A new member of the tandem-pore K⁺ (K₂P) channel family has been isolated from mouse testis complementary DNA. The new K₂P channel was named TRESK-2, as its amino acid sequence shares 65% identity with that of TRESK-1. Mouse TRESK-2 is a 394-amino acid protein and possesses four putative transmembrane segments and two pore-forming domains. TRESK-2 has a long cytoplasmic domain joining the second and third transmembrane segments and a short carboxyl terminus. In the rat, TRESK-2 mRNA transcripts were expressed abundantly in the thymus and spleen and at low levels in many other tissues, including heart, small intestine, skeletal muscle, uterus, testis, and placenta, as judged by Northern blot analysis. TRESK-2 mRNA was also expressed in mouse and human tissues. In COS-7 cells transfected with TRESK-2 DNA, a time-independent and noninactivating K⁺-selective current was recorded. TRESK-2 was insensitive to 1 mM tetraethylammonium, 100 μM aminaparin, 1 mM 4-aminopyridine, and 10 μM glibenclamide. TRESK-2 was inhibited by 10⁻⁶ M quinidine, 20 μM arachidonate and acid (pH 6.3) at 49, 43, and 23%, respectively. Single channel openings of TRESK-2 showed marked open channel noise. In symmetrical 150 mM KCl, the current-voltage relationship of TRESK-2 was slightly inwardly rectifying, with the single channel conductance 13 pS at +60 mV and 16 pS at −60 mV. In inside-out patches, TRESK-2 was unaffected by the intracellular application of 10 μM guanosine 5′-O-(thiotriphosphate). These results show that TRESK-2 is a functional member of the K₂P channel family and contributes to the background K⁺ conductance in many types of cells.

Mammalian potassium channels are divided into three structural classes based on the number of predicted transmembrane (TM) segments (two, four, or six) and pore-forming (P) domains (one or two). Searching the DNA data base for sequences homologous to previously cloned K⁺ channels led to identification of the class now referred to as tandem-pore or two-pore domain K⁺ (K₂P) channels (1–4). Each subunit of a K₂P channel possesses four TM segments and two P domains, and therefore two subunits are thought to assemble to form a functional dimeric K⁺ channel (4, 5). K₂P channels can be divided into six subfamilies based on amino acid homology: TWIK-1 and TWIK-2 (6–9); THIK-1 and THIK-2 (10); TASK-1, TASK-3, and TASK-5 (11–13); TALK-1, TALK-2, and TALK-2 (14–16); TREK-1, TREK-2, and TRAAK (17–20); and TRESK-1 (21). Many of these K₂P channel are able to form functional K⁺ channels when expressed in oocytes or mammalian cells. Those K₂P channels that form functional K⁺ channels exhibit interesting electrophysiological and pharmacological properties. For example, TASK-1 and TASK-3 are active at rest, highly sensitive to extracellular pH and oxygen concentration (22), and activated by volatile anesthetics (23, 24). TREK-1 and TREK-2 are activated by free fatty acids, protons, increased membrane tension, heat, and volatile anesthetics (25, 26). These channels are functionally expressed in many regions of the brain and in certain peripheral tissues, suggesting that they may be involved in a variety of biological functions including transduction of metabolic changes into electrical events. TALK-1 and TALK-2 are expressed in human pancreas and are active at rest, suggesting that they may regulate membrane-potential sensitive secretion of pancreatic hormones (15, 27).

