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Review

Two-pore domain K^+ channels—molecular sensors

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Abstract

Two-pore domain K^+ (K2P) channels have been cloned from a variety of species and tissues. They have been characterised biophysically as a 'background' K^+-selective conductance and are gated by pH, stretch, heat, coupling to G-proteins and anaesthetics. Whilst their precise physiological function is unknown, they are likely to represent an increasingly important family of membrane proteins.

Keywords: Potassium; Ion channel; Pore; pH sensitivity

1. Introduction

Potassium channels are ubiquitous, being found in cellular membranes throughout the plant and animal kingdoms. K^+ conductances are involved in the maintenance of fundamental cell properties such as growth, cell volume and membrane excitability. They also tend to dominate the cell conductance and are responsible for the maintenance of the negative resting membrane potential. In epithelia, the basolateral K^+ conductance permits recycling of potassium across the basolateral membrane to fuel the Na^+ pump, whereas apical K^+ channels allow K^+ excretion. The negative membrane potential generated by these K^+ movements also provides a supplementary driving force for the entry of Na^+ and co-transported solutes via channels and electrogenic secondary-active transport processes.

Since the introduction of molecular biology it has become apparent that there are three major families of K^+ channel: voltage-gated (Kv), inward rectifier (Kir) and two-pore domain (K2P) channels. Kv channels are activated by depolarisation, allowing the outward flow of K^+ in depolarised cells—these channels repolarise the membrane potential following an action potential. Kir channels on the other hand, preferentially conduct K ions in the inward direction, from the extracellular fluid into the cell. When the gradient for K^+ favours outward current flow, the pore of these channels becomes blocked by positively charged Mg^{2+} or polyamine molecules, leading to the rectification of current flow which gives this channel sub-family their name. Kir channels stabilise the membrane potential once it approaches the K^+ equilibrium potential. Current flow through K2P channels is determined predominantly by the prevailing electrochemical gradient for K^+, and *I/V* curves can be described by the GHK equation. Additionally, the open probability is independent of voltage, thus, these channels are thought to contribute to the resting membrane potential and are classified as background K^+ channels.

At first sight the structure of the three channel families is markedly different. Kv channels are comprised of six transmembrane (TM) domains, whereas Kir channels have only two and K2P channels have four (Fig. 1). All of these channel subunits have intracellular N- and C-termini, but there is little homology of the termini or the TM domains between groups. However, there is short stretch of about 20 amino acids known as the P (for pore forming) region which is highly conserved: it is this region which gives K^+ channels their remarkable ability to discriminate between K^+ and other cations with almost perfect distinction, yet facilitate the transmembrane passage of more than a million ions per second. The K2P channels have two P regions per subunit, whereas the others have one. The conserved sequence is T-X-G-X-G and is known as the 'K^+ channel signature sequence' [1,2]. Given this conserved amino acid sequence, the tertiary structure of K^+ channel pores is likely to be the same. In the case of the Kir channel KcsA, from

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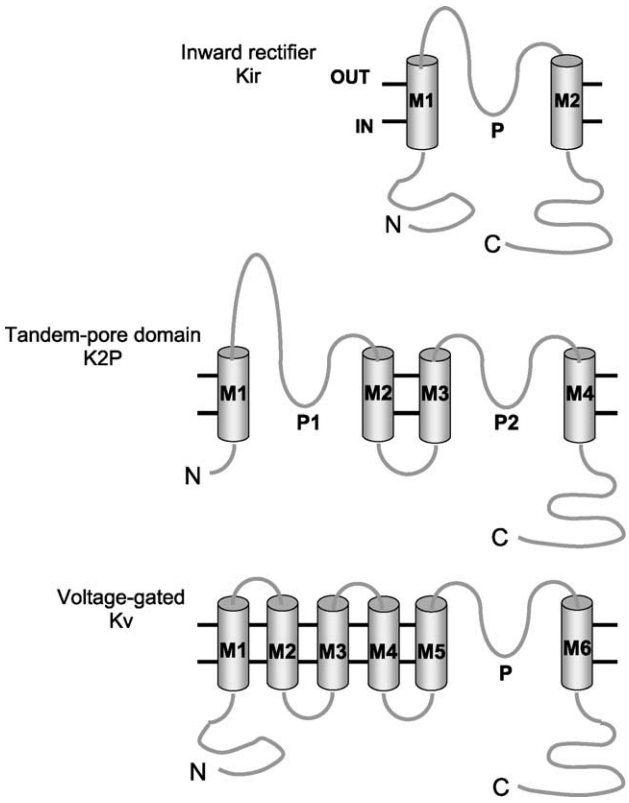

Fig. 1. Putative secondary structure of pore-forming subunits of major K^+ channel families. Transmembrane (TM) and pore (P) domains are numbered from the N terminus. K2P channels have an extended extracellular loop following the first TM domain. Functional channels comprise four P domains; i.e., either two (K2P) or four (Kir and Kv) subunits.

