Molecular and functional properties of two-pore-domain potassium channels

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Lesage, Florian, and Michel Lazdunski. Molecular and functional properties of two-pore-domain potassium channels. Am J Physiol Renal Physiol 279: F793–F801, 2000.—The two-pore-domain K⁺ channels, or K₂P channels, constitute a novel class of K⁺ channel subunits. They have four transmembrane segments and are active as dimers. The tissue distribution of these channels is widespread, and they are found in both excitable and nonexcitable cells. K₂P channels produce currents with unusual characteristics. They are quasi-instantaneous and noninactivating, and they are active at all membrane potentials and insensitive to the classic K⁺ channel blockers. These properties designate them as background K⁺ channels. They are expected to play a major role in setting the resting membrane potential in many cell types. Another salient feature of K₂P channels is the diversity of their regulatory mechanisms. The weak inward rectifiers TWIK-1 and TWIK-2 are stimulated by activators of protein kinase C and decreased by internal acidification, the baseline TWIK-related acid-sensitive K⁺ (TASK)-1 and TASK-2 channels are sensitive to external pH changes in a narrow range near physiological pH, and the TWIK-related (TREK)-1 and TWIK-related arachidonic acid-stimulated K⁺ (TRAAK) channels are the first cloned polyunsaturated fatty acids-activated and mechanogated K⁺ channels. The recent demonstration that TASK-1 and TREK-1 channels are activated by inhalational general anesthetics, and that TRAAK is activated by the neuroprotective agent riluzole, indicates that this novel class of K⁺ channels is an interesting target for new therapeutic developments.

two-pore-domain channels; mechanosensitivity; anesthetics

POTASSIUM CHANNELS ARE PROTEIN complexes that form K⁺-selective pores in biological membranes. They allow the passive transport of K⁺ through membranes. They play a major role in the control of K⁺ homeostasis and cell volume but also in physiological functions that are associated with modifications of the electrical membrane potential such as neurotransmitters and hormone secretion and neuronal and muscular excitability. A wide variety of K⁺ currents have been recorded in vivo that can be distinguished according to their functional and pharmacological properties.

A considerable cloning effort during the last ten years has revealed the structure of many of these channels. They are multimers of hydrophobic subunits that form the iconic pore itself, often associated with accessory subunits (for review, see Refs. 9 and 19). More than 60 pore-forming subunits have now been cloned in mammals. They are classified into three groups according to their membrane topology. The largest group comprises subunits that contain a hydrophobic core with six transmembrane segments (6TMS) and one pore (1P) domain. This domain is directly involved in the formation of the selectivity filter that pro-
vides the specificity for K⁺ transport. The second family is formed by pore-forming subunits having only 2TMS and 1P domain. The extensive characterization of these two types of cloned subunits both in vitro and in vivo, as well as the isolation of associated regulatory subunits, has allowed the reconstitution of many different types of K⁺ channels such as voltage-gated K⁺ channels, Ca²⁺-dependent K⁺ channels, ATP-sensitive K⁺ channels, G protein-coupled K⁺ channels, and inward rectifiers.

The last group of K⁺-selective pore-forming subunits corresponds to proteins with 4TMS and 2P domains, instead of one as for the other K⁺ channel families. This unique feature is at the origin of their name, 2P channel subunits. GenBank accession nos. are indicated in brackets. TWIK-1 and TWIK-2, Tandem of P domains in Weak Inward rectifier K⁺ channels; TREK-1 and TREK-2, Tandem of P domains in Weak Inward rectifier K⁺ channels; TASK-1 and TASK-2, TWIK-related Acid-Sensitive K⁺ channels; KCNK6 and KCNK7, Silent subunits. Seven or eight homologous channels have already been identified, depending on the fact that KCNK6 and KCNK7 may be products of orthologous genes. This dendrogram was established with the use of ClustalW and Treeview software, and the scale bar is in arbitrary units. GenBank accession nos. are indicated in brackets. TWIK-1 and TWIK-2, Tandem of P domains in Weak Inward rectifier K⁺ channels; TASK-1 and TASK-2, TWIK-related Acid-Sensitive K⁺ channels; TREK-1, TREK-Related K⁺ channel; TRAAK, TWIK-Related Arachidonic Acid-stimulated K⁺ channel.

