Two cDNAs encoding novel K⁺ channels, THIK-1 and THIK-2 (tandem pore domain halothane inhibited K⁺ channel), were isolated from rat brain. The proteins of 405 and 430 amino acids were 58% identical to each other. Homology analysis showed that the novel channels form a separate subfamily among tandem pore domain K⁺ channels. The genes of the human orthologs were identified as human genomic data base entries. They possess one intron each and were assigned to chromosomal region 14q24.1–14q24.3 (human (h) THIK-1) and 2p22–2p21 (hTHIK-2). In rat (r), THIK-1 (rTHIK-1) is expressed ubiquitously; rTHIK-2 expression was found in several tissues including brain and kidney. In situ hybridization of brain slices showed that rTHIK-2 is strongly expressed in most brain regions, whereas rTHIK-1 expression is more restricted. Heterologous expression of rTHIK-1 in Xenopus oocytes revealed a K⁺ channel displaying weak inward rectification in symmetrical K⁺ solution. The current was enhanced by arachidonic acid and inhibited by halothane. rTHIK-2 did not functionally express. Confocal microscopy of oocytes injected with green fluorescent protein-tagged rTHIK-1 or rTHIK-2 showed that both channel subunits are targeted to the outer membrane. However, coinjection of rTHIK-2 did not affect the currents induced by rTHIK-1, indicating that the two channel subunits do not form heteromers.

The family of tandem pore domain potassium (2P K⁺)1 channels can be divided into several subfamilies: (i) the acid-sensitive 2P K⁺ channels TASK-1 to -3 (1–6), (ii) the mechanosensitive channels TASK-1, TASK-2, and TRAAK (7–11) and (iii) the weakly inward rectifying 2P K⁺ channels TWIK-1, TWIK-2, and TASK-3. All 2P K⁺ channels have four transmembrane regions (M1-M4) and two typical pore-forming regions (P1 and P2) including the K⁺ selectivity filter consensus sequence TxG[YF]G (12) and a large extracellular loop between M1 and P1. The extracellular loop is thought to participate in dimerization of subunits, which in some 2P K⁺ channels may involve disulfide bond formation (16). The N terminus of the 2P K⁺ channels is usually very short, whereas the C-terminal domain is much larger and determines many functional properties.

Relatively little is known about the function of 2P K⁺ channels in vivo. Recently, endogenous currents with properties similar to those of TASK channels have been found in the heart (17), in arterial chemoreceptor cells (18), in zona glomerulosa cells of the adrenal cortex (19), in cerebellar granular cells (20), and in motoneurons (21). TASK-1 currents have been shown to be coupled to the activation of thyrotropin-releasing hormone receptor 1 (TRH-R1; Ref. 21) and angiotensin II receptors (AT1a; Ref. 19); thus, TASK-1 channels are regulated also by mechanisms other than extracellular pH. Furthermore, another member of this subfamily, TASK-3, is activated by depolarization and modulated by extracellular divalent cations (5).

With TREK-1, TREK-2, and TRAAK, three mechanosensitive channels have been cloned (10, 22). Other factors that have been reported to modulate the activity of these mechanosensitive channels include heat (TREK-1; Ref. 23), lysophospholipids (TREK-1, TRAAK; Ref. 24), arachidonic acid (TRAAK; Ref. 7), and intracellular pH (TREK-1; Ref. 25). The third subfamily of 2P K⁺ channels so far comprises TWIK-1 and TWIK-2, both of which are expressed in multiple tissues (12, 13, 15). TWIK-1 shows weak inward rectification with symmetrical K⁺ concentrations (12). The current carried by TWIK-2 is heat-sensitive and shows rapid time-dependent inactivation at 37 °C (15). Both channels may contribute to setting the resting membrane potential, but their specific function in various tissues is not yet clear.