Streptomyces lividans, the structure has been resolved at the molecular level by X-ray crystallography [3,4]. KcsA is a multimeric channel, composed of four identical subunits assembled around a central ion-conducting pore. On the basis of the above and much other experimental work (summarised in Ref. [5]), it is currently thought that all functional K^+ channels are multimeric complexes comprising four P-domains which may be identical (homomeric) or different (heteromeric). Thus, functional Kv and Kir channels would contain four subunits, whereas K2P channels would comprise two subunits [6–8].

2. K2P channels

The first 2P-domain K^+ channel to be discovered was TOK-1 from the yeast *Saccharomyces cerevisiae* [9]. This channel has eight TMS and two P-domains in tandem in one subunit, and permits only outward current. However, this is the only channel to have been described thus far with eight TM domains and there are apparently no homologues in the genome of *C. elegans*. Functional 2P-domain channels have since been cloned from *Drosophila melanogaster* [10], *C. elegans* [11], and mammals (Tables 1 and 2). These channels all have four TM- and two P-domains. A characteristic of mammalian channels is that in the absence of a concentration gradient, they pass current equally well in both directions across the membrane. Given the normal concentration gradients, with high K^+ inside and low K^+ outside the cell, outward currents are larger than inward currents because of the availability of ions. This appears as an outward rectification of the currents that disappears if the external K^+ concentration is raised to cytosolic levels (for an example, see Ref. [12] for linear currents from TASK-1 expressed in oocytes in symmetrical K^+). There are over 50 putative K2P channels in *C. elegans* [11], which is about half of the animal's putative K^+ channel gene complement. In just 5 years, 14 subunits of these channels have been cloned in mammals, raising the possibility that many more mammalian K2P channels may await discovery.

2.1. Nomenclature

Nomenclature has become somewhat confused, with use of common names, usually given by their discoverers, and the names approved by the Human Genome Nomenclature Committee (HGNC; http://www.gene.ucl.ac.uk/nomenclature). Since the K2P field is relatively new, and several of the channels were cloned in parallel, there has inevitably been some duplication, e.g. TASK-1 has also been called cTBAK. The common names generally describe the main pharmacological or functional characteristic. For example, TWIK-1 stands for *T*wo pore domain *W*eak *I*nward rectifying *K*$^+$ channel and TASK-1 for *T*WIK-related *A*cid-*S*ensitive *K*$^+$ channel, and the number stands for the chronological order that related channels have been cloned. Channels within the same functional group may not be closely related (Fig. 2): TASK-1 and TASK-2, while both sensitive to extracellular acid have only about 30% identity. TASK-2 is actually a closer relation to TREK-1 than it is to TASK-1. Not only has there been duplication of common names, there are also identical genes with multiple names, for example TWIK-2 is also known as KCNK-6 and KCNK-8 [8]. Notwithstanding the above, for the remainder of this paper K2P-domain channels will be named using their common name, whilst their gene names can be obtained from Tables 1 and 2.

2.2. Structure

As well as the obvious implications from the structural similarities to KcsA, there is some experimental evidence supporting the notion that K2P channels exist as dimers. TWIK-1 has a cysteine residue on the large extracellular loop between M1 and P1 at position 69. It was thought that two cysteine residues, one from each α-subunit, formed a di-sulfide bridge, thus stabilising the channel. When C69 was mutated to a serine, currents were absent, and it was assumed that the channel could not dimerise [13]; this was also true for TWIK-2 [14]. However, this mechanism cannot hold for all K2P channels since TASK-1 does not possess an

Table 1
Whole-cell rectification and localisation of mammalian TASK channels

Name	Synonym	Whole cell rectification	Cloned from	Location in each species	References
TASK-1	KCNK-3 (cTBAK-1)	GHK	Human, Mouse, Rat	$NB^{H\&R}$: Pancreas, placenta, brain, lung, prostate, heart atrium, kidney, uterus, small intestine, colon, skin, testis, skeletal muscle, stomach $RT\text{-}PCR^{M\&RB}$: Heart atrium and ventricle; ^{R}glomerulus and proximal tubule of kidney nephron ISH^{M}: Brain; cerbral cortex, CA1–CA4 pyrimidal cell layer, granule cells of dentate gyrus, habenula, paraventricular thalamic nuclei, amyloid nuclei, substantia nigra and Purkinje and granular cells of cerebellum. Heart; atrium IC^{RB}: Atrium and ventricle	$^{H\&M}$[15]; ^{R}[16]; ^{M}[35]; ^{R}[38]; ^{RB}[46]
TASK-2	KCNK-5	GHK	Human	NB^{H}: Kidney cortical distal tubule and collecting duct, pancreas, liver, placenta, small intestine $RT\text{-}PCR^{M}$: Brain, heart, skeletal muscle, colon, lung, uterus	[34]
TASK-3	KCNK-9 (KT3.2)	GHK	Guinea Pig, Human, Rat	$RT\text{-}PCR^{GP\&R}$: Brain, heart ventricle, kidney, liver, colon, stomach, testis, skeletal muscle, trace in heart atrium and aorta	^{R}[54]; $^{GP\&H}$[32]
TASK-4	KCNK-17 (TALK-2)	GHK	Human	RT-PCR: Liver, lung, placenta, pancreas, small intestine, aorta, heart atrium, colon, ovary, peripheral blood leukocytes, prostate, spleen, testis and thymus, small extent in brain	[31]
TASK-5	(KT3.3)	No current	Human	RT-PCR: Adrenal gland, skeletal muscle, placenta, brain, testis, thyroid, salivary gland, pancreas	[55]

