Cerebrovascular responses in mice deficient in the potassium channel, TREK-1


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Submitted 22 January 2010; accepted in final form 30 March 2010

ACTIVATION OF POTASSIUM (K) CHANNELS on endothelium and/or vascular smooth muscle constitutes an important step in many mechanisms of vascular dilation (6, 11, 25, 36). Traditionally, K channels from two distinct families were known to serve in this dilator role. One family is characterized by protein subunits having two transmembrane-spanning domains and one pore domain. K channels in this family include inwardly rectifying (K<sub>a</sub>) and ATP-sensitive (K<sub>a,ATP</sub>) K channels. A second family consists of protein subunits having six transmembrane-spanning domains and one pore domain. Members of this family include delayed rectifier or voltage-activated (K<sub>V</sub>) and calcium-activated (K<sub>Ca</sub>) K channels. Both of these K channel families require the combination of four protein subunits, each contributing one pore domain, to form a fully functional K channel.

More recently, a third family of K channels has been suggested to be involved with dilations. This family consists of protein subunits having four transmembrane-spanning domains and two pore domains for each subunit. Thus, the family has been given the name, two-pore domain K channels (K<sub>2P</sub>). Unlike the other two families, K<sub>2P</sub>s require only two protein subunits, each contributing two pore domains, to form a functional channel. To date, 15 mammalian genes coding for K<sub>2P</sub> have been identified (16).

At least 10 members of the K<sub>2P</sub> family are expressed in the vascular system. They include TWIK-1, TWIK-2, TREK-1, TREK-2, TRAAK, TASK-1, TASK-2, TASK-3, TASK-4, and THIK-1 (3, 7, 14, 15, 17–19, 34, 38). Since there are no selective activators or inhibitors for any of the K<sub>2P</sub>, our understanding of their role is limited. Nevertheless, there are reports to suggest that TREK-1, TASK-1, and TASK-2 are involved with regulation of arterial diameter (3, 14, 15, 17–19). However, more studies are needed to fully demonstrate their function in arteries and arterioles.

The second K<sub>2P</sub> to be discovered, TREK-1 (gene name KCNK2), is of interest since it is highly expressed in the vascular system (5, 7, 14, 18, 34). In fact, message for TREK-1 is 10-fold more abundant in the middle cerebral artery of the rat than the next most abundant K<sub>2P</sub> (34). One group of investigators generated TREK-1 knockout (KO) mice (22) and studied vascular responses in basilar and mesenteric arteries (3, 15). These investigators reported that stimulation of endothelial NO production elicited by ACh in basilar arteries and ACh or bradykinin in mesenteric arteries was severely impaired or abolished in arteries from TREK-1 KO mice (3, 15). The authors suggested that the pathway for activating NO synthase is profoundly dysfunctional, likely through altered endothelial Ca<sup>2+</sup> regulation, in the absence of TREK-1 (3, 15).

These data suggest that TREK-1 is of fundamental importance in regulating NO production in endothelium. If true, then alterations in TREK-1 expression and/or function could be an important mechanism for impaired NO production in pathological states (39). Therefore, it is imperative to expand these studies involving TREK-1, endothelial NO production, and regulation of vascular tone.

The purpose of this study was to determine whether TREK-1 functions to regulate arterial diameter. First, we generated and validated a TREK-1 KO mouse line to be used in the subsequent studies. The mouse line was of major importance since there are no selective activators or inhibitors for TREK-1. Second, we determined the cardiovascular phenotype in anesthetized mice by comparing wild-type mice to TREK-1 KO mice.
Because the heart and blood vessels express TREK-1, it is possible that any dysfunction, if found, would be a result of blood pressure or other cardiac phenotypic differences. Third, we compared endothelium-mediated dilations in aortas, basilar arteries, and middle cerebral arteries from wild-type and TREK-1 KO mice. Fourth, we studied K$^+$ currents elicited by polyunsaturated fatty acids in single smooth muscle cells isolated from cerebral arteries of wild-type and TREK-1 KO mice.

