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Modulation of Native TREK-1 and Kv1.4 K^+ Channels by Polyunsaturated Fatty Acids and Lysophospholipids

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Abstract. The modulation of TREK-1 leak and Kv1.4 voltage-gated K^+ channels by fatty acids and lysophospholipids was studied in bovine adrenal zona fasciculata (AZF) cells. In whole-cell patch-clamp recordings, arachidonic acid (AA) (1–20 μM) dramatically and reversibly increased the activity of bTREK-1, while inhibiting bKv1.4 current by mechanisms that occurred with distinctly different kinetics.

bTREK-1 was also activated by the polyunsaturated cis fatty acid linoleic acid but not by the trans polyunsaturated fatty acid linolelaidic acid or saturated fatty acids. Eicosatetraynoic acid (ETYA), which blocks formation of active AA metabolites, failed to inhibit AA activation of bTREK-1, indicating that AA acts directly. Compared to activation of bTREK-1, inhibition of bKv1.4 by AA was rapid and accompanied by a pronounced acceleration of inactivation kinetics. Cis polyunsaturated fatty acids were much more effective than trans or saturated fatty acids at inhibiting bKv1.4. ETYA also effectively inhibited bKv1.4, but less potently than AA. bTREK-1 current was markedly increased by lysophospholipids including lysophosphatidyl choline (LPC) and lysophosphatidyl inositol (LPI). At concentrations from 1–5 μM, LPC produced a rapid, transient increase in bTREK-1 that peaked within one minute and then rapidly desensitized. The transient lysophospholipid-induced increases in bTREK-1 did not require the presence of ATP or GTP in the pipette solution. These results indicate that the activity of native leak and voltage-gated K^+ channels are directly modulated in reciprocal fashion by AA and other cis unsaturated fatty acids. They also show that lysophospholipids enhance bTREK-1, but with a strikingly different temporal pattern. The modulation of native K^+ channels by these agents differs from their effects on the same channels expressed in heterologous cells, highlighting the critical importance of auxiliary subunits and signaling. Finally, these results reveal that AZF cells express thousands of bTREK-1 K^+ channels that lie dormant until activated by metabolites including phospholipase A_2 (PLA_2)-generated fatty acids and lysophospholipids. These metabolites may alter the electrical and secretory properties of AZF cells by modulating bTREK-1 and bKv1.4 K^+ channels.

Key words: TREK-1 — K^+ channels — Kv1.4 — Arachidonic acid — Fatty acids — Adrenal

Introduction

Bovine adrenal zona fasciculata (AZF) cells express two types of K^+-selective ion channels. These include a voltage-gated, rapidly inactivating bKv1.4 channel and a bTREK-1 leak-type K^+ channel (Mlinar & Enyeart, 1993; Mlinar, Biagi & Enyeart, 1993; Enyeart, Xu & Enyeart, 2000; Enyeart et al., 2002). The bTREK-1 channel sets the resting membrane potential and functions pivotally in the regulation of cortisol secretion by ACTH and AngiotensinII (AII). Specifically, activation of ACTH or AII receptors is coupled to inhibition of bTREK-1 channels leading to depolarization-dependent Ca^{2+} entry and cortisol secretion (Mlinar et al., 1993; Enyeart, Mlinar & Enyeart, 1993, 1996). bTREK-1 channels are also regulated by ionic and metabolic factors. They are activated by intracellular ATP and acidification, and inhibited by Ca^{2+} (Gomora & Enyeart, 1998; Enyeart et al., 1997, 2002). The specific function of bKv1.4 channels in AZF cell physiology is not clear. However, the activity of bKv1.4 channels is reciprocally linked to that of bTREK-1 channels through metabolic and hormonal factors (Enyeart et al., 2001).

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Fig. 1. Reciprocal modulation of bKv1.4 and bTREK-1 K^+ currents by AA. Whole-cell K^+ currents were recorded from bovine AZF cells in response to voltage steps to +20 mV applied from a holding potential of −80 mV with or without 10-s depolarizing prepulses to −20 mV, as indicated. Cells were superfused with AA while recording currents at 30 s intervals. (*A*) AZF cell was superfused with AA (5 μM) while recording combined bKv1.4 and bTREK-1 currents. Traces show currents at indicated times after initiating superfusion of AA. bKv1.4 and bTREK-1 amplitudes are plotted against time at right. (*B*) Separate effects of AA in $I_{bKv1.4}$ and $I_{bTREK-1}$. K^+ currents were recorded with (*left traces*) and without (*middle traces*) depolarizing prepulses in control saline and 15 min after superfusing the cell with AA (20 μM), as indicated. $I_{bKv1.4}$ was obtained by digital subtraction of $I_{bTREK-1}$ from the combined $I_{bKv1.4}$ + $I_{bTREK-1}$ current (*right traces*).

The hormonal and metabolic factors that regulate K^+ channels in AZF cells are only partially understood. ACTH and cAMP inhibit bTREK-1 through at least one A-kinase-independent pathway (Enyeart et al., 1996). Inhibition of bTREK-1 by AII occurs through an ATI receptor, but appears to be independent of phospholipase C-derived second messengers (Mlinar, Biagi & Enyeart, 1995; Xu & Enyeart, 2001). The precise signaling pathway linking AT1 receptors to bTREK-1 channels has not been identified.

Several studies indicate that ACTH and AII effects in adrenocortical cells are mediated through PLA_2-generated products of glycerophospholipids including AA, lysophospholipids, and other AA-derived metabolites (Kojima, Kojima & Rasmussen, 1985; Cymeryng et al., 1995; Yamazaki et al., 1996; Wang et al., 2000). In this regard, AA and lysophospholipids modulate the activity of many native and cloned leak and voltage-gated K^+ channels (Ordway, Singer & Walsh, 1991; Keros & McBain, 1997; Colbert & Pan, 1999; Lesage & Lazdunski, 2000; Patel & Honore, 2001).

AA increases the activity of native unidentified background K^+ channels in cardiac and smooth muscle, and CNS neurons (Kim & Clapham, 1989; Ordway, Walsh & Singer, 1989; Ordway et al., 1991; Kim et al., 1995). Molecular cloning showed that AA activated only three of the more than one dozen cloned background channels of the four transmembrane spanning/two pore (4TMS/2P) variety. These included TREK-1, TREK-2, and TRAAK (Fink et al., 1998; Bang, Kim & Kim, 2000; Maingret et al., 2000; Patel & Honore, 2001). Inhibition of several 4TMS/2P K^+ channels by AA has also been observed (Patel & Honore, 2001).

AA, as well as other polyunsaturated fatty acids, inhibit native and cloned voltage-gated K^+ channels (Ordway et al., 1991; Villarroel & Schwarz, 1996; Keros & McBain, 1997; Colbert & Pan, 1999). Interestingly, in some cases, the modulatory action of these fatty acids on specific native K^+ channels differs markedly from their effects on the same cloned channels expressed in *Xenopus* oocytes and cell lines (Villarroel & Schwarz, 1996; Keros & McBain, 1997; Peri, Wible & Brown, 2001).

PLA_2-generated lysophospholipids such as LPC and LPI enhance the activity of cloned 4TMS/2P K^+ channels, including TREK-1 and TRAAK (Maingret et al., 2000). However, the modulation of native background K^+ channels by lysophospholipids has not been reported. Overall, a large number of patch-clamp studies in neurons, muscle cells, and heterologous expression systems have shown that fatty acids and phospholipids modulate native and cloned K^+ channels. The modulation of K^+ channels in AZF cells by fatty acids and lysophospholipids has not been reported. Further, the effects of these same agents on TREK-1 and Kv1.4 channels in their native environment, where auxiliary subunits are present and functioning, have not been examined.

We have characterized the effects of fatty acids and lysophospholipids on bTREK-1 and bKv1.4 K^+ currents in bovine AZF cells. Cis polyunsaturated fatty acids were found to modulate the activity of the background and voltage-gated channels in reciprocal fashion. Lysophospholipids enhanced bTREK-1 activity by a mechanism that rapidly desensitized. In some cases, the effects of these agents on native channels differ significantly from those reported previously on the same channels expressed in heterologous cells.

Materials and Methods

Materials

Tissue culture media, antibiotics, fibronectin, and fetal calf sera (FCS) were obtained from Invitrogen (Carlsbad, CA). Coverslips were from Bellco (Vineland, NJ). Phosphate-buffered saline (PBS), enzymes, 1,2 *bis*-(2-aminophenoxy)ethane-*N*,*N*,*N*′,N″-tetraacetic acid (BAPTA), ATP, AMP-PNP, ACTH (1-24), cAMP, 8-pcpt cAMP, AA, linoleic acid, linolelaidic acid, ETYA, LPC, and LPI were from Sigma (St. Louis, MO).

Isolation and Culture of AZF Cells

Bovine adrenal glands were obtained from steers (age range 2 to 3 years) within 1 h of slaughter at a local slaughterhouse. Fatty tissue was removed immediately and the glands were transported to the laboratory in ice-cold PBS containing 0.2% dextrose. Isolated AZF cells were obtained and prepared as previously described (Enyeart et al., 1997). After isolation, cells were either resuspended in DMEM/F12 (1:1) with 10% FBS, 100 U/ml penicillin, 0.1 mg/ml streptomycin and the antioxidants 1 μM tocopherol, 20 nM selenite and 100 μM ascorbic acid (DMEM/F12+) and plated for immediate use, or resuspended in FBS/5% DMSO, divided into 1-ml aliquots, each containing ~4×10^6 cells, and stored in liquid nitrogen for future use.

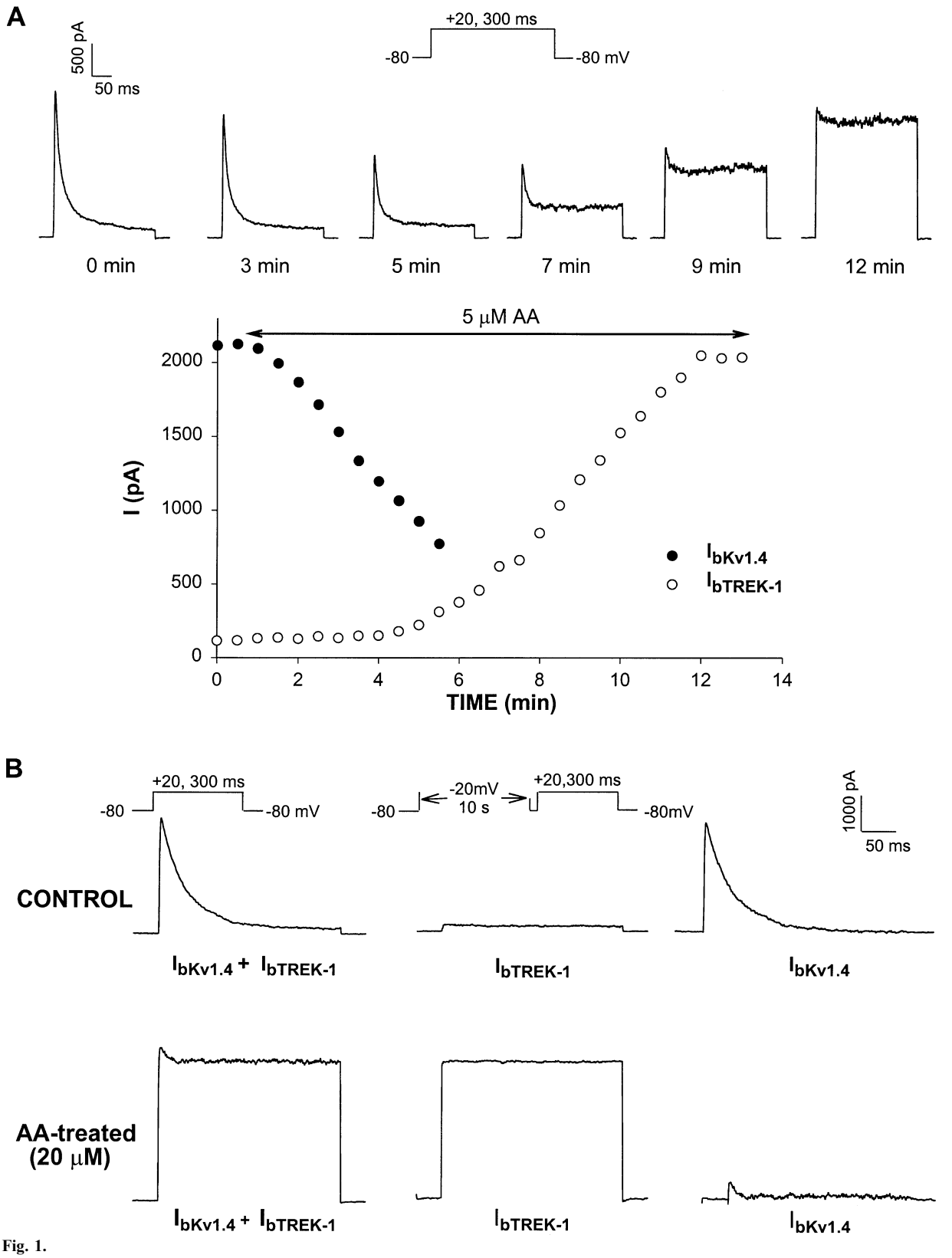


Fig. 1.