Synonyms are those approved by HGNC (http://www.gene.ucl.ac.uk/nomenclature). NB, Northern blot; ISH, In situ hybridisation; RT-PCR, reverse transcriptase - polymerase chain reaction. GHK = follows Goldman rectification ([8] and/or listed reference). The following abbreviations indicate the species from which a channel was cloned or used in localisation studies: ^{GP}Guinea pig, ^{H}human, ^{M}mouse, ^{R}rat, ^{RB}rabbit.

analogous cysteine residue, yet TASK-1 injection into *Xenopus* oocytes or transfection of mammalian cells produces acid-sensitive currents [12,15–17]. Whilst there is evidence that TASK-1 exists as a homodimer [12], it has also been shown recently that a tandem construct of TASK-1 and TASK-3 yielded functional channels with properties intermediate to those two channel types [18]. Heteromerisation would permit greater functional diversity, but it is not a uniform property of K2P channels, since heterodimer formation was not observed between THIK-1 and THIK-2 [19].

Both pore regions in a single K2P channel subunit have the conserved TXGXG motif, so dimerisation of two such subunits might provide the structural framework for a K^+ channel with similar selectivity and permeation properties to KcsA. However, many K2P channels have a phenylalanine (F) in the GXG motif of the selectivity filter in the second pore domain (highlighted in Table 3) instead of tyrosine (Y). In homomeric channels, loss of this residue would lead to loss of K^+-selectivity. However, in K2P channels interaction between residue X in the GXG motif and a conserved aspartate (D) residue in the second pore domain immediately following the GXG motif can determine ion-selectivity and gating properties. Thus, the deviation from the conventional GYG pore motif allows for more varied permeation and gating properties in K2P channels [20].

Table 2
Whole-cell rectification and localisation of functional mammalian K2P channels

Name	Synonym	Whole cell rectification	Cloned from	Location in each species	Reference
TWIK-1	KCNK-1	No current	Human	NB: Heart, brain, placenta, lung, liver, kidney, pancreas	[51]
TWIK-2	KCNK-6	No current	Human	NB^{H}: Placenta, pancreas, heart, colon, spleen, peripheral blood leukocytes, lung, liver, kidney, thymus, brain	[52]
TREK-1	KCNK-2	GHK^{M} or voltage-dependent^{R}	Human, Mouse, Rat	NB and ISH^{M}: Brain, lung, kidney, heart, skeletal muscle	$^{M\&H}$[22]; $^{R\&H}$[24]
TREK-2	KCNK-10	GHK	Human, Rat	NB^{R}: Brain, pancreas $RT\text{-}PCR^{R}$: cerebellum, spleen, testis	[26]
TRAAK	KCNK-4	GHK	Human, Mouse	NB^{M}: Brain, skeletal muscle, liver, lung, kidney, testis RT-PCR and ISH^{M}: Brain, spinal cord, retina	^{M}[25]; ^{H}[27]
THIK-1	KCNK-13	In (weak)	Human, Rat	$RT\text{-}PCR^{R}$: Muscle, heart, testis, spleen, lung, brain, kidney, liver, stomach ISH^{R}: Many brain areas including Hippocampus, thalamus and olfactory bulb granule cell layer	[19]
TALK-1	KCNK-16	GHK	Human	NB: Pancreas	[53]

Synonyms are those approved by HGNC (http://www.gene.ucl.ac.uk/nomenclature/). NB, Northern blot; ISH, in situ hybridisation; RT-PCR, reverse transcriptase-polymerase chain reaction. GHK = follows Goldman rectification (Ref. [8] and/or listed references). The following abbreviations indicate the species from which a channel was cloned or used in localisation studies: ^{GP}Guinea pig, ^{H}human, ^{M}mouse, ^{R}rat.