**METHODS**

All studies were approved by the Institutional Animal Care and Use Committee at the Baylor College of Medicine. **TREK-1 KO mice.** Gene targeting and generation of the TREK-1 mouse line were performed in conjunction with the Texas Institute of Genomic Medicine (Houston, TX). The gene for TREK-1, KCNK2, spans ~136 kbp of the mouse genome, having eight exons (Fig. 1A). For the mutant gene, a 4 kbp of KCNK2 consisting of the second exon (excluding the first 13 bp), all of the second intron, all of the third exon, and the first 23 bp of the third intron was replaced by a β-galactosidase/neomycin selection cassette (Fig. 1A). The mice were generated on a mixed background of C57BL6J and SV129 strains.

**PCR genotyping and reverse transcriptase-PCR.** PCR genotyping was conducted using tail DNA (see the online supplemental material and Supplemental Fig. 1A). Reverse transcriptase-PCR was conducted on RNA extracted from brain, aorta, and cerebral arteries (Fig. 1B). All primer pairs spanned at least two exons to avoid amplification of genomic DNA. See the online supplemental material for further details. The PCR products were sequenced by the fluorescent dye-terminator sequencing technique (SeqWright, Houston, TX).

**Real-time RT-PCR.** Relative expression of other K$_{2p}$s and the large-conductance Ca-activated K channel (BK$_{Ca}$) was measured in
cerebral arteries (mid-cerebral cerebral arteries and basilar artery) and aorta from three adult male TREK-1 KO mice and three adult male WT mice to determine whether there was upregulation of other channels in TREK-1 KO mice. TREK-2 (KCNK10) and TRAAK (KCNK4) were chosen since they are closely related mechanosensitive K_\text{app} (16). TASK-1 (KCNK3) and TWIK-2 (KCNK6) were chosen because they are highly expressed in cerebral arteries (34). Additionally, relative expression of TWIK-1 (KCNK1) and BK_{Ca} (KCNMA1) was measured. After reverse transcription, cDNA was quantified from cerebral arteries and aorta by real-time PCR using SYBR Green PCR Master Mix on an Applied Biosystems 7000 Sequence Detection System. More details of the method and the primers used are presented in the online supplemental material. The efficiency of each primer set was assessed using cDNA from mouse brain and determined to be >95%. Relative expression of each gene was normalized to GAPDH with the following equation: Relative expression = 2^{\frac{\Delta \Delta Ct}{Ct_{GAPDH}}} and Ct = the cycle threshold.

Immunoprecipitation. Immunoprecipitation of TREK-1 was performed as previously described (49). Briefly, TREK-1 from mouse or rat brain homogenates was immunoprecipitated using two C-terminal goat anti-TREK-1 antibodies (100 μl of each, Santa Cruz C-20 and E-19; Santa Cruz Biotechnology, Santa Cruz, CA) and protein G Sepharose beads (GE Healthcare, Piscataway, NJ). TREK-1 was eluted from the beads, and protein was separated by electrophoresis. Membranes were probed overnight with the CT#67 antibody (1:400 dilution, SA Goldstein, University of Chicago), a rabbit polyclonal antibody directed against C-terminal amino acids 371–396 of rat TREK-1 (49). These 26 amino acids of the C-terminal are identical to antibody directed against C-terminal amino acids 371–396 of rat TREK-2 (51). The lower band of the doublet represents alternative splicing produced two bands as expected (51) that differed by ~200 bp (Fig. 1B). Expression of KCNK2 mRNA was determined using whole brains from KCNK2^{+/+} and KCNK2^{-/-} mice (n = 5 each). The schematic location and direction of the primers and the results of the PCR are shown in Fig. 1B. Primer sets spanning any part of exons 2 or 3 failed to amplify a product in KCNK2^{-/-} mice, while the expected amplicons were detected in the KCNK2^{+/+} and KCNK2^{-/-} mouse brains (see Fig. 1B and Supplemental Fig. 1 in the online version of this article). Alternative splicing produced two bands as expected (51) which was used in the immunoprecipitation protocol. Rats were used as positive controls, as previously reported (49). The bands around 34 kDa represent Protein G, which was used in the immunoprecipitation protocol. Rats were used as positive controls, as previously reported (49). The Western blot of brain homogenates from WT mice and rats show two prominent bands at ~50 kDa (Fig. 1C). The upper band of the doublet represents one or both splice variants for TREK-1 (51). The lower band of the doublet represents alternatively translated TREK-1 (49). Of note, the bands near 50 kDa are absent in brain homogenates of TREK-1 KO mice.