In this paper we describe the cloning of the first two members of a novel subfamily of 2P K⁺ channels. One of these channels, rTHIK-1, was found to be expressed in all tissues tested. Heterologous expression of rTHIK-1 in Xenopus oocytes induced a current that could be activated by arachidonic acid and inhibited by the volatile anesthetic halothane. The second novel 2P K⁺ channel, THIK-2, is closely related to THIK-1 (58% identity at the amino acid level) but could not be functionally expressed. THIK-2 was strongly expressed in several tissues including stomach, liver, and kidney and was particularly abundant in the brain.
Synthetic oligonucleotides were chosen from the open reading frame with least homology to other known sequences to minimize cross-hybridization. Antisense oligonucleotides designed for a minimal tendency of forming hairpins and self-dimers were as follows (base position on coding strand indicated). rTHIK-2: i) (834–879) 5'-TATGGTGCAAGGCTGGCTGCACGGA-3' (GenBank accession number AY074680); ii) (1280–1328) 5'-GCTCTTTGCTCCTGCTTCTCCTCCACGACTGTCACGTATGTTCCCAAGAA-3' (rTHIK-1: i) (1074–1177) 5'-GTCGCCAGCATCTGGTCACCTTCCCTGCTTCTCAG-3' and (ii) (1280–1328) 5'-GCTACCTGTGTGCTCCTGCCTCCACCCTGCTGTGTCACGTA-3'. Oligonucleotides were 3' end-labeled with [32P]-dATP or [32P]-dCTP (PerkinElmer Life Sciences, 1200/1000 Ci/mmol) by terminal deoxynucleotidyltransferase (Roche Molecular Biochemicals) and used for hybridization at concentrations of 2–10 pg/µl (4 × 10^6 cpm/10 µl of hybridization buffer/slide). Slides were air-dried and hybridized for 20–24 h at 43 °C in 100 µl of buffer containing 50% formamide, 10% dextran sulfate, 50 mM DTT, 0.3 M NaCl, 30 mM Tris-HCl, 4 mM EDTA, 1× Denhardt's solution, 0.5 mg/ml denatured salmon sperm DNA, 0.5 mg/ml polyadenylic acid, and the labeled probe. After hybridization, slides were washed 2 × 30 min in 1× SSC (0.15× NaCl and 0.015× sodium citrate) plus 50 µM β-mercaptoethanol, 1 h in 1× SSC at 60 °C, and 10 min in 0.1× SSC at room temperature. Specimens were then dehydrated, air-dried, and exposed to Kodak Biomax x-ray film for 14–28 days. For cellular resolution, selected slides were dipped in photographic emulsion Kodak NTB2, incubated for 4–12 weeks, and then developed in Kodak D-19 for 2.5 min. For identification and confirmation of brain structures with bright- and dark-field optics, sections were Nissl-counterstained with cresyl violet. Controls sections were (a) digested with RNase A (50 ng/ml) for 30 min at 37 °C before hybridization or (b) hybridized with a probe containing a 20–50-fold excess of unlabelled oligonucleotides. These control hybridizations resulted in a complete loss of specific hybridization signal.

**RESULTS**

**Cloning of Two Novel 2P K⁺ Channels**

Two cDNA populations coding for novel 2P K⁺ channels were isolated from two rat cDNA libraries. The first channel was isolated from a rat brain and a rat heart library and was named rTHIK-1 for tandem pore domain halothane inhibited K⁺ channel. The second, closely related channel was isolated only in the rat brain library and was named rTHIK-2 despite the fact that it was not functional when expressed in Xenopus oocytes. Sequence analysis of the isolated cDNA clones revealed complete open reading frames (Fig. 1) for rTHIK-1 (2032 bp) and rTHIK-2 (1900 bp). The nucleotide sequences predict proteins of 405 (rTHIK-1) and 430 (rTHIK-2) amino acids, respectively, which show the typical features of 2P K⁺ channels: four transmembrane regions, two pore-forming regions, a large extracellular linker between M1 and P1, a short N termius and a larger C termius. As indicated in Fig. 1, A and B, several putative intracellular phosphorylation sites for protein kinase A, protein kinase C, and casein kinase were found in both sequences. In the M1-P1 linker region, rTHIK-1 harbors two putative glycosylation sites (N-[P]-[S/T]-[P]), whereas rTHIK-1 possesses only one. The rTHIK-2 protein is significantly longer at the N termius including three putative protein kinase C phosphorylation sites and several repetitive amino acid elements.