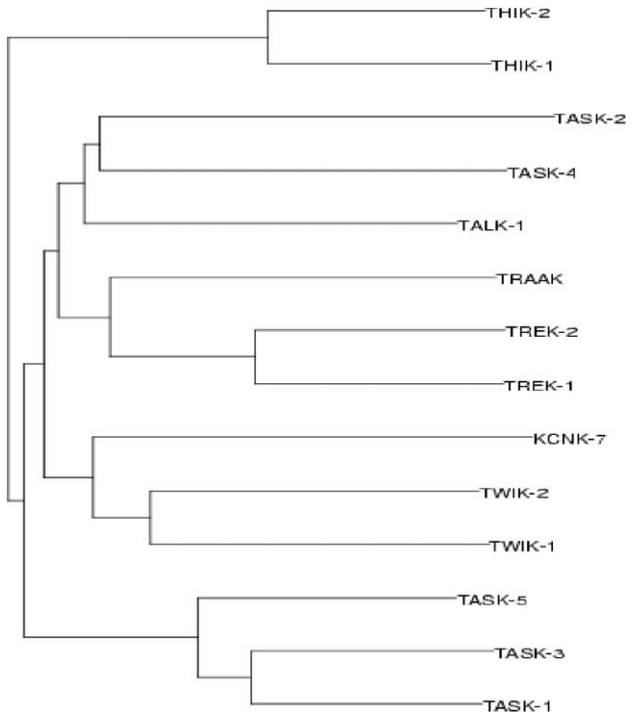

Fig. 2. Phylogenic tree of cloned human 2P-domain K^+ channels. Note that TASK channels are divided into two families. Alignments performed using Clustal NJ Plot software http://igbmc.u-strasbg.fr:8080/DbClustal/dbclustal.html).

2.3. Distinguishing features and pharmacology

The pharmacological sensitivities of mammalian K2P channels are varied, but generally they are relatively insensitive to classical K^+ channel blockers such as TEA^+, Ba^{2+} and Cs^+ (Table 4). TASK-1 channels are reportedly selectively inhibited by submicromolar concentrations of the endocannabinoid anandamide [21]. A number of novel regulatory properties have been uncovered. For example, TREK-1 is stretch-activated [22] and heat-sensitive [23], where a sevenfold increase in current accompanies a 10 °C increase in temperature, compared to a twofold increase in TASK-1 current. Heat-sensitivity is removed by intracellular cyclic adenosine monophosphate (cAMP), and by mutation of serine (S) at position 333 to alanine, where serine is a site for phosphorylation by protein kinase A [23]. Another interesting property of TREK-1 is that it changes from a leak channel to a voltage-gated channel upon phosphorylation of S333 [24]. In addition, TREK-1, TREK-2, TRAAK and THIK-1 are stimulated by internally applied arachidonic acid and polyunsaturated fats [19,22,25–27].

K2P channels are generally sensitive to anaesthetics, and usually show differential sensitivity to inhalation (e.g., halothane, isoflurane) and local (e.g., lidocaine, bupivicaine) agents. Table 5 summarises the published information on K2P anaesthetic sensitivity. TWIK-2 and TRAAK showed no responsiveness to anaesthetics [14,28]. The anaesthetic sensitivity of K2P channels lends further credence to their putative role as major determinants of the cell membrane potential and membrane excitability.

3. Task channels

TASK-1 and TASK-3 mRNA transcripts are found in neural tissue (Table 1), while TASK-2 is highly expressed in the kidney, and TASK-4 is ubiquitously expressed, but to a lesser extent in neural tissue. The four functional TASK channels (TASK-5 is nonfunctional) have different sensitivities to extracellular pH. Fig. 3 shows predicted H^+ dose–response curves for the channels in a low K^+ solution from currents at around +50 mV. The curves were plotted using the Hill Equation (Eq. (1)) with the values shown in Table 6.

$$\frac{I}{I_{MAX}} = \frac{1}{1 + \left(\frac{[H^+]}{K_D}\right)^n} \quad (1)$$

Where $[H^+]$ is the hydrogen ion (H^+) concentration, K_D is the dissociation constant that describes half-maximal block, and n is the Hill coefficient that describes the minimum amount of ions that may bind to a channel to cause block. It seems from these curves that only TASK-1 and TASK-2 have pK's which would allow regulation by the extracellular pH under normal physiological conditions (shown by the shaded vertical bar) unless the cell membrane in which the TASK channel was expressed resided in a specialised tissue or tissue compartment. Indeed, even for TASK-1, the predicted modulation by extracellular pH would be modest. The low pK of TASK-3 would suggest that it would be permanently open, but it is expressed in the stomach where the lumen pH is around 4.5 and the pH in the gastric pits themselves may be less than one [29]. In the absence of knowing the precise localisation of TASK-3 within the gastric epithelium its role remains speculative. TASK-4 has an extremely alkaline pK, and is expressed, amongst other places (Table 1), in the pancreas where the pH within the lumen of the secretory ducts is approximately 0.5 pH units alkaline to plasma [30]: even if the channels were located on the apical membrane of the duct cells the pK of TASK-4 may lie outside the range encountered physiologically. The physiological relevance of TASK-4 in particular is not clear, although co-expression with other channel subunits may alter its pH-sensitivity [31].

At least part of the molecular mechanism underlying pH-sensing involves a histidine residue which lies immediately distal to the first P domain of TASK-1 and TASK-3. Mutation of this amino acid causes a large reduction of pH-sensitivity [12,32]. This inhibition by protons is voltage-insensitive (Fig. 4), showing that protons do not enter the selectivity filter to cause block, but must exert their effect by titration of an amino acid(s) facing the extracellular medium. Mutation of this residue in TASK-1 to either the charged residue aspartate (H98D) or the neutral amino acid

Table 3
Pore domain amino acid sequence alignment of selected cloned K^+ channels

