Bovine adrenal zona fasciculata (AZF) cells express a background K⁺ channel (IAC) that sets the resting potential and acts pivotally in ACTH-stimulated cortisol secretion. We have cloned a bTREK-1 (KCNK2) tandem-pore K⁺ channel cDNA from AZF cells with properties that identify it as the native IAC. The bTREK-1 cDNA is expressed robustly in AZF cells and includes transcripts of 4.9, 3.6, and 2.8 kb. In patch clamp recordings made from transiently transfected cells, bTREK-1 displayed distinctive properties of IAC in AZF cells. Specifically, bTREK-1 currents were outwardly rectifying with a large instantaneous and smaller time-dependent component. Similar to IAC, bTREK-1 increased spontaneously in amplitude over many minutes of whole cell recording and was blocked potently by Ca²⁺ antagonists including penfluridol and mibefradil and by 8-(4-chlorophenylthio)-cAMP. Unitary TREK-1 and IAC currents were nearly identical in amplitude. The native IAC current, in turn, displayed properties that are specific to TREK-1 K⁺ channels. These include activation by intraacellular acidification, enhancement by the neuroprotective agent riluzole, and outward rectification. bTREK-1 current differed from native K⁺ current only in its lack of ATP dependence. In contrast to IAC, the current density of bTREK-1 in human embryonic kidney-293 cells was not increased by raising pipette ATP from 0.1 to 5 mM. Further, the enhancement of IAC current in AZF cells by low pH and riluzole was facilitated by, and dependent on, ATP at millimolar concentrations in the pipette solution. Overall, these results establish the identity of IAC K⁺ channels, demonstrate the expression of bTREK-1 in a specific endocrine cell, identify potent new TREK-1 antagonists, and assign a pivotal role for these tandem-pore channels in the physiology of cortisol secretion. The activation of IAC by ATP indicates that native bTREK-1 channels may function as sensors that couple the metabolic state of the cell to membrane potential, perhaps through an associated ATP-binding protein.

Bovine AZF¹ cells express a novel K⁺-selective channel (IAC) that functions pivotally in the regulation of cortisol secretion (1–3). IAC displays a number of properties typical of background or leak-type channels that function in setting the resting membrane potential of various cells. However, native IAC channels also exhibit a combination of properties not observed previously in a single type of K⁺ channel. In whole cell patch clamp recordings, IAC appears as a non-inactivating, outwardly rectifying K⁺ current that displays weak voltage dependence and grows spontaneously over many minutes (1, 3, 4). At the single channel level, the conductance and rectifying properties of these channels depend strongly on the presence of divalent cations (5). In symmetrical K⁺, with physiological concentrations of Ca²⁺ and Mg²⁺, the channels are outwardly rectifying, whereas they become nearly ohmic in the absence of divalent cations (4, 5).

In whole cell patch clamp recordings, IAC is inhibited potently by ACTH and angiotensin II, the two peptide hormones that regulate cortisol secretion physiologically (1, 6). IAC channels are also inhibited by paracrine factors, including ATP and adenosine, which act through specific G protein-coupled nucleotide receptors (7, 8).

Second messengers synthesized or released upon activation of these receptors, including Ca²⁺ and cAMP, inhibit IAC channels. However, inhibition appears to involve atypical signaling pathways, because responses to peptides and second messengers are insensitive to inhibition of cAMP and Ca²⁺-activated protein kinases (3, 5, 6, 9).

Among background K⁺ channels, IAC channels are unique in their activation by hydrolyzable and non-hydrolyzable forms of ATP, applied intracellularly at physiological concentrations through a recording pipette (4). These background channels are also activated by UTP, GTP, and by inorganic polyphosphosphate (PPP) (4, 5).

IAC channels also display a unique pharmacological profile. Specifically, these channels are relatively insensitive to many standard K⁺ channel antagonists, including 4-AP and TEA (respective IC₅₀ values of 2.8 and 24.3 μM), whereas they are blocked by others such as quinidine (IC₅₀ = 24.1 μM) (10). Surprisingly, IAC K⁺ channels are blocked potently by the Ca²⁺ antagonists penfluridol and mibefradil at far lower concentrations (respective IC₅₀ values of 0.20 and 0.50 μM) (10, 11).

Although IAC K⁺ channels function as background or leak K⁺ channels that set the resting potential of AZF cells, their aggregate properties do not match the profile of any K⁺ channel.
nel yet described. Regardless, these channels appear to function critically in the physiology of cortisol secretion. In this capacity, they act as sensors that integrate hormonal and metabolic signals and couple these to depolarization-dependent Ca²⁺ entry.

Because of their distinctive properties as a metabolic sensor and role in cortisol secretion, it will be important to determine the molecular identity of Iₐc channels. In this regard, K⁺-selective ion channels in mammals can be divided into several families, based on structure and membrane topology. Voltage-gated K⁺ channels include a major subunit that consists of one pore domain and six transmembrane segments (1P/6TMS) (12). A second family that consists of channel subunits containing a single pore domain and two membrane-spanning segments (1P/2TMS) includes inward rectifiers many of which contribute to the resting potential and some of which are induced directly by intracellular ATP (12–14).

K⁺-selective leak or background channels comprise the third and most recently described family of K⁺ channels. In mammals, the largest group of leak K⁺ channels possesses distinctive subunits that consist of two pore domains and four transmembrane segments. These 2P/4TMS channels are typically non-inactivating K⁺ channels that set the resting potential in a range of cells (15–17). Since 1995, more that a dozen members of the 2P/4TMS family have been identified and characterized by molecular cloning and patch clamp techniques (15–17).

Although similar in structure, 2P/4TMS K⁺ channels can be distinguished based on a variety of functional properties including unitary conductance, rectification, sensitivity to mechanical stimulation, pH and temperature, modulation by cAMP, fatty acids, membrane phospholipids, general anesthetics, and the neuroprotective agent riluzole (15–19). These leak-type channels also show variable sensitivity to standard K⁺ channel blockers (15–17). At least one member of this family is reported to acquire voltage-dependent gating upon phosphorylation (20).

Although Iₐc functions in AZF cells as a classic leak-type K⁺ channel, its distinctive properties are not duplicated in any of the 2P/4TMS K⁺ channels yet described. In particular, direct modulation of K⁺ channels by ATP has only been demonstrated in K⁺ channels of the 1P/2TMS type, which are inhibited rather than activated by this nucleotide (13, 14).

We have cloned a 2P/4TMS channel TREK-1 (KCNK2) K⁺ channel cDNA from AZF cells that appears to code for Iₐc channels. When expressed in cell lines, bTREK-1 currents share several distinctive features with native Iₐc currents. Evidence is presented that indicates that, in AZF cells, bTREK-1 channels acquire additional properties including ATP sensitivity.