Relative expression of K\textsubscript{2,\textgamma} and BK\textsubscript{Ca}. Figure 2 shows the relative expression of BK_{Ca} and K\textsubscript{2,\textgamma} in aorta and cerebral arteries of WT and TREK-1 KO mice (n = 3 each). Note that mutation of KCNK2 did not alter the expression of TREK-2, TRAAK, TASK-1, TWIK-1, TWIK-2, or BK_{Ca}. Figure 2 also shows the relative abundance of mRNA for these K\textsubscript{2,\textgamma} and
BK<sub>Ca</sub>. TREK-1 is ∼100-fold more abundant in the cerebral arteries than in the aorta of WT mice.

**Cardiovascular phenotype.** Table 1 shows a number of cardiovascular indices obtained from WT and TREK-1 KO mice. Heart rate and blood pressure did not differ between the two genotypes. Peak ventricular pressure and +dP/dt<sub>max</sub>, indicators of cardiac systolic function, were not significantly different between genotypes. Similarly, −dP/dt<sub>max</sub> and tau (time constant for left ventricle relaxation), which are measures of cardiac diastolic function, were similar. Pulse wave velocity was not significantly changed in the TREK-1 KO mice.

Isoflurane, a potent dilator and a nonspecific activator of TREK-1 (41), was used to determine whether the absence of TREK-1 affected the coronary flow reserve. The peak blood flow velocities in the left main coronary arteries were similar in WT and TREK-1 KO mice. The addition of isoflurane (1 and 2.5%) to the ventilatory gas increased peak blood flow velocities in the left main coronary arteries were similar in WT and TREK-1 KO mice. The peak blood pressure to the same degree in each genotype in a concentration-dependent manner (data not shown).

**Middle cerebral arteries.** Resting diameters of MCAs after pressurization to 75 mmHg for WT and TREK-1 KO mice were 153 ± 4 μm (n = 25) and 152 ± 4 μm (n = 26), respectively. The constrictor responses to cumulative doses of phenylephrine (0.1–100 μM) were not significantly different between the two genotypes (P = 0.32, n = 9 and 10 for WT and TREK-1 KO mice, respectively; data are not shown). Each artery was preconstricted with 3 μM PE, to luminally applied ATP in the absence (n = 10 for each genotype) and the presence of l-NAME and indomethacin (indo) (n = 8 or 9 per genotype). *P < 0.05 compared with corresponding genotype receiving 10<sup>−5</sup> M ATP without inhibitors; **P < 0.05 compared with corresponding genotype receiving l-NAME and indomethacin.