Approximately 120×10^6 cells were obtained by enzymatic dissociation of 6 bovine adrenal glands. Cells were plated in either 60-mm dishes or 35-mm dishes containing 9 mm^2 glass coverslips. Dishes or coverslips were treated with fibronectin (10 μg/ml) at 37°C for 30 minutes, then rinsed with warm, sterile PBS immediately before adding cells. Cells were plated in DMEM/F12+ and were maintained at 37°C in a humidified atmosphere of 95% air-5% CO_2.

Solutions and Bath Perfusion

For recording whole-cell K^+ currents, the standard pipette solution was (in mM) 120 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 11 BAPTA, 5 MgATP and 200 μM GTP, with pH buffered to 7.2 using KOH. With this composition, free $[Ca^{2+}]$ was determined to be 2.3×10^{-8} M using the "Bound and Determined" program (Brooks & Storey, 1992). Pipette solutions were filtered through 0.22 micron cellulose acetate filters. The external solution consisted of (in mM) 140 NaCl, 5 KCl, 2 CaCl, 2 $MgCl_2$, 10 HEPES and 5 glucose, pH 7.35, using NaOH. Drugs were applied by bath perfusion, controlled manually by a six-way rotary valve.

Recording Conditions and Electronics

AZF cells were used for patch-clamp experiments 2–12 hours after plating. Coverslips with cells were transferred from 35-mm culture dishes to the recording chamber. Cells with diameters of 10–15 μM and capacitances of 8–15 pF were used for recording. Patch electrodes with resistances of 1–3 megohms were fabricated from 0010 glass (Corning) using a Brown-Flaming Model P-87 microelectrode puller (Sutter Instruments, Novato, CA). Access resistance during recording estimated from the transient-cancellation controls of the patch-clamp amplifier was 2–5 megohms. The combination of access resistance and cell capacitance yielded voltage-clamp time constants of <100 μs.

Whole-cell currents were recorded at room temperature (22–24°C) following the procedure of Hamill et al. (1981), using a List EPC-7 (List-Medical, Darmstadt, Germany) patch-clamp amplifier. Pulse generation and data acquisition were done using a personal computer and PCLAMP software with TL-1 interface (Axon Instruments, Burlingame, CA). Currents were digitized at 2–10 KHz after filtering with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA). Linear leak and capacity currents were subtracted from current records using summed scaled hyperpolarizing steps of 1/2 to 1/4 pulse amplitude. Data were analyzed using PCLAMP 6.04 (CLAMPAN and CLAMPFIT) and SigmaPlot (version 5.0) software.

Measurement of bTREK-1 and bKv1.4 K^+ Currents

The absence of time- and voltage-dependent inactivation of the bTREK-1 K^+ current allowed it to be easily isolated for measurement in whole-cell recordings, using either of two voltage clamp protocols. When voltage steps of 300 ms duration were applied from a holding potential of −80 mV to a test potential of +20 mV, bTREK-1 could be selectively measured near the end of the voltage step, where the rapidly inactivating bKv1.4 K^+ current had completely inactivated. Alternatively, bTREK-1 was selectively activated with an identical voltage step, after a 10 s prepulse to −20 mV had fully inactivated the A-type K^+ current (*see* Figure 1*B*). When combined K^+ currents were present, bKv1.4 amplitude was measured at its maximum value, after digital subtraction of bTREK-1 recorded with an inactivating prepulse.

Results

In whole-cell patch-clamp recordings from bovine AZF cells, voltage steps from a holding potential of −80 mV to a test potential of +20 mV activate both voltage-gated bKv1.4 and bTREK-1 K^+ channels. With pipette solutions containing 1 mM ATP at pH 7.1, the noninactivating bTREK-1 current is weakly expressed, while the inactivating bKv1.4 current is prominent (Fig. 1*A*).

AA produced distinctive reciprocal modulation of these two K^+ currents. As illustrated in Fig. 1*A*, AA (5 μM) rapidly inhibited the transient bKv1.4 K^+ current, while it produced, after a delay of several minutes, a gradual increase in the non-inactivating component, presumed to be bTREK-1. In this cell, AA inhibited bKv1.4 to 34% of its control value within 4 minutes, at which time bTREK-1 had not begun to increase. Between 4 and 12 minutes, bTREK-1 increased from its original amplitude of 150 pA to a stable maximum of 2030 pA.

Although AA rapidly inhibited bKv1.4, the inhibition typically did not reach a steady state before it was obscured by the growth of the non-inactivating bTREK-1 current. A more accurate measure of the time-dependent inhibition of bKv1.4 by AA was obtained by combining the standard voltage-clamp protocol with a second protocol designed to selectively inactivate the bKv1.4 current with a depolarizing prepulse (Fig. 1*B*).

Digital subtraction of bTREK-1 from the combined current allowed bKv1.4 to be accurately measured, even after AA had produced large increases in bTREK-1 (Fig. 1*B*, right traces). In the experiment illustrated, a 20 min exposure to AA (20 μM) increased bTREK-1 27-fold over its control value (140 pA to 3820 pA), while inhibiting bKv1.4 by 85% (3070 pA to 450 pA). Overall, with respect to time course, AA (10 μM) inhibited bKv1.4 with a $t_{1/2}$ of 2.0 ± 0.1 min (n = 8), while in these same cells, bTREK-1 increased to a maximum value with a $t_{1/2}$ of 7.0 ± 1.0 min.

The AA-induced increase in bTREK-1 current was concentration-dependent and easily reversible. In the experiment illustrated in Fig. 2*A*, bTREK-1 was nearly undetectable while recording for 5 minutes in control saline (trace *1*). Superfusion of the cell with AA (10 μM) increased bTREK-1 to 2500 pA within 11 minutes (trace *2*). Upon switching to control saline, bTREK-1 returned to its control value after 10 minutes (trace *3*). However, the AA-induced inhibition of bKv1.4 was not significantly reversed at this time.

In this and other figures, the increase in bTREK-1 current was accompanied by a disproportionate increase in current noise compared to that observed for bKv1.4 alone. This occurs because the unitary conductance of bTREK-1 is several-fold larger than that of bKv1.4.

Overall, AA caused significant increases in bTREK-1 current at concentrations of 5–20 μM (Fig. 2*B*). At 10 μM, AA increased bTREK-1 current density from a control value of 9.4 ± 1.3 pA/pF to 112.4 ± 12.0 pA/pF (n = 28). At a concentration of 20 μM, AA produced significantly larger increases in bTREK-1, but at this concentration, gigohm seals were typically lost before stable maximum values were attained (Fig. 2*B*).