**EXPERIMENTAL PROCEDURES**

**Materials**

Tissue culture media, antibiotics, fibronectin, and fetal bovine sera were obtained from Invitrogen. Culture dishes were purchased from Corning Glass (Corning, NY). Coverslips were from Belloco (Vineland, NJ). Phosphate-buffered saline, enzymes, ACTH (1–24), AMP-PNP (lithium salt), 2,3-bis(2-aminophenoxy)ethane-N,N',N″,N‴-tetraacetic acid, riluzole, forskolin, and 8-ppt-cAMP, glibenclamide, and LaCl₃ were from Sigma. Penfluridol was obtained from Janssen Pharmaceuticals (Beersel, Belgium). p3-CD8 clone was kindly provided by Dr. Brian Seed, Department of Genetics, Massachusetts General Hospital, Boston, MA. Rat TREK-2 cDNA was kindly provided by Dr. Brian Seed, Department of Genetics, Massachusetts General Hospital, Boston, MA. Rat TREK-2 cDNA was kindly provided by Dr. D. Kim, Dept. of Physiology and Biophysics, Finch University of Health Sciences, The Chicago Medical School, Chicago, IL. HEK-293 and CHO-K1 cells were obtained from ATCC (Manassas, VA).
1% SDS for 30 min with a final wash at 65 °C with 0.1 x saline/sodium phosphate buffer/EDTA. 1% SDS for 20 min. Autoradiograms were obtained by exposing the blots for 2 to 5 h to Eastman Kodak Co. X-Omat AR film at −70 °C.

**Patch Clamp Experiments**—Whole cell patch clamp recordings of Kv currents were made from AZF cells or bTREK-1-transfected HEK-293 cells. The standard pipette solution was 120 mM KCl, 2 mM MgCl₂, 1 mM CaCl₂, 20 mM HEPES, 11 mM 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid, 5 mM MgATP, and 200 μM GTP, with pH buffered to 7.2 using KOH. Deviations from these solutions with respect to nucleotide and pH are described in the text. The external solution consisted of 140 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, 10 mM HEPES, and 5 mM glucose, pH 7.35, using NaOH. All solutions were filtered through 0.22-μm cellulose acetate filters.

AZF cells were used for patch clamp experiments 2–12 h after plating. Typically, cells with diameters of <15 μm and capacitances of 8–15 pF were selected for recording. Coverslips were transferred from 35-mm culture dishes to the recording chamber (volume, 1.5 ml), which was perfused continuously by gravity at a rate of 3 ml/min. Drugs were applied externally by bath perfusion controlled manually by a six-way rotary valve. Patch electrodes with resistances of 2.0–5.0 MΩ were fabricated from Corning 0010 glass (WPI, Sarasota, FL). K⁺ perfused with saline/sodium phosphate buffer/EDTA, transfected cells were identified based on decoration with the streptavidin–biotin–peroxidase system. Whole cell bTREK-1 K⁺ currents in transfected cells were recorded as described for AZF cells. Smaller cells with capacitances of 10–15 pF were selected for recording.

Pulse generation and data acquisition were done using a personal computer and PCLAMP software with a TL-1 interface (Axon Instruments, Inc., Union City, CA). Currents were digitized using an 8-pole Bessel filter (Frequency Devices, Haverhill, MA). Linear leak and capacitance currents were subtracted from current records using hyperpolarizing steps of 1/2 to 1/4 amplitude. Data were analyzed and plotted using PCLAMP 5.5 and 6.04 (Clamp and Clampfit) and SigmaPlot (version 5.0).

**RESULTS**

**bTREK-1 in AZF Cells**—Previous studies using whole cell and single channel patch clamp recording showed that IA_C channels resembled 2P4TMS channels of the TREK-1 and TRAAK with regard to single channel conductance and rectification (5, 16). Degenerate primers were designed using the P1 and M2 regions of bTREK-1 and hTRAAK as templates, and 3’ RACE PCR was performed using AZF cell double-stranded cDNA generated from poly(A)+ mRNA. A 750-bp product was obtained that showed 92% nucleotide sequence identity to human TREK-1. No PCR products were obtained that were homologous to hTRAAK. Specific primers were designed from this TREK-1-like product for extension in the 5’ direction using nested 5’ RACE PCR. Combining the 3’ and 5’ RACE products yielded a 1534-nt cDNA (GenBank™ accession number AY148474). This coding sequence consists of an open reading frame of 411 amino acids. The predicted amino acid sequence for bTREK-1 is given in Fig. 1, with the four transmembrane regions noted. The bovine sequence AAN37591-1 shows 99% protein sequence identity to human TREK-1 (protein ID, NP_055032.1), 96% identity to rat TREK-1 (protein ID, AAL95708), and 96.4% to mouse TREK-1 (Protein ID, XP_123605).

Northern blot analysis of poly(A)+ mRNA showed that bTREK-1 mRNA is expressed highly in the bovine adrenal cortex. A 1415-bp DNA probe that included the full-length coding region of bTREK-1 hybridized to separate 4.9-, 3.6-, and 2.8-kb mRNA transcripts isolated from AZF cells (Fig. 1B, left lane). To eliminate the possibility that these transcripts could be the result of the full-length TREK-1 probe hybridizing to mRNA coding for homologous channels present in the adrenal cortex, a TREK-1-specific probe consisting of a 206-bp BamHI fragment from the 5’ untranslated end of bTREK-1 (right) labeled with 32P-dCTP was hybridized to size-fractionated poly(A)+ mRNA (7 μg) extracted from AZF cells. Northern blot analysis was carried out as described under “Methods.” Bands correspond to transcripts of ~4.9, 3.6, and 2.8 kb.

**Properties of bTREK-1 Currents**—Properties of bTREK-1 currents were studied in transiently transfected HEK-293 cells. Successfully transfected cells were identified based on decoration with anti-CD-8 antibody-coated beads, as described under “Methods.” In whole cell patch clamp recording, bTREK-1 was remarkably similar in several respects to the native IA_C current in bovine AZF cells. Specifically, in physiological saline bTREK-1 appeared as a non-inactivating current that increased spontaneously in amplitude to a maximum value over many minutes of whole cell recording (Fig. 2A).

In the experiment illustrated in Fig. 2A, during 17 min of recording, bTREK-1 amplitude doubled from its initial value of ~1250 pA. This spontaneous time-dependent increase in the amplitude of bTREK-1 current was observed in nearly all of the more than 75 cells tested. It occurred at pipette solution pHs ranging from 6.4 to 7.3 and was independent of ATP concentrations between 0.1 and 5.0 mM. Further, this phenomenon was not specific to HEK-293 cells. The growth of bTREK-1 current with time was also observed in transfected CHO-K1 cells (data not shown). Although the time-dependent growth of IA_C in whole cell recordings has been established as a hallmark of this current in AZF cells (1, 3, 4), this phenomenon has not been reported previously for other native or cloned TREK-1 channels.
time-dependent component that could be fit with a single exponential (Fig. 2, A (left) and B). In physiological saline, bTREK-1 is outwardly rectifying (Fig. 2 B).

Comparative Pharmacology of IAC and Cloned bTREK-1—If native IAC and cloned bTREK-1 K+ channels are identical then they should display a similar pharmacological profile. The pharmacology of 2P/4TMS K+ channels, including TREK-1, is currently being investigated. Murine and human TREK-1 channels are relatively insensitive to standard organic K+ channel antagonists, including TEA and 4-AP (23, 24). High concentrations of these two drugs are also required to inhibit native IAC channels (10). However, IAC channels are inhibited potently by several organic Ca2+ antagonists, including diphenylbutylpiperidines (DPBPs) and mibebradil (10, 11).