The most recently identified member of the K₂P channel family is TRESK-1 (TWIK-related spinal cord K⁺ channel), named so because its mRNA was expressed solely in the human spinal cord (21). When expressed in L929 cells, TRESK-1 has been reported to form a functional K⁺ channel that has properties of a background (or leak) K⁺ channel. Thus, TRESK-1 shows basal activity at physiological membrane potentials, activates rapidly upon depolarization (and shows little inactivation), and is likely to contribute to the resting membrane potential in those cells that express it. As each K₂P channel subfamily has at least two members, we hypothesized that there may exist other genes that encode TRESK isoforms. When the GenBank™ data base was searched for sequences homologous to TRESK-1, we found within mouse chromosome 19 (accession number AC139040) a DNA sequence that shared 65% amino acid identity with TRESK-1 and two K⁺ channel signature sequences (TVGG and TIFG) in the two pore-forming regions. We named this potentially novel K₂P channel TRESK-2. The aim of this work was therefore to clone TRESK-2, which could be a new member of the TRESK subfamily, and study its tissue expression and electrophysiological properties. Our results show that TRESK-2 is a functional member of the K₂P channel family. TRESK-2 mRNA was expressed in many tissues and was particularly in abundance in rat spleen and thymus. Transfection of TRESK-2 DNA into COS-7 cells produced functional K⁺ channels that were active at rest and did not inactivate or desensitize. Thus, TRESK-2 is a new member of the tandem-pore K⁺ (K₂P) channel family that may contribute to the background K⁺ conductance in many types of cells.
possesses properties of a background K⁺ channel that serves to set the resting membrane potential in those cells that express it.

**MATERIALS AND METHODS**

cDNA Cloning—A BLASTn search using the TRESK-1 K⁺ channel sequence revealed a homologous sequence present in mouse chromosome 19 (GenBank™ accession number AC139040) and a mouse sequence (accession number XM285304) that was similar to that in chromosome 19. The mouse DNA sequence was not obtained from cDNA derived from mRNA but was predicted by automated computational analysis from an annotated genomic sequence (NT-039692) using the GeneScanR method (GNOMON). Therefore, it was uncertain whether the DNA sequence represented by the two accession numbers encoded a functional protein. To obtain the full-length TRESK-2 cDNA, we used several sets of primers complementary to the start and end of the coding sequences. The first strand cDNA from mouse testis was prepared and used for reverse transcriptase (RT)-PCR using the Superscript system (Invitrogen). We were successful in obtaining two overlapping DNA fragments using two sets of primer pairs. The first primer pair (5'-ATGGAGCGTGAGGACACCTGAG-3' and 5'-AAAAGGAGGTCGATGACACCTGAG-3') yielded a 544-bp fragment, and the second primer pair (5'-CCCTCTACATGCTTGGCTCTCAG-3' and 5'-GTAGGTTCACGGAAATGCCGGAAACCTCTCCATTTACCAAGG-3') yielded a 725-bp DNA fragment. PCR was performed for 35 cycles at 94 °C for 45 s, 60 °C for 1 min, 72 °C for 2 min, and finally at 72 °C for 10 min with Taq polymerase. The two overlapping DNA fragments were amplified by PCR to obtain the full-length TRESK-2 sequence. One DNA fragment containing the entire open reading frame could also be obtained using a pair of primers (5'-ATGGAGCGTGAGGACACCTGAG-3' and 5'-GCCGGAACCCTCTCCATTTACCAAGG-3'). The final amplified DNA product that contained the full open reading frame was subcloned into the pCR2.1 TOPO vector by TA cloning and sequenced on both strands using the dideoxynucleotide chain termination method.

**Northern Blot Analysis and RT-PCR**—Rat multiple tissue Northern blots consisting of 15 different tissues were purchased from Seegene, Inc. (Seongnam, Korea). The membrane was prehybridized for 30 min at 60 °C and then hybridized for 1 h at 60 °C in ExpressHyb solution (Clontech, Palo Alto, CA) with 32P-labeled TRESK-2 cDNA following the manufacturer’s protocol. The membrane was rinsed with solution containing 2 × SSC (3.0 M sodium chloride and 0.3 M sodium citrate) and 0.1% SDS for 30 min at room temperature. A second washing was performed in solution containing 0.2 × SSC and 0.1% SDS for 30 min at 55 °C. The membrane was exposed to an x-ray film and developed at 16 h later. The membrane was probed again with 32P-labeled glyceraldehyde-3-phosphate dehydrogenase DNA.

