Lysosphospholipids Open the Two-pore Domain Mechano-gated K⁺ Channels TREK-1 and TRAAK*

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The two-pore (2P) domain K⁺ channels TREK-1 and TRAAK are opened by membrane stretch as well as arachidonic acid (AA) (Patel, A. J., Honére, E., Maingret, F., Lesage, F., Fink, M., Duprat, F., and Lazdunski, M. (1998) EMBO J. 17, 4283–4290; Maingret, F., Patel, A. J., Lesage, F., Lazdunski, M., and Honére, E. (1999) J. Biol. Chem. 274, 26691–26696; Maingret, F., Fosset, M., Lesage, F., Lazdunski, M., and Honére, E. (1999) J. Biol. Chem. 274, 1381–1387. We demonstrate that lysosphospholipids (LPs) and platelet-activating factor also play a protective role during ischemia and inflammation.

The recently discovered family of mammalian two pore (2P)¹ domain K⁺ channels consists so far of seven members (TWIK-1, TWIK-2, TREK-1, TRAAK, TASK-1, TASK-2, and KCNK6) (4–12). Although these subunits share the same structural motif with four transmembrane segments, 2P domains, an extended M1P1 external loop (60–70 residues), and both amino and carboxyl termini intracellularly, they only share 25–50% identity. TWIK-1 and TWIK-2 encode weak inward rectifiers that are inhibited by internal acidosis but stimulated by protons, whereas TRAAK is also opened by membrane stretch, cell swelling, and shear stress (1–3). At atmospheric pressure, basal activity is negligible, and channels are opened by convex curvature of the plasma membrane (3). Mechano-gating does not require the integrity of the cytoskeleton, and the activating force is apparently directly coming from the bilayer (3). Mechanical activation of TREK-1 and TRAAK is mimicked by polyunsaturated fatty acids such as arachidonic acid (AA) and by the anionic amphiphile trinitrophenol (1, 3, 4). Recently, we showed that TREK-1, but not TRAAK, is also opened by inhalational anesthetics including chloroform, ether, halothane, and isoflurane as well as by mild intracellular acidosis (2, 13). In mouse tissues, TREK-1 is ubiquitous, with strong expression in brain and heart, whereas mouse TRAAK is restricted to the central nervous system, the spinal cord, and the retina (4, 6). In human tissues, TREK-1 is at low level or even absent from heart, whereas TRAAK is also detected in the placenta, testis, prostate, and small intestine (13). In the present report, we demonstrate that extracellular LPs as well as PAF mimic the effect of stretch and are potent openers of the neuronal 2P domain K⁺ channels TREK-1 and TRAAK.

EXPERIMENTAL PROCEDURES

COS cell culture, transfection, site-directed mutagenesis and electrophysiology procedures have been extensively described elsewhere (1–4).

Cell Culture—COS-7 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. The 2P domain K⁺ channel cDNAs were subcloned into the pRSV-CDS vector and transfected using the DEAE-dextran procedure. Cells were visualized 48 h after transfection using the anti-cDNA antibody-coated bead method.

Solution—For whole-cell experiments, bath solution (EXT) contained 150 mM NaCl, 5 mM KCl, 3 mM MgCl₂, 1 mM CaCl₂, 10 mM Hepes, pH 7.4 with NaOH, and pipette solution (INT) contained 150 mM KCl, 3 mM MgCl₂, 5 mM EGTA and 10 mM Hepes, pH 7.2 with KOH. For cellattached and inside-out experiments, the bath solution was INT, and the pipette contained EXT solution (5 mM KCl). All chemicals were obtained from Sigma except ginkolide B, trans-1,3-dioxolane, lyso-PAF, methyl arachidonyl fluorophosphonate, 7,7-dimethyl-1,2,3,4-tetrahydronaphthalene, and RHC-80267 were obtained from Biomol. Stock solutions were kept at –20 °C and renewed weekly. AA and arachidonoyl triluoromethyl ketone were dissolved in ethanol at the concentration of 100 mM. Saturated fatty acids, PAF, lyso-PAF, and PAF receptor antagonists ginkolide B and trans-1,3-dioxolane were dissolved at the concentration of 10 mM in ethanol. LPCs, LPA, LPI, choline, and Gd³⁺ were dissolved in water at the concentration of 10 mM. LPS and LPE were either saturated with fatty acids or dissolved at the concentration of 10 mM in 10% ethanol. Amiloride was dissolved at the concentration of 1 mM in water.