Fig. 2. Properties of bTREK-1 K+ current in HEK-293 cells. Whole cell K+ currents were recorded from HEK-293 cells that had been transiently co-transfected with pCR®3.1-Uni-bTREK-1 and P3-CD8 cDNAs. Cells decorated with anti-CD8 antibody-coated beads were selected for patch clamping (see “Methods”). Mock-transfected cells were transfected with the expression plasmid pCR®3.1-Uni (no insert) and P3-CD8 cell surface antigen. A, time-dependent increase in bTREK-1 currents. K+ currents were activated by voltage steps to +20 mV applied at 30-s intervals from a holding potential of −80 mV. Traces and associated graph illustrate currents recorded from a single cell at indicated times after initiating whole cell recording. B, bTREK-1 current-voltage relationship. Whole cell K+ currents were recorded from bTREK-1-transfected HEK-293 and control cells in response to voltage steps applied from −80 mV in 10-mV increments to test potentials between −70 and +60 mV. Current traces from bTREK-1-transfected and control cells and associated I-V plots are shown.

Fig. 3. Penfluridol blocks bTREK-1 in transfected cells. K+ currents were recorded from bTREK-1-transfected HEK-293 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 maximum value, cells were superfused with penfluridol at concentrations between 0.1 and 2.5 μM. A, current traces and associated plot of bTREK-1 current amplitudes for a cell superfused with 2.5 μM penfluridol. Numbers on traces correspond to those on graph at right. B, penfluridol on current-voltage relationship. Whole cell K+ currents were recorded from transiently transfected HEK-293 cells in response to voltage steps applied from −80 mV to test potentials between −50 and +40 mV, before and 6 min after superfusing the cell with 2.5 μM penfluridol. Current amplitudes are plotted against test potential. C, inhibition curve for penfluridol constructed from experiments as in A. Fraction of unblocked bTREK-1 current is plotted against penfluridol concentration. Data were fit with an equation of the form, I/I max = 1/[1 + B(IC50)]X, where B is the penfluridol concentration, IC50 is the concentration that produced half-maximal inhibition, and X is the Hill slope. The IC50 estimated from the fit is 3.5 × 10−7 m. Data are normalized mean values ± S.E. of the indicated number of measurements.
In the present study, we found that the DPBP penfluridol and mibefradil also potently inhibit cloned bTREK-1 channels expressed in mammalian cell lines. DPBPs such as penfluridol inhibit IAC $K^+$ with IC$_{50}$ values of 0.2–0.4 $\mu$M (10). Penfluridol inhibited bTREK-1 expressed in HEK-293 cells with an IC$_{50}$ of 0.35 $\mu$M (Fig. 3A and C). Inhibition of bTREK-1 by penfluridol was independent of voltage at test potentials of −40 to +40 mV (Fig. 3B). bTREK-1 was also inhibited by a second DPBP, pimozide (data not shown).

Mibefradil inhibits IAC $K^+$ channels with an IC$_{50}$ of 0.5 $\mu$M (11). This drug also inhibited cloned bTREK-1 channels with similar potency (Fig. 4A). At concentrations of 0.5 and 2.5 $\mu$M, mibefradil inhibited bTREK-1 by 52 ± 15% ($n = 4$) and 81 ± 8.3% ($n = 9$), respectively. Other agents including La$^{3+}$ and glibenclamide inhibited bTREK-1 less potently but at concentrations similar to those that inhibited native IAC (Fig. 4, B, C, and E).

Cloned TREK-1 channels and bovine IAC channels are inhibited by cAMP (3, 23). The membrane-permeable cAMP analog 8-pcpt-cAMP inhibited native IAC and cloned bTREK-1 channels with equal effectiveness (Fig. 4, D and E).

bTREK-1 and ATP—Despite these similarities, bTREK-1 expressed in HEK-293 cells differed from native IAC in that bTREK-1 activity was independent of intracellular ATP. Specifically, in whole cell recordings with patch pipettes containing 1 mM MgATP, bTREK-1 reached a maximum current density of 116.7 ± 28.7 pA/pF ($n = 8$), compared with 111.3 ± 25.2 pA/pF ($n = 9$) when pipettes containing 5 mM MgATP.

Unitary bTREK-1 and IAC Currents—Unitary bTREK-1 currents were recorded from transfected CHO-K1 cell membrane patches in the inside-out configuration and compared with unitary IAC currents recorded from AZF cells under the same conditions. Amplitude analysis of single channel bTREK-1 and native IAC currents showed that these were nearly identical in size (Fig. 5, A and B). In the experiment illustrated unitary bTREK-1 and IAC currents measured at +30 mV had amplitudes of 3.57 and 3.60 pA, respectively. Solutions in contact with the cytoplasmic membrane face in these recordings contained 0.1 mM ATP. In excised inside-out patches activity of IAC and bTREK-1 channels were both independent of ATP.

Activation of IAC Current in AZF Cells by Intracellular Acidification—When expressed in HEK-293 cells, bTREK-1 current
displayed several distinctive properties of the native $I_{AC}$ of AZF cells. Experiments were done to determine whether $I_{AC}$, in turn, possessed well established properties of TREK-1 channels cloned previously. These include activation by acidification and the neuroprotective agent riluzole (18, 25).

Extensive patch clamp and molecular cloning studies have identified only two types of $K^+$ channels in bovine AZF cells, a rapidly inactivating, voltage-gated Kv1.4-type current and the non-inactivating $I_{AC}$ current (1, 4, 26, 27). Although present at low density upon initiating whole cell recordings, $I_{AC}$ often grows dramatically over a period of minutes provided that ATP is present at millimolar concentrations in the pipette solution (1, 4, 5).

The absence of time- and voltage-dependent inactivation of the $I_{AC}$ current allows it to be isolated and measured in whole cell recordings, using either of two voltage-clamp protocols. When voltage steps of 300 ms duration are applied from a holding potential of $-80$ mV, $I_{AC}$ can be measured near the end of a voltage step when the transient current has inactivated (Fig. 6, left traces). Alternatively, $I_{AC}$ can be activated selectively after a 10-s prepulse to $-20$ mV has fully inactivated the Kv1.4-type current (Fig. 6, right traces).

Of the 2P/4TMS $K^+$ channels described thus far, TREK-1 and TREK-2 channels (KCNK2 and KCNK10) are unique in the pronounced increase in activity observed in response to lowering intracellular pH (25, 28). To determine whether the activity of the ATP-dependent $I_{AC}$ current was enhanced by cytoplasmic acidification, $K^+$ currents were recorded with pipette solution that was supplemented with 5 mM MgATP at pH levels between 6.2 and 7.5.