For RT-PCR, total RNAs from mouse, rat, and human tissues were isolated and reverse transcribed with oligo(dT) primer using the aldehyde-3-phosphate dehydrogenase DNA. The first strand cDNA was used for reverse transcriptase (RT)-PCR using the SuperScript system (Invitrogen). We were successful in obtaining two overlapping DNA fragments using two sets of primer pairs. The first primer pair (5'-ATGGAGCGTGAGGACACCTGAG-3' and 5'-AAAAGGAGGTCGATGACACCTGAG-3') yielded a 544-bp fragment, and the second primer pair (5'-CCCTCTACATGCTTGGCTCTCAG-3' and 5'-GTAGGTTCACGGAAATGCCGGAAACCTCTCCATTTACCAAGG-3') yielded a 725-bp DNA fragment. PCR was performed for 35 cycles at 94 °C for 45 s, 60 °C for 1 min, 72 °C for 2 min, and finally at 72 °C for 10 min with Taq polymerase. The two overlapping DNA fragments were amplified by PCR to obtain the full-length TRESK-2 sequence. One DNA fragment containing the entire open reading frame could also be obtained using a pair of primers (5'-ATGGAGCGTGAGGACACCTGAG-3' and 5'-GCCGGAACCCTCTCCATTTACCAAGG-3'). The final amplified DNA product that contained the full open reading frame was subcloned into the pCR2.1 TOPO vector by TA cloning and sequenced on both strands using the dideoxynucleotide chain termination method.

**Cloning of Mouse TREK-2**—A DNA sequence homologous to TRESK-1 was identified in mouse chromosome 19 (GenBank™ accession number AC139040). We named the new sequence TRESK-2 and cloned its cDNA from mouse testis by RT-PCR. The open reading frame of mouse TRESK-2 contains 1182 nucleotides and encodes a 394-amino acid polypeptide with a calculated molecular mass of 43 kDa (Fig. 1A). Hydrophobicity analysis of the amino acid sequence shows that TRESK-2 belongs to the Kᵢp family because it reveals four putative TM domains and two pore-forming domains (Fig. 1B). The predicted membrane topology of TRESK-2 is similar to other Kᵢp channels, with amino and carboxyl termini located at the intracellular side of the membrane. Like TRESK-1, TRESK-2 has a short amino-terminal region, an extended intracellular loop between M2 and M3, and a short carboxyl-terminal region (Fig. 1C). One N-glycosylation site in the M1–P1 linker region (Asn-83) and four potential phosphorylation sites for protein kinase A in the cytoplasmic region are present. Three protein kinase A consensus sites are located in the intracellular loop between M2 and M3 (Ser-175, Ser-192, Ser-264), and one is located near the carboxyl tail (Ser-391). A putative phosphorylation site for protein kinase C is also located at the carboxyl tail (Thr-377).

Alignment of amino acid sequences of TRESK-1 and TRESK-2 is shown in Fig. 2A. TRESK-2 shares 63% overall amino acid identity and 75% similarity with TRESK-1. The amino acid sequences within the TM and pore regions are nearly identical between TRESK-1 and TRESK-2. The major differences can be found in the amino terminus, the extracellular loop between M1 and P1, the proximal half of the long intracellular loop, and the carboxyl terminus. The carboxyl terminus of TRESK-2 is 14 amino acids longer than that of TRESK-1 in which the carboxyl terminus is only ~14 amino acids long. TRESK-2 shares low amino acid identity (~25%) with other members of the Kᵢp family, such as TREK and TASK. Comparison of the full coding sequence of TRESK-2 with that of mouse chromosome 19 shows that TRESK-2 is made up of three exons (801, 134, and 256 bp) and two intervening sequences of 9.3 and 5.4 kb (Fig. 2B). This is similar to human TRESK-1, which also consists of three exons and two introns located in human chromosome 10 (21). In the mouse expressed sequence tag data base, seven cDNA fragments with amino acid sequences identical to those of TRESK-2 were identified. Thus, TRESK-2 is a new member of the Kᵢp channel family and a second member of the TRESK subfamily. Fig. 2C shows a phylogenetic tree consisting of 16 mammalian Kᵢp channels that have been cloned so far.