Because of its unique conservation between subunits belonging to the 6TMS/1P and 2TMS/1P classes, the pore domain was used extensively to identify, from public DNA sequence databases, new sequences potentially coding for novel K⁺ channel subunits. This approach resulted in the cloning of the 8TMS/2P channel TOK1 from yeast (20, 29, 47, 64) and the human 4TMS/2P channel TWIK-1 (30). Subsequently, seven TWIK-1-related subunits were cloned by degenerated PCR and by computational mining of DNA databases (7, 10, 13, 14, 22, 28, 31, 44, 48, 51) (Table 1). These subunits are 307–499 amino acid residues long and share a common structural organization as shown in Figure 1B. A dendrogram of the K₂P channel cloned in humans. Seven or eight homologous channels have already been identified, depending on the fact that KCNK6 and KCNK7 may be products of orthologous genes. This dendrogram was established with the use of ClustalW and Treeview software, and the scale bar is in arbitrary units. GenBank accession nos. are indicated in brackets. TWIK-1 and TWIK-2, Tandem of P domains in Weak Inward rectifier K⁺ channels; TASK-1 and TASK-2, TWIK-related Acid-Sensitive K⁺ channels; TREK-1, TREK-Related K⁺ channel; TRAAK, TWIK-Related Arachidonic Acid-stimulated K⁺ channel; h, human; m, mouse.

Fig. 1A. The major structural features are the four potential transmembrane segments (M1–M4), the 2P domains (P1 and P2), short NH₂-terminal and long COOH-terminal cytoplasmic parts, and an extended extracellular loop between M1 and P1. Outside the pore domains, these subunits do not share significant sequence homologies with the 6TMS/1P and 2TMS/1P subunits. Figure 1B shows a dendrogram deduced from the sequence alignment of the K₂P subunits cloned from mice and humans. The sequence homology between these subunits is usually low (not exceeding 45% for the human subunits), except between TWIK-1 and TWIK-2 (58%) and between TREK-1 and TRAAK (54%). This sequence conservation is associated with a conservation of some of the functional properties. However, this is not always the case. For example, TASK-1 and TASK-2 have similar functional properties (see Table 2) but are not particularly sequence related (<33% of homology). This indicates that sequence comparison is

![Fig. 1](http://ajprenal.physiology.org/)

### Table 1. Chromosomal locations of K₂P channel genes

<table>
<thead>
<tr>
<th>Channel</th>
<th>Gene</th>
<th>Chromosomal Location</th>
<th>Reference No.</th>
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<tr>
<td>TWIK-1</td>
<td>KCNK1</td>
<td>1q41-42</td>
<td>35</td>
</tr>
<tr>
<td>TREK-1</td>
<td>KCNK2</td>
<td>1q41</td>
<td>32</td>
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<tr>
<td>TASK-1</td>
<td>KCNK3</td>
<td>2p23</td>
<td>32</td>
</tr>
<tr>
<td>TRAAK</td>
<td>KCNK4</td>
<td>11q13</td>
<td>34</td>
</tr>
<tr>
<td>TASK-2</td>
<td>KCNK5</td>
<td>6p21</td>
<td>48</td>
</tr>
<tr>
<td>TWIK-2</td>
<td>KCNK6</td>
<td>19q13</td>
<td>18</td>
</tr>
<tr>
<td>KCNK7</td>
<td></td>
<td>11q13</td>
<td>51</td>
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</tbody>
</table>

K₂P, 2-pore-domain K⁺ channel; TWIK-1 and TWIK-2, Tandem of P domains in Weak Inward rectifier K⁺ channels; TASK-1 and TASK-2, TWIK-related Acid-Sensitive K⁺ channels; TREK-1, TWIK-Related K⁺ channel; TRAAK, TWIK-Related Arachidonic Acid-Stimulated K⁺ channel.
not sufficient for predicting the functional properties of K_{2P} channels.