As shown in Fig. 6, the time-dependent development of the non-inactivating $K^+$ current was enhanced markedly and selectively at successively lower pH levels. Overall, $I_{AC}$ current density varied from a minimum of $14.6 \pm 1.7$ pA/pF ($n = 15$) at pH 7.4 to $118.3 \pm 9.2$ pA/pF ($n = 8$) at pH 6.2 (Fig. 6, A and B). Cooperative Activation of $I_{AC}$ by pH and ATP—The magnitude of $I_{AC}$ $K^+$ current recorded in the presence of 5 mM ATP was enhanced markedly at low pH levels. Conversely, the enhancement of $I_{AC}$ by acidification was found to depend on the concentration of ATP in the pipette solutions.

The cooperative actions of ATP and pH on $I_{AC}$ expression are

FIG. 6. pH-dependent activation of $I_{AC}$ $K^+$ current. 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 using patch pipettes containing standard solutions at pH levels between 6.2 and 7.4. A, time-dependent expression of $I_{AC}$ current at intracellular pH levels of 6.2 and 7.4. Current traces recorded with (right) and without (left) depolarizing prepulses immediately after initiating whole cell recording (I) and after $I_{AC}$ had reached a stable maximum amplitude (II). $I_{AC}$ amplitude with (○) and without (●) depolarizing prepulses are plotted against time at right. B, maximum average $I_{AC}$ current densities (expressed as pA/pF) were determined from experiments as in A at seven different pH levels and plotted against pH. Values are mean ± S.E. of indicated number of determinations.
illustrated in Fig. 7. When \( \text{I}_{\text{AC}} \) \( K^+ \) currents were recorded at pH 6.5 (Fig. 7A) or pH 7.0 (Fig. 7B), the time-dependent increase in \( \text{I}_{\text{AC}} \) was ~4-fold greater in the presence of 5.0 mM ATP compared with 0.5 mM ATP. Overall, with 0.5 ATP in the pipette lowering pH from 7.5 to 7.0 and 6.5 increased maximum \( \text{I}_{\text{AC}} \) current density from a value of 11.7 ± 3.4 pA/pF (n = 4) to 13.1 ± 3.0 pA/pF (n = 4) and 40.4 pA/pF (n = 5), respectively. By comparison, with pipettes containing 5.0 mM ATP, reducing pH from 7.5 to 7.0 and 6.5 increased maximum \( \text{I}_{\text{AC}} \) current density from 15.0 ± 3.1 (n = 7) to 63.5 ± 8.4 pA/pF (n = 5) and 114 ± 9.8 pA/pF (n = 6), respectively (Fig. 7C).

**Effects of Penfluridol, ACTH, and Riluzole on pH-activated \( K^+ \) Current**—The cooperative enhancement of the background \( K^+ \) current of AZF cells by acidity and ATP suggest that both agents act on the same channels. They suggest further that \( \text{I}_{\text{AC}} \) is a TREK-1 type \( K^+ \) channel that acquires ATP sensitivity when expressed in its native cell type.

If the pH-activated \( K^+ \) current and \( \text{I}_{\text{AC}} \) are one and the same, selective \( \text{I}_{\text{AC}} \) antagonists such as penfluridol should inhibit the pH-activated current with similar potency. Accordingly, penfluridol (2.5 \( \mu \)M) inhibited the non-inactivating \( K^+ \) current recorded at pH 6.4 by 85 ± 5% (n = 5) (Fig. 8A).

ACTH inhibits \( \text{I}_{\text{AC}} \) current in AZF cells at subnanomolar concentrations (1). This peptide hormone also inhibited the acid-stimulated \( K^+ \) current potently in these cells. In four experiments, ACTH (200 pm) completely inhibited the non-inactivating \( K^+ \) current activated at pH 6.4 with pipette solutions containing ATP at either 0.5 or 5.0 mM (Fig. 8B).

In addition to low pH, cloned human and rat TREK-1 \( K^+ \) channels are activated by the neuroprotective agent riluzole (2-amino-6-(trifluoromethoxy) benzothiazole) (18). However, the riluzole-induced increase in \( K^+ \) current through cloned TREK-1 channels was reported to be transient and followed by inhibition, in response to a secondary rise in intracellular cAMP (18).

If the pH-activated \( K^+ \) current of AZF cells is because of TREK-1 channels, then riluzole should also modulate this current. In the experiment illustrated in Fig. 8C, whole cell currents were recorded at pH 6.4 with 0.5 mM ATP in the pipette. After the pH-dependent current had reached maximum amplitude, AZF cells were superfused with saline containing 100 \( \mu \)M riluzole, whereas continuing to monitor currents at 30-s intervals.

Riluzole produced a pronounced increase in the non-inactivating \( K^+ \) current, which reached a maximum within several minutes and then declined toward the control value. In a total...
of eight cells, riluzole increased the non-inactivating current to 331 ± 59% of its control value. In six of these cells, the non-inactivating $K^+$ current declined to 71.9 ± 6.8% of its maximum value after 10 min in the continued presence of riluzole. In the remaining two cells, the riluzole-induced increase was sustained, with no transient component. The riluzole-induced increase in non-inactivating $K^+$ current is consistent with the notion that the drug acts on native TREK-1-type $K^+$ channels. The variation in the kinetics of the response likely reflects differences in the extent of dilution of intracellular components (see below). The inhibition of the acid-stimulated current by penfluridol and ACTH, and its enhancement by riluzole are consistent with the hypothesis that $I_{AC}$ is a pH and ATP-dependent $b$TREK-1 current expressed in AZF cells.

Rectifying Properties of Native and Transfected $b$TREK-1 Channels—If the ATP- and pH-dependent background $K^+$ currents of AZF cells are because of $K^+$ flux through $b$TREK-1 channels, then each of these currents should display similar voltage-dependent rectification. The rectifying properties of non-inactivating $K^+$ currents in AZF cells, activated by acidification of pipette solution to pH 6.4 or by 5 mM ATP were compared with those of $b$TREK-1 currents in transfected HEK-293 cells using ramp voltage protocols. Fig. 9A shows non-inactivating $K^+$ currents recorded in response to 2-s linear ramp voltages, applied between +60 and −140 mV. Each of the three currents displayed strong outward rectification, crossing the voltage axis near the Nerst equilibrium potential for $K^+$. Scaling of the three currents using factors derived from the maximum current amplitudes measured at +60 mV resulted in three nearly superimposable traces (Fig. 9B). This result suggests that these currents result from $K^+$ flow through identical channels.

Enhancement of ATP-dependent $I_{AC}$ by Riluzole—If the ATP-dependent $I_{AC}$ $K^+$ channels are of the TREK-1 type, then riluzole should increase the amplitude of $I_{AC}$ in AZF cells. In the experiments illustrated in Fig. 10A, whole cell recordings were made with pipettes containing 5 mM ATP at pH 7.1. When $I_{AC}$ reached a stable maximum amplitude, cells were superfused with riluzole at concentrations between 1 and 100 $\mu$M. Riluzole produced a concentration-dependent, reversible increase in non-inactivating component of $K^+$ current that was indistinguishable from $I_{AC}$ (Fig. 10). At maximally effective concentrations of 75 and 100 $\mu$M, riluzole increased this current to 418 ± 19% ($n = 6$) and 410 ± 39% ($n = 18$) of its control value (Fig. 10B).

