A TREK-1–Like Potassium Channel in Atrial Cells Inhibited by β-Adrenergic Stimulation and Activated by Volatile Anesthetics

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Abstract—Many members of the two-pore-domain potassium (K+) channel family have been detected in the mammalian heart but the endogenous correlates of these channels still have to be identified. We investigated whether I_{KAA}, a background K+ current activated by negative pressure (stretch) and by arachidonic acid (AA) and sensitive to intracellular acidification, could be the native correlate of TREK-1 in adult rat atrial cells. Using the inside-out configuration of the patch-clamp technique, we found that I_{KAA}, like TREK-1, was outwardly rectifying in physiological K+ conditions, with a conductance of 41 pS at +50 mV. Like TREK-1, I_{KAA} was reversibly activated by clinical concentrations of volatile anesthetics (mmol/L, chloroform 0.18, halothane 0.11, and isoflurane 0.69). In cell-attached experiments, I_{KAA} was inhibited by chlorophenylthio-cAMP (500 μmol/L) and also by stimulation of β-adrenergic receptors with isoproterenol (1 μmol/L). In addition, TREK-1 mRNAs were detected in all cardiac tissues, and the TREK-1 protein was immunolocalized in isolated atrial myocytes. Such a background potassium channel might contribute to the positive inotropic effects produced by β-adrenergic stimulation of the heart. It might also be involved in the regulation of the atrial natriuretic peptide secretion. (Circ Res. 2001;89:336-342.)

Key Words: potassium channels • volatile anesthetics • β-adrenergic receptor • heart cells

Cardiac potassium (K+) channels have extensively been studied for years. In the working atrial and ventricular myocytes, they belong to the following two major families: (1) the voltage-dependent channels (I_{ca}, I_{kr}, I_{ks}, etc), which are involved in the different repolarizations phases of the cardiac action potential (AP), and (2) the background K+ channels, which stabilize the resting membrane potential at a hyperpolarized level and regulate AP duration in physiological conditions (I_{ks}). During sympathetic stimulation of the heart (I_{KAdo}), or during pathological events such as ischemia (I_{KACh}), etc (for a review, see Carmeliet1).

During the last decade, a large number of K+ channel α subunits were cloned and further characterized in expression systems such as Xenopus oocytes and mammalian cell lines (COS, HEK, etc). Some of these cloned channels were proposed as molecular substrates for the endogenous cardiac K+ currents on the basis of their physical and pharmacological properties. For instance, Kv4.2 is suggested to be a molecular correlate of I_{ca}, whereas HERG and KvLQT1/5k correspond to I_{kr} and I_{ks}, respectively.2–6 IRK1 (Kir2.1) generates a current similar to that of I_{ks}, whereas GIRK1 (Kir3.1) forms a complex with GIRK4 (Kir3.4) to reproduce I_{Kmax}.7,8 Kir6.2 coassembles with the sulfonylurea receptor SUR2A to reconstitute the cardiac K_{ATP} channel.9,10 However, not all cloned K+ channel α subunits shown to be expressed in the heart have been associated with an endogenous channel. This is particularly the case for the expanding family of mammalian two-pore-domain potassium (K_{2P}) channels.11 Of the 11 functional members that have now been cloned, the following seven were detected in the mammalian heart, at low or high levels, by Northern and reverse transcriptase–polymerase chain reaction (RT-PCR) experiments: TWIK-1 (human), TWIK-2 (human and rat), TREK-1 (mouse), TASK-1 (human, mouse, and rat), TASK-3 (rat), THIK-1 (rat), and TALK-2 (human).12–23

TREK-1 is one of the most interesting members of the K_{2P} channel family. It is activated by changes in membrane tension (stretch), by arachidonic acid (AA) and other polyunsaturated fatty acids, and by intracellular acidification.15,24,25 It looks similar to a background potassium channel that was previously recorded in adult rat atrial myocytes.26 This channel, also sensitive to intracellular acidosis and activated by stretch, produces an outwardly rectifying current, named I_{KAA}, on application of AA. Despite the lack of specific effectors for TREK-1, this K_{2P} channel presents an interesting property; it is activated by volatile anesthetics,27 whereas all cardiac potassium channels studied until now are either inhibited or not sensitive to these agents (I_{ca}, I_{kr}, I_{ks}, etc) and I_{KATP},3,4,13,14 with the exception of I_{KATP}, which is activated by halothane.35 The purpose of this work is to