Pore-forming K^+ channel subunits with 4TMS and 2P domains have also been identified in Drosophila (17) and Caenorhabditis elegans (59). In the nematode, >50 genes may encode K^+ channels belonging to this family. Because a total of 70–80 genes encode for potential pore-forming K^+ channel subunits in this animal model, the K_{2P} channels form the largest class. Sequence homology between nematode K_{2P} subunits, and between them and the human subunits, is low, with usually <35% of amino acid similarity. Except for TASK-1, no K_{2P} channel orthologs can be clearly identified between human and C. elegans (33). This is also the case for the K_{2P} channels in Drosophila, where three genes are related to TASK-1, whereas the eight others do not seem specially related to any human channel (unpublished observations). Whether a very large family of K_{2P} genes exists in mammals as in C. elegans will soon be verified, thanks to the human genome program. By mining the databases containing extracellular K^+ forming K_{2P} may encode K^+ channels belonging to this family. Because a total of 70–80 genes encode for potential pore-forming K^+ channel subunits in this animal model, the K_{2P} channels form the largest class. Sequence homology between nematode K_{2P} subunits, and between them and the human subunits, is low, with usually <35% of amino acid similarity. Except for TASK-1, no K_{2P} channel orthologs can be clearly identified between human and C. elegans (33). This is also the case for the K_{2P} channels in Drosophila, where three genes are related to TASK-1, whereas the eight others do not seem specially related to any human channel (unpublished observations). Whether a very large family of K_{2P} genes exists in mammals as in C. elegans will soon be verified, thanks to the human genome program. By mining the databases containing extracellular K^+ forming K_{2P} channels, the prediction of their secondary structure is always an amphipathic a-helix. This suggests that the ability of this domain to self-interact is a property that is common to all K_{2P} channels. The sequence of the M1P1 interdomain that is sufficient to promote the self-dimerization of fusion proteins. Secondary structure analysis of this domain predicts that it forms an amphipathic a-helix with a regular occurrence of charged residues and large apolar residues. This pattern is typical of the interdigitating helices. Cysteine 69, which is part of the self-interacting domain, is implicated in the formation of the interchain disulfide bond. Replacing this cysteine with either a serine residue in TWIK-1 (36) or an alanine residue in TWIK-2 (7, 30, 31) results in the loss of functional expression.

Finally, the extracellular location of the M1P1 domain of TWIK-1 was verified both by demonstrating the covalent dimerization of TREK-1 and TRAAK (7, 30, 31) results in the loss of functional expression. Since the original characterization of TWIK-1, these observations have been extended to the other K_{2P} channels. All cloned subunits except TASK-1 contain a cysteine residue at a position equivalent to cysteine 69 of TWIK-1 and all these subunits except TASK-1 are able to form covalent homodimers when heterologously expressed in insect or COS cells (unpublished observations). The covalent dimerization of TREK-1 and TRAAK was also observed in synaptic membranes. Despite low-sequence conservation between the M1P1 domains of the different K_{2P} channels, the prediction of their secondary structure is always an amphipathic a-helix. This suggests that the ability of this domain to self-interact is a property that is common to all K_{2P} channels. The sequence of the M1P1 extracellular domain in the different K_{2P} channels is very variable. Besides its role in the dimerization, this extracellular domain might well bind regulatory factors or extracellular ligands that would participate to the control of activity of this particular class of channels. However, data supporting such a role are not yet available.

Table 2. Comparison of the functional characteristics of the K_{2P} channels

<table>
<thead>
<tr>
<th></th>
<th>Electrophysiology</th>
<th>Pharmacology</th>
<th>Regulation</th>
<th>Reference(s)</th>
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</thead>
<tbody>
<tr>
<td>TWIK-1</td>
<td>336aa Inward rectifier</td>
<td>34 pS</td>
<td>Ba^{2+}/quinidine</td>
<td>[H^+], PKC activators</td>
</tr>
<tr>
<td>TWIK-2</td>
<td>313aa Inward rectifier</td>
<td>ND</td>
<td>General anesthetics</td>
<td>[H^+], PKC activators</td>
</tr>
<tr>
<td>TASK-1</td>
<td>395aa GHK rectifier</td>
<td>14 pS</td>
<td>Zn^{2+}/Local anesthetics</td>
<td>[H^{+}r, pIC_{50} = 7.3</td>
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<tr>
<td>TASK-2</td>
<td>499aa GHK rectifier</td>
<td>60 pS</td>
<td>Quinidine Local anesthetics</td>
<td>[H^{+}r, pIC_{50} = 8.3]</td>
</tr>
<tr>
<td>TREA-K</td>
<td>411aa Outward rectifier</td>
<td>100 pS</td>
<td>Quinidine/Gd^{3+}/cationic membrane cup formers</td>
<td>PKA and PKC activators, [Na^{+}_r, [H^+]_i, membrane stretch</td>
</tr>
<tr>
<td>TRAA-K</td>
<td>393aa GHK rectifier</td>
<td>45 pS</td>
<td>Gd^{3+}/cationic membrane cup formers</td>
<td>Membrane stretch</td>
</tr>
</tbody>
</table>

aa, Amino acid; ND, not determined; PUFA, polyunsaturated fatty acid; GHK, Goldman-Hodgkin-Katz; [H^{+}], and [H^{+}]_i, intracellular and extracellular H^+ concentration, respectively; P_{0.5} pressure to induce half-maximal activation.