**Tissue Distribution of TRESK-2**—To determine the tissue expression of TRESK-2 mRNA in the rat, Northern blot analysis was performed using rat multiple tissue blots. Northern blot data show that TRESK-2 mRNA is expressed predominantly in the spleen and thymus (Fig. 3A). The strongest signals are observed...
at −4.2 and −7.5 kb, and a weak signal is observed at −1.6 kb. The three bands are also present in other tissues (heart, lung, small intestine, skeletal muscle, uterus, and placenta), although at much lower levels. As expected, RT-PCR yielded a TRESK-2 DNA fragment (544 bp) from the first strand cDNA prepared from rat spleen, thymus, pancreas, and kidney. To determine

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**Fig. 1. DNA and amino acid sequences of mouse TRESK-2, hydrophobicity analysis, and predicted membrane topology.**

A. DNA and amino acid sequences of mouse TRESK-2 with transmembrane (TM) segments and pore-forming regions underlined are shown. Potential N-glycosylation site (Asn-83) is indicated by a square. Protein kinase A sites are circled, and a protein kinase C site is underlined in bold.

B. Hydrophobicity analysis using the Kyte-Doolittle algorithm shows four potential membrane-spanning segments (M1–4), two pore-forming domains (P1, P2), a long cytoplasmic loop between M2 and M3, and a short carboxy terminus.

C. Predicted membrane topology of TRESK-2, extracellular N-glycosylation (N-Gly), and intracellular protein kinase A (PKA) and C (PKC) sites are shown.
whether TRESK-2 mRNA is also expressed in mouse and human tissues, we performed RT-PCR on cDNAs prepared from spleen, thymus, and testis from mouse tissue and spinal cord, testis, placenta, and pancreas from human tissue. Sequencing of the DNA products of RT-PCR obtained using the same TRESK-2-specific primers confirmed that TRESK-2 mRNA was also expressed in these mouse and human tissues (Fig. 3B). Clearly, these results show that in human, TRESK-2 mRNA is more widely expressed than TRESK-1 mRNA, which was detected only in the spinal cord among the 23 tissues examined (21).

Whole-cell Current Recordings—To determine whether TRESK-2 forms a functional K⁺ channel, we first recorded whole-cell currents from COS-7 cells transfected with plasmids containing TRESK-2 and GFP DNA fragments. In physiological bath solution containing 5 mM KCl (pH 7.3), the membrane potential of cells under the whole-cell configuration was held at −80 mV, and then voltage steps (−120 to +60 mV; 2-s duration) or a ramp pulse (−120 to +60 mV; 1-s duration) was applied. In control cells transfected with plasmid containing GFP alone, voltage steps from a holding potential of −80 mV to various potentials (−120 to +60 mV) elicited very small currents (<0.2 nA at +60 mV; n = 10; Fig. 4A). In cells transfected with TRESK-2/GFP DNA, the same voltage step produced large currents (2–3 nA at +60 mV; n = 20; Fig. 4A) with reversal potentials near −85 mV, indicating that TRESK-2 formed a functional K⁺ channel in the plasma membrane of COS-7 cells. The activation of current by step depolarization was nearly instantaneous, and very little or no inactivation was observed. Ramp pulses of 1-s durations applied from −120 mV to +60 mV showed a mildly outwardly rectifying K⁺ current (Fig. 4A). Thus, TRESK-2 exhibits properties of a background/leak K⁺ current similar to those of other K₂P channels.