**Sequence comparison** identified the human orthologs of rTHIK-2 in dbase sequence entries AC009600 and of rTHIK-1 in two overlapping dbase data base entries (AL535074, AL137128). In accordance with the human nomenclature committee (HUGO), the two novel channel genes were named KCNK12 (THIK-2) and KCNK13 (THIK-1), respectively. The human channel subunits are 98% (rTHIK-2) and 84% (rTHIK-1), identical to their rat orthologs. rTHIK-1 and rTHIK-2 are 61.8% identical and 67.5% similar to each other, whereas only about 25–35% identity was found to other 2P K⁺ channels (Fig. 2B). Interestingly, the intracellular M2-M3 linker was 16–20 amino acids longer than that of other cloned 2P K⁺ channels. The cytosolic C-terminal domains of the THIK channels are 57% identical to each other (see supplemental material), whereas no significant
homology was found to other C-terminal domains of 2P K⁺ channel. The pore regions of both THIK channels are nearly identical (no mismatch in a stretch of 13 amino acids in the first pore region and only one mismatch in the second pore), whereas there is considerable difference to other 2P K⁺ channels (Fig. 2C). From the homology scores, the unique structural features of THIK channels and the phylogenetic clustering (Fig. 2A), it is obvious that THIK 1 (KCNK13) and THIK-2 (KCNK12) form a novel subfamily among the 2P K⁺ channels. Supplemental material (multiple sequence alignment excluding the unrelated C-terminal domains and pairwise alignment of THIK-1 and THIK-2 C-terminal domains) is available in the on-line version.

Analysis of the entries of the human genome databases showed that both genes described here have a similar structure, with a single large intron of 48.1 kilobases (KCNK12) and 121.5 kilobases (KCNK13), respectively, splitting the coding region at the first pore GYG motif. This intron is conserved in all mammalian 2P K⁺ channel genes (KCNK1-10) cloned so far. Chromosomal localization assigned KCNK12 to the human chromosomal region 2p22–2p21 between markers WI-10633 and SGC34238. KCNK13 was assigned to chromosomal region 14q24.1–14q24.3 between markers IB3608 and SGC30527, which is very close to the human TREK-2 (KCNK10) gene. The results of the chromosomal assignments are summarized in Table I. The tissue distribution of the rTHIK-1 and rTHIK-2 was obtained by reverse transcription-PCR analysis. Intron-spanning primers were used to avoid false positive results due to genomic contamination. rTHIK-1-specific products were amplified from all tissues tested. In contrast, rTHIK-2 expression was found in brain, lung, kidney, liver, stomach, and spleen but not in skeletal muscle, heart, and testis (Fig. 3).