				Genbank accession number
Kv channels				
Kv1.1	D A F W W A V V	S M T T V G Y G	D M Y	*NM 00217*
Kv2.1	A S F W W A T I	T M T T V G Y G	D I Y	*NM 004975*
Kv3.1	I G F W W A V V	T M T T L G Y G	D M Y	*NM 004976*
Kir channels				
ROMK-1	S A F L F S L E	T Q V T I G Y G	F R C	*NM 000220*
IRK-1	A A F L F S I E	T Q T T I G Y G	F R C	*AF153820*
GIRK-1	S A F I F F I E	T E A T I G Y G	Y R Y	*NM 002239*
KcsA	R A L W W S V E	T A T T V G Y G	D L Y	*Z37969*
K2P channels				
TWIK-1	S A L F F A S T	V L S T T G Y G	H T V	*NM 002245*
	E S F Y F C F I	S L S T I G **L** G	D Y V	
TRAAK	S A F F F S G T	I I T T I G Y G	N V A	*NM 247042*
	E A I Y F V I V	T L T T V G **F** G	D Y V	
TREK-1	S S F F F A G T	V I T T I G F G	N I S	*NM 014217*
	D A I Y F V V I	T L T T I G **F** G	D Y V	
THIK-1	G A F Y F V G T	V V S T I G F G	M T T	*AF 287303*
	D S L Y F C F V	A F S T I G **F** G	D L V	
TASK-1	G S F Y F A I T	V I T T I G Y G	H A A	*AF 065163*
	Q A Y Y Y C F I	T L T T I G **F** G	D Y V	
TASK-2	N A M I F A A T	V I T T I G Y G	N V A	*NM 003740*
	E G L Y Y S F I	T I S T I G **F** G	D F V	
TASK-3	G S F Y F A I T	V I T T I G Y G	H A A	*NM 016601*
	H A Y Y Y C F I	T L T T I G **F** G	D Y V	
TASK-4	G S F F F S V S	T I T T I G Y G	N L S	*NM 031460*
	E G F Y F A F I	T L S T V G **F** G	D Y V	
TASK-5	G S F Y F A I T	V I T T I E Y G	H A A	*AF 294352*
	H A Y Y Y C F I	T L T T I G **F** G	D F V	

The K^+ channel pore signature sequence is boxed. Reference numbers at right are Genbank accession numbers (http://www.ncbi.nlm.nih.gov/entrez). Hydrophobic Phenylalanine (F) or Leucine (L) residues in the selectivity filter of the second pore domain of some K2P channels are highlighted (see text).

Table 4
Pharmacological properties of K2P channels

Name	Classical blockers					Physiological sensitivity				PKA activators via ↑ internal [cAMP]				PKC activators
	Quinine 100 μM	TEA^+ 1 mM	4-AP 1 mM	Cs^+ 100 μM	Ba^{2+} 100 μM	Stretch	AA 10 μM	Acid INTERNAL pH 6	Acid EXTERNAL pH 6	IBMX 1 mM	Forskolin 10 μM	IBMX + Forskolin	$cAMP^{\pm}$	PMA 40 nM
TASK-1	X	X	X	↓	X	X	X	X	↓	X	X			X
TASK-2	↓	X	X	X	↓		X	X	↓	X	X	X	X	X
TASK-3	↓	X		X	X		X	X	↓			X		X* or ↓**
TASK-4	X	X	X	X	↓		X	X	↓			X		X
TWIK-1	↓	↓	X	X	↓	X	X	↓	X		X		X	↑
TWIK-2	X				↓			↓	X			X	X	↑
TREK-1	X	X		X	↓	↑***	↑	↑				↓		↓
TREK-2		X			↓	↑	↑	↑		↓			↓	X
TRAAK		X	X	X	X	↑****	↑	X	X	X	X	X		X
THIK-1							↑	X	↓					
TALK-1	↓	X	X	X	↓		X	X	↓	X	X	X	X	X
TALK-2	↑	X	X	X	↓		X	X	↓	X	X	X	X	X

A reagent is considered effective if it changes the measured parameter from normal by 30%.
Key: ↓ = currents inhibited. ↑ = currents increased. X = no effect. Blank cell = no experiment reported. AA = arachidonic acid, TEA^+ = tetraethylammonium, 4-AP = 4-amino pyridine, PKC = phosphkinase C, IBMX = 1-methyl-3-isobutylxanthine, PMA = phorbol-12-myristate-acetate. $^{\pm}$8-Bromo-cAMP is a permeant form of externally applied cAMP and is used to assess effects of intracellular cAMP. Experiments where the effects of AA were investigated also included experiments investigating the effects of polyunsaturated fats, which gave similar results. Saturated fats had no effect.
References: TASK-1 = [15]; TASK-2 = [34]; TASK-3 = [32]; *[56]; **[57]; TASK-4 = [31]; TWIK-1 = [51]; TWIK-2 = [14]; TREK-1 = [22]; *** also heat activated [23]; TREK-2 = [26]; TRAAK = [25] and ****[27]; THIK-1 = [19]; TALK-1 = [53].