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dimethyllecosadienoic acid, methyl arachidonyl fluorophosphonate, bromophenacyl bromide, and diacylglycerol lipase inhibitor RHC-80267 were dissolved in MeSO at concentrations of 100 mM. GTPγS was dissolved in saline at the concentration of 100 μM. Cytochalasin D was dissolved at a concentration of 1 mg/ml in MeSO. Colchicine was dissolved in saline at the concentration of 0.5 mM.

Mutations—Polymerase chain reaction was used to generate aminoterminal deletions in TREK-1 by introducing a methionine just before Val-47. A carboxyl terminus deletion was generated by introducing a stop codon at Gly-308. The external loop MIP1 of mouse TREK-1 (amino acids 68–135) was substituted with the loop of human TASK-1 (amino acids 30–85).

Data Analysis—For whole-cell recordings, values of control currents were subtracted from the currents recorded in the presence of the various lipids. Results are presented as means of density current associated with their standard errors (S.E.). Cell capacitance was determined by hyperpolarization pulses of 10 mV from a holding potential of −80 mV. The number of experiments are indicated on each graph.

RESULTS

The biophysical and pharmacological properties of TREK-1 were investigated in transiently transfected COS cells. Cells expressing TREK-1 were voltage-clamped at 0 mV in the whole-cell patch clamp configuration, and lipids were applied in the bath as illustrated in Fig. 1A. As previously reported, TREK-1 was activated by polyunsaturated fatty acids including AA (10 μM) (Fig. 1A) (1). Similarly, 3 μM LPC (C14:0) opened TREK-1 and induced a strong outward current (Fig. 1A). Onset and offset kinetics for LPC activation were fast compared with AA (Fig. 1B). In physiological K⁺ conditions, the LPC-induced current displayed an outward-going rectification and reversed at −81 ± 3 mV (n = 6) (Fig. 1C). In symmetrical K⁺ conditions, the reversal potential was shifted to 0 mV, as expected for a K⁺-selective channel, and rectification was maintained (Fig. 1D). Activation of TREK-1 was dose-dependent, with a threshold concentration of 30 nM (Fig. 1E). Concentrations higher than 10 μM could not be tested since they produce irreversible cell leakage. Current stimulation was stable during continuous application of LPC up to 15 min (not shown). 10 μM LPC had no significant effect on mock-transfected or TASK-1- or TASK-2-expressing cells (Fig. 1F).

LPC (C18:1), lyso-PAF (C16:0), and PAF (C16:0) at the concentration of 10 μM induced a comparable stimulation of both TREK-1 and TRAAK (Fig. 2). The phospholipid PC, which contains two acyl chains (C18:0 or C18:1), and LPA, (C18:1), which lacks the head group, were ineffective on both channels at 10 μM (Fig. 2). Choline (100 μM; n = 7) as well as saturated fatty acids (10 μM) including myristate (C14:0), palmitate (C16:0), stearate (C18:0), and arachidate (C20:0) had no effect on TREK-1 and TRAAK (n = 6; data not shown). The PAF (C16:0) dose-effect curve was similar to that of LPC (C14:0), and the threshold concentration was 30 nM (Fig. 2C, inset). In Fig. 3, A–B, we examined the effect of four LPS with different polar heads (ethanolamine, serine, choline, and inositol) and comparable acyl chains (C16:0-C18:0). LPI and LPS are negatively charged, whereas LPC and LPE are neutral. LPE and LPS only weakly activated TREK-1 and TRAAK, whereas LPC and LPE produced strong activations (Fig. 3B). The activation of TREK-1 and TRAAK by LPC was also critically dependent on the length of the acyl chain (Fig. 3, C–D). C6:0 and C10:0 LPC were only weakly effective, whereas C14:0 and C18:0 LPC were strong openers of TREK-1 and TRAAK (Fig. 3D). Both saturated and unsaturated LPC long acyl chains (C18:0 and C18:1) were equally effective (Fig. 3D). Simultaneous application of LPC and AA produced reversible and additive opening of TREK-1 (Fig. 4).

