Mechano- or Acid Stimulation, Two Interactive Modes of Activation of the TREK-1 Potassium Channel*

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TREK-1 is a member of the novel structural class of K⁺-selective channel. Moreover, TASK-1 is extremely sensitive to intracellular acidosis. Mutagenesis experiments identify the carboxyl-terminal region of TREK-1 as critical for the integration of both mechanical and acidic stimuli.

EXPERIMENTAL PROCEDURES
The cDNA cloning, mutational strategy, cell culture, transfection, and electrophysiology procedures have been previously described elsewhere (2, 16). Briefly, murine TREK-1 and TRAAK cDNAs were cloned into pCRES-CD8 vector (1, 7). COS cells were transfected with the DEAE dextran procedure. The positive cells were visualized using the anti-CD8 antibody-coated bead method (2). Mutant TREK-1 Δ46 was deleted at Thr-368, [Thr-322, 89 at Thr-322, 89 instead of 10 m M Hepes) for 10 min (2). The NH₄Cl prepulse solution contained 20 m M NH₄Cl, pH 7.4, with NaOH, and pipette solution (INT) contained 150 m M KCl, 3 m M MgCl₂, 5 m M EGTA, and 10 m M Hepes, pH 7.2, with KOH. The EXT-K⁺-rich solution contained 150 m M KCl instead of 150 m M NaCl. The HCO₃⁻ solution used to induce intracellular acidosis was made by substituting 90 m M NaCl with 90 m M NaHCO₃. A K⁺-rich HCO₃⁻ solution was made by substituting 90 m M KCl with 90 m M KHCO₃. For cell-attached experiments, the EXT solution contained 150 KCl instead of 150 NaCl, and the pipette contained the EXT solution (150 NaCl). To induce intracellular acidosis, 90 m M KHCO₃ was substituted for KCl (31). CO₂-rich solution was prepared by bubbling CO₂ in an EXT KCl solution containing 25 m M HCO₃⁻ instead of 10 m M Hepes) for 10 min (pH 6.0). The NH₄Cl prepulse EXT solution contained 20 m M NH₄Cl substituting for 20 m M KCl. For inside-out experiments, the pipette solution was EXT, and the bath solution was INT. For acidic (pH 5.0–6.0) INT solutions, Hepes was substituted with Mes, and for basic (pH 8.0) INT solution, Hepes was substituted with Tris. Hepes INT solutions at both acidic and basic pH gave similar results (not shown). Mechanical stimulation was applied through an open loop pressure.

The suppression of the channel's sensitivity to TASK-1 opening with subsequent K⁺ influx and hyperpolarization.

The near completion of the sequencing of the nematode Caenorhabditis elegans genome recently identified more than 80 K⁺ channel genes divided into three major structural classes: (i) the inward rectifiers with two TMS and a single P domain; (ii) the Shaker types with six TMS and a single P domain comprising the voltage-gated Kvs, the calcium-activated Slo, the calcium-regulated SK, the Eag/Erg, and the KcI channels; and (iii) the two P types with 4TMS being the largest structural class (about 50 genes) (4–6). Despite an overall similar 4TMS/2P structure, the sequence identity between these channels is very low (less than 30%) (5, 6).

The mammalian family of 4TMS/2P K⁺ channel comprises TWIK-1, TWIK-2, TASK-1, TASK-2, TREK-1, and TRAAK (1, 2). TREK-1 is opened by intracellular acidification (31) as shown that internal acidification opens TREK-1. Indeed, lowering pH shifts the pressure-activation relationship toward positive values and leads to channel opening at atmospheric pressure. The pH-sensitive region in the carboxyl terminus of TREK-1 is the same that is critical for inward rectifiers with two TMS and a single P domain; AA, arachidonic acid; Mes, 4-morpholineethanesulfonic acid; NPo, number of channels × open channel probability.

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¶ The abbreviations used are: TMS, transmembrane segment(s); AA, arachidonic acid; Mes, 4-morpholineethanesulfonic acid; NPo, number of channels × open channel probability.