<table>
<thead>
<tr>
<th></th>
<th>Behavior</th>
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<th>Blockers</th>
<th>Openers</th>
<th>Regulation</th>
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<td>Membrane stretch</td>
<td>14, 37</td>
</tr>
</tbody>
</table>
THE WEAK INWARD RECTIFIERS TWIK-1 AND TWIK-2

When expressed in heterologous expression systems, both TWIK-1 and TWIK-2 produce constitutive K⁺ currents of weak amplitude (7, 30, 31). These currents are quasi-instantaneous and nonactivating. A saturation of outward currents is observed for high depolarization indicating a weak inward rectification. TWIK-1 has a unitary conductance of 34 pS in symmetrical 140 mM K⁺ (30). As expected for a time-independent current active at all potentials, its expression is associated with a setting of the resting potential close to the K⁺ equilibrium potential (E_K) (30).

TWIK-1 but not TWIK-2 is blocked by Ba²⁺, quinine, and quinidine (50 μM > IC₅₀ > 100 μM). Both channels are slightly or not sensitive to the classic K⁺ channel blockers tetraethylammonium (TEA), 4-aminopyridine (4-AP), and Cs⁺. The regulatory properties are similar between the two channels (7, 30). They are regulated in opposite ways by activators of protein kinase C (PKC) and by acidification of the internal medium. PKC activation increases the TWIK currents, whereas acidification inhibits them. For TWIK-1, it has been demonstrated that these effects are indirect (30). The mutation of the unique consensus site for PKC phosphorylation does not modify the sensitivity to agents that activate PKC, and the inhibition by acidification is not seen in the inside-out patch configuration when the internal side of the channel is faced to the acidic medium. TWIK-1 and TWIK-2 are not sensitive to changes in extracellular pH and to treatments that activate protein kinase A (PKA).

The TWIK channels have widespread tissue distribution in adult mice (1, 31) and humans (Fig. 2). They are present in all examined tissues except in skeletal muscle. Together with their functional properties, their wide distribution suggests that these channels could be involved in the control of background K⁺ conductances in many cell types. Similar currents have been recorded in pancreatic acinar cells, where they maintain the resting membrane potential (52, 53). Like TWIK-1 and TWIK-2, these currents are inhibited by intracellular acidity, and they are not sensitive to TEA and 4-AP. However, their insensitivity to Ba²⁺ suggests that they are more probably carried by TWIK-2 than TWIK-1 (52). Weak inward rectifiers have also been reported in hepatocytes (56). Moreover, in these cells, intracellular acidification is known to cause a depolarization associated with the inhibition of a quinine-sensitive K⁺ conductance (4). Finally, compared with TWIK-2, TWIK-1 is highly expressed in the brain. In situ hybridization (31) indicates a distribution that is restricted to a few regions, and the strongest signals were seen in hippocampus and in cerebellar granule and Purkinje cells. TWIK-1 is expected to have a major role in the maintenance of the resting potential of neuronal cells that express it.

THE BASELINE ACID-SENSITIVE TASK-1 AND TASK-2 CHANNELS

TASK-1 was the first cloned mammalian K⁺ channel to produce currents with all the characteristics of background or baseline conductances (10, 22, 28). These currents are time and voltage independent: they are instantaneous with voltage changes (they do not display activation, inactivation, or deactivation kinetics), and their current-voltage relationships fit the curves predicted from the constant field theory for simple electrodiffusion through an open K⁺-selective pore. TASK-1 currents show an outward rectification in physiological asymmetric K⁺ conditions that is not observed in symmetric K⁺ conditions. The rectification can be approximated by the Goldman-Hodgkin-Katz current equation that predicts a curvature of the current-voltage relationships in asymmetric K⁺ conditions. Unlike TASK-1, TASK-2 currents display rapid activation kinetics (48). These kinetics are fitted with a single exponential characterized by time constants of 60 ms at +50 mV. Despite this difference, TASK-2, like TASK-1, shows no rectification other than that predicted by the Goldman-Hodgkin-Katz current equation and lacks intrinsic voltage sensitivity. TASK-1 and TASK-2 currents are highly flickering and have unitary conductances of 14 and 60 pS, respectively, in symmetric 150 mM K⁺ (24, 28, 48).

