

C12

Identification of the determinants for volatile general anaesthetic modulation of TASK 2PK channels

A.J. Caley, I. Andres-Enguix and N.P. Franks

Biophysics, Imperial College London, London, UK

Several of the two-pore domain K⁺ channels have been shown to be activated by clinically relevant concentrations of volatile (Patel *et al.* 1999) and gaseous (Gruss *et al.* 2004) general anaesthetics. Consequently, they have been proposed to play a role in inducing the general anaesthetic state.

We have been studying the TASK like channels with the aim of identifying the critical regions necessary for anaesthetic modulation. It has been shown that both the TASK-1 and TASK-3 channels are potentiated by halothane. However we have found that the volatile anaesthetic chloroform (1.98 mM) causes an activation of TASK-3 (82 ± 11%; n = 17) in contrast to the inhibition that is observed on TASK-1 (27 ± 5%; n = 5) (errors given as s.e.m.). This difference can be exploited to identify the relevant regions responsible for chloroform activation. Furthermore, if we hypothesise that halothane and chloroform share an overlapping site of action then this approach may provide information about the halothane modulatory site.

Previously the halothane binding site on TASK-3 was proposed to be the 243-248 VLRFLT region at the end of the fourth transmembrane (TM) domain (Talley & Bayliss, 2002). This region is highly conserved between TASK-3 and TASK-1, with only the lysine residue present in TASK-3 being replaced by a methionine in TASK-1. We investigated this residue using a point mutation to change this region from TASK-3 to TASK-1. We found, however, that the activation caused by chloroform on this L247M mutant (84 ± 10%; n = 4) was not significantly different (P < 0.01) to the wildtype channel. Due to the high sequence identity between TASK-1 and TASK-3 we were able to construct a series of chimeras between the channels to identify the region that was critical for chloroform activation. We found that the intracellular loop between TM2 and TM3 was one of the key determinants for the chloroform activation.

In a parallel study (Andres-Enguix *et al.* in preparation) we have recently cloned the *Lymnaea stagnalis* K⁺ channel responsible for the I_{K(AN)} current (Franks & Lieb, 1988). A sequence comparison between the TASK-1, TASK-3 and the *Lymnaea* channels was used to identify individual residues which are important for anaesthetic activation.

Franks NP & Lieb WR (1988). *Nature* **333**, 662-664.

Gruss M *et al.* (2004). *Mol Pharmacol* **65**, 443-452.

Patel AJ *et al.* (1999). *Nat Neurosci* **2**, 422-426.

Talley EM & Bayliss DA (2002). *J Biol Chem* **277**, 17733-17742.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C13

Modulation of K_{2p}2.1 by mGlu4: a molecular and pharmacological study

S. Cain and T. Bushell

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, UK

Two-pore potassium ion channels (K_{2p}s) are a sub-group of the potassium ion channel superfamily which are proposed to contribute to the setting and modulation of the neuronal resting membrane potential (Goldstein *et al.* 2001). We have shown previously that the group III metabotropic glutamate receptor subtype 4 (mGlu4) induces robust potentiation of the K_{2p}2.1 (TREK-1) current in expression systems and cultured striatal neurons, via a cAMP dependant mechanism (Cain *et al.* 2005). In the present study, we have investigated the potential phosphorylation sites involved in this potentiation.

Perforated patch whole cell recordings were made from Chinese hamster ovary (CHO) cells stably transfected with human mGlu4 cDNA and transiently transfected with human K_{2p}2.1 or K_{2p}2.1 mutant channel cDNA. Cells were held at -70mV and voltage ramps (-120mV to 40mV, 500ms) were applied every 30s. All drugs were added via the perfusate. Data are expressed as mean ± S.E.M. and statistical significance determined using Student's paired t tests, n = number of neurons.