Table 1. Cardiovascular indices in WT and TREK-1 KO mice

<table>
<thead>
<tr>
<th>Cardiovascular Index</th>
<th>Units</th>
<th>WT</th>
<th>TREK-1 KO</th>
</tr>
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<tbody>
<tr>
<td>Heart rate</td>
<td>bpm</td>
<td>346 ± 33</td>
<td>336 ± 23</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>mmHg</td>
<td>97 ± 5</td>
<td>94 ± 8</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>mmHg</td>
<td>70 ± 4</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>Mean arterial blood pressure</td>
<td>mmHg</td>
<td>83 ± 4</td>
<td>75 ± 9</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>mmHg</td>
<td>26 ± 2</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>Peak left ventricular pressure</td>
<td>mmHg</td>
<td>100 ± 3</td>
<td>104 ± 6</td>
</tr>
<tr>
<td>+dP/dt&lt;sub&gt;max&lt;/sub&gt;</td>
<td>mmHg/s</td>
<td>9879 ± 1439</td>
<td>9497 ± 886</td>
</tr>
<tr>
<td>−dP/dt&lt;sub&gt;max&lt;/sub&gt;</td>
<td>mmHg/s</td>
<td>−13755 ± 3257</td>
<td>−10337 ± 1020</td>
</tr>
<tr>
<td>Tau</td>
<td>s</td>
<td>8.1 ± 1.4</td>
<td>7.5 ± 0.8</td>
</tr>
<tr>
<td>Pulse wave velocity</td>
<td>cm/s</td>
<td>568 ± 109</td>
<td>654 ± 170</td>
</tr>
</tbody>
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Values are expressed as means ± SE; n = 5 for each genotype. WT, wild type; KO, knockout.
TREK-1 KO mice (n = 4 for each genotype; data not shown).

**Basilar artery.** Because we found no genotypic differences in endothelium-mediated dilations in the MCA, we repeated previous studies using the basilar artery (3). This included pressurization of the artery without luminal flow and administration of dilating agents to the extraluminal bath in an identical concentration. Resting diameters after pressurization to 75 mmHg were not significantly different between genotype [214 ± 7 μm and 223 ± 7 μm for basilar arteries from WT and TREK-1 KO mice, respectively (n = 16 for each group, P = 0.38)]. Constrictions to endothelin-1 (ET-1) were similar in basilar arteries from WT and TREK-1 KO (n = 5; see Supplemental Fig. 2).

Figure 4 (left) shows dilations of the basilar arteries to 10^{-5} M ACh when preconstricted with 10 nM ET-1. Blocking NO production with l-NAME decreased the dilation to ACh (P = 0.009) and the combination of l-NAME and indomethacin further reduced the dilation (P < 0.001); however, the genotype of the basilar artery had no significant effect on the responses to ACh (n = 5 or 6 per group).

Basilar arteries in this study (Fig. 4, left) were from mice on a mixed genetic background of C57BL6J and SV129 strains. Fig. 4 (right) shows that after four backcrosses onto a C57BL6J background, basilar arteries from TREK-1 KO mice diluted to ACh, and these dilations were not significantly different from the dilations of WT littermate mice (n = 5 for each group).

Figure 5 shows dilations to α-linolenic acid (ALA), a nonspecific dilator of TREK-1. The dilations in basilar arteries from WT and TREK-1 KO mice were not significantly different (P = 0.83; n = 5 for each group). The BKca blocker, penitrem A (200 nM), was present in these studies since α-linolenic acid and other polyunsaturated fatty acids also activate BKca. At the end of each experiment 10^{-5} M MAHMA NONOate, an NO donor, was administered. Dilations to MAHMA NONOate were similar in basilar arteries from WT and TREK-1 KO mice (n = 5 for each genotype, data not shown).

**Aorta.** Isometric constrictions to phenylephrine and endothelium-mediated relaxations to carbachol (a cholinergic agonist) were similar in WT and TREK-1 KO mice (see Supplemental Fig. 3 and supplemental material online).

**Electrophysiology studies.** CVSMC sizes, as measured by cell capacitance, were similar between the genotypes. The capacitance, which is directly related to cell surface area, was 12.0 ± 0.4 pF (n = 50) and 12.4 ± 0.3 pF (n = 56) for CVSMCs from WT and TREK-1 KO, respectively (P = 0.41). Whole cell currents recorded in CVSMCs from WT and TREK-1 KO mice were almost identical (Fig. 6A and Supplemental Fig. 4). Figure 6A shows the effects of 10^{-5} and 10^{-4} M α-linolenic acid on whole cell currents in CVSMCs from WT and TREK-1 KO mice. The vehicle for α-linolenic acid, ETOH, had no effect on current when given alone (data not shown). Interestingly, CVSMCs from TREK-1 KO showed an enhanced response to 10^{-4} M α-linolenic acid compared with WT. Repeated-measures ANOVA revealed a significant interaction between genotype and membrane potential (P = 0.002; n = 7 and 4 for WT and TREK-1 KO, respectively).