The cationic cup former membrane modifying agent chlorpromazine completely and reversibly reversed LPC activation of TREK-1 (1 μM; n = 5) (Fig. 5A). Interestingly, chlorpromazine had no effect on TRAAK (n = 6) (not shown). Blockers of stretch-sensitive channels such as amiloride (2 mM) and Gd³⁺ (10 μM) (14) similarly reduced LPC stimulation of TREK-1 and TRAAK (Fig. 5, B–C) (n = 6). Basal TREK-1 activity, unlike.
TRAAK, was inhibited by protein kinase A and protein kinase C stimulation (1, 4, 6). Similarly, activation of TREK-1 by LPC was rapidly reversed by 500 μM 8-(4-chlorophenylthio)-cAMP (n = 5) and 400 nM phospholipase D-myristate 13-acetate addition (n = 3) (Fig. 6). Protein kinase A-mediated inhibition was slowly reversible over a 30-min wash, whereas protein kinase C inhibition was completely irreversible (Fig. 6).

Fig. 7 A shows that LPC added to the bath rapidly activated TREK-1 channels in the cell-attached patch configuration. In comparison, AA in the same configuration was weakly active and required several minutes of application to open the channels isolated by the patch pipette (Fig. 7 A). In the excised inside-out patch configuration, LPC decreased basal activity, whereas AA reversibly and strongly opened TREK-1 (Fig. 7 B). Moreover, intracellular LPC application reversed the opening induced by internal AA in the inside-out patch configuration (Fig. 7 C). In the outside-out patch configuration, LPC was only very weakly effective, whereas AA fully activated TREK-1 (Fig. 7 D). Similar results were obtained with TRAAK (not shown).

LPC activation of TREK-1 and TRAAK in the whole cell configuration was insensitive to the PAF receptor antagonists ginkolide B (10 μM; n = 4) and trans-1,3-dioxolane (10 μM; n = 4), to the phospholipase A2 inhibitors arachidonyl trifluoromethyl ketone (30 μM; n = 5), bromophenacylbromide (100 μM; n = 5), 7,7-dimethyleicosadienoic acid (15 μM; n = 5), and methyl arachidonyl fluorophosphonate (15 μM; n = 5), and to the diacylglycerol lipase inhibitor RHC-80267 (30 μM; n = 12). In the outside-out patch configurations, intracellular ATP (5 mM; n = 3) and GTP (1 mM; n = 7) did not affect LPC activation. In the inside-out patch configuration, the addition of intracellular GTPγS (100 μM; n = 3) or intracellular calcium (3 μM free calcium; n = 7) did not open channels. Cells treated for 8 h with the cytoskeleton-disrupting agents colchicine (500 μM) and cytchalasin D (3 μg/ml) displayed a similar sensitivity to LPC compared with control cells (n = 6).

Site-directed mutagenesis was used to investigate the respective role of amino and carboxyl termini as well as the external M1P1 loop in TREK-1 channel activation by LPC as well as AA (Fig. 8). Deletion of the amino terminus and exchange of external loops between TREK-1 and TASK-1 had no effect on AA and LPC activation (Fig. 8B). However, partial deletion of the TREK-1 carboxyl terminus strongly reduced both AA and LPC activation (Fig. 8B).