TASK-1 and TASK-2 are relatively insensitive to Ba²⁺, Ca²⁺, TEA, and 4-AP. TASK-2 (and to a lesser extent, TASK-1) is blocked by quinine (IC₅₀ = 22 μM) and quinidine (65% of inhibition at 100 μM). Zn⁺ is a better blocker of TASK-1 (IC₅₀ = 175 μM) than of TASK-2 (<15% of inhibition at 100 μM). Both TASK channels are inhibited by the local anesthetics lidocaine and bupivacaine, bupivacaine being the more potent blocker (IC₅₀ = 65 μM for TASK-1 and 81% of inhibition of TASK-2 at 1 mM) (25, 28, 48). TASK-1 was recently shown to be opened by volatile general anesthetics, halothane and isoflurane, at concentrations used in human general anesthesia (41).
The essential property of TASK currents is their extreme sensitivity to variations in external pH in a narrow physiological range (10, 24, 28, 48). As much as 90% of the maximal TASK-1 current is recorded at pH 7.7 and only 10% at pH 6.7. The pH value for 50% of inhibition is 7.3 at 0 mV (10). The sensitivity of TASK-2 is less sharp, with 90% of the current at pH 8.8 and 10% at pH 6.5. The pH value for 50% of inhibition is 8.3 at 0 mV (48). In both cases, the inhibition and activation produced no modification of current kinetics. The pH effects are due to a variation in the number of active channels and not in the single-channel conductances (48). TASK channels are insensitive to application of PKC activators, and only TASK-1 has been shown to be decreased by application of PKA activators (28).

The distribution of TASK channels is more restricted than the TWIK expression pattern (Fig. 2). However, TASK-1 and TASK-2 are present in many different tissues (pancreas, placenta, kidney, lung, liver, ovary, prostate, and small intestine), where they are supposed to contribute to maintenance of resting membrane potentials and/or to K+ transport associated with recycling or secretion. Both channels are present in nonexcitable tissues, but only TASK-1 is present in brain and heart. In rodent heart, TASK-1 is mainly expressed in atrial myocytes. In the brain, its expression is neuronal. The literature on baseline or leak K+ channels is not abundant compared with that on other types of K+ channels. This probably originates from the fact that they are difficult to study. They are voltage and time independent, and they have no specific pharmacology. Background K+ channels have been recorded in Bufo smooth muscle cells (39), in rat ventricular myocytes (2) and carotid bodies (5), and in bovine adrenocortical cells (12). They have also been recorded in different preparations of neuronal cells, in invertebrates, in Aplysia sensory neurons (55), leech AP neurons (43), Lymneae neurons (15), and lobster stretch receptor neurons (57), as well as in vertebrates, in bullfrog sympathetic ganglia (27), Xenopus myelinated nerve (26) and demyelinated axons (61), guinea pig submucosal neurons (54), and rat hippocampal (23, 45, 46) and premotor respiratory neurons (58). All these channels are quasi-instantaneous and noninactivating. They are also not gated by potential and exhibit outward rectification in physiological K+ conditions. When determined, their single-channel behavior is flickering. The majority of these currents are insensitive to TEA and 4-AP, and Ba2+ differentially affects them. TASK-1 could be a major contributor to these background conductances in excitable cells. In addition to maintenance of resting potential, it could also play a role in the modulation of electrical activity of these cells. The modulation of TASK-1 by external protons probably has important implications for its physiological function. Stimulus-elicited pH shifts have been characterized in a variety of neural tissues by using extracellular pH-sensitive electrodes. Electrical stimulation of Schaeffer collateral fibers in the hippocampal slice, or light stimulation of the retina or parallel fibers in cerebellum, produces pH shifts corresponding to bursts of H+ or OH-, creating small pH variations from the external physiological pH value of 7.4 (up to 0.3 pH unit in the alkaline or acidic direction). The variations might actually be larger in range or shorter in time course in the vicinity of the synaptic cleft. TASK-1 contains a potential site of interaction with synaptic proteins containing PDZ-domains, suggesting that it could be located at synapses. The strong modulation of TASK-1 by external pH favors the idea that extracellular variations in H+ concentrations can be a modulator of neuronal activity.

THE UNSATURATED FA- AND STRETCH-ACTIVATED TREK-1 AND TRAAK CHANNELS

TREK-1 and TRAAK have unique functional properties and represent the first cloned polyunsaturated FA and stretch-activated K+ channels. Like TASK-1, these channels produce instantaneous currents, which are outwardly rectifying in physiological K+ gradient. In high symmetric K+, TRAAK currents are linear like those of TASK-1, but TREK-1 still presents an outward rectification for strong hyperpolarizations. TREK and TRAAK channels are highly flickering, and their unitary conductances are 100 and 45 pS, respectively, in symmetric 150 mM K+ (13, 14).