Arachidonic acid is one of the most widely used tools used to activate TREK-1 (10, 20, 33, 51). Figure 6B shows whole cell currents before and after the addition of 10^{-5} M arachidonic acid. Tetraethylammonium (TEA; 10 mM), a blocker of BKca that does not inhibit TREK-1 (42), was present in all experimental conditions. Arachidonic acid increased the whole cell currents in both CVSMCs for both genotypes. However, unlike α-linolenic acid, there was no significant difference between currents from WT and TREK-1 KO mice (P = 0.88; n = 9 and 7, respectively).

Figure 7 shows the whole cell currents after the addition of α-linolenic acid (n = 12 each for group) or arachidonic acid (n = 7 WT and n = 6 KO) when 200 nM penitrem A (PA), a BKca blocker, was present in the bath. Penitrem A not only prevented the increase in currents elicited by α-linolenic or arachidonic acid.
acid, but the whole cell currents actually decreased upon application of either α-linolenic or arachidonic acid. Inhibition of voltage-sensitive K channels by polyunsaturated fatty acid may account for the decreased current (9). In the absence of penitrem A, the decrease in whole cell current due to the inhibition of voltage-sensitive K channels is masked by the much larger increase in BKCa currents. The vehicle for penitrem A, DMSO, had no effect on whole cell currents when administered alone (data not shown). Similar to penitrem A, 200 nM iberiotoxin, another selective BKCa blocker, prevented the increase in currents upon administration of 10^{-4} M α-linolenic acid or 10^{-5} M arachidonic acid (data not shown).

**DISCUSSION**

The purpose of this study was to determine whether TREK-1 functions to regulate arterial diameter. Because there are no selective activators or inhibitors, we generated mice that did not express TREK-1. From our results, we conclude that regulation of arterial diameter is not altered in mice lacking TREK-1. This conclusion is based on the following observations:

1. Dilator responses were similar in arteries from WT and TREK-1 KO mice (Figs. 3B, 4, and 5, and Supplemental Fig. 3B). In addition, the increase in blood velocity in the left main coronary artery was similar in WT and TREK-1 KO mice after the addition of isoflurane, a nonselective activator of TREK-1 (41), to the ventilatory gas.

2. Freshly isolated CVSMCs did not show TREK-1-like currents (Figs. 6 and 7, and Supplemental Fig. 4). Our conclusion is strengthened and amplified by the confirming results from arteries of multiple origins or vascular beds, the use of multiple vasoactive agents, and the electrophysiological studies.

We report that dilator responses were not altered in arteries from TREK-1 KO mice. These studies include velocity measurement in the left main coronary artery to isoflurane (data not shown), dilations of the middle cerebral artery to ATP (Fig. 3B), dilations of the basilar to ACh (Fig. 4), dilations of the basilar artery to α-linolenic acid (Fig. 5), and relaxations of the aorta to carbachol (Supplemental Fig. 3B). Not only were the responses similar in these arteries from WT and TREK-1 KO mice, but the NO and EDHF components were identical in the middle cerebral arteries (Fig. 3B), and the NO and cyclooxygenase-dependent components were identical in the basilar arteries of WT and TREK-1 KO mice (Fig. 4). Given that we used only one or two concentrations of the dilating agents, it is possible that we missed subtle differences in the concentration response curves of the dilators.