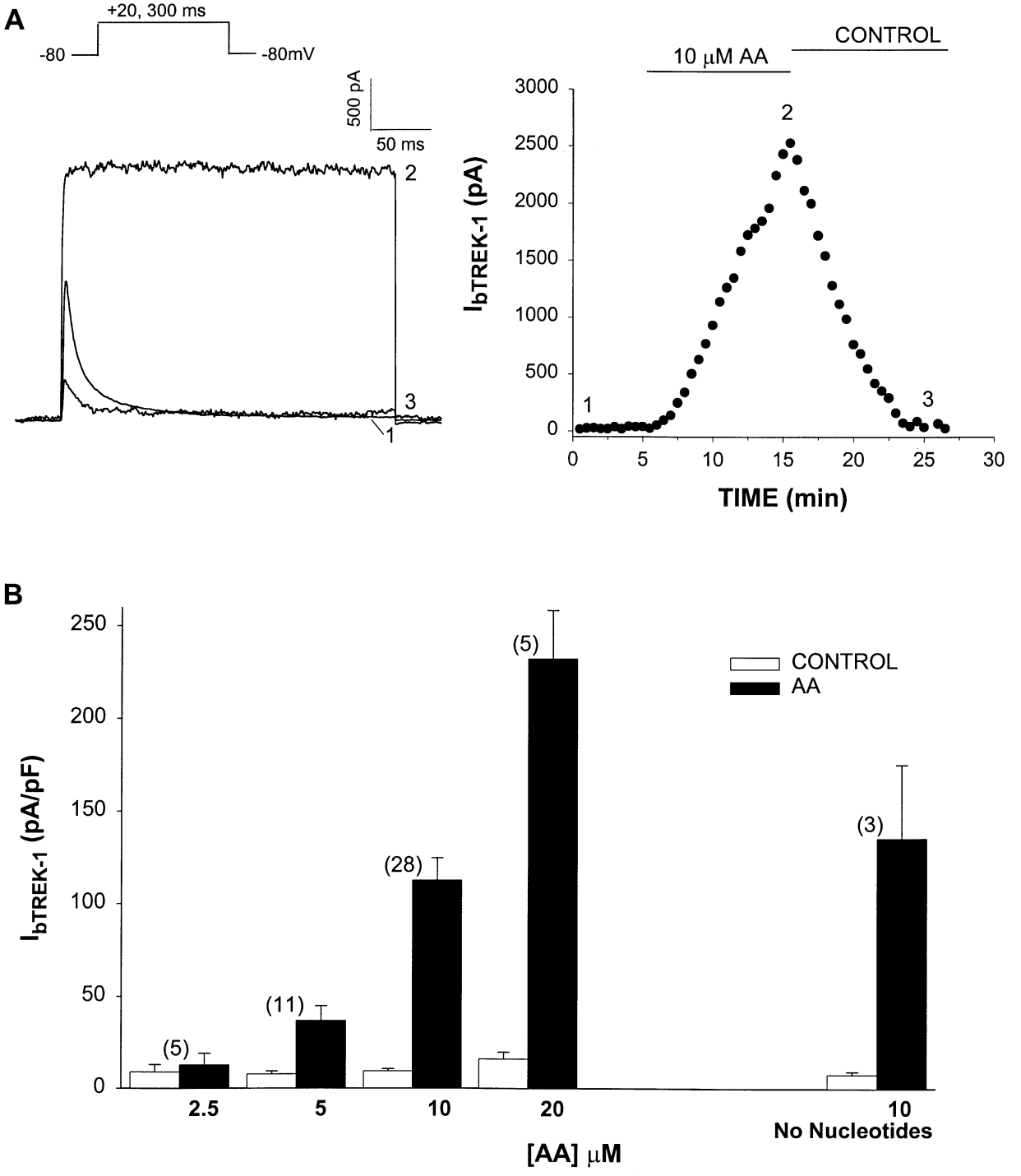
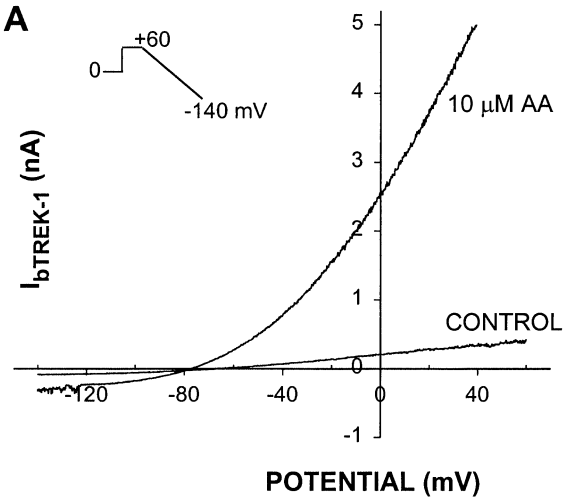
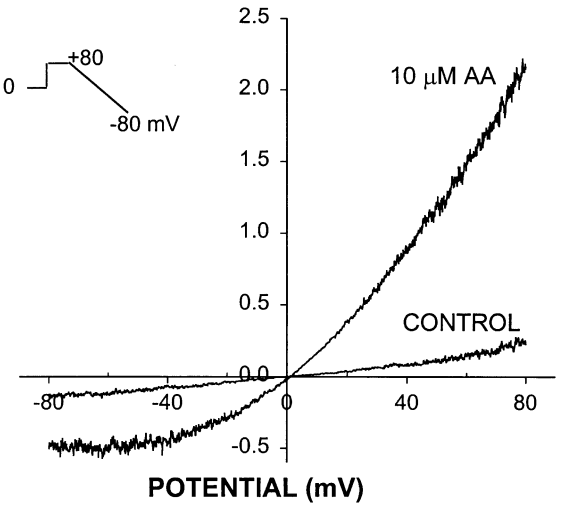
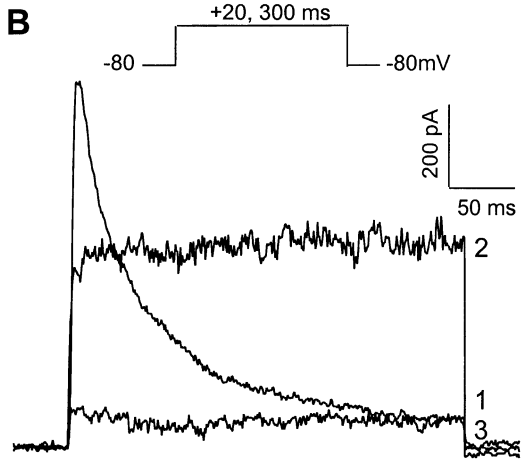
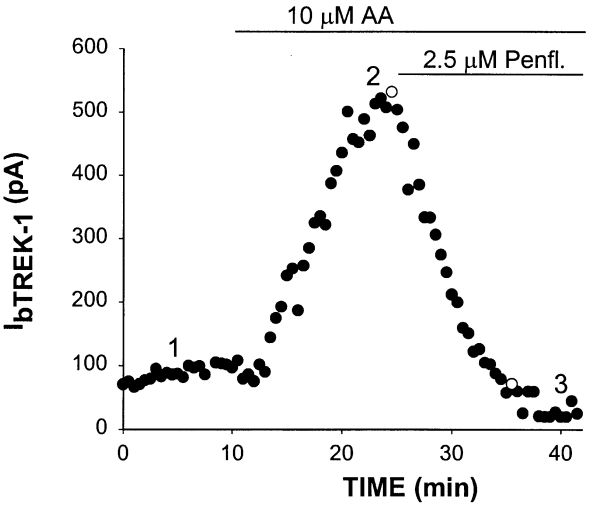


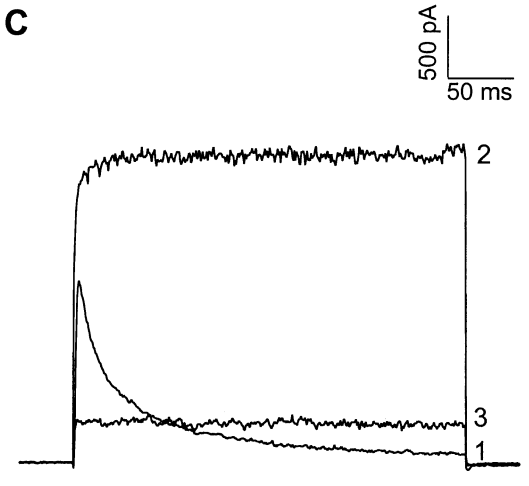
Fig. 2. Concentration, nucleotide dependence, and reversibility of AA activation of bTREK-1 current. K^+ currents were recorded at 30 s intervals in response to voltage steps to +20 mV from a holding potential of −80 mV. When bTREK-1 reached a stable maximum value, cells were superfused with AA at concentrations ranging from 2.5 to 20 μM. Recordings were made with standard pipette solutions (1 mM MgATP, 200 μM GTP) or in the absence of added nucleotides. (*A*) Reversibility of AA-mediated effects on bTREK-1 and bKv1.4. Cell was superfused with AA (10 μM) for 10 min before switching back to control saline. bTREK-1 amplitudes are plotted against time at right. Numbers on graph correspond to traces shown at left. (*B*) Concentration and nucleotide-dependent effects of AA on $I_{bTREK-1}$ current density were obtained from experiments as shown in (*A*). $I_{bTREK-1}$ current densities were determined by dividing the maximum current amplitude by the cell capacitance. Values are mean ± SEM for the indicated number of determinations. Effect of AA (>2.5 μM) is significant, both in the presence and absence of nucleotides ($P < 0.005$ according to a paired Student's *t*-test).


A
+60
0
-140 mV
10 μM AA
CONTROL
$I_{bTREK-1}$ (nA)
POTENTIAL (mV)


+80
0
-80 mV
10 μM AA
CONTROL
POTENTIAL (mV)


B
+20, 300 ms
-80
-80mV
200 pA
50 ms
2
1
3


10 μM AA
2.5 μM Penfl.
$I_{bTREK-1}$ (pA)
TIME (min)


C
500 pA
50 ms
2
3
1

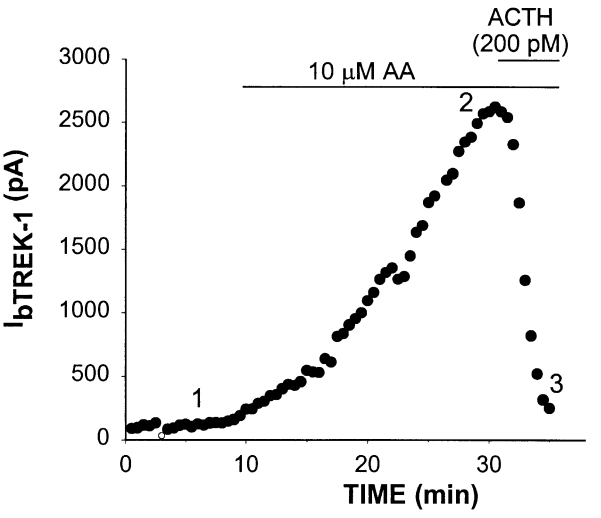

ACTH
(200 pM)
10 μM AA
$I_{bTREK-1}$ (pA)
TIME (min)

Fig. 3.

Fig. 3. Rectification, ion selectivity, and inhibition of AA-activated current by penfluridol and ACTH. (*A*) Rectification and selectivity. Linear voltage ramps of −100 mV/s were applied from a holding potential of 0 mV to potentials between +60 and −140 mV in standard external solution (*left traces*) or external solution containing 150 mM KCl (*right traces*) before and after superfusing cells with AA (10 μM). (*B*) Inhibition of AA-activated current by penfluridol. K^+ currents were recorded in response to voltage steps from −80 mV to +20 mV with (*empty circles*) or without (*filled circles*) depolarizing prepulses. After recording K^+ currents in saline, AZF cells were sequentially superfused with saline containing AA (10 μM), followed by one containing AA (10 μM) and penfluridol (2.5 μM). Numbers on current traces at left correspond to those at right, where TREK-1 amplitude is plotted against time. (*C*) Inhibition of AA-activated current by ACTH (aa1-24) (200 pM). After recording K^+ currents in saline, AZF cells were sequentially superfused with saline containing AA (10 μM) followed by one containing AA (10 μM) and ACTH (200 pM). Numbers on current traces at left correspond to those at right, where TREK-1 amplitude is plotted against time.

Activation of bTREK-1 K^+ channels by AA did not require the presence of ATP or GTP in the patch pipette. With no added nucleotides, AA (10 μM) increased bTREK-1 current density from its control value of 7.8 ±1.6 pA/pF (n = 3) to 135 ± 40 pA/pF (Fig. 2*B*).

The AA-activated Channel is bTREK-1

Patch-clamp and molecular cloning studies indicate that bovine AZF cells express a single type of leak K^+ channel, bTREK-1 (Mlinar et al., 1993; Enyeart et al., 1997; Enyeart et al., 2002). However, it is possible that these cells express leak channels other than TREK-1 that could be activated by AA. Experiments were done to establish the identity of the current activated by AA. The AA-activated current in AZF cells was identified as a K^+-selective current with properties indistinguishable from bTREK-1. With standard pipette and external solutions, ramp voltage protocols applied over a range of +60 to −140 mV showed that AA (10 μM) activated a strongly outward-rectifying current that reversed near the theoretical Nernst equilibrium potential for a K^+-selective channel (Fig. 3*A*, left traces).

When AA was applied with symmetrical 150 mM KCl on each side of the membrane, the AA-activated current was weakly outward-rectifying and reversed direction near 0 mV as expected for a K^+-selective channel (Fig. 3*A*, right traces). Thus, with respect to ionic selectivity and rectification, the AA-activated current resembles both the native bTREK-1 current and that recorded from recombinant channels expressed in cell lines (Mlinar et al., 1993; Enyeart et al., 1997; Enyeart et al., 2002).

The AA-activated K^+ current was effectively blocked by penfluridol, a drug that potently blocks native and cloned bTREK-1 channels (Gomora & Enyeart, 1999; Enyeart et al., 2002). In the experiment illustrated in Fig. 3*B*, K^+ currents were recorded in control saline (trace *1*), after superfusing the cell with AA (10 μM) (trace *2*), and after superfusing AA (10 μM) and penfluridol (2.5 μM) in combination (trace *3*). Since AA and penfluridol selectively inhibit bKv1.4 and bTREK-1, respectively, these two drugs together inhibited both bKv1.4 and bTREK-1 current almost completely (trace *3*). Similar results were obtained in each of three experiments.

cAMP has long been regarded as the principal second messenger for ACTH. ACTH and cAMP inhibit native and cloned bTREK-1 K^+ channels (Enyeart et al., 1996, 2002). If the current activated by AA is bTREK-1, it should be potently inhibited by ACTH. In the experiment illustrated in Fig. 3*C*, superfusion of the cell with 10 μM AA increased the noninactivating K^+ current from an initial value of 90 pA (trace *1*) to a maximum value of 2600 pA (trace *2*). In the continued presence of AA, superfusion of ACTH (200 pM) reduced bTREK-1 to near its control value within 5 min (trace *3*), providing further evidence that the AA-enhanced current is bTREK-1. Similar results were obtained in each of 3 experiments.