**Fig. 2.** Cone-shaped lysophospholipids open TREK-1 and TRAAK. **A**, TREK-1 whole-cell recording measured at 0 mV. 3 μM LPC (C18:1), 10 μM LPA (C18:1), 10 μM PC (C18:0), and 3 μM PAF (C16:0) were added to the bath for 30 s as illustrated by horizontal bars. **B**, structure of the lipid molecules. **C**, summary of the effects of various lipids at the concentration of 10 μM for 1 min on TREK-1 and TRAAK measured at 0 mV. The inset shows the dose-effect curve for PAF (C16:0) activation of TREK-1. Increasing concentrations of PAF were applied at 0 mV in the whole cell configuration for a duration of 1 min, with a washout after each application. The number of experiments is indicated.

**Fig. 3.** LPs with large polar heads and long hydrophobic acyl chains open TREK-1 and TRAAK. **A**, schematic illustrating the shape of the lipid molecules with increasing size polar heads. **B**, summary of the effects of LPs with increasing size polar heads on TREK-1 and TRAAK. Lipids were applied at the concentration of 10 μM for 1 min at a holding potential of 0 mV. pA/pF, picocamperes/picofarads. **C**, schematic illustrating the shape of the lipid molecules with increasing acyl chain length. **D**, summary of the effects of LPs with increasing size acyl chains. Lipids were applied at the concentration of 10 μM for 1 min at a holding potential of 0 mV. The number of experiments is indicated.
TREK-1 and TRAAK were opened by various lipids including polyunsaturated FAs, LPs, and PAF. Previous reports have suggested that lipids, independently of their metabolic conversion, may directly modulate stretch-sensitive ion channel function by either interacting with the channel protein or by partitioning into the bilayer (1, 3, 14–27). Micromolar concentrations of AA open TREK-1 and TRAAK with onset and offset kinetics that are in the order of minutes (Refs. 1, 3, and 4 and the present report). The relatively low affinity of AA for channel activation as well as the slow kinetics suggest that the accessibility to the “AA site” is limited. Insertion of FAs in the membrane may be required to directly reach the active site of the channel protein or may alter fluidity, tension, curvature, or phospholipid-channel interaction, which in turn may open the mechano-gated ion channels (1, 3). We have previously demonstrated that the anionic amphipath trinitrophenol mimics the effect of AA and opens TREK-1 and TRAAK channels (1, 3). These results were interpreted qualitatively on the basis of the bilayer couple hypothesis, assuming that the mechanosensitivity derives entirely from interactions within the bilayer and is independent of the cytoskeleton (1, 3, 23, 28). Anionic amphipaths would preferentially insert in the outer leaflet (because of the natural asymmetric distribution of negative charges in
the inner leaflet) and generate a convex curvature of the membrane that would open TREK-1 and TRAAK (1, 3).

Both TREK-1 and TRAAK are also opened by neutral amphipaths such as LPC, lyso-PAF, and PAF. The interpretation based on the bilayer couple model may thus be challenged as neutral amphipaths (such as LPC), which may distribute evenly at equilibrium in both leaflets, also mimic the effect of negatively charged compounds and open TREK-1 and TRAAK. An alternative hypothesis is to consider the shape rather than the charge of the molecule (15, 16, 21). LPC has a large polar head with a diameter of about 5.2 nm and a thin hydrophobic acyl chain tail with a cross-section estimated to be 1.8–2.0 nm and can be pictured as a cone with its base at the polar interface (29–32). Hydration of the polar head will also contribute to increase the conic shape of the molecule. Compounds with a small polar head and a large hydrophobic tail such as AA are considered as inverted cones with the base of the cone at its hydrophobic tail (30–32). The cone shape hypothesis assumes that amphipaths distribute mainly in a single layer that is deformed in opposite directions by cone (LPC)- and inverted-cone (AA)-shaped lipids (16, 21, 30–32). This hypothesis also implies that the potency of cones is proportional to the size of the polar head rather than their charge. LPI and LPC (large head groups) are much more potent openers of TREK-1 and TRAAK than LPE and LPS (small head groups). LPC is neutral, whereas LPI is negatively charged, demonstrating that the effect of LPs is independent of the charge of the molecule. Therefore, the structural requirements for effective activations seem to be the presence of a large head group independently of its charge and a sufficiently hydrophobic domain. The data are apparently in favor of the cone shape hypothesis for LPs, but this hypothesis does not account for activation of TREK-1 and TRAAK by inverted cones such as AA.