Ion selectivity of TRESK-2 was studied further in large outside-out patches by recording the reversal potentials at various external [K⁺] after applying voltage ramps from −120 mV to +20 mV (Fig. 4B). The reversal potential shifted to the right as
[K⁺] in the bath solution increased, as was expected of an ion channel that is permeable to K⁺ but not to Cl⁻. A plot of the reversal potential as a function of [K⁺]₉ₑ₉ₒ₉ showed a slope of 58 ± 2 mV/10-fold change in [K⁺]ₑ₉ₒ₉ close to the calculated Nernst value of 59 mV at 22 °C shown as a dotted line in Fig. 4C. These results show that TRESK-2 is a K⁺-selective channel similar to other two-pore domain K⁺ channels.

**Single Channel Recordings**—To study the single channel properties of TRESK-2, cell-attached patches were formed on COS-7 cells in a 150 mM KCl bath solution. The openings of channels with properties different from those of endogenous channels normally found in COS-7 cells were observed in nearly all cells transfected with TRESK-2/GFP DNAs but never in cells transfected with GFP DNA alone. Therefore, identifying the functional expression of TRESK-2 in the plasma membrane was unambiguous. The openings and closings of several channels in a typical cell-attached patch is shown in Fig. 5A. The channels were active at all membrane potentials tested (−100 mV to +100 mV) and showed no inactivation or desensitization in cell-attached and inside-out patches. Using cells with lower expression levels and therefore showing only one level of opening, we recorded channel currents obtained here could be slightly underestimated because of the inadequate resolution of channel currents. The outward currents recorded during the depolarized state showed less noisy openings. The current-voltage relationship, plotted using values obtained from amplitude histograms obtained at each membrane potential, was slightly inwardly rectifying in symmetrical 150 mM KCl (Fig. 5E). The open probability of TRESK-2 was relatively insensitive to changes in membrane potential. These findings show that TRESK-2 is a relatively small conductance background K⁺ channel with single channel properties that are distinct from those other K₂P channel subfamilies. These single channel characteristics should help to identify the native K⁺ channel encoded by TRESK-2.

**Modulation by Pharmacological Agents**—We examined the effects of various pharmacological agents and changes in pH on TRESK-2 expressed in COS-7 cells. Under whole-cell conditions, extracellular application of 3 mM Ba²⁺ produced a 38 ± 4% decrease in outward current, typical of the low sensitivity observed with nearly all K₂P channels to this divalent cation (Fig. 6A). K⁺ channel blockers, such as tetraethylammonium (1 mM), 4-aminopyridine (1 mM), and apamin (100 nM), caused no significant effects on TRESK-2 current (n = 5 each, p > 0.05). Quinidine (100 μM) produced a near complete block of TRESK-2. At 10 μM, quinidine blocked TRESK-2 current by 49 ± 6%. In inside-out patches, the application of arachidonic acid (20 μM) produced a 43 ± 6% reduction of TRESK-2 current.
Tandem-pore Domain K⁺ Channel

Fig. 4. Whole-cell current in COS-7 cells. A, whole-cell currents were recorded from cells transfected with plasmid containing GFP only or with that containing TRESK-2/GFP. Cell membrane potential was held at −80 mV, and voltage steps from −120 to +60 mV were applied at 20-mV intervals for 2-s durations. In other cells, voltage ramps from −120 to +60 mV were applied for 1-s durations. Pipette and bath solutions contained 150 and 5 mM K⁺, respectively. Peak currents determined at the end of the 1-s pulse from the whole-cell currents were plotted as a function of membrane potential. Each bar represents the mean ± S.D. of 10 experiments. B, whole-cell currents of TRESK-2 at different extracellular K⁺ concentrations are shown. Note the rightward shift of the reversal potential as [K⁺]o is increased. C, reversal potentials from five whole-cell experiments were determined and plotted as a function of [K⁺]o. Experimental values were fitted by linear regression. Slope, 58 mV/decade.