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generating system and monitored at the level of the patch pipette throughout the experiment by a calibrated pressure sensor. This system provides a stable pressure pulse (16). Pressure-effect relationships were fitted with Boltzmann equations. AA was dissolved in ethanol at a concentration of 100 mM, flushed with argon, and kept at −20 °C for 1 week. All chemicals were obtained from Sigma.

RESULTS

TREK-1 cDNA was transiently transfected in COS cells, and channel activity was recorded using the whole cell patch clamp configuration. In a cell voltage-clamped at 0 mV, AA superfusion induces a strong outward current (Fig. 1A). In the same cell, repetitive application of 90 mM HCO$_3^-$, which produces intracellular acidification (31, 32), mimics AA stimulation (5.5 ± 0.5-fold increase, n = 29 at 0 mV). Both AA and HCO$_3^-$ are ineffective on control mock-transfected cells (0.5-fold increase, n = 7) (Fig. 1B). Moreover, substitution of 90 mM NaCl by an equivalent concentration of sodium gluconate does not mimic the activation of TREK-1 by NaHCO$_3$ (n = 6). The I-V curve of the current induced by HCO$_3^-$ shows a prominent outward going rectification in physiological K$^+$ conditions and reverses at the predicted $E_K$ value of −80 mV (Fig. 1C). When external Na$^+$ is substituted with K$^+$, the reversal potential shifts to 0 mV, and the I-V curve remains outwardly rectifying (Fig. 1D). AA similarly activates TRAAK, while HCO$_3^-$ is ineffective (0.9 ± 0.1, n = 17) (Fig. 1E).

The activation of TREK-1 by the addition of HCO$_3^-$ is also observed at the single channel level in the cell-attached patch configuration (n = 13) (Fig. 2A). In this configuration, the pH of the external solution bathing channels under recording is clamped by the pipette medium (pH 7.2), and channel modulation is expected to be due to intracellular effects. In this experiment, the activity of the mechanogated channel TREK-1 is recorded both at atmospheric pressure and during the application of a −66 mm Hg pressure stimulation (Fig. 2A, inset). At atmospheric pressure, channel activity (NPo) is very low (0.80 ± 0.22, n = 27). Both the resting and the pressure-induced activities are reversibly stimulated by the HCO$_3^-$ addition (Fig. 2A). Superfusion of a CO$_2$-rich solution, which also produces a strong intracellular acidification (32), leads to TREK-1 opening in the cell-attached patch configuration (n = 15) (Fig. 2B). Again, both basal and stretch-induced activities are strongly stimulated. The current induced by CO$_2$ is outwardly rectifying and reverses at −80 mV (Fig. 2B, inset). Another classical approach to alter pH$_i$-regulated mechanisms is the NH$_4$Cl prepulse technique, which relies on the greater membrane permeability for NH$_4^+$ than for NH$_3^+$ ions (32). The addition of NH$_4$Cl (producing intracellular alkalinization) does not affect TREK-1 channel activity, while washout of NH$_4$Cl (producing intracellular acidosis) strongly stimulates TREK-1 channel activity (n = 6) (Fig. 2C). The current activated by NH$_4$Cl withdrawal similarly displays a strong outward rectification (Fig. 2C).

The effects of intracellular acidification were also studied on excised inside-out patches expressing TREK-1 (Fig. 3). Channel activity was recorded at both atmospheric pressure and during membrane stretch. Gradual intracellular acidification from 7.2 to 5.0 induces channel opening at atmospheric pressure (Fig. 3, A and B). NPo is strongly increased, while the single channel conductance is gradually decreased by internal acidosis (Fig. 3B). Half-maximal activation is induced at pH 6.0, and a drop of 0.7 pH unit already produces a significant increase in channel activity (Fig. 3B). A −42 mm Hg stretch induces a robust channel opening at intracellular pH between 7.2 and 6.0, although it fails to further open TREK-1 at pH 5.0 (Fig. 3A). The pressure-activity relationship of TREK-1 is presented in Fig. 3C. At physiological intracellular pH 7.2, the pressure-activity relationship is described by a Boltzmann function with a pressure required for half-maximal activity ($P_{0.5}$) of −36.8 ± 2.1 mm Hg (n = 13). Progressive lowering in pH$_i$ gradually shifts the $P_{0.5}$ toward positive values, leading to constitutive channel activity under atmospheric pressure at pH 5.0 (Fig. 3C and D). Half-maximal effect is observed at pH 5.9 (Fig. 3D).