FIG. 1. cDNA and amino acid sequence of rTHIK-1 (A) and rTHIK-2 (B). Transmembrane regions are shown in black, and pore regions are marked gray. In addition, putative glycosylation sites (h), protein kinase A phosphorylation sites (a), protein kinase C phosphorylation sites (t), and casein kinase phosphorylation sites (Δ) are indicated below the amino acid sequence. Consensus sites were identified using the program PROSITE.
In Situ Hybridization of Rat Brain—The mRNA distribution of rTHIK-1 and rTHIK-2 in the adult rat brain as detected by in situ hybridization is highly differential, with little overlap (summarized in Table II). Two different antisense probes were used for either subunit. The resulting labeling profiles were identical for each subunit, indicating that the probes were specific. rTHIK-2 mRNA was found to be widely expressed in most brain regions, with highest levels in the cerebellar granule cell layer, the mitral and granule cell layers of the olfactory bulb (Fig. 4E), and in the anterodorsal and anteroventral nuclei of the thalamus (Fig. 4F). Strong signals were also found in the thalamic ventral posterior nuclei (Fig. 4G), cortex, hippocampus, the pontine nucleus, the red nucleus (Fig. 4E), the oculomotor nucleus, and some nuclei of the amygdala. In the brainstem, elevated levels were found in all trigeminal sensory nuclei, in the tegmental and reticular nuclei, and in the ventral cochlear nuclei. rTHIK-2 mRNA was also found in some non-neuronal cells such as the ependymal lining of the ventricles. rTHIK-2 was found to be absent from the substantia nigra, gracile nuclei, inferior olive, and from most parts of the caudate putamen and septal nuclei as well as all white matter pathways.

In contrast, rTHIK-1 mRNA expression was found to be rather weak and restricted to only a few brain regions and nuclei (Fig. 4, A–D). Substantial expression levels were detected only in the granule cell layer of the olfactory bulb, in the olfactory tubercle, the lateral septum (Fig. 4A), and in distinct hypothalamic and thalamic nuclei (ventromedial hypothalamic nucleus, lateral mamillary nucleus, reticular nucleus, reuniens nuclei (Fig. 4, B and C)). Other rTHIK-1-positive structures were only partially labeled. The cortex, for example, was positive only in layer II, the striatum was labeled only in the caudal part, and the dentate gyrus granule cell layer of the dentate gyrus exhibited particularly high mRNA levels in the caudal ventral part (Fig. 4D). Similarly, within a nuclear group, only...
specific subnuclei were positive, e.g., the dorsal subnucleus of the lateral septum (Fig. 4A), the magnocellular part of the red nucleus (Fig. 4D), a subnucleus of the lateral habenula (Fig. 4, A and C), one lateral parabrachial subnucleus, and the ventral nucleus of the cochlear nuclei.

**Heterologous Expression in Xenopus Oocytes**—At day 2 after injection of rTHIK-1 cRNA into *Xenopus* oocytes, large currents were recorded using the two-electrode voltage clamp (Fig. 5A), which were not seen under control conditions. The steady-state amplitude of the currents measured at \(160 \text{ mV} \) was 21.85 ± 4.45 \( \mu \text{A} \) (\( n = 7 \)) in the presence of 2 mM external K\(^+\). Current activation in response to depolarizing voltage steps was not...
In contrast, the currents measured in *Xenopus* oocytes after injection of rTHIK-2 (Fig. 5D) were not significantly different from those of noninjected or water-injected oocytes (n = 10). To test whether both channel proteins were translated and targeted to the surface membrane, rTHIK-1 and rTHIK-2 were tagged with EGFP at the N terminus. 48 h after injection of EGFP-rTHIK-2, confocal microscopy showed strong membrane fluorescence that was very similar to that found after injection of EGFP-rTHIK-1 (Fig. 6A). Thus rTHIK-2 subunits appear to be targeted to the outer membrane. The reason for the lack of functional expression of rTHIK-2 was further studied by constructing chimeric subunits in which the first part of the rTHIK-2-coding region (M1-P1-M2) was fused to the second half of rTHIK-1 (M3-P2-M4) and *vice versa*. Injection of the chimeric cRNAs did not induce any current in *Xenopus* oocytes.