Fig. 5. TRESK-2 single channel currents. A, a current tracing typically observed from a cell-attached patch of a COS-7 cell transfected with TRESK-2 DNA shows several channels that open in bursts. B, current tracings from an inside-out patch showing single channel openings at various membrane potentials in symmetrical 150 mM KCl. C, amplitude histogram obtained from channel openings at −60 mV shows a single peak. D, duration histogram obtained from channel openings at −60 mV could be fitted with a single exponential function. E, a current-voltage relationship of TRESK-2 in 150 mM KCl is shown.

cAMP (100 µM) or 1-methyl-3-isobutylxanthine (100 µM), which elevates intracellular [cAMP] also failed to alter TRESK-2 current (n = 5; p > 0.05). An increase in temperature of the perfusion solution from 24°C to 37°C produced no significant change in TRESK-2 activity (n = 5; p > 0.05). Thus, TRESK-2 does not appear to be modulated by phosphorylation by protein kinases A and C or heat.

DISCUSSION

We have cloned a new member of the K₂P channel family and studied its tissue expression and electrophysiological properties. We named it TRESK-2 because it shares 63% amino acid identity with TRESK-1, which was cloned recently (21). The cloning of TRESK-2 brings the number of mammalian K₂P channels to a total of 16. These 16 members can be divided into six subfamilies based on the amino acid identities of the K₂P channels. Although division into subfamilies is not based on function, certain members within each subfamily possess similar functional properties, such as those in the TASK and TREK subfamilies. TRESK-1 and TRESK-2 also appear to possess similar electrophysiological and pharmacological properties. TRESK-1 and TRESK-2 are structurally unique among members of the K₂P channels in that both contain extended intracellular loops between TM2 and TM3 and have short carboxyl-terminal segments. All other K₂P channels have short intracellular loops and long carboxyl termini. TRESK-1 and TRESK-2 are also kinetically unique in that they possess single channel properties that are distinct from those of other K₂P channels described so far.

TRESK-1 was expressed solely in the spinal cord in human
tissue; hence its coined name, TWIK-related spinal cord K\(^+\) channel (21). TRESK-1 mRNA expression in rat or mouse tissues has not yet been reported. In human spinal cord, TRESK-1 mRNA could not be detected by Northern blot analysis and only a very faint band was detected by RT-PCR. This indicated that TRESK-1 mRNA expression in the spinal cord was very low or that only a small subset of cells express it (21). In contrast, TRESK-2 mRNA transcript was expressed abundantly in rat thymus and spleen and could be detected in many other tissues by Northern blot analyses. Although we have not performed Northern blot analysis on mouse tissues, TRESK-2 could be detected by RT-PCR in several mouse tissues that we have tested (testis, spleen, and thymus). Searching the GenBank\(^{TM}\) DNA data base for the TRESK-1 sequence did not identify any expressed sequence tags, whereas searching for the TRESK-2 sequence showed seven expressed sequence tags. This also suggests that TRESK-2 is probably more abundantly and widely expressed than TRESK-1. Interestingly, we were unable to find a human ortholog of TRESK-1 in the GenBank\(^{TM}\) data base. This was surprising because human, rat, and mouse orthologs of all other K\(_{\text{2P}}\) channels could be identified in the DNA data base. This prompted us to determine whether the mouse TRESK-2 that we cloned is a novel member of the K\(_{\text{2P}}\) channel family or an ortholog of human TRESK-1. Our RT-PCR studies using human tissues (spinal cord, pancreas, placenta, and testis) and sequencing of the DNA fragments clearly showed that TRESK-2 mRNA is expressed in these human tissues. Therefore, both TRESK-1 and TRESK-2 mRNA transcripts are expressed in human tissue, indicating that they are different members of the K\(_{\text{2P}}\) channel family sharing 65% amino acid identity.