Specificity of Activation by Fatty Acids

A number of cloned and native background K^+ currents are activated by AA and other free fatty acids, both saturated and unsaturated (Patel & Honore, 2001). In addition to AA, we found that the polyunsaturated fatty acid cis-linoleic acid (cis-9,cis-12-octadecadienoic acid) also enhanced bTREK-1, while it inhibited bKv1.4 (Fig. 4*A*, *C*). Overall, linoleic acid (10 μM) increased bTREK-1 current density 9-fold, from 13.7 ± 5 pA/pF (n = 5) to 125 ± 16 pA/pF. Linoleic acid also inhibited bKv1.4 (*data not shown*). In contrast, the trans unsaturated fatty acid linolelaidic acid (trans-9,trans-12-octadocadienoic acid) and the saturated fatty acid, stearic acid (octadecanoic acid) each failed to significantly increase bTREK-1 (Figure 4*B*, *C*). Linolelaidic and stearic acid also failed to significantly inhibit bKv1.4 (*data not shown*).

Experiments with free fatty acids proved that cis polyunsaturated fatty acids effectively activated native bTREK-1 K^+ current, while trans unsaturated and saturated fatty acids were inactive in this respect. The structure/activity relationship for bTREK-1 activation by fatty acids raised the possibility that bTREK-1 activation was mediated by an oxygenated metabolite. Specifically, cis unsaturated fatty acids are substrates for lipoxygenase and cyclooxygenase that, respectively, convert these fatty acids to the

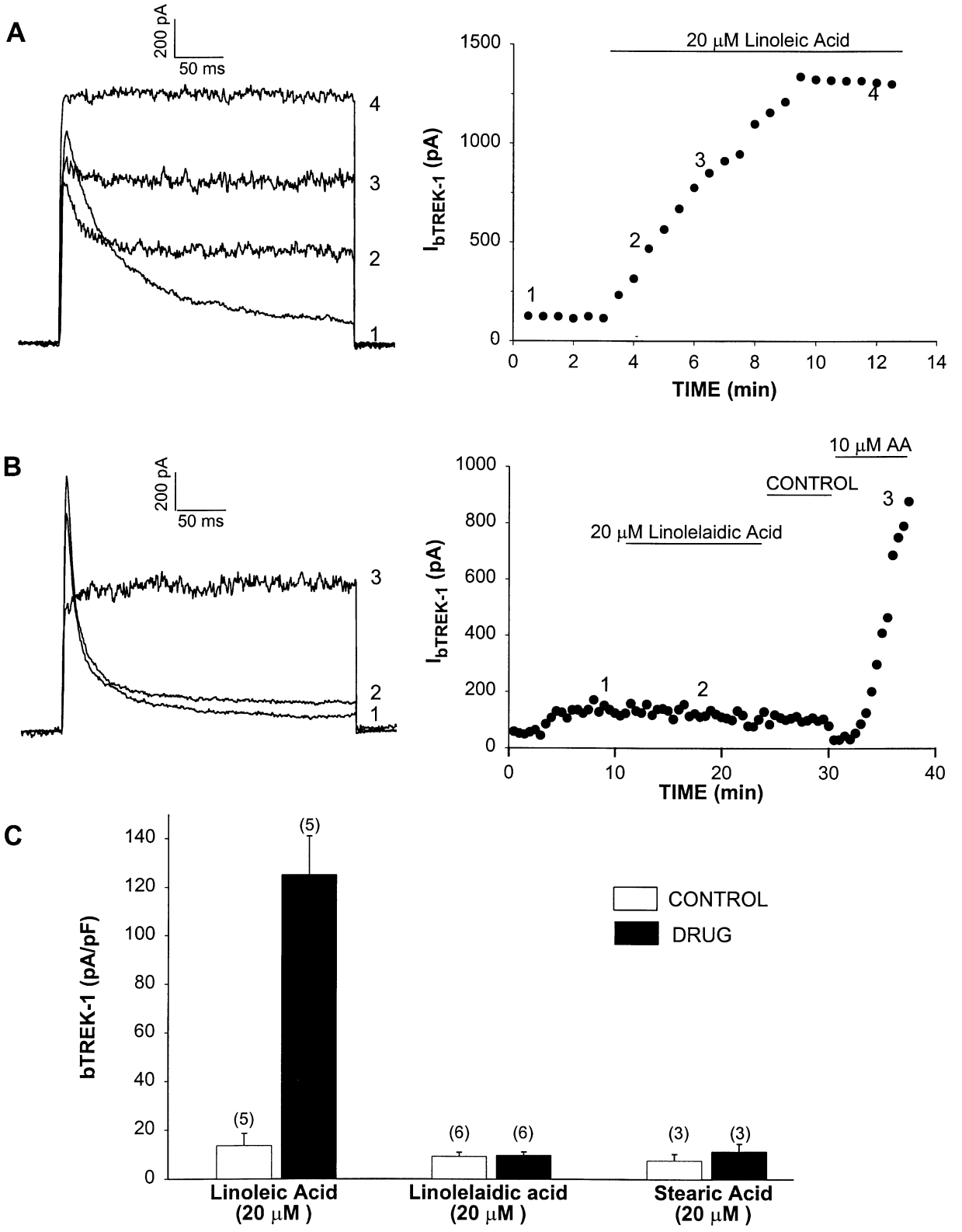


Fig. 4. Activation of bTREK-1 by polyunsaturated fatty acids. Whole-cell K^+ currents were recorded from AZF cells in response to voltage steps to +20 mV applied at 30 s intervals from a holding potential of −80 mV. When bTREK-1 reached a stable value in control saline, the cell was superfused with linoleic acid, linolelaidic acid, or stearic acid and AA, as indicated. Numbers on graphs at right correspond to numbers on traces at left. (*A*) Effect of linoleic acid (20 μM). (*B*) Effect of linolelaidic acid (20 μM) and AA (10 μM). (*C*) Summary data for experiments as in (*A*) and (*B*). Values are mean ± SEM of bTREK-1 current densities for the indicated number of determinations before and after exposure to the indicated fatty acid. Effect of linoleic acid is significant ($P < 0.001$).

active metabolites, including leukotrienes and prostaglandins (Ordway et al., 1991). Fatty acids that lack at least one cis double bond are not substrates for these enzymes (Ordway et al., 1989).

ETYA, the acetylene analog of AA that inhibits cyclooxygenase, lipoxygenase, and cytochrome P450 enzymes was used to determine whether AA activation of bTREK-1 was mediated by an oxygenated metabolite (Capdevila et al., 1988). When applied to AZF cells at 40 μM, ETYA failed to block AA (10 μM)-induced increases in bTREK-1 current (Fig. 5*A*, *C*).

Experiments with ETYA indicated that the activation of bTREK-1 by AA is direct and not mediated by oxygenated metabolites. In this regard, in bovine AZF cells, ACTH (1 pM) has been reported to activate the 15-lipoxygenase pathway, resulting in the synthesis of 15(S)-HPETE (Yamazaki et al., 1996). However, 15(S)-HPETE (150 nM) failed to increase bTREK-1 current in cells where subsequent superfusion of AA produced large increases in bTREK-1 (Fig. 5*B*, *C*). Overall, these results indicate that activation of bTREK-1 by polyunsaturated fatty acids is direct and not mediated through an oxygenated metabolite.

Activation of bTREK-1 by Lysophospholipids

In addition to AA, the activation of PLA_2 results in the production of lysophospholipids including LPC and LPI from glycerophospholipids. These lysophospholipids have been reported to produce sustained activation of cloned TREK-1 and TRAAK K^+ channels (Maingret et al., 2000). LPC and LPI were found to rapidly increase bTREK-1 current in whole-cell recordings (Fig. 6*A*, *B*, and *C*). However, the increase of native bTREK-1 current was transient and rapidly desensitized. In the experiment illustrated in Fig. 6*A*, LPC (5 μM) increased bTREK-1 from approximately 100 pA to 500 pA within 2 min of exposure to the lipid. During the next 2 min, bTREK-1 current returned to its control amplitude, despite the continued presence of LPC. Subsequent application of LPC was ineffective at enhancing bTREK-1, indicating complete desensitization (*data not shown*).

LPC-induced increases in bTREK-1 current were observed at concentrations from 1 to 5 μM (Figure 6*C*). However, at LPC concentrations above 2 μM, seals were frequently lost. At a maximally effective concentration of 2 μM, LPC increased bTREK-1 current density from 11.3 ± 1.7 to 46.5 ± 0.9 pA/pF ($n = 16$). Compared to AA, LPC was significantly more potent, but somewhat less effective at activating bTREK-1 K^+ channels.

When AZF cells were exposed to LPC and AA in combination, bTREK-1 increased with a temporal pattern that was rapid in onset, and sustained in duration (Fig. 6*B*). No transient component was detectable. Thus, the kinetics of the combined response shared features produced by either agent alone. The lysophospholipids also differed from the polyunsaturated fatty acids in that they did not significantly inhibit bKv1.4 at concentrations that enhanced bTREK-1 (Fig. 6*A*).

In some cell types, LPC activates multiple protein kinases, including MAP kinases, tyrosine kinases, protein kinase C, and C jun N-terminal kinase (Fang et al., 1997; Tian et al., 1998; Bassa et al., 1999). To determine whether LPC-induced increases in bTREK-1 required activation of one or more protein kinases, whole-cell recordings were made with pipette solutions containing no added nucleotide or the nonhydrolyzable ATP analog AMP-PNP.

In the absence of nucleotides or when AMP-PNP (1 mM) was substituted for ATP in the pipette solution, LPC again produced a rapid, transient increase in bTREK-1 current. In the experiment illustrated in Fig. 7*A*, LPC (2 μM) increased bTREK-1 current 8-fold within 2 min, after which bTREK-1 rapidly returned towards its control value.

Ramp-voltage protocols applied between +60 and −140 mV on the same cell also showed the transient activation of an outwardly rectifying current that was indistinguishable from bTREK-1 (Fig. 7*B*). Overall, the LPC-induced increases in bTREK-1 triggered in the absence of nucleotides or presence of AMP-PNP were larger in magnitude, but similar in kinetics to those observed with ATP in the pipette (Fig. 7*C*). Also, as previously reported, Fig. 7*C* shows that AMP-PNP (1 mM) is more effective than ATP (1 mM) in activating bTREK-1 (Xu & Enyeart, 2001).

Effect of Fatty Acids on bKv1.4 Currents

Fatty acids inhibited bKv1.4 K^+ currents with a structure/activity profile that resembled that for the enhancement of bTREK-1 by these same agents. AA and cis polyunsaturated fatty acids effectively inhibited bKv1.4 current, while trans, unsaturated and saturated fatty acids were much less effective. AA (10 μM) inhibited bKv1.4 by 69.7 ± 4.2% ($n = 9$), while linolelaidic acid (20 μM) and stearic acid (20 μM) produced little or no inhibition (Fig. 8*A*, *B*, *D*).

In contrast to its lack of effect on bTREK-1, ETYA did inhibit bKv1.4, but was significantly less potent than AA in this respect (Fig. 8*C*, *D*). ETYA (20 μM) reduced bKv1.4 by 31.1 ± 4.2% ($n = 5$).