Some voltage-activated (Kv) or inwardly rectifying (Kir) K⁺ channel subunits that do not functionally express as homomers can modify the activity of other subunits by heteromerization. Therefore we investigated the possibility that rTHIK-1 and rTHIK-2 subunits might assemble to form heterodimeric channels. When rTHIK-1 and rTHIK-2 cRNAs were injected at equimolar amounts (n = 5), all macroscopic current properties were virtually indistinguishable from rTHIK-1 currents, and current amplitudes were not significantly different (Fig. 6, B and C). Furthermore, the currents induced by injection of rTHIK-1 were unaffected by injection of larger amounts of rTHIK-2 cRNAs (ratio 1:5). The apparent absence of heteromerization of rTHIK-2 and rTHIK-1 subunits suggests that rTHIK-2 is not a regulatory subunit of the rTHIK-1 conductance.

**Regulation of rTHIK-1 Channels**—To test for functional similarities to other members of the two pore domain K⁺ channel family, we studied the modulation of rTHIK-1 channel activity by various experimental interventions such as extracellular acidification, application of the polyunsaturated fatty acid arachidonic acid, or application of the volatile anesthetic halothane (Fig. 7A). The outward current carried by rTHIK-1 was

| THIK-1 | THIK-2 |
|--------|--------|
| Superior colliculus superficial layer | ++ |
| Inferior colliculus | + |
| Central gray | ++ |
| Red nucleus | + |
| Occulomotor nucleus | ++ |
| Lateral lemniscus nuclei | ++ |
| Substantia nigra | ++ |
| Pontine nucleus | ++ |
| Reticulotegmental nucleus | ++ |
| Superior olive (lateral) | ++ |
| Mesencephalic trigeminal nucleus | ++ |
| Spinal trigeminal nucleus | ++ |
| Principal sensory trigeminal nuclei | ++ |
| Dorsal tegmental nuclei | ++ |
| Ventral tegmental nuclei | ++ |
| Parabrachial nuclei, lateral | ++ |
| Lateral reticular nucleus | ++ |
| Motor nuclei (M05, -7, -12) | ++ |
| Cochlear nuclei, dorsal | ++ |
| Cochlear nuclei, ventral anterior | ++ |
| Cochlear nuclei, ventral posterior | ++ |
| Solitary nucleus | ++ |
| Inferior olive | ++ |

*Structure is only partly positive.*
only weakly inhibited by extracellular acidification to pH 6, which is in marked contrast to the pronounced pH sensitivity of TASK-1 (KCNK3; half-maximal block at pH 7.38), as illustrated in Fig. 7B. Lowering the pH to 4.5 inhibited the outward current carried by rTHIK-1 by 34 ± 8% (n = 5). Similar to TASK-3, proton block of rTHIK-1 occurred with a fast time course (Fig. 7A) and was independent of the membrane potential (Fig. 7B). The sensitivity of rTHIK-1 to intracellular pH was tested by the “rebound acidification” technique. 20 mM NH₄Cl was applied for 5 min and then removed, which should decrease the intracellular pH by about 1 unit. This intervention produced no measurable change in the current carried by...
rTHIK-1 (n = 3), indicating that rTHIK-1 is not modulated by intracellular pH.

Since TREK-1 has been reported to be heat-sensitive (23), we also studied the temperature dependence of rTHIK-1. Raising the temperature from 22 to 37 °C increased the current amplitude by a factor of 1.6 (n = 3), in agreement with van’t Hoff’s rule, whereas TREK-1 is augmented by a factor of 10 under the same conditions (23). These findings suggest that, unlike TREK-1, rTHIK-1 is not a heat-sensitive channel. Lysoosphatidylcholine (3 μM) has also been shown to activate TREK-1 and TRAAK (24). Application of 10 μM lysoosphatidylcholine to Xenopus oocytes expressing rTHIK-1 induced only a very minor (up to 20%) increase in current amplitude, which may be attributable to a nonspecific effect of the lysoospholipid.