TRESK-2, similar to TRESK-1, forms a functional K\(^+\) channel in COS-7 cells. As with many other K\(_{\text{2P}}\) channels, TRESK-2 is insensitive to inhibition by typical K\(^+\) channel blockers (triethanolamine, Ca\(^{2+}\), 4-aminopyrine) and is blocked only at millimolar concentrations of Ba\(^{2+}\). Quinidine, a nonspecific blocker of many K\(^+\) channels (including those of K\(_{\text{2P}}\) channels), strongly inhibits TRESK-1 and TRESK-2. The inhibitory effect of arachidonic acid on TRESK-2 is similar to those observed with TASK-1 and TASK-3, although the physiological significance of such inhibition is not yet known (12, 28). It is clear from these findings that the pore properties of many K\(_{\text{2P}}\) channels, including TRESK-2, are different from those of voltage-gated (Kv) and inwardly rectifying (Kir) K\(^+\) channels that are highly sensitive to Ba\(^{2+}\) and tetraethylammonium, which block the channels by entering the permeation pathway. From the effects observed with 13 different molecules, it appears that the pharmacological profiles of TRESK-1 and TRESK-2 are somewhat similar to those of other K\(_{\text{2P}}\) channels, particularly with respect to K\(^+\) channel blockers. We observed no striking effects of any agents on TRESK-2 that might help us to distinguish between TRESK-2 and other K\(^+\) channels. In a recent report, human TRESK was found to be modulated by calcineurin, a Ca\(^{2+}\)/calmodulin-dependent protein phosphatase, and this effect was inhibited by FK506, an inhibitor of calcineurin (29). Whether other K\(_{\text{2P}}\) channels are also regulated by such a mechanism needs to be studied in the future.

To be able to identity TRESK-2 from other K\(^+\) channels, it
is therefore crucial to determine its single channel characteristics. Our results show that TRESK-2 is a small conductance K⁺ channel (~16 pS), which opens in short bursts and exhibits rapid kinetics of openings and closings. The apparent single channel conductance and mean open times of TRESK-2 that we report here are likely to be slight underestimates of the true values because of highly noisy openings, which are difficult to resolve adequately, particularly in the inward current direction. Nevertheless, the single channel properties of TRESK-2 are unique among K⁺ channels and should help to identify native K⁺ channels that are functional correlates of TRESK-2. Although the single channel properties of TRESK-1 are not described in the earlier study (21), a current tracing shown in one figure suggests that TRESK-1 may behave like TRESK-2 and is also a relatively low conductance K⁺ channel (~15 pS in symmetrical 150 mM KCl). Therefore, it may be difficult to distinguish between TRESK-1 and TRESK-2 if they are expressed in the same cell. Our Northern blot studies show that TRESK-2 mRNA is expressed in many tissues in the rat. Therefore, a native K⁺ channel with the single channel kinetics described above is most likely to be encoded by TRESK-2. However, the single channel properties of TRESK-1 and TRESK-2 and their tissue distribution in mouse and rat need to be studied further to determine their similarities and differences.

Based on electrophysiological studies, TRESK-2 exhibits all the hallmarks of a background or leak K⁺ channel. Thus, TRESK-2 is active across the physiological range of membrane potential and activates and deactivates rapidly with little or no inactivation with voltage changes, and the reversal potential is therefore crucial to determine its single channel characteristics. A rise in the intracellular [Ca²⁺] signal is a critical event that leads to the activation of lymphocytes and their phagocytic activity (30). Several voltage-gated and Ca²⁺-activated K⁺ channels and their role in Ca²⁺ influx and lymphocyte activation have been reported (31). TRESK-2 could also be involved in these processes by opposing forces that tend to depolarize the cell. TRESK-2 could promote Ca²⁺ influx by helping to keep the resting membrane potential at negative potentials in nonexcitable cells.

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