Table 5
Anaesthetic-sensitivity of mammalian K2P channels

Name	Inhalation anaesthetic sensitivity	References	Local anaesthetic sensitivity	References
TASK-1	POTENTIATED BY: Halothane^{MR} isoflurane^{M} ACTIVATED BY: Halothane^{M}	[28]^{M}, [59]^{R}	INHIBITED BY: Mepivacaine, lidocaine, (R)-ropivacaine, (L)-ropivacaine, tetracaine, bupivacaine and etidocaine	[58]^{H}
TASK-2	POTENTIATED BY: Halothane, isoflurane, desflurane, enflurane, chloroform	[60]^{H}	INHIBITED BY: Lidocaine, bupivacaine	[34]^{H}
TASK-3	POTENTIATED BY: Halothane INHIBITED BY: Alphaxolone NO EFFECT: Pentobarbitol, ketamine	[56]^{H}	INHIBITED BY: Lidocaine, bupivacaine	[54]^{R}
TASK-4	No information		INHIBITED BY: Lidocaine, bupivacaine	[31]^{H}
TWIK-1	No information		INHIBITED BY: Bupivacaine	[58]^{H}
TREK-1	ACTIVATED BY: Halothane POTENTIATED BY: Chloroform, halothane, isoflurane	[28]^{M}	INHIBITED BY: Bupivacaine, tetracaine	[43]^{H}
TREK-2	No information		NO EFFECT: Lidocaine, bupivacaine	[26]^{R}
THIK-1	INHIBITED BY: Halothane	[19]^{H}	No information	
TALK-1	INHIBITED BY: Halothane NO EFFECT: Isoflurane	[53]^{H}	INHIBITED BY: Chloroform	[53]^{H}
TALK-2	INHIBITED BY: Halothane ACTIVATED BY: Isoflurane	[53]^{H}	INHIBITED BY: Chloroform	[53]^{H}

ACTIVATED BY = activates an inactive channel (outside-out patch); POTENTIATED BY = augments currents already active without anaesthetic. ^{H}Human, ^{M}Mouse, ^{R}Rat. TWIK-2 and TRAAK showed no response when exposed to anaesthetics [14,28].

asparagine (H98N) gave a reduction in pH-sensitivity (Fig. 4) [33]. However, whilst the pH-sensitivity has been markedly reduced it is notable that the channel is still sensitive to the external pH. This remaining pH sensitivity cannot be due to titration of N98 since this is a neutral residue incapable of binding protons. Thus, removal of H98 uncovers some other pH sensing mechanism in TASK-1, which in WT channels is overridden by the higher pH sensitivity of H98. Consistent with the idea of an additional pH-sensing mechanism independent of H98, TASK-2 and TASK-4 do not possess an equivalent histidine residue, and yet are sensitive to extracellular pH [34,31]. The similarities between TASK-1 and TASK-3 on the one hand and TASK-2 and TASK-4 on the other, which were evident from their sequence homology (Fig. 2), are also evident in terms of their pH-sensitivity. Inspection of Fig. 3 and Table 6 shows that the Hill coefficient for TASK-1 and TASK-3 is greater than 1, indicating positive co-operativity in the pH-sensing machinery, whereas this is not the case for TASK-2 and TASK-4 where the Hill coefficient is not greater than unity. Similarly, TWIK-1 does have a residue analogous to H98 but the channel is not pH-sensitive. Therefore, H98 on its own is not enough to confer pH-sensitivity on TASK-1 channels, and must work in conjunction with some other residue or region within the channel. This seems not to be the case for TASK-3, where H98 appears to confer full pH sensitivity [32]. In summary, there must be at least two different mechanisms underlying pH-sensitivity in TASK channels, one involving a pore-neighbouring His residue in conjunction with another part of the channel, and another mechanism independent of this His residue.

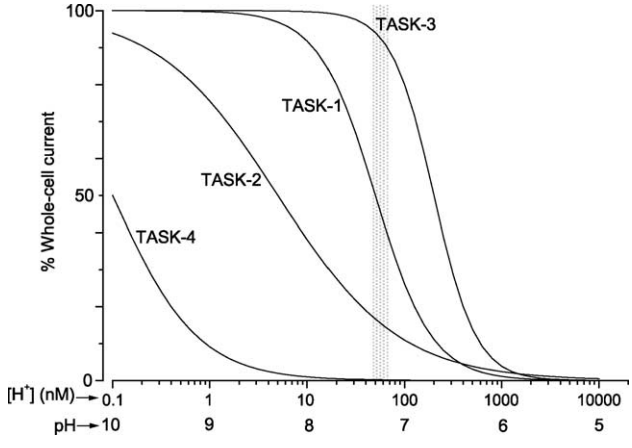

Fig. 3. H^+ dose–response curves for the four functional TASK channels. Curves are predicted using the Hill equation (Eq. (1)) from Hill coefficients and K_D values derived from the literature (Table 6). The shaded region identifies the normal pH range of extracellular fluid.