In CHO cells co-expressing wild-type K_{2p}2.1 (K_{2p}2.1_{WT}) and mGlu4, the mGlu group III selective agonist L-(+)-2-amino-4-phosphonobutyric acid (L-AP4; 10μM) induced potentiations of 65.1 ± 14.3% (n=11; p<0.05). Mutation of K_{2p}2.1 serine 333 (reported as a protein kinase A phosphorylation site) to alanine (K_{2p}2.1_{S333A}) significantly attenuated L-AP4 induced potentiations to 11.9 ± 3.6% (n=7; p<0.01), whilst mutation of K_{2p}2.1 serine 300 (reported as a protein kinase A/C phosphorylation site) to alanine (K_{2p}2.1_{S300A}) significantly attenuated L-AP4 induced potentiations of the K_{2p}2.1 current to 34.2 ± 5.2% (n=4; p<0.05). Furthermore, following mutation of both S333 and S300 to alanine (K_{2p}2.1_{S333A/S300A}) L-AP4 induced potentiations were completely abolished (2.7 ± 2.9%; n=4; p>0.05). Finally, mutating K_{2p}2.1 serine 351 (reported as a protein kinase G phosphorylation site) to alanine (K_{2p}2.1_{S351A}), resulted in L-AP4 induced potentiations of 75.2 ± 15.6% (n=4; p>0.05) which were not significantly different from controls (p>0.05).

These data indicate that the robust potentiation of K_{2p}2.1 by activation of mGlu4 is mediated via modulation of the PKA pathway with both S300 and S333 playing a significant role. However, modulation of the PKG pathway does not appear to be involved. Thus, our increased understanding of the second messenger systems underlying this modulation may help elucidate the role of receptor induced activation of K_{2p}s in controlling neuronal excitability.

Cain SM & Bushell TJ (2005). *Soc Neurosci Abstr* **153.19**.

Goldstein SA, Bockenhauer D, O'Kelly I & Zilberberg N (2001). *Nat Rev Neurosci* **2**, 175-184.

Supported by the Medical Research Council, Glaxo-SmithKline, Stevenage, UK and Wyeth Research, Princeton, USA.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Effects of deletion of TASK-1 on the electrical properties of mouse dorsal vagal neurones

S.E. Hopwood¹ and S. Trapp¹

¹Dept of Anaesthesia, Pain Medicine & Intensive Care, Imperial College London, London, UK and ²Biophysics Section, Imperial College London, London, UK

The dorsal vagal nucleus expresses high levels of mRNA for the two-pore-domain K^+ channels TASK-1 and TASK-3 in rodents [1,2]. We have previously demonstrated that rat dorsal vagal neurones (DVN) express functional TASK-1-containing K^+ channels that are inhibited by 5-HT and modulated by pH_o changes [3]. Here we investigate their role in murine DVN using a recently developed TASK-1 KO mouse model [2].

Brainstem slices (200 μ m) were obtained from wild-type (WT; juvenile and adult) and TASK-1 KO (adult) C57Bl6 mice. Following recovery at 34°C for 60 min, slices were maintained in standard $NaHCO_3$ -buffered ACSF at RT. Whole-cell recordings were established using borosilicate glass electrodes (3–6 M Ω , filled with (in mM) 120 K-gluconate, 1 NaOH, 1 MgCl₂, 1 CaCl₂, 10 HEPES, 5 BAPTA, 2 K₂ATP, pH 7.3. Recordings were performed in HEPES-buffered ACSF [3]. Solutions of halothane were made up as fractions of a saturated solution at room temperature. The concentration of the saturated solution was taken to be 17.5 mM. Reservoirs containing the halothane solutions were sealed with a rigid plastic float, and all tubing and valves were made of polytetrafluoroethylene. All values are given as mean \pm 1 s.e.m.

DVN from juvenile WT mice had a resting potential (E_m) of -62 ± 3 mV ($n=16$) and 56% were spontaneously active. DVN from adult mice had an E_m of -72 ± 3 mV ($n=14$) and were not spontaneously active. The holding current at -20 mV (I_{H-20}) was 63 ± 8 pA ($n=14$) for juvenile and 85 ± 8 pA ($n=28$) for adult DVN. E_m and I_{H-20} were not significantly different between adult WT and

TASK-1 KO DVN. I_{H-20} of both WT and KO was unaffected by ZnCl₂ (100 μ M; $n=4$ each), suggesting the absence of homomeric TASK-3 channels.

Next we investigated the pH-sensitivity of I_{H-20} . Consistent with a role for TASK-1, acidification (pH 6.3) reduced I_{H-20} by 70 ± 26 pA in WT. The underlying current had a reversal potential of -94 ± 1 mV and was well described by the Goldman-Hodgkin-Katz (GHK) equation ($n=5$). In contrast, this current was not observed in TASK-1 KO ($n=9$). Raising pH from 7.3 to 8.5 elicited an outward current of 165 ± 28 pA ($n=10$) in WT and 167 ± 50 pA ($n=4$) in TASK-1 KO. However, the reversal potential of this current was -72 ± 4 mV and -71 ± 4 mV, respectively, and the current did not follow the GHK equation.

