta. 1851:844-856.

Honore E (2007), The neuronal background K2P channels: focus on TREK1 Nat Rev Neurosci 8(4): 251-261

Jouen-Tachoire TRH et al. (2026). Structural determinants for GPCR-mediated inhibition of TASK K2P channels by diacylglycerol and its dysfunction in disease. EMBO J 45(7):2400-2412

Ma L et al. (2013). A novel channelopathy in pulmonary arterial hypertension. N Engl J Med. 369(4): 351-361.

Mathie A (2007). Mammalian K2P channels and their regulation by G protein coupled receptors. J Physiol 578: 377-385.

Mathie A, Veale EL (2015) Two pore domain potassium channels: potential therapeutic targets for the treatment of pain. Pflügers Archiv - Eur J Physiol 467: 931-943

Mathie A et al. (2021). Two-pore domain potassium channels as drug targets: Anesthesia and beyond. Annu Rev Pharmacol Toxicol 61: 401 - 420.

Mathie A et al. (2024). The contribution of genetic sequencing information to the identification and functional characterization of two-pore domain (K2P) channels as viable therapeutic targets. In: Ion Channels as Targets in Drug Discovery, 199-220. Stephens G & Stevens E (eds), Springer, Cham.

Proks P et al. (2021) Norfluoxetine inhibits TREK-2 K2P channels by multiple mechanisms including state-independent effects on the selectivity filter gate. J Gen Physiol. 153(8):e202012812.

Ragozzino D et al. (1997). Functional properties of neuronal nicotinic acetylcholine receptor channels expressed in transfected human cells. Eur J Neurosci 9(3): 480-488

Rodstrom KEJ et al. (2020). A lower X-gate in TASK channels traps inhibitors within the vestibule. Nature 582: 443–447

Rust G et al. (1994). Expression of neuronal nicotinic acetylcholine receptor subunit genes in the rat autonomic nervous system. Eur J Neurosci 6(3): 478-485

Sands SB, Barish ME (1991). Calcium permeability of neuronal nicotinic acetylcholine receptor channels in PC12 cells. Brain Res 560(1-2): 38-42.

Unwin N (2005). Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution and physiological relevance. J Mol Biol. 346(4): 967-989.

Wickman K Clapham DE (1995) Ion channel regulation by G proteins. Physiol Rev. 75: 865-885.

Yamada M et al. (1998). GIRK channels in the heart and brain: physiology and pharmacology. Pharmacol Rev. 50: 723-760.

Appendix (removed due to Copyright Restrictions)