Application of arachidonic acid to the bath solution induced a rapid increase in the outward current carried by rTHIK-1 channels (Fig. 7A). This effect could be washed out within 5 min. In the presence of 5 μM arachidonic acid, the current was increased by 85 ± 24% (n = 5) at +30 mV. As can be seen from Fig. 7C, the current activated by arachidonic acid was outwardly rectifying. It reversed at the calculated potassium equilibrium potential when the external K⁺ concentration was altered. The concentration dependence of the effect of arachidonic acid on rTHIK-1 could be described by a Kᵣ of 0.98 μM and a Hill coefficient of 1.97 (Fig. 7C). The effects of arachidonic acid reported here are similar to the effects found in TREK-1 (KCNK2) and TRAAK (KCNK4) (7, 9, 24).

Two of the known 2P K⁺ channels, TREK-1 and TASK-1, are activated by the volatile anesthetic halothane (27). Surprisingly, our whole-cell recordings in Xenopus oocytes showed that the current carried by rTHIK-1 was rapidly and reversibly inhibited by halothane (Fig. 7, A and D). When the membrane potential was held at +30 mV, application of 5 mM halothane reduced rTHIK-1 currents by 56 ± 5% (n = 5). The fit of the concentration-effect curve gave a Kᵣ of 2.83 mM and a Hill coefficient of 1.06. Under the same experimental conditions, application of chloroform (1 mM) had no effect (n = 3; data not shown).

**DISCUSSION**

THIK-1 and THIK-2 are the first two members of a novel 2P K⁺ channel subfamily. Although they show the typical features of other 2P K⁺ channels, such as four transmembrane regions, two pore-forming regions, and a large extracellular M1-P1 linker region, the two novel channels are only about 25–35% identical to the other known 2P K⁺ channels but 61.8% identical to each other. One notable difference is the larger cytosolic M2-M3 linker region, containing three (rTHIK-2) or one (rTHIK-1) putative phosphorylation site(s). In addition, the THIK-2 protein has an unusual N terminus containing several repeats of proline, arginine, and cysteine residues. The C-terminal domain of THIK-1 and THIK-2 shows no significant homology to other mammalian 2P K⁺ channels.

The reversal potential of the current induced by injection of rTHIK-1 cRNA in Xenopus oocytes followed the calculated K⁺ equilibrium potential when external K⁺ was changed. This suggests that the THIK channels are mainly permeable to K⁺ ions. The whole-cell current induced by heterologous expres-
sion of rTHIK-1 displayed outward rectification at physiologically external K\(^+\) and weak inward rectification with approximately symmetrical K\(^+\) concentrations. It could be activated by arachidonic acid (K\(_d\), 0.98 \(\mu\)M; Hill coefficient, 1.97) and inhibited by halothane (K\(_d\), of 2.8 mM; Hill coefficient, 1.06). Chloroform had no effect on rTHIK-1. Another 2P K\(^+\) channel, TWIK-2, which is almost absent in the brain, has recently been found to be inhibited by both halothane and chloroform (15). In contrast, both TREK-1 and TASK-1 are activated by halothane and isoflurane, and TREK-1 is additionally activated by chloroform and diethyl ether (27). TREK-1, TASK-1, and THIK-1 are all expressed in specific regions of the brain. The findings reported here suggest that the effects of volatile anesthetics on the brain may be more complex than hitherto assumed. We are aware of the fact that the IC\(_{50}\) for the effects of halothane on rTHIK-1 is higher than the EC\(_{50}\) for the anesthetic effects in \(\text{in vivo} \) (\(\sim 250 \mu\)M; Ref. 28). Nevertheless, we decided to name the new channels tandem pore-domain halothane inhibited K\(^+\) channels because this discriminates them from some of the other 2P K\(^+\) channels.