The modulation of channel activity by intracellular acidification was studied on deleted TREK-1 mutants in the inside-
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out patch configuration. We used a protocol including two acidic steps to pH 6.0 and 5.0 at both atmospheric pressure and during a -66 mm Hg stretch (Fig. 4, B–D). Lowering internal pH from 7.2 to 6.0 reversibly induces TREK-1 wild type opening at atmospheric pressure and slightly potentiates internal pH from 7.2 to 6.0, and stimulation of the stretch-induced activity is only observed at pH 5.0 (Fig. 4, D and E, fifth panel). No channel activity is detected with Δ113 at both pressures and at all pH conditions tested (n = 7).

Progressive deletion of the cytosolic region of TREK-1 shifts the pressure-activity curve toward more negative values, leading to less sensitive mutant channels (Fig. 5, A–C). For instance, the relationship for Δ103 is about 60 mm Hg more negative compared with that of TREK-1 at pH 7.2 (Figs. 3C and 5C). Lowering pH to 6.0 and then 5.0 gradually shifts the relationship toward more positive values and strongly stimulates channel activity elicited by pressure (Figs. 4D and 5C).

Unlike TREK-1, TRAAK, the other stretch- and AA-sensitive member of the 4TMS/2P K+ channel family (P0.5 = -46 ± 2 at pH 7.2; n = 7) is quite insensitive to intracellular acidosis (Figs. 1E and 6, A and D). However, the high pH sensitivity of TREK-1 can be transferred to TRAAK when the proximal C-terminal regions are exchanged (Fig. 6, B, C, and E). As observed for TREK-1, internal acidosis shifts the pressure-activity curve toward positive values, resulting in the opening of the TRAAK/TREK-1 chimera at atmospheric pressure (P0.5 = -40 ± 3, n = 7; P0.5 = -32 ± 5, n = 4; P0.5 = -26 ± 3, n = 4, at pH 7.2, 6.0, and 5.0, respectively). Moreover, HCO3 superfusion induces a strong stimulation of the whole cell membrane current of the TRAAK/TREK-1 chimera (3.8 ± 0.4, n = 10), although it has no effect on TRAAK (0.9 ± 0.1, n = 17) (Fig. 1E).

**DISCUSSION**

Mammalian mechano-gated K+ channels have been previously described in atrial and ventricular cardiac myocytes, in neurons from mesencephalic and hypothalamic areas of the brain as well as in kidney (17–20, 29). Negative pressure applied to cell-attached patches activates K+ channels (17–20). The pressure to induce half-maximal activation is between -12 and -18 mm Hg at +40 mV. I-V curves are outwardly rectifying, and single channel conductances are 94 and 143 picoeinemens at +60 mV in symmetrical K+ for cardiac and brain cells, respectively. Openings induced by stretch are typically bursty and flickery. The probability of these channels to open at a fixed pressure is voltage-dependent with a higher opening at depolarized potentials. Both cardiac and neuronal channels are similarly opened by AA and other lipophilic compounds in the micromolar range (17–20). AA activation is found in cells treated with cyclo-oxygenase and lipoxygenase inhibitors, indicating that AA itself can directly activate these channels. Unsaturated fatty acids (linoleic, linolenic, and docosahexaenoic acids) but not saturated fatty acids also activate these channels. A very important property of these native mechan gated arachidonic-sensitive K+ channels is that they are also stimulated by lowering cytoplasmic pH over the range 7.2–5.6 (17, 20), and the channels are more sensitive to pressure at acidic intracellular pH.

Two recently cloned 4TMS/2P channels, TREK-1 and TRAAK (1, 2, 7, 16), have many of the properties of endogenous mechan gated K+ channels. Both channels are activated by shear stress, cell swelling, and membrane stretch (2, 16). Moreover, TREK-1 and TRAAK are opened by AA, linoleic, linolenic, and docosahexaenoic acids but are resistant to saturated fatty acids (2, 7). The single channel conductance of about 100 pS at +50 mV in symmetrical K+, the outward going rectification, the bursty and flickery openings, and the voltage dependence are identical to described endogenous channels (2, 16–20).