Although riluzole (50–100 $\mu$M) induced rapid 2- to 8-fold increases in the amplitude of the non-inactivating $K^+$ current in each of 33 cells tested, the temporal pattern of the responses varied widely (Fig. 10A). In the majority of cells (21/33) the riluzole-induced increase was characterized by distinct transient and sustained components (Fig. 10A, middle panel). In four cells, the transient increase in current was followed by near complete inhibition of the non-inactivating current (Fig. 10A, left panel). In these cells, a second application of riluzole, after washing with control saline, failed to produce any increase.

When riluzole was applied to the remaining 12 cells, it induced a sustained increase in current with no detectable decrement during at least 10 min of exposure (Fig. 10A, right panel). In these cells, a second application of riluzole was again effective at enhancing the non-inactivating $K^+$ current.

The varying temporal pattern of the riluzole-induced responses was correlated with the measured series resistance of the recording pipette. In the cells where riluzole-induced increase in current was sustained, series resistance averaged 3.6 ± 0.3 meghohms ($n = 12$). By comparison, in experiments where the riluzole response displayed a distinct transient component, averaged series resistance was more than twice as great (8.6 ± 0.7 meghohms) ($n = 21$). These results suggest that the inhibitory component of the riluzole response is dependent on the continued presence of cytoplasmic contents that are lost more rapidly when currents are recorded with low resistance pipettes.

The varying transient and sustained components of the riluzole-induced increases in non-inactivating current could suggest that this drug enhances the activity of two different $K^+$ channels in AZF cells. Several lines of evidence indicate that this is not the case and that riluzole enhances current flow only through pre-activated $I_{AC}$ channels. Ramp voltage protocols applied before and after exposing
AZF cells to riluzole showed that the transient and sustained components of non-inactivating K⁺ current induced by this drug were indistinguishable from IAC. In Fig. 10C (left), ramp voltage protocols were applied after the K⁺ current reached a maximum value in control saline (control), immediately after the current reached a maximum value in riluzole (Riluzole max) and after a steady state value was reached (Riluzole SS).

Scaling of the three currents using factors derived from the maximum current amplitudes measured at +60 mV and plotting these on a separate set of axis yielded three current traces as indicated. B, current traces in A were scaled by multiplying each of the currents by an appropriate scaling factor determined from the maximum value of each current measured at +60 mV.

In whole cell recordings made with pipette solutions containing 5 mM ATP, IAC currents reached much larger maximum values, whereas riluzole produced far greater absolute increases in a non-inactivating component of K⁺ current that was not distinguishable from IAC (Fig. 11, A (left traces) and B).

By comparison, when recordings were made with pipettes containing 5 mM ATP, IAC currents reached much larger maximum values, whereas riluzole produced far greater absolute increases in a non-inactivating component of K⁺ current that was not distinguishable from IAC (Fig. 11, A (left traces) and B).

The non-hydrolyzable ATP analog AMP-PNP is more potent than ATP as an activator of IAC K⁺ channels (5) (4). Accordingly, with 1 mM AMP-PNP in the recording pipette, IAC reached current densities severalfold greater than those observed with 1 mM ATP. Further, in these experiments, riluzole again produced much larger increases in non-inactivating K⁺ current in the presence of 1 mM AMP-PNP, compared with 1 mM ATP (Fig. 11, A (right panel) and B).

In addition to nucleotide triphosphates, PPP activates IAC effectively in whole cell recordings (5). With pipettes containing 1 mM ATP and 5 mM PPPi, IAC current density was severalfold larger than that observed with ATP alone. Accordingly, riluzole produced 6-fold larger absolute increases in non-inactivating K⁺ current in the presence of PPPi and ATP, compared with ATP alone (Fig. 11B). Overall, these results are consistent with the hypothesis that riluzole enhances only the activity of IAC K⁺ channels that have been pre-activated by ATP and PPPi.

Inhibition of Riluzole-activated K⁺ Current by ACTH and cAMP—Results demonstrating cooperative effects of ATP, PPP, and riluzole on expression of background K⁺ current in AZF cells suggest that these agents all enhance only the activity of IAC channels and that these are bTREK-1 channels. However, the possibility that multiple K⁺ channels are involved has not been excluded. For example, TRAAK-type K⁺ channels exhibit some properties of IAC, including activation by riluzole (18, 29). In this regard, unlike native IAC and cloned TREK-1 channels, TRAAK channels are not inhibited by cAMP (29). If riluzole activates TRAAK in AZF cells, this current would be resistant to inhibition by ACTH and cAMP.

ACTH was found to inhibit all non-inactivating K⁺ currents completely in AZF cells, including that induced by riluzole. In the experiment illustrated in Fig. 12A, IAC reached a stable maximum value before the cell was superfused with 100 μM riluzole, which produced a 3-fold enhancement of the current. Superfusion of the cell with ACTH (200 pM) in the continued presence of riluzole produced complete inhibition of the non-inactivating current. Nearly identical results were obtained in each of four experiments.

In other experiments, IAC was allowed to grow to a stable maximum amplitude before sequentially perfusing 200 pM ACTH and 100 μM riluzole, respectively. After complete inhibition of IAC by ACTH, riluzole was totally ineffective in activating any current (Fig. 12B). Similar findings were obtained in three other experiments.

cAMP was equally effective as ACTH in suppressing riluzole-induced K⁺ currents. In the experiment illustrated in Fig. 12C, IAC was permitted to reach a maximum amplitude before sequentially superfusing a membrane-permeable cAMP analog (8-pcpt-cAMP; 500 μM), followed by riluzole (100 μM). After complete inhibition of IAC current by 8-pcpt-cAMP, riluzole failed to produce any increase in K⁺ current.

When 8-pcpt-cAMP was applied intracellularly through the recording pipette, IAC was suppressed, and riluzole was again ineffective (Fig. 12D). In a total of six similar experiments, riluzole increased IAC from a control value of 53.6 ± 7.6 pA (n = 6) to a final value of only 68.8 ± 6.1 pA. These experiments demonstrate that the riluzole activated K⁺ channel is identical.
to $I_{AC}$ with respect to sensitivity to inhibition by cAMP and ACTH.

Effect of Penfluridol on Riluzole-activated Current—Penfluridol is a potent antagonist of both native $I_{AC}$ and cloned bTREK-1 currents. If the riluzole-activated current in AZF cells is composed only of $I_{AC}$, then it should be inhibited by this drug with similar potency. In the experiment shown in Fig. 13A, $I_{AC}$ was allowed to approach a maximum value (trace 2) before superfusing with saline containing riluzole (100 $\mu$$M$), which produced transient (trace 3) and sustained (trace 4) increases in the non-inactivating current. Subsequent superfusion of saline containing riluzole and penfluridol (2.5 $\mu$$M$) resulted in near complete inhibition of the remaining non-inactivating K$^{+}$ current (trace 5). Overall, in four similar experiments, penfluridol inhibited the sustained K$^{+}$ current by $92.6 \pm 5.8\%$.