In addition to inhibiting bKv1.4, AA and ETYA accelerated the inactivation kinetics of this current. In the experiments illustrated in Fig. 9*A*, bKv1.4 currents were recorded in response to a 40 ms voltage step, before and after superfusing cells with AA (5 μM) or ETYA (40 μM), as indicated. In addition to

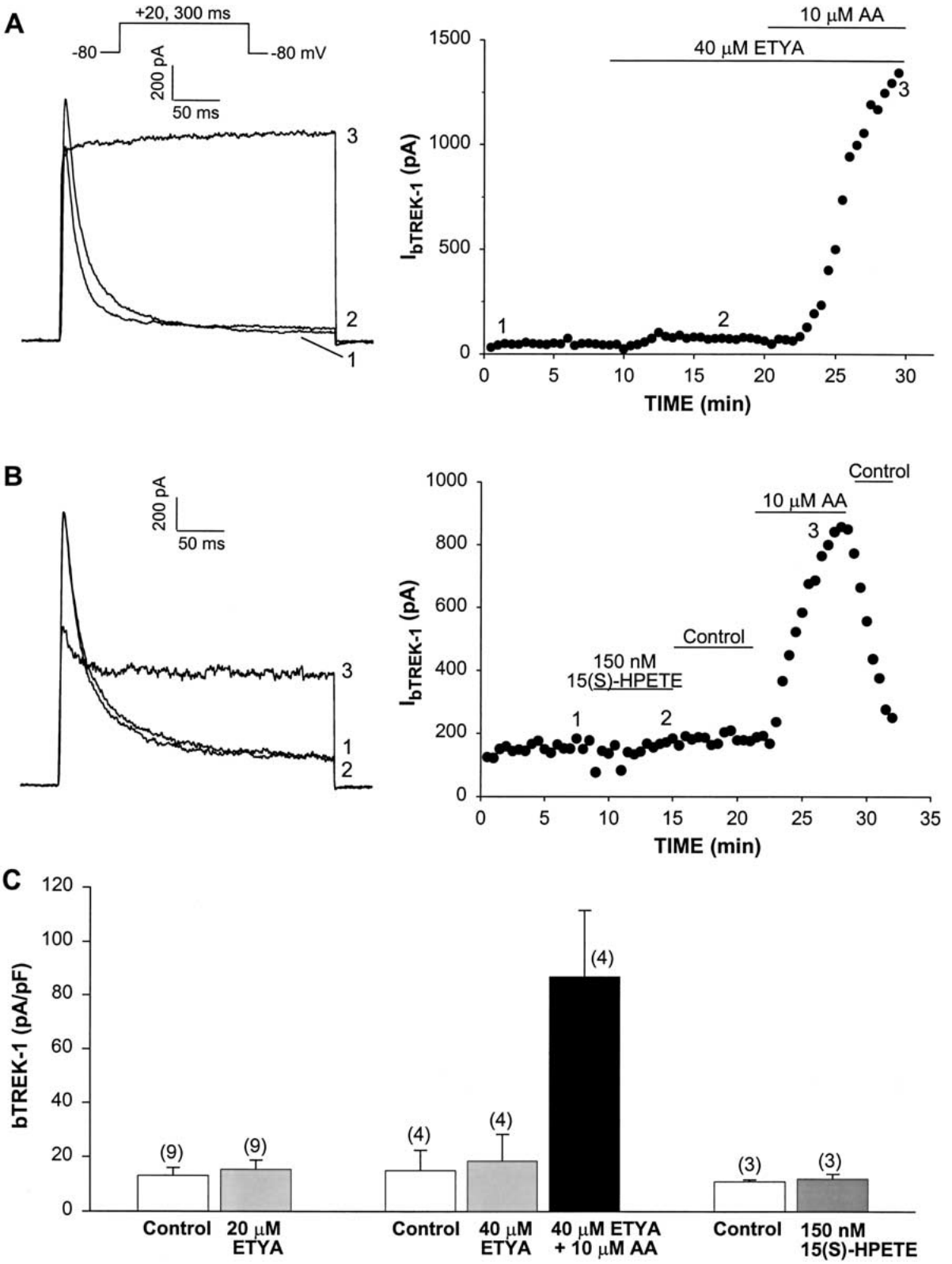


Fig. 5.

reducing bKv1.4 to 49.1% of its control value, AA decreased the inactivation time constant (τ_i) from 19.1 to 13.1 ms (Fig. 9*A*, *left traces*). ETYA had a similar effect, reducing τ_i from a control value of 20.4 ms to 13.9 ms. Scaling current traces recorded in the presence of ETYA or AA to their control amplitude clearly demonstrated that these agents enhanced inactivation kinetics without altering the kinetics of

Fig. 5. AA directly activates bTREK-1 K^+ current. Whole-cell K^+ currents were recorded from AZF cells in response to voltage steps to +20 mV applied at 30 s intervals from −80 mV. (*A*) Effect of ETYA on AA activation of bTREK-1. After recording K^+ currents in control saline, the cell was superfused with solution containing ETYA (40 μM) before switching to one containing ETYA and AA (10 μM). bTREK-1 amplitudes are plotted against time at right. Numbers on graph correspond to numbered traces on left. (*B*) Effect of 15 (S)-HPETE on bTREK-1. After recording currents in control saline, cell was superfused with saline containing 15 (S)-HPETE (150 nM) and then separately by AA(10 μM). bTREK-1 amplitudes are plotted against time at right. Numbers on graph correspond to numbered traces on left. (*C*) Summary data for experiments as in (*A*) and (*B*). Values are mean ± SEM of bTREK-1 current densities for the indicated number of determinations before and after exposure to indicated drugs. Effect of 40 μM +10 μM AA is significant ($P < 0.001$).

activation (Fig. 9*A*). Overall, AA (5 μM) reduced τ_i from a control value of 20.3 ± 2.1 ms ($n = 7$) to 15.5 ± 1.2 ms ($n = 7$). ETYA (40 μM) reduced τ_i from 19.8 ± 1.4 ms to 17.3 ± 1.2 ms ($n = 4$).

The inhibitory effects of AA on bKv1.4 and acceleration of inactivation kinetics were observed over a wide range of test potentials. In Fig. 9*B* and 9*C*, bKv1.4 currents were recorded at test potentials from −50 to +50 mV, before and after superfusing AA (5 μM). At potentials between −20 mV and +50 mV, where bKv1.4 could be accurately measured, AA inhibited this current by 35 to 44%. At test potentials between +10 and +50 mV, where τ_i could be accurately measured, AA reduced τ_i uniformly to a nearly constant voltage-independent value (Fig. 9*C*).

Discussion

This study showed that polyunsaturated fatty acids produce reciprocal modulation of native bTREK-1 and bKv1.4 K^+ currents, including a pronounced increase in bTREK-1 and a corresponding decrease in bKv1.4. Lysophospholipids also activated bTREK-1, but this effect rapidly desensitized. The actions of fatty acids and lysophospholipids on these native K^+ channels differ fundamentally in several respects from their reported effects on the same cloned channels expressed in oocytes or cell lines. In addition, these results reveal that AZF cells express large quantities of dormant bTREK-1 K^+ channels whose activity might be increased or decreased by physiological or pharmacological agents. Modulation of these channels could produce significant changes in the electrical and secretory properties of these cells. It is unlikely that AA or lysophospholipids synthesized in response to ACTH or AII would contribute to depolarization-dependent secretion, since activation of bTREK-1 channels would hyperpolarize AZF cells.

In previous studies, we reported the activation of native bTREK-1 channels by intracellular ATP at concentrations greater than 1 mM and by acidification of the pipette solution (Enyeart et al., 1997; Hille, 2001). However, with respect to magnitude, AA is easily the most effective agent for enhancing bTREK-1 current. In this regard, there is little doubt that the current activated by AA is bTREK-1. The AA-activated current is identical to the ATP- and pH-activated native bTREK-1, and to cloned bTREK-1 with respect to rectification and inhibition by penfluridol and ACTH (Enyeart et al., 1997; Hille, 2001). Further, of the more than one dozen cloned 4TMS/2P channels, only TREK channels are activated by AA, and inhibited by cAMP, the principal second messenger of ACTH (Patel et al., 1998; Maingret et al., 2000; Patel & Honore, 2001).

The pronounced activation of bTREK-1 K^+ channels by AA was observed in the absence of pre-activation by intracellular ATP or acidification of the pipette solution. In this respect, the enhancement of bTREK-1 by AA differs significantly from activation by the neuroprotective agent riluzole, which is effective only when bTREK-1 channels are pre-activated by low pH or ATP concentrations above 1 mM (Duprat et al., 2000; Enyeart et al., 2002). The effectiveness of AA in the absence of intracellular nucleotides indicates that enzyme signaling pathways requiring ATP hydrolysis, including kinases and ATPases, are not involved in this response.

Direct Activation of bTREK-1 by AA and Other Polyunsaturated Fatty Acids

Even though the activation of bTREK-1 by AA was relatively slow in onset, the failure of ETYA (40 μM) to block the enhancement of the current by AA indicates that AA acts directly. At concentrations less than 10 μM, ETYA inhibits each of the three enzymatic pathways that are responsible for converting AA into active metabolites, including prostaglandins, leukotrienes, and epoxides (Capdevila et al., 1988). AA has also been reported to directly activate cloned TREK-1 channels based on its activation of unitary TREK-1 channels, when applied to the cytoplasmic surface of inside-out patches (Maingret et al., 2000).

The activation of native TREK-1 K^+ channels in AZF cells by AA also resembled the activation of unidentified outwardly rectifying K^+ channels in neonatal rat atrial cells, where activation was slow in onset and unaffected by antagonists of enzymes that convert AA to active metabolites (Bean, 1984). Interestingly, TREK-1 K^+ channels recently have been shown to be expressed in cardiac atrial myoytes (Fink et al., 1996; Terrenoire et al., 2001).