The mechano-gated K+ channel TREK-1 is opened, like native cardiac and neuronal mechano-sensitive K+ channels (17–
acids may indeed be involved.

Deletion and chimeric analysis indicate that the pH-sensitive region of TREK-1 is located in the carboxyl-terminal region between Val-298 and Thr-368 (Δ113–Δ46 region). Further deletion in this carboxyl-terminal region impairs activation by stretch, AA, and pH, demonstrating its critical importance for channel function (2). The sensitivity to pH is conferred to TRAAK when the proximal carboxyl terminus of TREK-1 is exchanged with TRAAK, demonstrating that the region between Δ46 and the fourth TMS is necessary and sufficient to provide pH sensitivity. Progressive deletions of the carboxyl terminus of TREK-1 show that pH sensitivity as well as mechanogating is gradually altered. These results indicate that the whole segment (Val-298 to Thr-368) is probably involved in acidic and stretch modulation and that several amino acids may indeed be involved.

TREK-1 is modulated by a variety of mechanical stimuli such as stretch, swelling, and shear stress and by a variety of chemical stimuli including AA, ligands producing cAMP-dependent phosphorylation, and acidic stimuli (2, 3). TREK-1 is therefore an example of molecular integrator. Furthermore, mechanical activation of TREK-1 is enhanced by intracellular acidosis,
demonstrating that a response to one type of stimulus alters the sensitivity to others.

By integrating multiple stimuli, TREK-1 probably fulfills an essential physiological function in the nervous and cardiovascular systems. Under physiological conditions, effectors of TREK-1 activity are probably stretch, AA, and neurotransmitters or hormones that increase intracellular cAMP (2). It is unlikely that activation of TREK-1 by acidification of the intracellular medium is an important physiological stimulus, although one cannot eliminate transient variations below pH 7.0 (3). Protection against an exaggerated cellular Ca\(^{2+}\) invasion is one of the important roles of several types of K\(^{+}\) channels (34, 35). This has been particularly well demonstrated for the large conductance Ca\(^{2+}\)-activated K\(^{+}\) channels (K\(_{Ca}\)-channels of the BK type) as well as for ATP-sensitive K\(^{+}\) channels (K\(_{ATP}\) channels) (36–38). When Ca\(^{2+}\) invades a cell, one of the ways to resist further Ca\(^{2+}\) invasion (which would be deleterious), from voltage-sensitive Ca\(^{2+}\) channels and/or through NMDA receptors, is hyperpolarization. Hyperpolarization puts the cell membrane potential far from the threshold of voltage-sensitive Ca\(^{2+}\) channel activation and favors the NMDA receptor-associated Ca\(^{2+}\)-permeable channel blockade by Mg\(^{2+}\). It has been particularly well demonstrated that, when hypoxia, anoxia, or hypoglycemia occur, the associated decrease of intracellular ATP and the related increase of intracellular ADP result in the activation of K\(_{ATP}\) channels (34, 35, 39–41). This activation produced by inhibition of the energetic metabolism is protective in tissues where the channel is expressed such as in the heart or brain (35). In conditions of intracellular acidification, which would occur in many physiopathological situations such as brain or heart ischemia, opening of TREK-1 (K\(_{Hi}\) channel mode) may fulfill a similar protective role. TREK-1 channels in the K\(_{Hi}\) channel mode could work in concert with K\(_{Ca}\)-channels (BK type) and K\(_{ATP}\) channels. Cellular swelling, which also accompanies ischemia (42), would increase the effect of intracellular acidification on the activity of the TREK-1 channel (2). Ischemia in heart and brain is associated with a major K\(^{+}\) efflux (34, 35, 43). This efflux is supposed to be through K\(^{+}\) channels that open under these conditions (34, 35). However, specific blockers of K\(_{Ca}\)-channels or of K\(_{ATP}\) channels do not eliminate this efflux (43, 44). TREK-1 channels may thus constitute an important pathway for this K\(^{+}\) efflux.

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