In heterologous expression systems, TREK-1 and TRAAK currents have a low basal activity compared with the TASK channels. They can be strongly activated by application of AA (14, 34, 37, 42). This activation is reversible and concentration dependent. It is not prevented when the AA perfusion is supplemented with a mixture of inhibitors of the AA metabolism pathway, supporting the idea that the AA effect is direct and not due to another eicosanoid. This effect is specific to unsaturated FAs. Oleate, linoleate, arachidonate, eicosapentaenoate, and docosahexaenoate all strongly activate TREK-1 and TRAAK, whereas saturated FAs such as palmitate, stearate, and arachidate are ineffective. Another effective way for activating these channels is the application of stretch to the cell membrane. Both channels are activated by shear stress, cell swelling, and negative pressure (37, 38, 42). The pressure to induce half-maximal activation is −36 mmHg for TREK-1 and −46 mmHg for TRAAK. Disruption of the cytoskeleton by either biological or mechanical means (colchicine, cytochalasin, or membrane excision) potentiates the opening by membrane stretch. This result suggests that these channels are tonically repressed by the cytoskeleton but that their mechanogating does not require the integrity of the cytoskeleton. This also implies that the activating force is coming directly from the bilayer membrane. Moreover, agents that insert preferentially in one of the leaflets of the membrane and that modify the cell shape cause modification of the activity of these channels. The lipid bilayer anionic or neutral crenators open the channels, whereas the cationic cup formers inhibit both basal and stimulated activities (37, 42).
As expected for stretch-activated channels, TREK-1 and TRAAK are reversibly blocked by micromolar concentrations of Gd$^{3+}$. They are resistant to TEA and 4-AP and slightly sensitive to Ba$^{2+}$ at high concentrations. TREK-1 is blocked by quinidine (IC$_{50} = 100 \mu$M). Both channels are activated by riluzole, a neuroprotective agent used in the treatment of amyotrophic lateral sclerosis (11).

Compared with TRAAK, TREK-1 has additional features. Inhalational general anesthetics halothane and isoflurane activate TREK-1 as well as TASK-1 (41). However, unlike TASK-1, TREK-1 is also activated by chloroform and diethyl ether (41). In terms of regulation, TREK-1 but not TRAAK is inhibited by activators of PKC and PKA (13, 14). The phosphorylation site by PKA has been localized to the cytoplasmic COOH part of the channel (serine 333) (42). Finally, TREK-1 is opened by internal acidification. Lowering intracellular pH shifts the pressure-activation relationships toward positive values and leads to channel opening at atmospheric pressure (38). By mutagenesis, it has been shown that the COOH terminus of TREK-1 is critically involved in mechanogating, AA activation, and intracellular pH sensitivity (38, 42).

Human TREK-1 is mainly expressed in brain, ovary, and small intestine (Fig. 2). TRAAK is highly expressed in brain and placenta (34) (Fig. 2). This distribution is not strictly identical in the mouse, where TRAAK is specifically expressed in neuronal cells, whereas TREK-1 is present in more tissues than in humans (13, 14). Both channels are found in hippocampus, neocortex, cerebellum, brain stem nuclei, and olfactory bulb (13, 14). However, immunolocalization by specific antibodies has shown that the two channels have different subcellular locations. TRAAK is mainly present in soma and, to a lesser degree, in axons and dendrites (49), whereas TREK-1 is concentrated in dendrites in almost all neuronal types expressing the channel (unpublished observations).

TREK-1 shares many of the biophysical and pharmacological properties of the Aplysia $S$-type channel (42). This channel is expressed in sensory neurons of the mollusk, where it is known to play a major role in the regulation of synaptic transmission in Aplysia synapses (6, 55). Both channels are outwardly rectifying, time independent, and resistant to Ba$^{2+}$, TEA, and 4-AP, and they are opened by volatile general anesthetics (41, 60). AA and membrane stretch activate both channels, and they are blocked by serotonin via the PKA-cAMP pathway. The closure of the Aplysia channel by cAMP causes slow depolarization and a broadening of action potentials in the cell body. The enhanced excitability results in an augmentation of neurotransmitter release from sensory neurons. On the other hand, the opening of the channel by AA causes reduced excitability and lowering of neurotransmitter release. Background K$^+$ channels with properties similar to TREK currents were also recorded from mammalian neurons that are activated by application of baclofen, which binds to the GABAB receptor that is negatively coupled to adenylate cyclase (45, 58), or that are activated by application of volatile anaesthetics (60). Background K$^+$ channels activated by AA have been described in mammalian neurons (23, 46) and in heart (21) and smooth muscle cells (39). Our results suggest that TREK-1 and/or TRAAK underlies some of these currents. They also suggest that the distribution of this particular class of K$^+$ channels in the central nervous system is much more widespread than previously believed.