Experiments such as those illustrated in Fig. 13A did not eliminate the possibility that the transiently activated compo-

FIG. 10. Time- and concentration-dependent enhancement of $I_{AC}$ K$^{+}$ current by riluzole. Whole cell K$^{+}$ currents were recorded from bovine AZF cells at 30-s intervals in response to voltage steps to $+20$ mV with or without inactivating 10-s prepulse to $-20$ mV. After $I_{AC}$ reached a maximum, cells were superfused with 100 $\mu$$M$ riluzole. $I_{AC}$ amplitudes recorded with (C) and without (I) depolarizing prepulses are plotted against time for three separate cells. B, concentration dependence. $I_{AC}$ current amplitudes obtained from experiments as in A at riluzole concentrations from 1 to 100 $\mu$$M$ are expressed as percent of maximum value recorded in control saline. Values are mean $\pm$ S.E. of the indicated number of determinations. C, rectifying properties of riluzole-activated current. Left traces, linear voltage ramps of 100 mV/s were applied from a holding potential of 0 mV to potentials between $-60$ and $+140$ mV after $I_{AC}$ reached a maximum in control saline (control), after a maximum increase was reached in response to riluzole (Riluzole (max)), and after a steady increase in riluzole was achieved (Riluzole (SS)). Right traces, traces at left were scaled against the riluzole (max) trace, using the maximum value of each trace measured at $+60$ mV to determine the appropriate scaling factors.

FIG. 11. ATP- and PPP-dependent activation of $I_{AC}$ by riluzole. Whole cell K$^{+}$ currents were recorded from AZF cells at 30-s intervals in response to voltage steps to $+20$ mV with or without depolarizing prepulses, using patch pipettes containing MgATP (0.1, 0.5, or 5.0 $mM$), AMP-PNP (1 $mM$), or 5 $mM$ PPP, plus 1 $mM$ MgATP. When $I_{AC}$ reached a maximum amplitude, the cell was superfused with 100 $\mu$$M$ riluzole. A, K$^{+}$ current records made with pipettes containing MgATP or AMP-PNP at the indicated concentrations. Numbers on traces recorded with (bottom) and without (top) depolarizing prepulses correspond to currents recorded immediately after initiating whole cell recording (1), after $I_{AC}$ reached a maximum value in control saline (2), and after the maximum current in the presence of riluzole (3). B, the effect of riluzole in combination with ATP, AMP-PNP, and PPP on $I_{AC}$ current density was determined from experiments as in A above. $I_{AC}$ current density, expressed as pA/pF, was determined by dividing the maximum $I_{AC}$ current amplitude for each condition by the cell capacitance. Values are mean $\pm$ S.E. of the indicated number of determinations.
ponent of non-inactivating current induced by riluzole was a separate penfluridol-insensitive K$^{+}$/H11545 current. This possibility was excluded by experiments like that illustrated in Fig. 13 in which IAC was permitted to reach a maximum amplitude (trace 2) before subsequently superfusing the cell with saline containing 100 μM riluzole followed by 100 μM riluzole plus 200 μM ACTH. B, after IAC reached a maximum amplitude in control saline, cell was superfused sequentially with saline containing 100 μM riluzole followed by 100 μM riluzole plus 2.5 μM penfluridol. Penfluridol inhibited the IAC current completely (trace 3) and nearly eliminated any riluzole-induced increase (trace 4). In this experiment, the previously reported spontaneous time-dependent decrease in Kv1.4 current that accompanies the growth of IAC is evident after treatment with penfluridol (trace 3) (30). At this concentration, penfluridol blocks IAC selectively. Similar results were obtained in each of three experiments.

**DISCUSSION**

In this study, a b-TREK-1 K$^{+}$ channel cDNA was cloned and shown to be expressed robustly in bovine AZF cells. When bTREK-1 cDNA was transiently expressed in HEK-293 or CHO-K1 cells, the corresponding membrane current displayed prominent characteristics of the native IAC background K$^{+}$/H11545 current of AZF cells. Conversely, IAC displayed distinctive properties of cloned bTREK-1 channels (Table I). Overall, these results provide convincing evidence that IAC is a bTREK-1 channel expressed in AZF cells.
bTREK-1 codes for a 411-amino acid protein that is 96 and 99% homologous in amino acid sequence to mouse and human TREK-1 channels of the same length (24, 31). However, Northern blot analysis of TREK-1 transcripts from several mouse tissues and human brain identified a single 3.8-kb transcript (24, 31). In contrast, Northern blot analysis of AZF cell mRNA identified three bTREK-1 transcripts of 4.9, 3.6, and 2.8 kb. This result indicates that several splice variants of bTREK-1 channels may be expressed in bovine AZF cells. Multiple variants of a number of K+ channel subtypes are expressed in various tissues (32). Kv1.4 transcripts of 3.4 and 4.4 kb are expressed by bovine AZF cells (27).

**Comparison of bTREK-1 Currents to IAC**—The time-dependent increase in bTREK-1 current observed in transiently transfected HEK-293 and COS-1 cells had not been observed previously in whole cell recordings of cloned TREK-1 channels but is typical of IAC in AZF cells (1, 3–5). The molecular mechanism underlying this phenomenon is unknown, but dilution of an endogenous cytoplasmic inhibiting factor common to all three cell types appears likely. Whole cell bTREK-1 current waveforms resemble IAC currents with respect to kinetics of activation. Both consist of an instantaneous component and a smaller time-dependent fraction that activates with first order kinetics (3). This two-component current has also been described for cloned mouse TREK-1 expressed in a mammalian cell line (31).

The time-dependent component of bTREK-1 and IAC currents observed in response to membrane depolarization are indicative of voltage-dependent gating. In a previous study, we demonstrated a weak voltage-dependent activation of IAC in which open probability increased by 0.3 between test voltages of −40 and +40 mV (4). Recently, others have shown a voltage dependence activation of native and cloned mouse and rat TREK-1 channels that may or may not depend on the phosphorylation state of the channel (20, 33). Regardless, bTREK-1, IAC, and TREK-1 currents from several species display similar kinetics and voltage-dependent gating.

In physiological solutions, cloned bTREK-1 current displayed identical rectification with both the ATP- and pH-activated background K+ current recorded from AZF cells. This rectification is typical of that observed for mouse and human TREK-1 channels (24, 31).

bTREK-1 channels displayed a pharmacological profile similar to that described previously for IAC in AZF cells (10, 11). In particular, the potent inhibition of bTREK-1 and IAC currents by penfluridol and mibebradil establishes an interesting pharmacological similarity between the two channels. It also identifies these two Ca2+ channel blockers as the most potent antagonists of TREK-1 channels yet described. In this respect, penfluridol and mibebradil are at least 1000- to 10,000-fold more potent than K+ channel antagonists such as TEA and 4-AP (16, 23, 31). It will be interesting to determine whether these Ca2+ channel antagonists will also inhibit other tandem-pore K+ channels.