The volatile anaesthetic halothane (1 mM), a known activator of TASK, elicited an outward current at -20 mV in 9 and an inward current in 2 out of 16 WT DVN. In contrast, only an inward current (4/7) or no response to halothane was seen in TASK-1 KO DVN.

In agreement with our previous study in rat [3], the present data show that mice express functional acid-sensitive TASK-1-containing channels in DVN and that these are activated by halothane. Deletion of TASK-1 removes these channels, but no homomeric TASK-3 channels are observed [2]. In contrast to rat, mouse DVN exhibit an additional alkalinisation-induced outward current other than TASK.

Talley EM et al. (2001). J Neurosci 21, 7491–7505.

Aller et al. (2005) J Neurosci 25, 11455–11467.

Hopwood & Trapp (2005) J Physiol 568, 145–154.

This work was supported by an MRC Career Development Award to S.T. We thank W. Wisden and S.G. Brickley for providing TASK-1 KO mice.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA8

Posttranslational regulation of surface expression of the K_{2P} channels TASK-1 and TASK-3: role of ER-export signals, retention signals and accessory proteins

J. Daut

Physiology, Marburg University, Marburg, Germany

The two-pore-domain acid-sensitive potassium channels TASK-1 and TASK-3 play an important role in the central nervous system, in the heart, in smooth muscle and in many other tissues. Their surface expression is regulated not only at the transcriptional level but also at the posttranslational level. Both TASK-1 and TASK-3 have putative ER-retention signals near their N-terminal and C-terminal ends (1,2), but the functional relevance of these motifs is not yet clear. In addition, we have recently detected an ER export signal in the C-terminus of TASK-3 that promotes surface expression.

There are two important accessory proteins involved in the trafficking of TASK-1: 14-3-3 proteins (2,3), which also interact with TASK-3, and p11 (3,4), which is also denoted S100A10. 14-3-3 proteins bind to the C-terminal end of TASK-1, the essential 14-3-3 binding motif is RRx(S/T)x. Removal of the last amino acid prevents 14-3-3 binding and abolishes surface expression of the channel, indicating that interaction with 14-3-3 is necessary for passage through the secretory pathway.

The binding domain for p11 is in the proximal C-terminus of TASK-1 (4); removal of this domain strongly increased surface expression of TASK-1, indicating that binding of p11 causes retention/retrieval in the endoplasmic reticulum (ER). Live-cell imaging of GFP-tagged TASK-1 channels in transfected COS-7 cells showed that after 24 h wild-type TASK-1 was mainly localised to the ER, whereas TASK-1 mutants that were unable to interact with p11 were localised to the surface membrane. We found that ER-localisation of TASK-1 is mediated by a di-lysine retention signal, (K/H)xKxxx, at the C-terminus of p11 (4). Thus, p11 may act as a 'retention factor': binding of p11 to the channel causes ER-retention/retrieval. Our results suggest that surface expression of TASK-1 requires either dissociation of p11 or masking of the retention signal on p11.

Efficient ER export of correctly assembled ion channels is often achieved by masking of retention signals in one of the membrane protein subunits during the assembly process. The recruitment of a cytosolic retention factor is a second mechanism for post-translational regulation of surface expression.

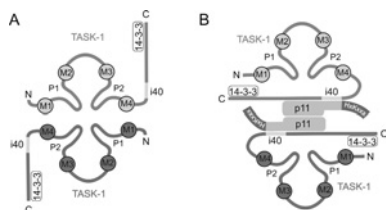


Figure 1. Topology of the interaction between TASK-1 and p11.

Girard C *et al.* (2002). *EMBO J* **21**, 4439-4448.

O'Kelly *et al.* (2002). *Cell* **111**, 577-588.

Rajan S *et al.* (2002). *J Physiol* **545**, 13-26.

Renigunta V *et al.* (2006). *Traffic* **7**, 168-181.