THE SILENT SUBUNITS KCNK6 AND KCNK7

The KCNK6 subunit has been cloned from mouse (51). It has the classic $4TMS/2P$ topology and contains a Ca$^{2+}$-binding EF hand motif. Although KCNK6 is able to dimerize as other functional K$_{ap}$ subunits when heterogously expressed in COS cells, it remains in the endoplasmic reticulum and is unable to generate ion channel activity at the cell surface. Mutagenesis experiments suggest that KCNK6 is not an intracellular channel but rather a subunit that needs to associate with a yet undiscovered partner to reach the plasma membrane (51). KCNK6 is mainly expressed in the embryo and in adult tissues such as eye, lung, and stomach. The highest level of expression is found in the eye, where in situ hybridization and immunohistochemistry showed that KCNK6 is only expressed in ganglion cells and in some neurons of the inner nuclear layer. In the mammalian retina, the first spontaneous Ca$^{2+}$ waves are observed at postnatal day 2 and are thought to result from Ca$^{2+}$ influx associated with a burst of action potentials seen in ganglion at this developmental stage. The early appearance of the KCNK6 in development, the fact that it has a Ca$^{2+}$-binding site potentially conferring Ca$^{2+}$ sensor properties, and its selective expression in ganglion cells suggest that this channel could play a role in the modulation of the electrical signal in the retina.

A human subunit, KCNK7, closely related to KCNK6, has been cloned (51). Despite 94% of sequence homology, KCNK7 and KCNK6 display several differences that question the possibility that these subunits are the products of orthologous genes in humans and mice. KCNK7 does not contain the EF hand motif of KCNK6, and its tissue distribution is wider than that of KCNK6, with the highest level of expression in peripheral blood leukocytes. In addition, a unique feature of KCNK7 is the presence of an unusual sequence in its second pore domain. An important element of the signature of K$^+$ channel function has long been recognized as being the pore domain GYG sequence. In the K$_{ap}$ channels, this GYG motif is replaced by GFG (TASK-1, TASK-2, TREK-1, and TRAAK) or GLG (TWIK-1 and TWIK-2). In KCNK7, a glutamic residue (GLE) is found instead of the strictly conserved glycine residue (GLG). This unusual sequence could be associated with a change in ionic selectivity. However, KCNK7, like KCNK6, failed to express channel activity by itself.
K\textsubscript{2P} CHANNELS IN THE KIDNEY

In the kidney, K\textsuperscript{+} channels are involved in the control of negative membrane potential, the regulation of cell volume, and K\textsuperscript{+} recycling or secretion. Several K\textsuperscript{+} conductances have been recorded in this tissue, with specific properties and locations in distinct cell types and membrane domains (for review, see Ref. 16). However, the corresponding molecular structures are not always clearly established. An important exception is ROMK, a 2TMS/1P channel that shares biophysical properties with a channel of cortical collecting ducts (CCDs) that constitutes the main way of K\textsuperscript{+} secretion in the principal cells. This secretion is essential for permitting sustained Na-K-2Cl cotransporter activity and renal Na\textsuperscript{+} reabsorption. Mutations in the ROMK gene cause Bartter's syndrome in humans, which is associated with salt wasting and hypokalemic alkalosis. Another small-conductance 2TMS/1P channel and two additional 6TMS/1P channels, cGMP gated (63) or glibenclamide sensitive (62), have been found in the kidney, but their exact roles are not completely understood.