In whole cell recordings from transfected HEK-293 cells, bTREK-1 currents were expressed independently of the concentration of ATP in the pipette solution. This is the single significant property distinguishing these cloned channels from native IAC channels. Direct activation by nucleotides or phosphates has not been reported for any cloned TREK-1 channel. It is possible that cloned TREK-1 channels lose their ATP dependence and sensitivity, because an ATP-binding protein similar to the KATP channel associated sulfonylurea receptor is missing in the host cell (34). If ATP binding to a TREK-associated inhibitory subunit frees this channel for activation, then absence of this protein in the host cell could explain the ATP-independent activity of bTREK-1 channels expressed in HEK-293. In this regard, we have been unable to demonstrate ATP-dependent activation of IAC channels in excised inside-out patches from AZF cells. In fact, on patch excision, channel activity typically increases spontaneously with time, regardless of the ATP concentration at the cytoplasmic membrane surface (5). This result indicates that ATP-mediated modulation of IAC activity requires AZF cell components that are lost after patch excision.

Relatedly, cloned TREK-1 channels are temperature-sensitive and activated dramatically by heat. The thermal activation of TREK-1 is lost on patch excision (35). It appears that the thermal sensitivity of TREK-1 channels also depends on factors present in an intact cell.

**Cooperative Activation of IAC** by H+ and ATP—ATP and pH acted cooperatively in promoting the activity of IAC channels. Expression of the native IAC current in the presence of 5 mM ATP was enhanced strongly at acidic pHs and nearly eliminated by raising the intracellular pH to 7.5 or above. These results suggest that IAC is a 2P/4TMS channel of the TREK variety. Of the tandem pore K+ channels cloned thus far, only murine and human TREK-1 and TREK-2 channels are activated by acidification of the cytoplasm (17, 25, 28). In this regard, it is unlikely that IAC is TREK-2, because TREK-2 is strongly inwardly rectifying in symmetrical K+ solutions and insensitive to 100 μM quinidine (28). By comparison, in symmetrical K+ solutions, IAC channels are nearly ohmic whereas they are blocked by quinidine with an IC50 of 26 μM (5, 10). Further, Northern blot analysis of AZF cell mRNA using a rat TREK-2 cDNA as a probe indicated that TREK-2 mRNA is not expressed in these cells.

The cooperative activation of IAC by pH and ATP was also evident in the ATP-dependent enhancement of IAC current by acidification. With pipettes containing ATP at a concentration of 0.5 mM, successive reductions in pH were far less effective at enhancing IAC than in the presence of 5 mM ATP.

There seems to be little doubt that the non-inactivating K+ current activated by ATP and/or acidification is due to a single type of K+ channel. In addition to the cooperative actions of ATP and pH described above, currents activated by either agent were inhibited by ACTH and penfluridol equivalently. Further, riluzole enhanced both pH- and ATP-activated K+ current in AZF cells. Finally, pH- and ATP-activated currents displayed identical rectification at potentials between +60 and −140 mV.

*2 J. J. Enyeart, L. Xu, S. Danthi, and J. A. Enyeart, unpublished observations.

**TABLE I**

| Characteristic                          | IAC | Cloned bTREK-1 |
|----------------------------------------|-----|---------------|
| Time-dependent increase in amplitude   | +   | +             |
| Instantaneous and time-dependent       | +   | +             |
| components                             |     |               |
| Outward rectification in physiological | +   |               |
| saline                                 |     |               |
| Activation by cytoplasmic acidification| +   | +             |
| Transient activation by riluzole       | +   | +             |
| Inhibition by CAMP                     | +   | +             |
| Block by Ca2+ antagonists penfluridol, | +   | +             |
| mibebradil, and La3+                   |     |               |
| Less sensitive to inhibition by TEA    | +   | +             |
| and 4-AP                               |     |               |
| ATP-dependent activation               | +   | −             |
Enhancement of I_{AC} by Riluzole—Several lines of evidence indicate that the riluzole-activated K⁺ current in AZF cells was I_{AC} and not a second non-inactivating K⁺ current. Specifically, the riluzole-activated current was indistinguishable from I_{AC} with regard to current waveform, voltage-dependent rectification, and inhibition by penfluridol, ACTH, and cAMP.

Further, the activation of non-inactivating K⁺ current by riluzole varied directly with the concentration of ATP in the pipette solution. At low ATP concentrations (<1 mM), I_{AC} was poorly expressed, and riluzole induced only very small absolute increases in I_{AC} current. These results are consistent with the hypothesis that riluzole enhances current through I_{AC} channels that have been pre-activated by ATP. The facilitatory effects of AMP-PNP and PPP, on riluzole-mediated increases in K⁺ current are also consistent with this model.

The identification of I_{AC} as the riluzole-activated K⁺ current in AZF cells provides further evidence that this current flows through bTREK-1 channels. Of the 2P4/TMS K⁺ channels riluzole has been reported to increase only the activity of TRAAK and TREK-1 (18). Of these two, only TREK-1 type channels are inhibited by cAMP (18, 31). Because cAMP and ACTH inhibited riluzole-induced increases completely in the non-inactivating current, it is unlikely that this current was TRAAK and consistent with the hypothesis that it is TREK-1. In addition, TRAAK has been shown to be a neuron-specific channel (15, 29).

The riluzole-induced increase in I_{AC} current was similar to that reported for activation of cloned rat TREK-1 channels with respect to concentration dependence but differed with respect to kinetics. The inhibition that followed transient activation of cloned TREK-1 channels has been attributed to a riluzole-induced increase in cAMP, subsequent to inhibition of phosphodiesterase (18). The temporal pattern of this response, and the inhibition in particular, would depend on the presence of required cytoplasmic components. Within this framework, the absence of the inhibitory component of the riluzole response that we observed with low resistance electrodes would occur through more rapid dilution of cytoplasmic contents by the pipette solution. We believe that the varying temporal pattern of the riluzole response in AZF cells is consistent with the activation of only I_{AC} (bTREK-1)-type channels.

Identity of I_{AC} and bTREK-1 Channels—In summary, we have cloned a bTREK-1 K⁺ channel cDNA that is expressed robustly in bovine AZF cells. When expressed in cell lines, bTREK-1 current displays many of the properties of the endogenous I_{AC} current of AZF cells. These include time-dependent growth in whole cell recordings, instantaneous and time-dependent components in response to depolarization, potent inhibition by penfluridol and mibebradil, similar rectification, and unitary current amplitude.

Native I_{AC} in turn displays essential properties of bTREK-1, as well as those of human, rat, and mouse TREK-1 channels expressed in cell lines (20, 24, 29). In addition to similarities mentioned previously in single channel conductance, rectification, and inhibition by cAMP, I_{AC} channels have now been shown to be activated by riluzole and intracellular acidification. In other experiments, we have observed that additional agents that activate TREK-1 channels, including unsaturated fatty acids, lysophospholipids, and the volatile anesthetic carbon monoxide also activate I_{AC} current in AZF cells. Overall, convincing evidence indicates that the cloned bTREK-1 channel is the primary subunit of AZF cell I_{AC} channels.