Supported by the Deutsche Forschungsgemeinschaft (SFB 593, TP A4).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA9

Regulation of the 2-pore domain potassium channel TRESK

P. Enyedi and G. Czirják

Department of Physiology, Semmelweis University, Budapest, Hungary

Members of the 2P (KCNK) channel subfamily are composed of two subunits, each possessing four transmembrane segments and two pore-domains. They express background/leak current, accordingly, they are open in a wide range of the membrane potential. A growing body of evidence indicates their important role in different cellular processes. Their activity can be regulated by extracellular factors i.e. by pH, temperature, hypoxia, membrane stretch etc., and by intracellular signalling mechanisms. The last member of the 2P potassium channel family, TRESK, was cloned from human spinal cord mRNA in 2003 (1), and subsequently from mouse cerebellum (2). While TRESK shows the characteristic structural features of 2P channel (two pore domains, and four transmembrane segments), it has an unusually long intracellular loop between the second and third TMS and a short C terminal intracellular tail. TRESK is regulated, like many other 2P channels; however, the mechanism of its regulation is unique in the 2P potassium channel family.

In order to study the electrophysiological properties and regulation of TRESK, we subcloned its cDNA into a *Xenopus* expression vector and expressed the channel in oocytes. TRESK current was measured by two electrode voltage clamp, and single channel patch clamping. The intracellular loop of the channel was expressed as a GST-fusion protein for experiments addressing protein-protein interactions.

TRESK was found to express a background potassium current, which was activated by calcium mobilizing agonists. Stimulation of the oocytes by coexpressed M1 muscarinic receptor increased the K^+ current 10.4 ± 1.4 -fold, ($n=5$). This effect could be mimicked by ionomycin and also by injection of saturated Ca^{2+} /EGTA buffer into the oocytes (6.2 ± 0.4 -fold ($n=57$) and 8.4 ± 1.3 -fold ($n=4$) stimulation, respectively). Under single channel conditions, however, application of high calcium to inside-out membrane patches failed to affect the channel activity. On the other hand, inhibitors of the calcium-activated protein phosphatase 2B (calcineurin, CN), cyclosporin or FK506 prevented the calcium induced activation. Coexpression of a truncated, constitutively active CN with TRESK in the oocytes elevated the basal current 3.3 ± 1.2 -fold ($n=16$), while the degree of stimulation in response to ionomycin was reduced from 6.3 ± 0.4 ($n=17$) to 2.2 ± 0.3 ($n=16$)-fold, indicating that the phosphatase activated the channel even in the absence of elevated calcium. Injection of a calcineurin inhibitory peptide into the oocytes, coexpressing constitutively active calcineurin with the channel, reduced the (elevated) basal current of TRESK, indicating that the catalytic

activity of calcineurin is necessary for the activation of the channel. As calcineurin is a serine/threonine phosphatase we mutated all of the intracellular Ser and Thr residues of the channel to alanine in order to localize the potential site of action of the phosphatase. The S276A mutant (of the intracellular loop), which mimics the dephosphorylated state, showed higher-than-normal basal activity (4.2 ± 0.7 -fold ($n=11$)), whereas the stimulation by calcium was diminished (only 1.22 ± 0.04 -fold activation ($n=11$)). On the other hand, the S276E mutant, mimicking the phosphorylated state, showed low basal activity, and at the same time its activation by ionomycin was also reduced.

A distinguished substrate of calcineurin is NFAT (nuclear factor of activated T cells). The dephosphorylation of NFAT, necessary for the activation and nuclear import of this transcription factor, requires the direct binding of calcineurin to a defined peptide motif of NFAT, which is distinct from the dephosphorylated site. The consensus sequence for this binding had been determined as PXIXIT. A similar sequence, PQIVID, was recognized in the intracellular loop of TRESK. Mutation of this sequence of the channel to PQIVIA, PQIVAD and PQAVAD progressively abolished the stimulation by ionomycin from the 7.5 ± 0.6 fold value of the wild type to 4.1 ± 0.3 , 1.6 ± 0.1 and 1.1 ± 0.1 -fold, respectively. The injection of a competing peptide "VIVIT" into the oocytes similarly reduced the activation of the current. These results suggest that calcineurin binds to the NFAT-like motif of TRESK and this interaction is necessary for the activation. The binding was confirmed also under *in vitro* conditions, using the purified GST/TRESK-loop protein. This protein, which was attached to glutathion agarose affinity matrix, bound constitutively active calcineurin. The interaction could be prevented also in this *in vitro* system by VIVIT peptide. The wild type calcineurin bound to the fusion protein with higher affinity in the presence of Ca²⁺/calmodulin, suggesting that the interaction of the two proteins is accelerated by the activation of the phosphatase *in vivo*.

Sano Y, Inamura K, Miyake A, Mochizuki S, Kitada C, Yokoi H, Nozawa K, Okada H, Matsushima H & Furuichi K (2003). *J Biol Chem* 278, 27406-27412.