Injection of rTHIK-2 cRNA in \(\text{Xenopus}\) oocytes did not produce any measurable currents. The lack of functional expression of rTHIK-2 was apparently not due to inadequate targeting, because confocal microscopy showed EGFP-tagged rTHIK-2 channels in the outer cell membrane (Fig. 6). To localize possible structural constraints in rTHIK-2 that prevent expression of functional channels, we constructed rTHIK-1/ rTHIK-2 and rTHIK-2/rTHIK-1 chimeras. However, since neither of the two chimeras was functional, the reason for the nonfunctional state of rTHIK-2 in \(\text{Xenopus}\) oocytes remains unclear. Another possibility is that rTHIK-2 might be a regulator of rTHIK-1 conductance by coassembling with rTHIK-1. This is unlikely, because whole cell currents produced by injection of rTHIK-1 cRNA were unaffected by coinjection even of 5-fold larger amounts rTHIK-2 cRNA. In \(\text{in situ}\) hybridization in the brain showed little overlap between rTHIK-1 and rTHIK-2, which also argues against heteromerization. In conclusion, the high expression of rTHIK-2 in cerebral cortex, hippocampus, and olfactory bulb and the specific expression in several nuclei (Fig. 6) support the idea that rTHIK-2 is functionally important in neurons, but the available experimental evidence suggests that rTHIK-2 requires an accessory subunit or specific intracellular ligands to form a conducting pore. The strong expression in lung, kidney, and stomach suggests that rTHIK-2 may also play a role in epithelial cells.

Both human THIK genes described here have only one very large intron, 121.5 kilobases in the THIK-1 gene (KCNK13) and 48.1 kilobases in the THIK-2 gene (KCNK12), that splits the
coding region of the first pore (GYG motif). In this respect, the THIK genes are similar to the TASK-3 gene (KCNK9). The genes of the TWIK and the TREK/TRAAK families possess several introns in the coding region (10, 29, 30), but an intron splitting the GYG motif of the first pore region is conserved in all mammalian 2P K+ channel genes cloned so far and, in addition, in most of the 2P K+ channels of *Caenorhabditis elegans* (31) and *Drosophila melanogaster*. Another interesting feature is the colocalization of 2P K+ channel genes at the same chromosomal region. We have shown that THIK-1 (KCNK13) maps to the same region on the long arm of chromosome 14 as TREK-2 (KCNK10). The other 2P K+ channel gene pairs known so far are TRAAK (KCNK5) and KCNK7 on chromosome 11q13 (29) and TWIK-1 (KCNK1) and TREK1 (KCNK2) on chromosome 11q41–43 (32). These data are consistent with a common ancestral gene for all 2P K+ channels that was subject to several duplication events.

As illustrated in Fig. 2, mammalian 2P K+ channels can be subdivided in five subfamilies: 1) TWIK/KCNK7, 2) TREK/TRAAK, 3) TASK-1/TASK-3, 4) THIK, and 5) TASK-2. These channels display a wide variety of electrophysiological and regulatory characteristics that are usually not confined to one of the subfamilies. The steady-state current voltage relation measured in the whole-cell configuration at symmetrical K+ concentrations was found to be inwardly rectifying (TWIK-1, TWIK-2, and THIK-1), outwardly rectifying (TREK-1, TASK-3), or linear (TREK-1, TASK-1, TRAAK). Some of the 2P K+ channels show a pronounced sensitivity to intracellular (TREK-1) or extracellular pH (TASK-1, TASK-2, TASK-3). The pharmacological profile of the 2P K+ channels is also very diverse and not related to subfamilies. Some 2P K+ channels are activated by volatile anesthetics (TREK-1, TASK-1); other channels are inhibited (TWIK-2, THIK-1). Some channels are activated by fatty acids such as arachidonic acid (TREK-1, TWIK, TWIK-2, THIK-1) or by phospholipids such as lysophosphatidylcholine (TREK and TRAAK). In addition, some of the 2P K+ channels are mechanosensitive (TREK-1, TREK-2, and TRAAK) or heat-sensitive (TREK-1). The most remarkable common characteristic of all 2P K+ channels known so far is that their regulation by physical and chemical stimuli is very complex. The difficulty in identifying their function is probably related to this complex regulation, which needs to be studied in the native cells in which the channels are expressed.

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