We observed increased currents in CVSMCs obtained from both WT and TREK-1 KO mice after administering either of the polyunsaturated fatty acids, α-linolenic, or arachidonic acid (Fig. 6). While polyunsaturated fatty acids activate TREK-1
(10, 20, 33, 51), they also can activate BKCa and the K\textsubscript{2pS}, TREK-2, TRAAK, TWIK-2, and THIK-1 (12, 27, 35, 40, 44, 46). Interestingly, the currents elicited by α-linolenic acid, but not arachidonic acid, were greater in CVSMCs from TREK-1 KO mice than those from WT mice. We have no explanation for this enhanced current for one polysaturated fatty acid, α-linolenic, but not another, arachidonic acid. Regardless of the magnitude of the current, selective BKCa inhibitors, penitrem A, or iberotoxin, completely abolished the α-linolenic and arachidonic acid currents in both genotypes (Fig. 7). We conclude that the polysaturated fatty acids that stimulated currents in CVSMCs from either WT or TREK-1 KO mice were a result of BKCa activation. Thus, TREK-1-like currents were not observed in CVSMCs from WT mice after blockade of BKCa and there were no differences in currents between the genotype. The electrophysiological studies reinforce the idea that TREK-1 is not involved with regulation of arterial diameter.

Our results in isolated basilar arteries differ from those of a previous study that used a different strain of TREK-1 KO mice (3). Although we found no differences in dilator responses in the basilar arteries from WT and TREK-1 KO mice, Blondeau et al. (3) reported that dilations elicited by ACh and α-linolenic acid were abolished or severely attenuated in basilar arteries from their strain of TREK-1 KO mice. Given this discrepancy, we felt that several pertinent points should be discussed.

One possibility for the discrepancy is that our mouse model is not a true TREK-1 KO line. Because our model was integral to these studies, it was thoroughly characterized in terms of gene structure, message transcribed, and protein translated. PCR genotyping and reverse transcriptase PCR confirmed the insertion of the β-galactosidase/neomycin selection cassette and the absence of exon 3 and most of exon 2 (Fig. 1, A and B). Finally, Western blot analysis, following immunoprecipitation, conclusively demonstrated that TREK-1 protein was absent in the KO mice (Fig. 1C). This is the first Western blot that has been published to date using TREK-1 KO mice. The antibodies used for the immunoprecipitation and Western blot should detect any TREK-1 being translated, regardless of which one of the multiple start codons was used to initiate protein translation. Thus, our studies conclusively demonstrate that our mouse line does not express TREK-1.

Different knockout strategies for K\textsubscript{c} channels have produced different phenotypes in mice (37). It is possible that the different strategies used to generate the two TREK-1 knockout strains account for the differences in the results in isolated basilar arteries. In our strategy, both the second and third exons of KCNK2 were deleted, removing all start codons. The earliest in-frame start codon of the mutated KCNK2 mRNA corresponds to a portion of the last transmembrane domain. Additionally, our knockout should express β-galactosidase and a neomycin selection protein (see Fig. 1A). It is possible that these two alien proteins could alter function in the cell; however, it is difficult to envision that their expression would restore TREK-1 function in the knockout arteries. The previous strategy for the TREK-1 knockout strain, which was used by Blondeau et al. (3), removed only the third exon (22). A start codon in exon 2 would be retained in the mutated TREK-1 with the possibility that a protein, 35 amino acids in length, could be expressed. This protein would consist of 15 amino acids from the N-terminal of TREK-1 and a 20-amino acid nonsense sequence coded by a frame-shift before an early stop codon. If translated, this peptide could bind and interfere with the post-translational modification of other K channels, particularly other K\textsubscript{2pS}. Similar scenarios have occurred where a truncation of one K channel isoform disrupted the expression of other K\textsubscript{2} isoforms by producing a dominant-negative effect (13, 37, 45). While this scenario is possible, we believe it to be unlikely. Even if this short peptide is expressed, a dominant-negative effect of this short peptide seems impractical given that it should not bind to critical segments of other K\textsubscript{2p} channels.