Czirják G, Tóth ZE & Enyedi P (2004). *J Biol Chem* 279, 18550-18558.

Supported by the Hungarian National Research Fund (OTKA T46954) and the Hungarian Medical Research Council (ETT-085/2003).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA10

Multiple modulatory mechanisms of neuronal K2P channels

D. Bayliss

Pharmacology, University of Virginia, Charlottesville, VA, USA

Background or 'leak' potassium currents contribute to establishing membrane potential and input resistance in central neurons, and thereby to determining intrinsic neuronal excitability. It is now clear that the so-called two-pore-domain K⁺

(K2P) channels have the requisite functional properties and neuronal distribution to account for native neuronal background K⁺ currents (Goldstein et al. 2001). Moreover, K2P channels appear richly endowed with modulatory potential, as dynamic changes in channel activity can be evoked by physicochemical factors, bioactive lipids, anesthetic compounds and neurotransmitters (Goldstein et al. 2001). In this talk, I will discuss our work on identification of native neuronal correlates of K2P channels and on molecular mechanisms by which they are modulated.

We used *in situ* hybridization to describe a differential distribution of multiple K2P channels in the central nervous system, suggesting that distinct subsets of K2P channels contribute to background K⁺ currents in different types of neurons. The pH-sensitive TASK-1 (K2P3) and TASK-3 (K2P9) channels are widely distributed in the brain, with overlapping and particularly prominent expression in cholinergic and aminergic neurons (Bayliss et al., 2003). We have recorded from these neurons in brain slice preparations and isolated native currents that have properties essentially identical to those of cloned TASK channels, in either homomeric or heteromeric configurations (Bayliss et al. 2003; Berg et al. 2004). For some cell types (i.e. motoneurons), we have used recently available knockout mice to verify that native background K⁺ currents are indeed due to TASK channels (unpublished).

In heterologous expression systems and neurons, TASK channels are activated by inhalation anesthetics and inhibited by neurotransmitters that signal via Gα_q-linked receptors. By using channel mutagenesis, we found that up- and down-modulation by anesthetics and neurotransmitters involves a shared channel determinant that includes a conserved domain at the membrane-cytoplasmic interface of the C terminus (Talley & Bayliss, 2002). In terms of signaling pathways interposed between Gα_q-linked receptors and TASK channels, we manipulated G protein expression and membrane PIP₂ levels in intact and cell-free systems to provide electrophysiological and biochemical evidence that TASK channel inhibition proceeds via a mechanism that is independent of phospholipase C (PLC), the typical effector for Gα_q, and instead involves close association of activated Gα_q subunits with the channels (Chen et al. 2006).

Overall, this work demonstrates the importance of TASK-1 and TASK-3 channel subunits to neuronal background K⁺ currents and describes novel mechanisms by which these channels are regulated to control neuronal excitability.

Bayliss DA, Sirois JE & Talley EM (2003). *Mol Interv* 3, 205-219.

Berg AP, Talley EM, Manger JP & Bayliss DA (2004). *J Neurosci* 24, 6693-6702.

Chen X, Talley EM, Patel N, Gomis A, McIntire WE, Dong B, Viana F, Garrison JC & Bayliss DA (2006). *Proc Natl Acad Sci U S A* 103, 3422-3427.

Goldstein SA, Bockenhauer D, O'Kelly I & Zilberberg N (2001). *Nat Rev Neurosci* 2, 175-184.

Talley EM & Bayliss DA (2002). *J Biol Chem* 277, 17733-17742.

Supported by NS33583 and GM66181.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA11

Oxygen, acid and metabolic sensing via TASK-like background K⁺ channels in arterial chemoreceptors

K. Buckler and R. Varas

Department of Physiology Anatomy and Genetics, University of Oxford, Oxford, UK

The type-1 cell of the carotid body is a chemoreceptor which detects blood oxygen, pH and CO₂ levels. In response to hypoxia or hypercapnic acidosis these cells undergo membrane depolarisation followed by the initiation of electrical activity, voltage gated calcium entry and neurosecretion. The initial depolarisation in response to both hypoxic and acidic stimuli appears to be mediated primarily through the inhibition of a resting background K⁺ conductance.

The channels responsible for this acid and oxygen sensitive K⁺ current display many of the characteristics of tandem-p-domain K⁺ channels and the TASK subgroup in particular. Specifically, the type-1 cell background K⁺ channels are relatively voltage insensitive and resistant to block by the classical K⁺ channel inhibitors TEA and 4-AP. They are, however, inhibited by acidosis, quinine, barium, external divalent cations (Mg²⁺, and Zn²⁺) and are activated by halothane (but not chloroform).