Figure 2 shows that all K\textsubscript{2P} subunits are expressed in human kidney, the most abundant being TWIK-1 and TASK-2. TWIK-1 is also highly expressed in rat kidney, where it was immunolocalized (8). TWIK-1 is present in the brush-border membrane of the proximal convoluted tubules, in the thick ascending limb of the loop of Henle, and in the collecting duct intercalated cells, with intracellular and apical localization. Another study has shown by RT-PCR that TWIK-1 is localized in the distal nephron in rabbit kidney (40). K\textsuperscript{+} channels with a relatively low conductance have been described in the apical membrane of CCD cells. In addition to the conductance (30–35 pS), they share with TWIK-1 a low sensitivity to TEA and an inhibition by internal acidosis. However, the sensitivity to PKC effectors and cAMP via PKA is different between TWIK-1 and these particular channels. Whether these differences are real or reflect a different cellular context of expression (native cells vs. oocytes) remains unknown. Nevertheless, the particular localization of TWIK-1 suggests that it could play a role in K\textsuperscript{+} secretion complementary to ROMK. TASK-2 has been localized by in situ hybridization in human kidney (48). It is present in cortical distal tubules and CCDs. The biophysical and pharmacological properties of TASK-2 do not fit those of the native K\textsuperscript{+} channels that have been identified there. A possibility would be that TASK-2 has not yet been recorded in kidney cells, which would not be surprising because of the difficulty in identifying this channel in the absence of a specific pharmacology. Another possibility would be that TASK-2 associates with yet unidentified pore-forming subunits or regulatory proteins to produce active channels in native cells with properties different from those of the cloned channel. As for TWIK-1, the high level of expression of TASK-2 suggests that it plays a significant, and maybe even an important, role in renal K\textsuperscript{+} transport.

The stretch-activated TREK-1 channel is also expressed in the kidney. Stretch-activated K\textsuperscript{+} currents have been recorded in tubules cells, and it has been proposed that these currents are important in regulating cell volume (50).

CONCLUSION

Background K\textsuperscript{+} channels have originally been described in myelinated nerve, where sequential application of TEA, 4-AP, and Cs\textsuperscript{+} removed different K\textsuperscript{+} conductances. However, after these treatments, axons still exhibited a pronounced outward rectification. A residual K\textsuperscript{+} background conductance that was outwardly rectifying, as expected from the constant field theory, was present in nerve to set the resting potential (3). TASK-1 is the perfect background or baseline K\textsuperscript{+} channel: it is time and voltage independent, constitutively active, and insensitive to TEA, 4-AP, and Cs\textsuperscript{+}. In terms of rectification, kinetics of activation, or basal activity, the other cloned K\textsubscript{2P} channels are not perfect background channels. However, they are very close to this “ideal” behavior and, as expected, are able to polarize the membrane potential. If the cloning of K\textsubscript{2P} channels has provided access to a class of background K\textsuperscript{+} channels, it has also provided access to the molecular characterization of the previously recognized functional class of K\textsuperscript{+} channels activated by FAs and stretch. Because of their functional diversity and their widespread distribution, K\textsubscript{2P} channels are expected to fulfill many physiological roles in addition to setting resting membrane potential. The elucidation of these roles will require finding a specific pharmacology for these channels to better analyze their roles in vivo. The identification of specific blockers and openers is also promising in terms of therapy. Until now, the only widely prescribed class of K\textsuperscript{+} channel drugs in clinical use was active on ATP-sensitive K\textsuperscript{+} channels. With the recent demonstration that TREK-1 and TASK-1 are activated by volatile general anesthetics, and TRAAK by the neuroprotective agent riluzole, K\textsubscript{2P} channels now appear as valuable targets for the rational development of new drugs.

NOTE ADDED IN PROOF

Two novel K\textsubscript{2P} channels, TASK-3 and TREK-2, have been cloned that are structurally and functionally related to TASK-1 and TREK-2, respectively (Kim Y et al. J Biol Chem 275: 9340–9347, 2000; Rajan S et al. J Biol Chem 275: 16650–16657, 2000; Lesage F et al. J Biol Chem. In press). Recent studies have shown that TASK-1 is important for the control of motoneuron and cerebellar granule cell excitability (Talley EM et al. Neuron 25: 399–410, 2000; Millar JA et al. Proc Natl Acad Sci USA 28: 3614–3618, 2000); for oxygen sensing in the carotid bodies [Buckler KJ et al. J Physiol (Lond) 15: 135–142, 2000]; and for the generation of a high resting membrane potential in adrenal glomerulosa cells (Czirjak G et al. Mol Endocrinol 14: 863–8764, 2000). In all these cases, TASK-1 is active at rest, and its closure by neurotransmitter, hypoxia, or hormone is associated with a depolarization of the cell membrane and increase in cell excitability. In addition, another recent study shows that rat TWIK-2 generates inactivating currents of large amplitude, suggesting a particular...
role for this channel type in cell electrogensis (Patel AJ et al. J Biol Chem. In press.).

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