Unlike the previous study (3), dilations to ACh were similar in basilar arteries from WT and TREK-1 KO mice. The TREK-1 KO mice used by Blondeau et al. (3) were on a C57BL6J background. Our mice were a mixed C57BL6J/SV129 background. Even after backcrossing our mixed strain to C57BL6J mice for four generations, we still observed robust dilations to ACh in basilar arteries from TREK-1 KO mice (Fig. 4). Four generations of backcrossing should produce mice that were on average 94% of the C57BL6J strain. Our backcrossing studies leave little room for strain differences to account for the differences in results of our study and the previous study (3).

Unlike the previous studies (3), dilations to the nonspecific activator of TREK-1, α-linolenic acid, were similar in basilar arteries from WT and TREK-1 KO mice (Fig. 5). Because polysaturated fatty acids, including α-linolenic acid, not only activate TREK-1 but also BKCa, we included pentrem A to block the confounding effects of BKCa activation. Contrary to our study, Blondeau et al. (3) reported that dilations to α-linolenic acid and other polysaturated fatty acids were either completely abolished or severely attenuated in arteries from TREK-1 KO mice, even without any BKCa blockers present in the preparation. Since BKCa are highly expressed in cerebral arteries (see Fig. 2 and Ref. 31) and are activated by polysaturated fatty acids, it would appear that BKCa blockers are somehow silenced in basilar arteries of TREK-1 KO mice used in the previous study (3).

In our study, we measured K\textsuperscript{+} currents in CVSMCs from WT and KO mice. We did not find TREK-1 or TREK-1-like currents in the WT mice. In fact, currents in WT and knockout were very similar. This observation is consistent with our conclusion that neither α-linolenic acid nor arachidonic acid dilates cerebral arteries by activating TREK-1 on smooth muscle. However, these electrophysiological studies in CVSMCs cannot address any altered function in endothelium that would involve NO production. While Blondeau et al. (3) reported differences in dilator responses in the basilar artery from WT and TREK-1 knockout mice to α-linolenic acid, no electrophysiological studies were included that demonstrated the presence and absence of TREK-1 currents in WT and TREK-1 KO mice, respectively.

Both our TREK-1 KO strain of mice and the previous strain (3, 15, 22) are normotensive. Given the severity of the endothelial dysfunction previously reported (3, 15), it is surprising that the TREK-1 KO mice used in the other studies were normotensive. However, it is possible that not all arteries, express TREK-1. Endothelial dysfunction to a selected number of arteries, which otherwise express TREK-1, may not be sufficient to affect blood pressure as a whole in the TREK-1 KO mice.
K channels, in general, are known to be involved with various cellular processes, including control of vessel diameter, regulatory and apoptotic volume regulation, cell migration, and proliferation (23, 24, 32). With the discovery that K\textsubscript{2p}s, including TREK-1, are expressed in arteries (3, 7, 14, 15, 17–19, 34, 38), the question arises regarding their function. In this study, we find no evidence that TREK-1 is involved with the regulation of arterial diameter. However, it must be pointed out that we find no evidence that TREK-1 is involved with the regulation of arterial diameter. While we cannot explain this discrepancy, we do wish to point out that a role for TREK-1 in the regulation of vascular diameter is controversial. TREK-1 could be involved with other cellular processes. For example, members of the TASK subgroup of the K\textsubscript{2p} family are involved with regulatory and apoptotic volume regulation (2, 3, 80). In the mouse embryo, apoptosis recruits a K channel whose characteristics closely resemble that of TREK-1 (50). One role that is particularly appealing is volume regulation since TREK-1 is a mechanosensitive ion channel (42, 43). Even without a change in the extracellular osmolarity, regulation of cell volume is required for normal cellular homeostasis. In addition, hormones often utilize volume regulation as a mechanism to exert their effects (29).

There is much to be learned regarding the functional role of K\textsubscript{2p}, in general, and TREK-1, specifically, in vascular tissues.

**REFERENCES**


**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.