In the cell-attached patch configuration, channel opening is induced within seconds when LPC is added to the bath outside of the patch pipette. However, patch excision produces a loss of channel activation by LPC, whereas AA is still able to maximally open channels. The loss of LPC activation after excision suggests the existence of a cytosolic factor, which may be involved in channel regulation. Channel activation by LPs is insensitive to PAF receptor antagonists and is not mimicked by LPA application, ruling out the eventual role of PAF and LPA receptors. External application of large size polar head cone-shaped LPs may release a cytosolic factor by either directly activating putative LPs membrane receptor(s) or may indi-
rectly affect membrane properties, thus activating a membrane-bound mechano-sensitive effector. Inhibition of AA re-leasing enzymes phospholipase A_2 and diacylglycerol lipase has no effect on LPC activation. The identity and the mechanism of release of the possible cytosolic factor, which may, similarly to AA, directly interact with the channels or affect membrane curvature, remain to be determined.

Deletional analysis indicates that the carboxyl terminus, but not the amino terminus and the external loop of TREK-1, is critical for LPC and AA activation. The same region was previously found to be important for stretch activation (2). The present data indicate that LPC activation requires the entire carboxyl terminus of TREK-1 and, thus, may involve the mechano-sensitivity of the channel.

During brain (and heart) ischemia two distinct phospholipase A_2 enzymes are at work. First, cytosolic phospholipase A_2 enzymes release FAs from phospholipids and, thus, generate massive amounts of intracellular LPs (for review, see Refs. 33, 34). Second, secretory phospholipase A_2-type II expression would produce internal LPC, which would close the channels and reverse AA activation. These results suggest that secretory (external LPs) and cytosolic phospholipase A_2 (internal LPs) might differentially regulate TREK-1 and TRAAK opening. Cell swelling (opener of TREK-1 and TRAAK) and intracellular acidosis (opener of TREK-1) occurring during ischemia will produce further opening of TREK 1 and TRAAK. Hyperpolarization via TREK-1 and TRAAK activation would tend to reduce Ca^{2+} influx via voltage-gated Ca^{2+} channels by setting the membrane potential to values below the voltage threshold for activation. Similarly, hyperpolarization is expected to reduce Ca^{2+} influx via the N-methyl-D-aspartate receptor-associated Ca^{2+}-permeable channel by promoting blockade by Mg^{2+}. Opening of the mechano-gated FAs/LPs/PAF-sensitive TREK-1 and TRAAK K^+ channels during ischemia may thus have an important neuro-protective effect.