3.1. Known biophysical characteristics of TASK-1 currents

The pharmacological and physiological sensitivities of TASK-1 (and other K2P channels) are shown in Table 4. However, there are some additional features. First, increases

Table 6
K_D and Hill coefficients for functional TASK channels

Channel	K_D (pH)	Hill coefficient	References
TASK-1	7.3	1.5	[15]
TASK-2	8.3	0.7	[34]
TASK-3	6.7	2.0	[54]
TASK-4	10	1.0	[31]

The values for TASK-4 are estimates that give a H^+ dose–response curve similar to that presented in Ref. [31].

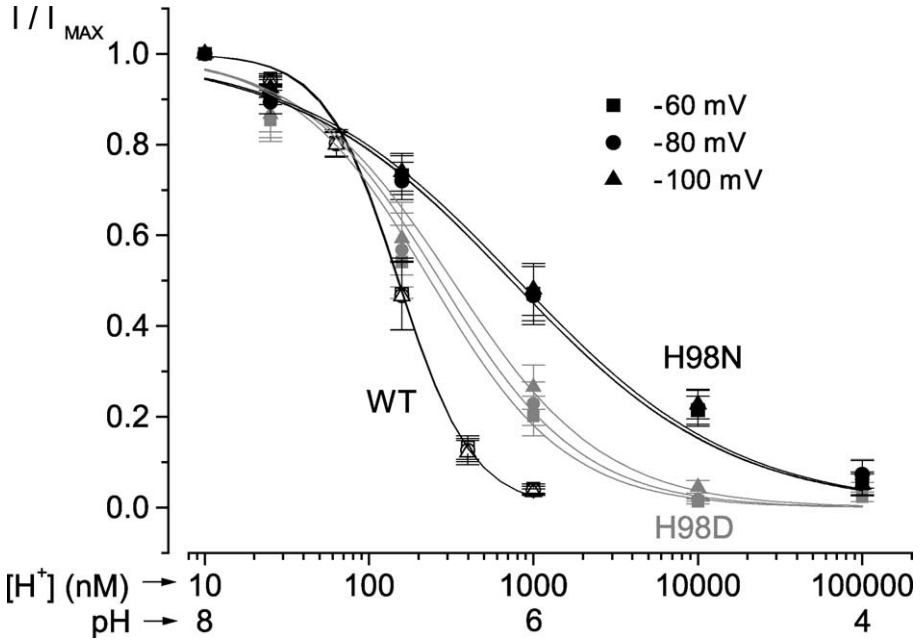

Fig. 4. pH-sensitivity of WT TASK-1 channels and H98D and H98N mutants. Normalised conductance is plotted against bathing-medium proton concentration and pH. Measurements were taken at −60, −80 and −100 mV as indicated. Data points are means ± S.E. (n = 6).

in external K^+ concentration causes outward currents to increase in magnitude [17]. Second, time-dependent gating has been observed [17]. Currents do not activate instantly, instead there is a time-dependent component that is altered by changes in external concentration of K^+ or H^+, or voltage. This has not been reported in previous publications [15,16,35] presumably because currents are activated so quickly that the onset of the currents is difficult to distinguish from the capacitance spike in electrophysiological recordings. Third, single channel conductance has been calculated to be between 12 and 16 pS with flickery kinetics [16,17,35,36].

3.2. Significance of TASK-1 in vivo

3.2.1. Distribution of TASK-1

TASK-1 is widely distributed amongst mammalian tissues. In particular, TASK-1 is found in brain cortical tissue [15,16,35], motoneurons [37] and cardiac tissue [15,16,35]. It is also found in epithelia, for example in the glomerulus of the kidney nephron [38].

3.2.2. Anaesthetic action in neural tissue and cardiac myocytes

Clinically relevant doses of some anaesthetics alter K2P properties by activating, augmenting or inhibiting currents (Table 5). With K2P channels expressed throughout neural tissue, it is reasonable to suggest that these channels may play a significant role in anaesthesia. In addition, TASK-1 is sensitive to volatile anaesthetics and is present in the heart, and may account for the depression of cardiac function in anaesthetised patients [8,28].

3.2.3. A background current in neural tissue

Neuronal excitability is altered by neuromodulators [5,8]. Talley et al. [37] used hypoglossal motoneurons from rat to show that a background K^+ conductance is created by TASK-1 channels. Acid, serotonin, norepinephrine, substance P and thyrotropin-releasing hormone directly inhibit the background conductance. Cloned TASK-1 channels expressed in HEK 293 cells are also inhibited by these substances. Cloned TASK-1 co-expressed with thyrotropin-releasing hormone receptor T1 ($G_{\alpha q/11}$-protein linked metabotropic receptor) was indirectly inhibited by the metabotropic glutamate receptor agonist 3,5,dihydroxyphenylglycine. This family of G-proteins are linked to phospholipase C (PLC) activation that leads to the production of diacylglycerol and IP_3. Neither of these products affects TASK-1 directly [15,16]. Rather PLC directly inhibits TASK-1 currents, without the need for the products of PLC activation [39]. Indeed, G-protein inhibition of a K^+ current in sympathetic neurons, the M current, also does not involve PLC activation [37]. It has been proposed that some channels (e.g.: GIRK-1/4, ROMK-1 and Kir2.1) directly bind phosphatidylinositol 4,5-bisphosphate (PIP_2)—a substrate in the PLC pathway—which increases their activity [40]. Perhaps it is a reduction of plasma membrane PIP_2—a consequence of PLC pathway activation—that causes channel inhibition.