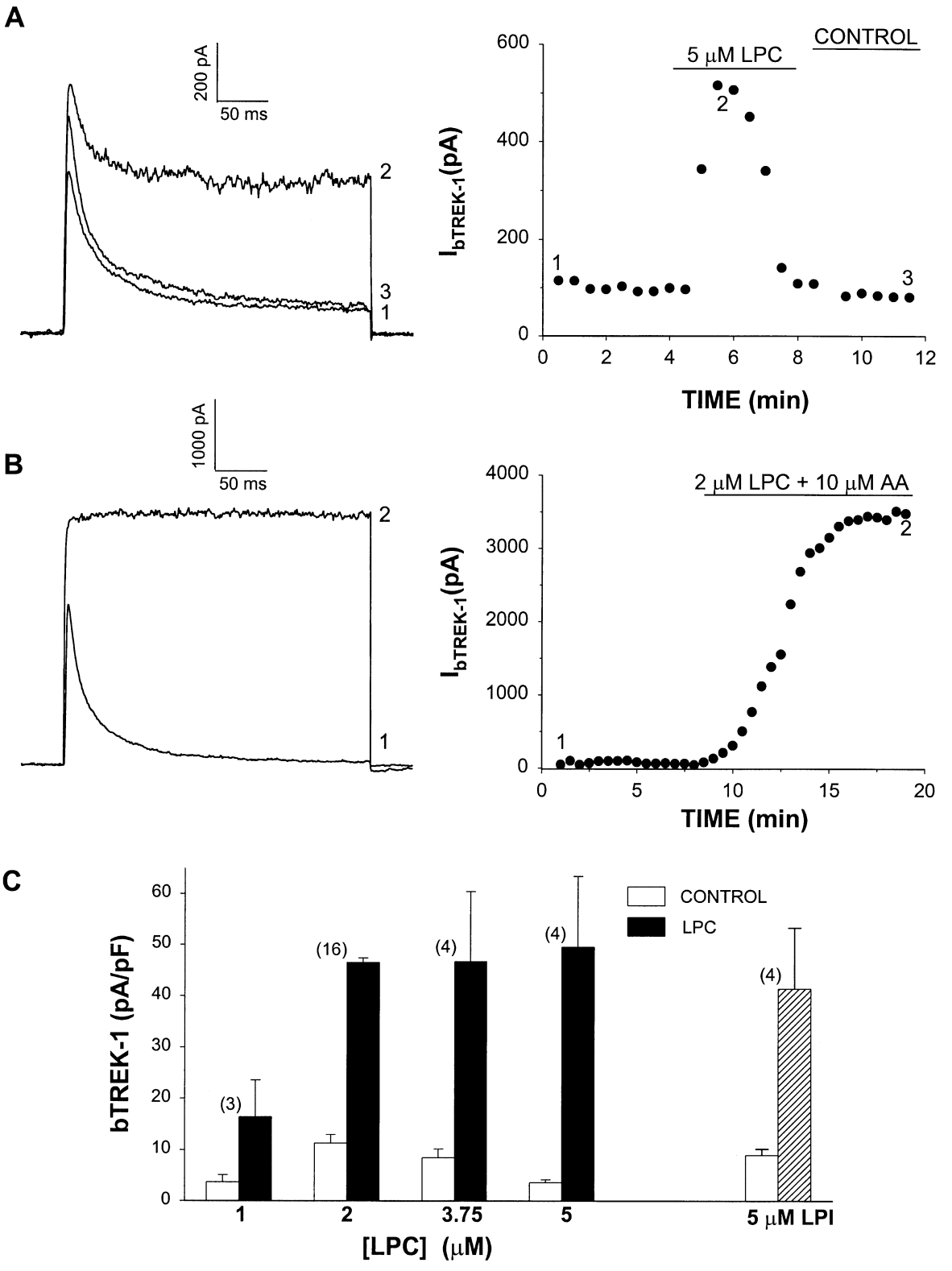


Fig. 6. Lysophospholipids activate bTREK-1. Whole-cell K^+ currents were recorded at 30 s intervals in response to voltage steps to +20 mV from a holding potential of −80 mV. Cells were superfused with LPC, LPI, or LPC + AA while recording currents at 30 s intervals. (*A*) Activation of bTREK-1 by LPC. Cell was superfused with LPC (5 μM) as indicated. bTREK-1 amplitudes are plotted against time at right. Numbers correspond to traces shown at left. (*B*) Activation of TREK-1 by LPC and AA. Cells were superfused with saline containing both LPC (2 μM) and AA (10 μM) as indicated. bTREK-1 amplitudes are plotted against time at right. Numbers correspond to traces at left. (*C*) Summary data for experiments as in (*A*) and (*B*). Values are mean ± SEM of bTREK-1 current densities for the indicated number of determinations before and after exposure to indicated agents. Effects of LPC and LPI are significant ($P < 0.001$).

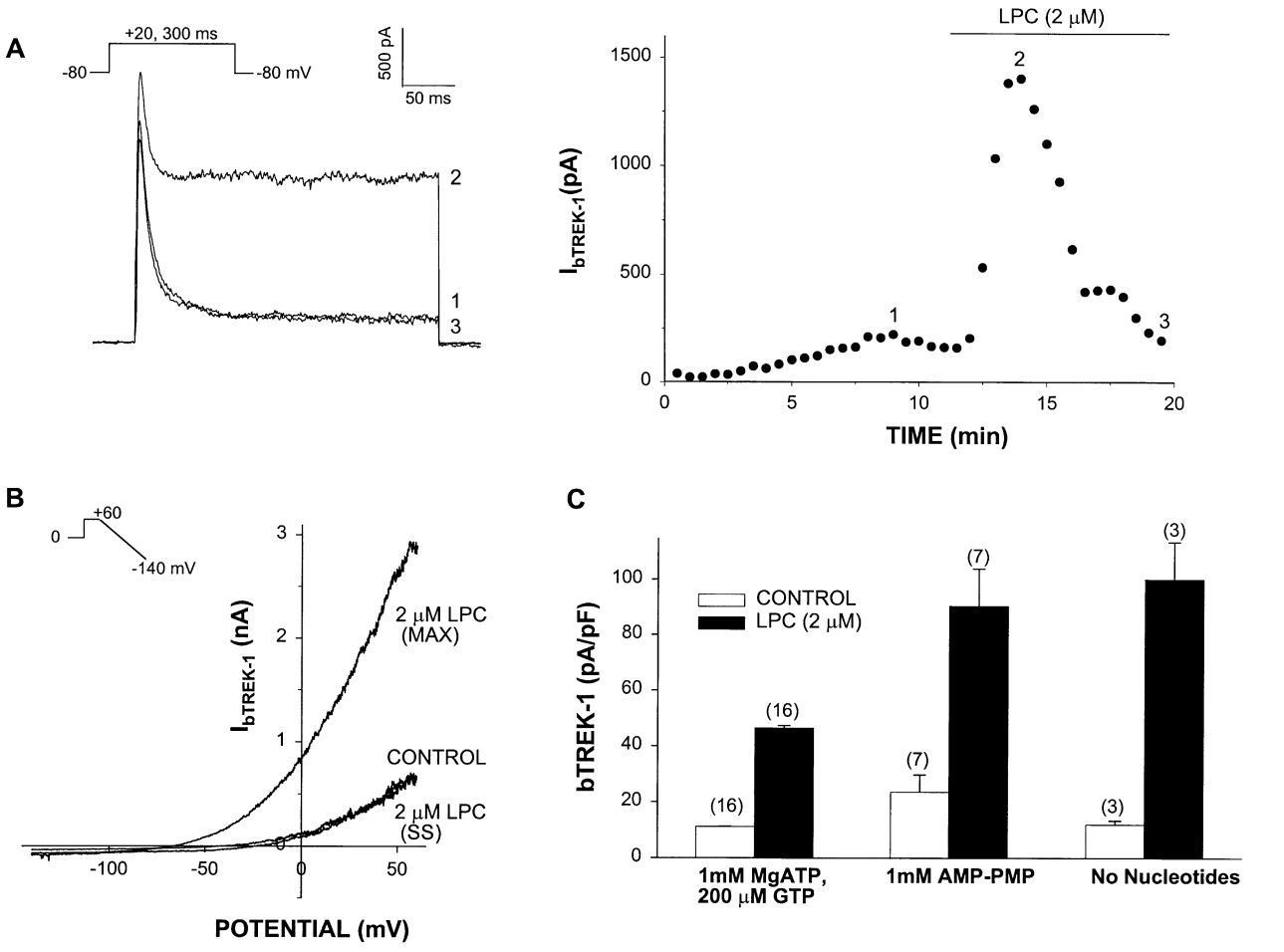


Fig. 7. Effect of AMP-PMP and nucleotides on LPC activation of bTREK-1. Whole-cell K^+ currents were recorded with pipette solution containing no addition, 1 mM AMP-PNP, or 1 mM MgATP and 200 μM GTP. (*A*) AMP-PNP-K^+ currents were recorded with pipette containing AMP-PNP (1 mM). When bTREK-1 reached a stable value, cell was superfused with LPC (2 μM) as indicated. Current amplitude is plotted on graph at right. Numbers correspond to those on traces at left. (*B*) Rectification: Linear voltage ramps of 100 mV/s were applied from a holding potential of 0 mV to potentials between +60 and −140 mV for the cell shown in (*A*). Currents were recorded in control saline, after maximum increase in LPC and after steady-state amplitude (*SS*) was attained in the presence of LPC as indicated. (*C*) Summary data for experiments as in (*A*) with pipette solutions containing MgATP + GTP, AMP-PNP, or no nucleotides as indicated. Values are mean ± SEM of bTREK-1 current densities for the indicated number of determinations. Effect of LPC (2 μM) for all 3 pipette solutions is significant ($P < 0.001$).

The effectiveness of only cis-unsaturated fatty acids as activators of bTREK-1 channels could suggest that fatty-acid metabolites were responsible for channel modulation. Cis-unsaturated, but not trans-unsaturated fatty acids or saturated fatty acids are substrates for cyclooxygenase and lipoxygenase enzymes (Ordway et al., 1989). However, activation of the native cardiac TREK-1-like and cloned TREK-1 channels show this same requirement for cis-unsaturated fatty acids, while apparently directly activating these channels (Bean, 1984; Patel et al., 1998).

Effect of Lysophospholipids on bTREK-1

LPI and LPC both activated bTREK-1, but with a temporal pattern distinct from that observed with AA. The temporal pattern for LPC activation of native bTREK-1 also differs significantly from that reported for activation of cloned TREK-1 channels (Maingret et al., 2000). Specifically, lysophospholipids including LPC and LPI were shown to activate cloned bTREK-1 channels much more rapidly than AA. However, activation of these cloned channels by lysophospholipids persisted for at least 15 min, with no diminution (Maingret et al., 2000). The rapid and complete desensitization of the native AZF-cell bTREK-1 channels in response to LPC or LPI stands in direct contrast to the persistent activation observed with cloned channels.

The molecular mechanisms that underlie the distinctly different temporal patterns for lysophospholipid activation of native and cloned TREK-1 channels are unknown. Typically, different responses observed for native and cloned channels re-

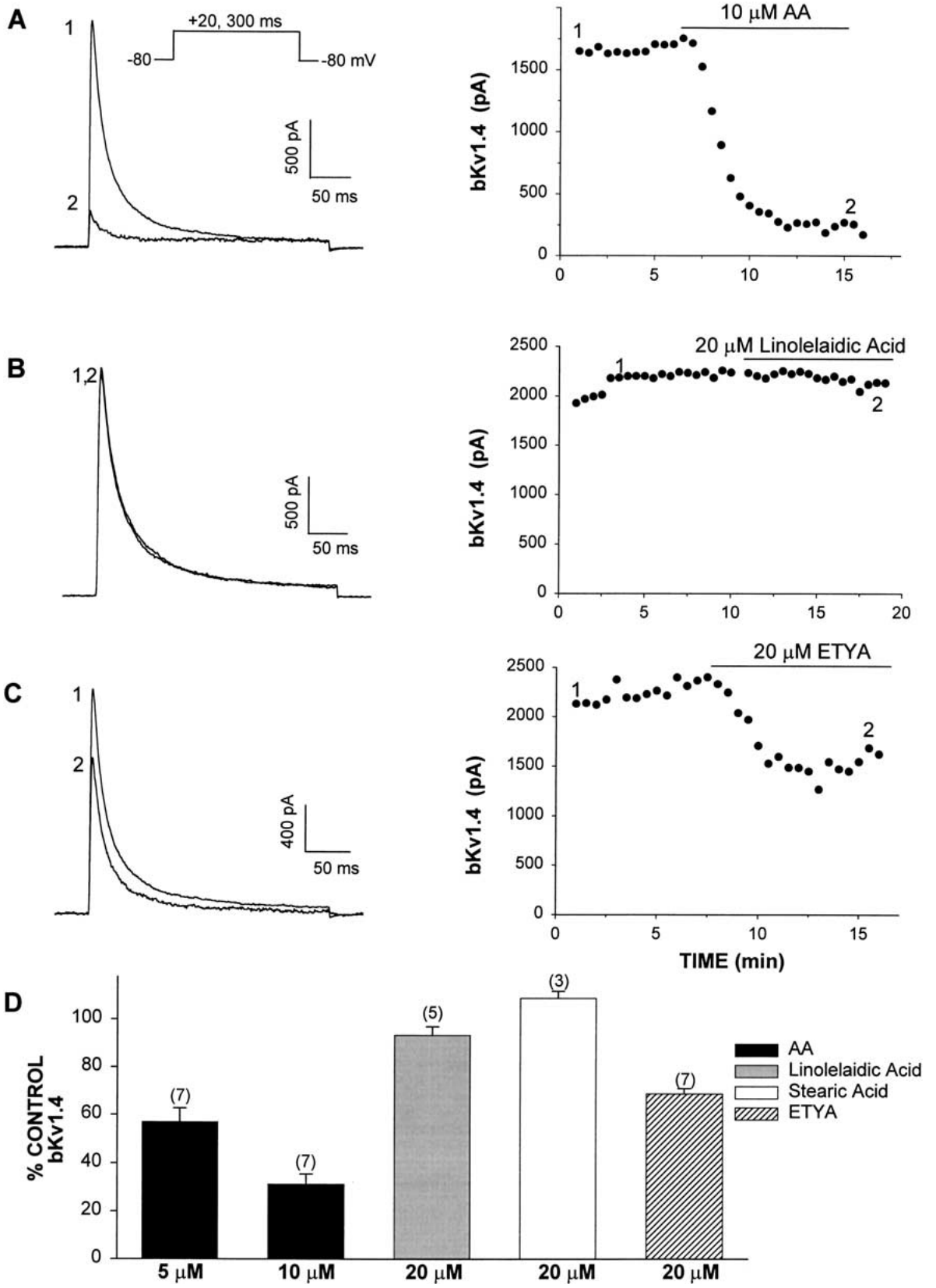

A
1
+20, 300 ms
-80
-80 mV
500 pA
50 ms
2
10 μM AA
bKv1.4 (pA)
B
1,2
20 μM Linolelaidic Acid
C
400 pA
20 μM ETYA
TIME (min)
D
% CONTROL bKv1.4
(7)
(5)
(3)
5 μM
10 μM
20 μM
AA
Linolelaidic Acid
Stearic Acid
ETYA

Fig. 8.