Significance—Earlier studies have shown that TREK-1 channels are expressed primarily in the CNS and in primary sensory neurons (31, 35). TREK-1 channels may also be present in heart, kidney, lungs, ovary, and stomach (16, 24, 31, 36). This is the first study demonstrating that TREK-1 channels are expressed by a specific endocrine cell.

Further, when combined with previous studies, the present work assigns a pivotal role to bTREK-1 in the physiology of corticosteroid secretion. Apparently, these channels set the resting potential of bovine AZF cells and couple ACTH and angiotensin II receptor activation to depolarization-dependent Ca²⁺ entry through T-type channels (1–3, 6). Because I_{AC} channels are also expressed in bovine glomerulosa cells where they are inhibited by ACTH and angiotensin II, it is also likely that bTREK-1 channels have a similar function in regulating aldosterone secretion (6). It will be interesting to determine whether TREK-1 is expressed in other steroid hormone-secreting cells.

Previous studies showed that TREK-1 K⁺ channels set the resting membrane potential and conferred sensitivity of the cell to diverse stimuli, including mechanical force, temperature, pH, fatty acids, phospholipids, and transmitters (25, 31, 35–37). The activation of I_{AC} by ATP and inorganic phosphate expands the role of TREK-1 channels to that of a metabolic sensor that may couple the metabolic state of the cell to membrane potential and electrical activity. In AZF cells, bTREK-1 channels may integrate both hormonal and metabolic signals linking these to depolarization-dependent Ca²⁺ entry and cortisol secretion.

REFERENCES

1. Mlinar, B., Biagi, B. A., and Enyeart, J. J. (1993) J. Biol. Chem. 268, 8640–8644
2. Enyeart, J. J., Mlinar, B., and Enyeart, J. A. (1993) Mol. Endocrinol. 7, 1031–1040
3. Enyeart, J. J., Mlinar, B., and Enyeart, J. A. (1996) J. Gen. Physiol. 108, 251–264
4. Enyeart, J. J., Gomora, J. C., Xu, L., and Enyeart, J. A. (1997) J. Gen. Physiol. 110, 679–692
5. Xu, L., and Enyeart, J. J. (2001) Am. J. Physiol. 280, C199–C215
6. Mlinar, B., Biagi, B. A., and Enyeart, J. J. (1995) J. Biol. Chem. 270, No. 36, 20942–20951
7. Xu, L., and Enyeart, J. J. (1999) J. Physiol. (Camb.) 521, 61–97
8. Xu, L., and Enyeart, J. J. (1999) Mol. Pharmacol. 55, 364–376
9. Gomora, J. C., and Enyeart, J. J. (1998) Am. J. Physiol. 275, C1536–C1537
10. Gomora, J. C., and Enyeart, J. J. (1999) J. Physiol. Exp. Thor. 290, 266–273
11. Gomora, J. C., and Enyeart, J. J. (1999) Mol. Pharmacol. 56, 1192–1197
12. Hille, B. (2001) Ion Channels of Excitable Membranes, Sinauer Associates, Inc., Sunderland, MA
13. Takano, M., and Noma, A. (1993) Progr. Neurobiol. 41, 21–30
14. Terzic, A., Tung, R. T., and Kurachi, Y. (1994) Cardiovasc. Res. 28, 746–753
15. Lesage, F., and Lazdunski, M. (2000) Am. J. Physiol. Renal. Physiol. 279, F693–F699
16. Goldstein, S. A., Bockenhauer, D., O’Kelly, I., and Zilberberg, N. (2001) Nat. Rev. Neurosci. 2, 175–184
17. Patel, A. J., and Honoré, E. (2001) Trends Neurosci. 24, 339–346
18. Duprat, F., Lesage, F., Patel, A. J., Fink, M., Romney, G., and Lazdunski, M. (2000) Mol. Pharmacol. 57, 906–912
19. Patel, A. J., Honoré, E., Lesage, F., Fink, M., Romney, G., and Lazdunski, M. (1999) Nat. Neurosci. 2, 422–426
20. Bockenhauer, D., Zilberberg, N., and Goldstein, S. A. (2001) Nat. Neurosci. 4, 486–491
21. Jurgens, M. E., Boland, L. M., Liu, Y., and Yellen, G. (1994) Biotechnol. 17, 876–881
22. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) Pfluegers Arch. 391, 85–100
23. Patel, A. J., Honoré, E., Maingret, F., Lesage, F., Fink, M., Duprat, F., and Lazdunski, M. (1998) EMBO J. 17, 4283–4290
24. Meadows, H. J., Benham, C. D., Cairns, W., Gloger, I., Jennings, C., Medhurst, A. D., Murdoch, P., and Chapman, C. G. (2000) Pfluegers Arch. 439, 714–722
25. Maingret, F., Patel, A. J., Lesage, F., Lazdunski, M., and Honoré, E. (1999) J. Biol. Chem. 274, 26691–26696
26. Bockenhauer, D., and Enyeart, J. J. (1990) J. Gen. Physiol. 102, 239–255
27. Enyeart, J. J., Xu, L., and Enyeart, J. J. (2000) J. Biol. Chem. 275, 34640–34649
28. Bang, H., Kim, Y., and Kim, D. (2000) J. Biol. Chem. 275, 17412–17419
29. Fink, M., Lesage, F., Duprat, F., Heurteaux, C., Reyes, R., Fosset, M., and Lazdunski, M. (1998) EMBO J. 17, 3297–3308
30. Enyeart, J. J., Xu, L., Gomora, J. C., and Enyeart, J. A. (2001) Mol. Pharmacol. 60, 114–125
31. Fink, M., Duprat, F., Lesage, F., Reyes, R., Heurteaux, C., and Lazdunski, M. (1996) EMBO J. 15, 6854–6862

*S. Danthi and J. J. Enyeart, manuscript in preparation.
32. Coetzee, W. A., Amarillo, Y., Chin, J., Chow, A., Lau, D., McCormack, T., Moreno, H., Nadal, M. S., Ozaita, A., Poingtney, D., Saganich, M., Vega-Saenz, D. M., and Rudy, B. (1999) *Ann. N. Y. Acad. Sci.* 868, 233–285

33. Maingret, F., Honore, E., Lazdunski, M., and Patel, A. J. (2002) *Biochem. Biophys. Res. Commun.* 292, 339–346

34. Aguilar-Bryan, L., Nichols, C. G., Wechsler, S. W., Clement, J. P. I., Boyd, A. E., III, Gonzalez, G., Herrera-Susa, H., Nguy, K., Bryan, J., and Nelson, D. A. (1995) *Science* 268, 423–429

35. Maingret, F., Lauritzen, I., Patel, A. J., Heurteaux, C., Reyes, R., Lesage, F., Lazdunski, M., and Honore, E. (2000) *EMBO J.* 19, 2483–2491

36. Terrenoire, C., Lauritzen, I., Lesage, F., Romey, G., and Lazdunski, M. (2001) *Circ. Res.* 89, 336–342

37. Maingret, F., Patel, A. J., Lesage, F., Lazdunski, M., and Honore, E. (2000) *J. Biol. Chem.* 275, 10128–10133