Similar experiments have demonstrated TASK-1 to be the background conductance in rat cultured cerebellar granule neurons (CGNs) [41]. The background K^+ current in these cells is called IK_{SO} (standing-outward K^+ current; [42]), and was identified as a TASK-1-mediated current using multiple lines of evidence. First, IK_{SO} was inhibited

by extracellular acid in a similar manner to cloned channels expressed in *Xenopus* oocytes. This distinguished the current from the pH-insensitive TREK-1. Second, the current was only transiently inhibited by 10 μM arachidonic acid in the same manner as cloned TASK-1 [43], while TREK-1 is exquisitely sensitive to arachidonic acid. Third, currents from slices of mouse brain were relatively voltage-independent, rapidly activating and non-inactivating, just as cloned TASK-1 currents are. Fourth, IK_{SO} was sensitive to extracellular Ba^{2+} over the same range as cloned TASK-1 channels in oocytes. Accompanying the electrophysiological data was RT-PCR data showing TASK-1 presence in cultured rat CGNs, and antibody labelling demonstrated TASK-1 in 7-day-old cultured CGNs, cultured glial cells and adult rat brain slices.

Brickley et al. [44] also demonstrated a background current with TASK-1-like properties in $GABA_A$ receptor-deficient CGN cells (lacking α6 and δ subunits) from mice. In these cells a TASK-1-like current increased to compensate for the lack of current usually stimulated by the tonic release of GABA from CGNs. Brickley also demonstrated that TASK-3 was not the channel responsible for the background current because the amount of TASK-3 mRNA declined after birth, yet both TASK-1 mRNA and current persisted.

3.2.4. A background current in cardiac tissue

A background current, I_{KP}, exists in cardiac myocytes [8,45]. Kim et al. [35] isolated a messenger RNA transcript from mouse heart that was identical to mouse TASK-1 [15] except for an additional nine amino acids in the N-terminus. These splice variants are not pharmacologically or biophysically distinct [36]. In cardiac myocytes, I_{KP} influences the amplitude and duration of the action-potential plateau, and consequently the duration of contraction [8]. TASK-1 and I_{KP} have similar properties [8] and TASK-1 has been electrophysiologically identified in rat heart ventricle myocytes [36]. RT-PCR and immunocytochemical studies have localised TASK-1 to both atrial and ventricular myocytes of the rabbit [46]. It is likely therefore that TASK-1 contributes to the background current in the heart.

3.2.5. Significance in other tissues

Native rat glomerulosa cells, oocytes injected with mRNA from rat glomerulosa cells, and oocytes co-injected with rat TASK-1 mRNA and the angiotensin II receptor, all displayed pH-sensitive and angiotensin II-sensitive K^+ currents [47]. In native tissue and mRNA-injected oocytes, pH-sensitivity was not as pronounced as that in TASK-1 injected oocytes. However, together with electrophysiological data from all-three cells types and Northern blot analysis, it was concluded that TASK-1 contributes to the background current in glomerulosa cells [47].

It has also been suggested that TASK-1 mediates the background current in rat carotid body [48]. These cells display many of the hallmarks of TASK-1 biophysics and pharmacology. First, the background current was insensitive to 10 mM TEA^+ or 5 mM 4-AP. Second, in symmetrical K^+ the current was linear and reversed at zero mV. Third, single channel activity was flickery and had a conductance of 14 pS, all as previously reported for TASK-1. Fourth, sensitivities to Ba^{2+}, pH and anaesthetics were all consistent with a TASK-1-like channel. In addition, in situ hybridisation confirmed the presence of TASK-1 mRNA in Type-1 cells. Interestingly, K^+ currents in cell attached patches were sensitive to a drop in oxygen, suggesting that TASK-1 may play a role in oxygen sensing in this tissue.

3.2.6. Cell volume regulation

The regulated decrease in cell volume after an increase in cell volume in Ehrlich ascites tumour cells was found to be partly due to an outward TASK-like K^+ current [49,50]. This current is sensitive to extracellular pH, is inhibited by H^+ in a voltage-independent manner, displays GHK rectification and is inhibited by clofilium [49,50]. The molecular identity of the channel responsible for this current is unresolved, but is believed to be either TASK-1 [50] or TASK-2 [49].

Further identification of the physiological roles of K2P channels will be aided by the identification of specific channel blockers which are currently lacking.

4. Summary

K2P channels are found throughout the animal kingdom and represent at least part of the background or leak conductance, and thus contribute to the maintenance of the resting membrane potential. Far from being passive leaks, as was once thought, these channels turn out to be diverse in terms of their structure and regulation. Given their finite open probability at the resting membrane potential, and their sensitivity to perturbations in the extracellular fluid composition (e.g., pH and oxygen tension), membrane stretch and phosphorylation state, these channels are able to act as cellular sensors and transducers, translating these diverse stimuli into a change in the membrane potential and leading predictable changes in membrane excitability.

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