Fig. 8. Inhibition of bKv1.4 by fatty acids. bKv1.4 currents were recorded at 30 s intervals in response to voltage steps to +20 mV from a holding potential of −80 mV. Currents were recorded with pipette solutions containing 200 μM cAMP to suppress bTREK-1. bKv1.4 K^+ currents were recorded in saline before superfusing cells with AA, ETYA, linolelaidic acid or stearic acid as indicated. (*A*,*B*,*C*) bKv1.4 current amplitudes are plotted against time at right. Numbers on graph correspond to current traces at left. (*D*) Summary data derived from experiments as in *A*,*B*, and C above. Values are mean ± SEM for bKv1.4 K^+ currents expressed as percent of control current for cells treated with each of the four agents at the indicated concentrations. Effects of AA and ETYA are significant ($P < 0.001$).

flect the presence of auxiliary subunits and signaling pathways in native cells that are absent in the transfected cell or cell line. Clearly, the rapid but transient lipid-induced increase in bTREK-1 activity observed in AZF cells occurs under conditions that are closer to those which occur in nature.

In this regard, the signaling pathways that underlie the rapid increase and desensitization of responses to lysophospholipids are unknown. In several cell types, it has been reported that the lysophospholipid effects are mediated through complex kinase cascades and G-coupled protein receptors (Fang et al., 1997; Bassa et al., 1999). However, it does not appear that the activation of native bTREK-1 channels by lysophospholipids involves G-protein-coupled receptors, protein kinases, or ATP hydrolysis, since the rapidly desensitizing responses were observed with pipette solutions containing no added nucleotides or only AMP-PNP. The rapid onset of the response could indicate a direct interaction with the channel.

Modulation of bKv1.4 by Fatty Acids

Several lines of evidence indicate that the reciprocal modulation of bKv1.4 and bTREK-1 K^+ currents by AA occurs through distinct receptors and/or signaling pathways. Most obvious were the differences in the kinetics of onset and reversibility. Inhibition of bKv1.4 current by AA was rapid in onset and poorly reversible, while activation of bTREK-1 occurred with a delay, and reversed rapidly.

The structural requirements for inhibition of bKv1.4 by fatty acids resembled those for activation of bTREK-1, with one notable exception. Specifically, cis-polyunsaturated fatty acids inhibited bKv1.4 and accelerated inactivation kinetics, while trans-unsaturated and saturated fatty acids were ineffective. However, in contrast to its ineffectiveness as an activator of bTREK-1, ETYA inhibited bKv1.4 and accelerated inactivation.

Although less potent than AA, the effective inhibition of bKv1.4 by ETYA indicates a significant difference in the receptors and/or signaling pathways by which fatty acids modulate the two currents. Since ETYA is an inhibitor of the enzymes that convert fatty acids to active metabolites, a direct action of this agent on bKv1.4 is likely.

In contrast to the inhibition of native bKv1.4 K^+ currents by AA that we observed, AA was reported to produce a slight increase in the amplitude of Kv1.4 currents expressed in *Xenopus* oocytes (Villarroel & Schwarz, 1996). AA also failed to increase the inactivation rate of Kv1.4 channels express in oocytes (Villarroel & Schwarz, 1996). The mechanism involved for these distinctly different results is unknown. However, it may be that the effects of AA are mediated through interaction of AA with an auxiliary subunit for Kv1.4 that is associated with the channel in AZF cells, but absent when the channel is expressed in *Xenopus* oocytes.

An auxiliary subunit for Kv1.4 (Kvβ2) that speeds inactivation has been identified (Peri et al., 2001). The Kvβ2 protein associated with Kv1.4 channels enhances the rate of inactivation. It is possible that AA acts by directly interacting with this protein to further enhance the rate of inactivation of native bKV1.4 channels in AZF cells. Within the context of this model, AA does not modulate the activity of Kv1.4 channels expressed in oocytes because these cells do not express this β subunit.

A remarkably similar situation has been observed for fast inactivating Kv4.2 K^+ currents expressed in hippocampal neurons. AA accelerates the rate of inactivation of native Kv4.2 channels expressed in neurons, but fails to alter inactivation of cloned Kv4.2 channels (Villarroel & Schwarz, 1996; Keros & McBain, 1997). However, a K^+-channel-interacting protein (KChIP) restored the modulation of Kv4.2 inactivation kinetics by AA when KChP and Kv4.2 were expressed in heterologous cells (Holmqvist et al., 2001). In AZF cells, Kvβ2 may function analogously to KChIP.

Inhibition and Inactivation

Although AA, as well as ETYA, inhibits peak bKv1.4 current and accelerates inactivation, it isn't yet clear that these two effects are separate and distinct. In a simplified gating scheme, bKv1.4 channels travel from closed to open, and finally, to the inactivated state during the course of a depolarizing voltage step.

$$C \xrightarrow{K_a} O \xrightarrow{K_i} I$$

By speeding inactivation kinetics (K_i) without altering activation kinetics (K_a), AA will reduce the maximum number of channels that are simultaneously open during a voltage step. Consequently, the peak current, which is proportional to the number of open

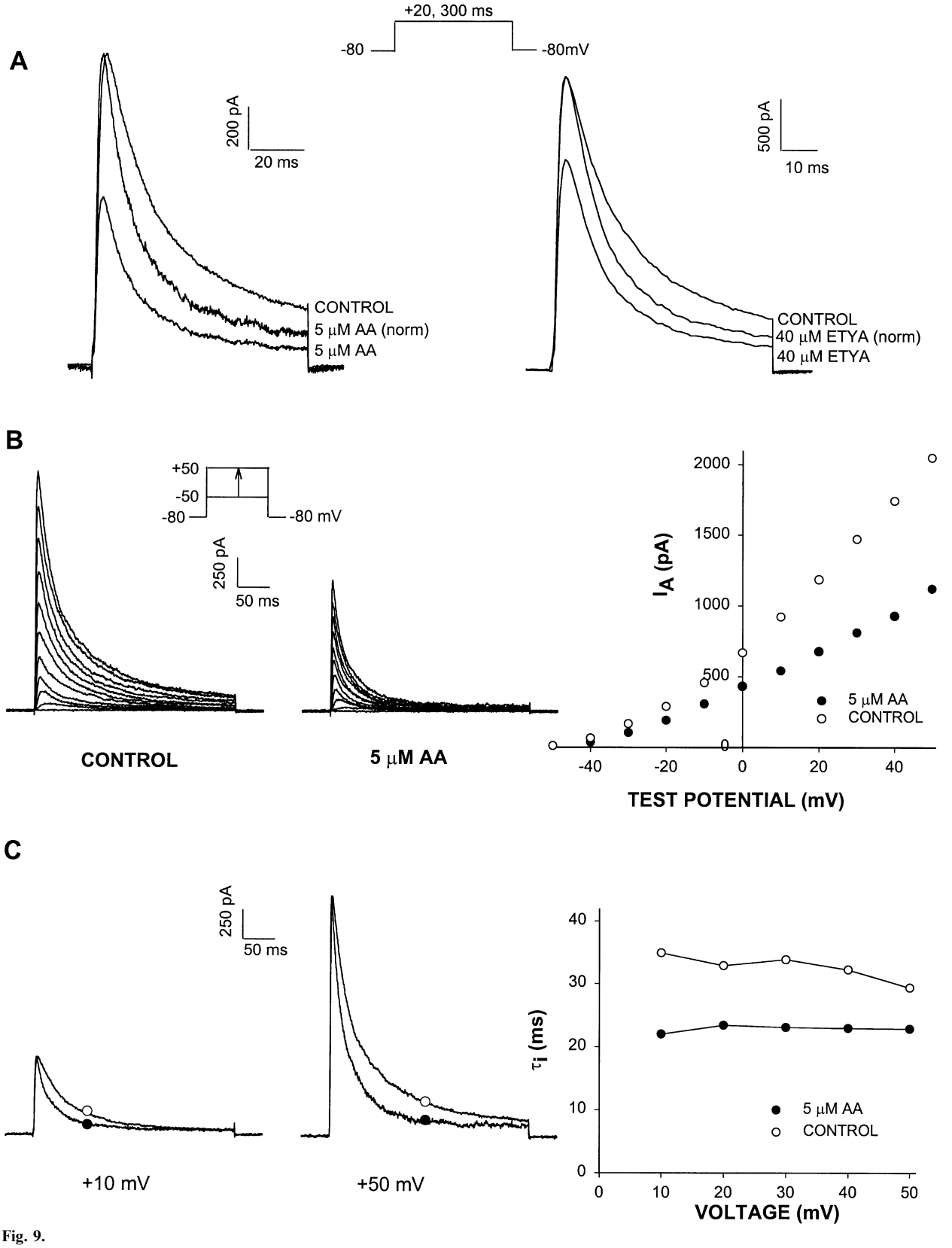

A
+20, 300 ms
-80
-80mV
200 pA
20 ms
CONTROL
5 μM AA (norm)
5 μM AA
500 pA
10 ms
CONTROL
40 μM ETYA (norm)
40 μM ETYA
B
+50
-50
-80
-80 mV
250 pA
50 ms
CONTROL
5 μM AA
I_A (pA)
2000
1500
1000
500
0
5 μM AA
CONTROL
-40
-20
0
20
40
TEST POTENTIAL (mV)
C
250 pA
50 ms
+10 mV
+50 mV
τ_i (ms)
40
30
20
10
0
5 μM AA
CONTROL
0
10
20
30
40
50
VOLTAGE (mV)

Fig. 9.

Fig. 9. Effect of fatty acids on inactivation kinetics. (*A*) Effect of AA and ETYA on bKv1.4 inactivation kinetics. bKv1.4 K^+ currents were recorded at 30 s intervals in response to voltage steps to +20 mV with pipettes containing 200 μM cAMP to suppress bTREK-1. After currents were recorded in control saline, cells were superfused with AA (5 μM) or ETYA (40 μM). Traces show control bKv1.4 currents, bKv1.4 currents after inhibition by AA (5 μM) (*left*) or ETYA (40 μM) (*right*) and scaled traces normalized to the control amplitude in the presence of either antagonist (*norm*). (*B*) Effect of AA on *I–V* relationship. bKv1.4 currents were activated from a holding potential of −80 mV to test potentials between −50 and +50 mV in control saline and after steady-state block by 5 μM AA. bKv1.4 current traces are shown at left. Peak current amplitudes are plotted against voltage at right. (*C*) Voltage-dependent effects of AA on bKv1.4 inactivation kinetics. Inactivating component of bKv1.4 currents shown in (*B*) were fit with an equation of the form: $y = y_o \exp(-t/\tau_i)$ to determine the inactivation time constant (τ_i). Time constants τ_is are plotted against test potential at right for test potentials of +10 to +50 mV. Traces show currents recorded at indicated test potentials before and after superfusing of AA (5 μM).

channels, will be reduced. The extent of current reduction would depend on the relative values of K_a and K_i. It is likely that the inhibition of Kv1.4 in AZF cells by AA is, at least in part, due to the accelerated inactivation kinetics.