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REFERENCES
1. Patel, A. J., Honoré, E., Maingret, F., Lesage, F., Fink, M., Duprat, F., and Lazdunski, M. (1996) EMBO J. 17, 4283–4290
2. Maingret, F., Patel, A. J., Lesage, F., Lazdunski, M., and Honoré, E. (1999) J. Biol. Chem. 274, 13811–13817
3. Maingret, F., Lesage, F., Lazdunski, M., and Honoré, E. (1999) J. Biol. Chem. 274, 13811–13817
4. Fink, M., Lesage, F., Duprat, F., Heurteaux, C., Reyes, R., Fosset, M., and Lazdunski, M. (1998) EMBO J. 17, 3297–3308
5. Duprat, F., Lesage, F., Fink, M., Reyes, R., Heurteaux, C., and Lazdunski, M. (1997) EMBO J. 16, 5464–5471
6. Fink, M., Duprat, F., Lesage, F., Reyes, R., Heurteaux, C., and Lazdunski, M. (1996) EMBO J. 15, 6854–6862
7. Lesage, F., Guillemaire, E., Fink, M., Duprat, F., Lazdunski, M., Romey, G., and Baraban, J. (1996) EMBO J. 15, 1004–1011
8. Salinas, M., Reyes, R., Lesage, F., Fosset, M., Heurteaux, C., Romey, G., and Lazdunski, M. (1999) J. Biol. Chem. 274, 11753–11760
9. Kim, D., Fujita, A., Horio, Y., and Kurachi, Y. (1998) Circ. Res. 82, 513–518
10. Leonoudakis, D., Gray, A. T., Winegar, B. D., Kindler, C. H., Harada, M., Taylor, D. M., Chavez, R. A., Forsayeth, J. R., and Yost, C. S. (1998) J. Neurosci. 18, 868–877
11. Chavez, R. A., Gray, A. T., Zhao, B. B., Kindler, C. H., Mazurek, M. J., Mehta, Y., Forsayeth, J. R., and Yost, C. S. (1999) J. Biol. Chem. 274, 7877–7892
12. Reyes, R., Duprat, F., Lesage, F., Fink, M., Farman, N., and Lazdunski, M. (1998) J. Biol. Chem. 273, 30863–30869
13. Patel, A. J., Honoré, E., Lesage, F., Fink, M., Romey, G., and Lazdunski, M. (1999) Nat. Neurosci. 2, 422–426
14. Hamill, O. P., and McBride, D., Jr. (1996) Pharmacol. Rev. 48, 231–252
15. Andersen, O. S., Nielsen, C., Maer, A. M., Lundback, J. A., Gooulain, M., and Koepe, R. N. (1999) Methods Enzymol. 294, 208–224
16. Casado, M., and Ascher, P. (1998) J. Physiol. (Lond.) 513, 317–330
17. Kim, D. H., Sladek, C. D., Lamas, C., and Mathiasen, J. R. (1995) J. Physiol. (Lond.) 484, 643–660
18. Kim, D. (1992) J. Gen. Physiol. 100, 1021–1040
19. Kim, D., and Duff, R. A. (1992) Circ. Res. 67, 1040–1046
20. Kim, D., and Clapham, D. E. (1989) Science 244, 1174–1176
21. Lundback, J. A., and Andersen, O. S. (1994) J. Gen. Physiol. 104, 645–673
22. Markin, V. S., and Martinac, B. (1991) Biophys. J. 50, 1120–1127
23. Martinac, B., and Kung, C. (1990) Nature 348, 261–263
24. Martinac, B. (1992) in Thermodynamics of Cell Surface Receptors (Jackson, M. B., ed) Vol. 1, pp. 327–352, CRC Press, Inc., Boca Raton, FL
25. Ordway, R. W., Walsh, J. V. J., and Singer, J. J. (1989) Science 244, 1176–1179
26. Ordway, R. W., Singer, J. J., Walsh, J. V., and Branco, J. E. (1991) Trends Neurosci. 14, 96–100
27. Petrou, S., Ordway, R. W., Hamilton, J. A., Walsh, J. J., and Singer, J. J. (1994) J. Gen. Physiol. 103, 471–486
28. Sheetz, M., and Singer, S. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4457–4461
29. Pascher, I., Lundmark, M., Nyholm, P. G., and Sundell, S. (1992) Biochim. Biophys. Acta 1113, 339–373
30. Cullis, P. R., and de Kruijff, B. (1979) Biochim. Biophys. Acta 559, 399–429
31. Chernomordik, L. V., Melikyan, G. B., and Chizmadzhev, Y. A. (1987) Biochim. Biophys. Acta 906, 309–325
32. Israelachvili, J. N., Mitchell, D. J., and Ninham, B. W. (1977) J. Physiol. Lond. 273, 313–330
33. Bazan, N. G., and Allan, G. (1996) J. Lipid Med. Cell Signal. 16, 321–330
34. Bazan, N. G., and Allan, G. (1996) J. Lipid Med. Cell Signal. 16, 321–330
35. Lauritzen, I., Heurteaux, C., and Lazdunski, M. (1994) Brain Res. 651, 353–356
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