We have recently observed that the background K⁺-channels of type-1 cells are also strongly inhibited by mitochondrial uncouplers, blockers of electron transport and blockers of ATP synthase. As in the case of hypoxia, this leads to rapid membrane depolarisation and calcium entry. Inhibitors of mitochondrial ATP synthesis have long been known to be very potent chemostimulants but it had been thought that their excitatory effects were due to calcium release from mitochondrial (and or other intracellular stores) rather than through changes in cell excitability. Inhibition of mitochondrial respiration was also observed to result in a loss of background K⁺ channel responsiveness to oxygen. These results suggest a fundamental link between cell energy status oxygen levels and the regulation of background K⁺ channel activity.

Recent studies in excised inside out patches have further revealed that these TASK-like background K⁺ channels are strongly activated (up to 8-fold) by cytosolic ATP with a K_{1/2} of approximately 2.3 mM. Changes in cytosolic ATP may therefore play an important role in the modulation of the activity of these channels both in response to changes in metabolism and oxygen.

This research was funded by the British Heart Foundation and the MRC.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA12

The functional consequences of altering two-pore potassium channel expression for the control of neuronal excitability

S. Brickley

Biophysics Section, Imperial College London, London, UK

Tandem pore potassium channels (K2P) set the resting membrane potential of many neuronal types due to the generation

of a potassium leak conductance. The leak conductance resulting from K2P expression also affects neuronal excitability by setting the input conductance. Based on in situ hybridization studies, the fifteen paralogous K2P genes have different, but often overlapping expression patterns. For example, adult cerebellar granule neurons (CGNs) express five K2P channel genes, TWIK-1, TASK-1, TASK-3, the TREK-2c splice variant and THIK-2 at high levels. However, identifying which K2P channels actually produce the leak conductance in any particular cell type is challenging, primarily because decisive pharmacological reagents are lacking. Thus, we believe that for the K2P family, gene knock-outs will prove essential for identifying the K2P channel composition found in vivo.

In this study we are primarily concerned with two K2P members: TASK-1 & TASK-3. The TASK-1 and -3 subunits are believed to form voltage independent non-inactivating K⁺ channels that are inhibited by acidic pH in the physiological range. In recombinant systems and in vivo they can assemble heteromERICALLY if the cell type expresses both genes at suitable levels; alternatively, they can function as homomers if either TASK-1 or TASK-3 dominates. Using a TASK-1 knockout approach we have recently demonstrated that in adult CGNs, the majority of channels contributing to the pH-sensitive component of the standing outward potassium current (IK(SO)) are heterodimers of TASK-1 and TASK-3. The TASK-1 KO strain has also recently been used to examine the subunit composition of TASK-like K⁺ channel populations found in other neuronal populations; e.g. thalamic relay neurons of the lateral geniculate nucleus and dorsal vagal neurons. All these neuronal populations express TASK-1 and TASK-3 mRNA. However, we see little functional evidence of functional TASK-1 containing K2P channels in the thalamus and the TASK-like conductance recorded from vagal neurons appears to be more consistent with a TASK-1 homodimeric population. This illustrates a complexity to TASK channel functional expression that makes it difficult to simply correlate mRNA patterns with expression of specific K2P types.

The ability of neurons to fire at high frequencies during sustained depolarisation is generally explained by the presence of voltage-gated ion channels. K2P channels are considered to be purely voltage independent channels with instantaneous activation/inactivation. However, CGNs lacking the TASK-3 type K2P channel exhibit marked accommodation of action potential firing in response to sustained depolarisation. We have examined the functional significance of TASK-3 channel expression in CGNs and, as expected, the magnitude of the standing outward leak conductance was significantly reduced in TASK-3 knockout mice. However, we also observed a reduction in the magnitude of a slowly inactivating conductance, not previously associated with K2P channel function. Examination of recombinant TASK-3 channel properties revealed that this K2P channel population does exhibit a kinetic component to its inactivation; a feature not shared by the closely related TASK-1 channel. We propose that the faster membrane time constant resulting from this TASK-3-mediated conductance enables CGNs to fire at high frequencies in response to sustained depolarization. This highlights a previously unappreciated consequence of K2P expression that will influence neuronal firing patterns in the CNS.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.