Physiological Significance of AA and Lysophospholipid Effects

The physiological significance of the reciprocal regulation of bTREK-1 and bKv1.4 K^+ channels by AA is unknown. AA and its metabolites have been implicated in the physiology of both ACTH- and AII-stimulated corticosteroid secretion (Kojima et al., 1985; Cymeryng et al., 1995; Yamazaki et al., 1996; Wang et al., 2000). In whole-cell recordings with pipettes containing 5 mM MgATP, bTREK-1 current amplitude spontaneously increases over many minutes of recording, while bKv1.4 decreases (Enyeart et al., 2001). The reciprocal change in the activity of these two K^+ channels could occur through a spontaneous increase in the AA content of the AZF cell membrane. Regardless of the mechanism, the physiological significance of this reciprocal regulation of these two K^+ channels is yet to be determined. AA also reciprocally alters the availability of transient and non-inactivating K^+ channels in hippocampal neurons (Colbert & Pan, 1999).

Overall, superfusion of AZF cells with AA revealed the presence of hundreds to thousands of leak-type K^+ channels, a large fraction of which are dormant until activated by AA. It is unclear why AZF cells express so many of these channels when only a small fraction would suffice to clamp the membrane potential near the K^+ equilibrium potential.

Since these channels function pivotally in coupling ACTH- and AII-receptor activation to membrane depolarization and Ca^{2+} entry, the activation of a large fraction of bTREK-1 channels could act as a brake on membrane depolarization. In this scheme, higher concentrations of ACTH or AII would be required to depolarize AZF cells when bTREK-1 open probability is high, thereby shifting the concentration response curves to the right. Endogenous activators of TREK-1-type channels such as AA could suppress membrane depolarization and corticosteroid secretion.

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References

Bang, H., Kim, Y., Kim, D. 2000. TREK-2, a new member of the mechanosensitive tandem-pore K^+ channel family. *J. Biol. Chem.* **275:**17412–17419

Bassa, B.V., Roh, D.D., Vaziri, N.D., Kirschenbaum, M.A., Kamanna, V.S. 1999. Lysophosphatidylcholine activates mesangial cell PKC and MAP kinase by PLCgamma-1 and tyrosine kinase-Ras pathways. *Am. J. Physiol.* **277:**F328–F337

Bean, B.P. 1984. Nitrendipine block of cardiac calcium channels: High affinity binding to the inactivated state. *Proc. Natl. Acad. Sci. USA* **81:**6388–6392

Brooks, S.P.J., Storey, K.B. 1992. Bound and determined: a computer program for making buffers of defined ion concentrations. *Anal. Biochem.* **201:**119–126

Capdevila, J., Gil, L., Orellana, M., Marnett, L.J., Mason, J.I., Yadagiri, P., Falck, J.R. 1988. Inhibitors of cytochrome P-450-dependent arachidonic acid metabolism. *Arch. Biochem. Biophys.* **261:**257–263

Colbert, C.M., Pan, E. 1999. Arachidonic acid reciprocally alters the availability of transient and sustained dendritic K^+ in hippocampal CA1 pyramidal neurons. *J. Neurosci.* **19:**8163–8171

Cymeryng, C.B., Paz, C., Dada, L., Cornejo, M., Neuman, M.I., Mele, P.G., Finkielstein, C., Solano, A.R., Mendez, C.F., Park, M. 1995. ACTH-dependent proteolytic activity of a novel phosphoprotein (p43) intermediary in the activation of phospholipase A2 and steroidogenesis. *Endo. Res.* **21:**281–288

Duprat, F., Lesage, F., Patel, A.J., Fink, M., Romey, G., Lazdunski, M. 2000. The neuroprotective agent riluzole activates the two P domain K^+ channels TREK-1 and TRAAK. *Mol. Pharmacol.* **57:**906–912

Enyeart, J.A., Xu, L., Enyeart, JJ. 2000. A bovine adrenocortical Kv1.4 K^+ channel whose expression is potently inhibited by ACTH. *J. Biol. Chem.* **275:**34640–34649

Enyeart, J.J., Gomora, J.C., Xu, L., Enyeart, J.A. 1997. Adenosine triphosphate activates a noninactivating K^+ current in adrenal cortical cells through nonhydrolytic binding. *J. Gen. Physiol.* **110:**679–692

Enyeart, J.J., Mlinar, B., Enyeart, J.A. 1993. T-type Ca^{2+} channels are required for ACTH-stimulated cortisol synthesis by bovine adrenal zona fasciculate cells. *Mol. Endo.* **7:**1031–1040

Enyeart, J.J., Mlinar, B., Enyeart, J.A. 1996. Adrenocorticotropic hormone and cAMP inhibit noninactivating K^+ current in adrenocortical cells by an A-kinase-independent mechanism requiring ATP hydrolysis. *J. Gen. Physiol.* **108:**251–264

Enyeart, J.J., Xu, L., Danthi, S., Enyeart, J.A. 2002. An ACTH- and ATP-regulated background K^+ channel in adrenocortical cells is TREK-1. *J. Biol. Chem.* **211:** 49186–49199.

Enyeart, J.J., Xu, L., Gomora, J.C., Enyeart, J.A. 2001. Reciprocal modulation of voltage-gated and background K^+ channels mediated by nucleotides and ACTH. *Mol. Pharmacol.* **60:** 114–123

Fang, X., Gibson, S., Flowers, M., Furui, T., Bast, R.C.J., Mills, G.B. 1997. Lysophosphatidylcholine stimulates activator protein 1 and the c-Jun N-terminal kinase activity. *J.Biol.Chem.* **272:**13683–13689

Fink, M., Duprat, F., Lesage, F., Reyes, R., Romey, G., Heurteaux, C., Lazdunski, M. 1996. Cloning, functional expression and brain localization of a novel unconventional outward rectifier K^+ channel. *EMBO J.* **15:**6854–6862

Fink, M., Lesage, F., Duprat, F., Heurteaux, C., Reyes, R., Fosset, M., Lazdunski, M. 1998. A neuronal two P domain K^+ channel stimulated by arachidonic acid and polyunsaturated fatty acids. *EMBO J.* **17:**3297–3308

Gomora, J.C., Enyeart, JJ. 1998. Ca^{2+} depolarizes adrenal cortical cells through selective inhibition of an ATP-activated K^+ current. *Am. J. Physiol.* **275:**C1526–C1537

Gomora, J.C., Enyeart, JJ. 1999. Mibefradil potently blocks ATP-activated K^+ channels in adrenal cells. *Mol. Pharmacol.* **56:**1192–1197

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Hille, B. 2001. Ion Channels of Excitable Membranes. Sinauer Associates, Sunderland, MA

Holmqvist, M.H., Cao, J., Knoppers, M.H., Jurman, M.E., Distefano, P.S., Rhodes, K.J., Xie, Y., An, W.F. 2001. Kinetic modulation of Kv4-mediated A-current by arachidonic acid is dependent on potassium channel interacting proteins. *J. Neurosci.* **21:**4154–4161

Keros, S., McBain, C.J. 1997. Arachidonic acid inhibits transient potassium currents and broadens action potentials during electrographic seizures in hippocampal pyramidal and inhibitory interneurons. *J. Neurosci.* **17:**3476–3487

Kim, D., Clapham, D.E. 1989. Potassium channels in cardiac cells activated by arachindonic acid and phospholipids. *Science* **244:**1174–1179

Kim, D., Sladek, C.D., Aguado-Velasco, C., Mathiasen, J.R. 1995. Arachidonic acid activation of a new family of K^+ channels in cultured rat neuronal cells. *J. Physiol.* **484:**643–660

Kojima, I., Kojima, K., Rasmussen, H. 1985. Possible role of phospholipase A2 action and arachidonic acid metabolism in angiotensin II-mediated aldosterone secretion. *Endocrinology* **117:**1057–1066

Lesage, F., Lazdunski, M. 2000. Molecular and functional properties of two-pore-domain potassium channels. *Am. J. Physio.* **279:**F793–F801

Maingret, F., Lauritzen, I., Patel, A.J., Heurteaux, C., Reyes, R., Lesage, F., Lazdunski, M., Honore, E. 2000. TREK-1 is a heat-activated background K^+ channel. *EMBO J* **19:**2483–2491

Maingret, F., Patel, A.J., Lesage, F., Lazdunski, M., Honore, E. 2000. Lysophospholipids open the two-pore domain mechano-gated K^+ channels TREK-1 and TRAAK. *J. Biol. Chem.* **275:**10128–10133

Mlinar, B., Biagi, B.A., Enyeart, J.J. 1993. A novel K^+ current inhibited by ACTH and Angiotensin II in adrenal cortical cells. *J. Biol. Chem.* **268:**8640–8644

Mlinar, B., Biagi, B.A., Enyeart, J.J. 1995. Losartan-sensitive AII receptors linked to depolarization- dependent cortisol secretion through a novel signaling pathway. *J. Biol. Chem.* **270:**20942–20951

Mlinar, B., Enyeart, J.J. 1993. Voltage-gated transient currents in bovine adrenal fasciculata cells II: A-type K^+ current. *J. Gen. Physiol.* **102:**239–255

Ordway, R.W., Singer, J.J., Walsh, J.V.J. 1991. Direct regulation of ion channels by fatty acids. *Trends Neurosci.* **14:**96–100

Ordway, R.W., Walsh, J.V., Singer, J.J. 1989. Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. *Science* **244:**1176–1179

Patel, A.J., Honore, E. 2001. Properties and modulation of mammalian 2P domain K^+ channels. *Trends Neurosci.* **24:**339–346

Patel, A.J., Honore, E., Maingret, F., Lesage, F., Fink, M., Duprat, F., Lazdunski, M. 1998. A mammalian two pore domain mechano-gated S-like K^+ channel. *EMBO J* **17:**4283–4290

Peri, R., Wible, B.A., Brown, A.M. 2001. Mutations in the Kv beta 2 binding site for NADPH and their effects on Kv1.4. *J. Biol. Chem.* **276:**738–741

Terrenoire, C., Lauritzen, I., Lesage, F., Romey, GL., Lazdunski, M. 2001. A TREK-1-like potassium channel in atrial cells inhibited by beta-adrenergic stimulation and activated by volatile anesthetics. *Circ. Res.* **89:**336–342

Tian, Y., Smith, R.D., Balla, T., Catt, K.J. 1998. Angiotensin II activates mitogen-activated protein kinase via protein kinase C and Ras/Raf-1 kinase in bovine adrenal glomerulosa cells. *Endocrinology* **139:**1801–1809

Villarroel, A., Schwarz, T.L. 1996. Inhibition of the Kv4 (Shal) family of transient K^+ currents by arachidonic acid. *J. Neurosci.* **16:** 2522–2532

Wang, H., Walker, S.W., Mason, J.I., Morley, S.D., Williams, B.C. 2000. Role of arachidonic acid metabolism in ACTH-stimulated cortisol secretion by bovine adrenocortical cells. *Endo. Res.* **26:**705–709

Xu, L., Enyeart, J.J. 2001. Properties of ATP-dependent K^+ channels in adrenocortical cells. *Am. J. Physiol.* **280:**C199–C215

Yamazaki, T., Higuchi, K., Kominami, S., Takemori, S. 1996. 15-lipoxygenase metabolite(s) of arachidonic acid mediates adrenocorticotropin action in bovine adrenal steroidogenesis. *Endocrinology* **137:**2670–2675