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establish the presence of the TREK-1 protein in the heart using specific antibodies to demonstrate that TREK-1 is the channel responsible for $I_{KAA}$ and that $I_{KAA}$-like TREK-1, 24,27 is activated by clinical concentrations of volatile anesthetics and is inhibited by increases of cAMP that activate protein kinase A (PKA) and that can be triggered by β-adrenergic stimulation.

**Materials and Methods**

**Cell Isolation**

Male Wistar rats (200 to 300 g) were anesthetized by inotrperitoneal injection of sodium pentobarbital (90 mg/kg). Heart dissociation was performed following a previously described protocol. Oxygenated bicarbonate-buffered solution, mixed with 0.06% collagenase (type II, Worthington) and 0.03% hyaluronidase (type IV-S, Sigma) for 20 to 45 minutes and containing the following, was used (in mmol/L): NaCl 125, KCl 5, MgSO4 2, KH2PO4 2, NaHCO3 25, and glucose 10. Isolated myocytes were stored at 4°C in modified KB medium containing the following (in mmol/L): potassium glutamate 90, KH2PO4 30, MgCl2 1, pyruvic acid 5, taurine 20, glucose 20, and HEPES 10 (pH adjusted to 7.2 with KOH).

**Electrophysiological Experiments**

Whole-cell, inside-out, and cell-attached configurations of the patch-clamp technique were performed on atrial cells at room temperature (20°C to 22°C) with a RK300 patch-clamp amplifier (Bio-Logic). Pipettes (3 to 5 MΩ) were Sylgard-coated when used for single-channel experiments. The standard intracellular solution contained the following (in mmol/L): KCl 150, MgCl2 2, EGTA 5, and HEPES 10 (pH adjusted to 7.2 with KOH). For cell-attached experiments, the same solution without EGTA was used as bathing solution. The basic extracellular solution contained the following (in mmol/L): NaCl 145, KCl 5, MgSO4 2, and HEPES 10 (pH adjusted to 7.2 with NaOH). AA, lysophosphatidylcholine (LPC), chlorophenylthio-cAMP (CPT-cAMP), glibenclamide, and tetraethylammonium (TEA) were all purchased from Sigma-Aldrich Chimie. The solutions containing volatile anesthetics were prepared as previously described.27 Negative pressure was applied to the pipette via a calibrated syringe. Whole-cell recordings were digitized and analyzed by pClamp software. Single-channel recordings, filtered at 3 kHz, were digitized using a DAT recorder (Bio-Logic) and further analyzed with Biopatch software. Maximum open probabilities of a single channel ($P_{open}$ measured over 5 seconds every 20 or 30 seconds, during a burst of openings) and amplitude histograms were determined using filtered signals at 1 kHz. Results, expressed as mean±SEM, were considered as significant when $P<0.05$ with the Student $t$ test.

**Reverse Transcriptase–Polymerase Chain Reaction**

Adult rat hearts were dissected, and total RNAs from four different tissues (atrium, septum, left ventricle, and right ventricle) were extracted by the guanidinium isothiocyanate method. Five micrograms of RNA was reverse transcribed in a final volume of 40 μL. In a first step, these cDNAs were used as template for PCR by using primers deduced from this sequence with mouse TREK-1 over 145 amino acids. In a second step, the DNA corresponding to rat TREK-1 DNA was subcloned and then extracted by the guanidinium isothiocyanate method. Five microsomal preparations were used as template for PCR amplification: sense primer, 5′-GGATGGGCAGTGTTAGCTGGA-3′; and reverse primer, 5′-GCCCTGGACGCCATCTAC-3′. One microliter of each sample was used as template. PCR conditions were 32 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C. The amplified fragments were transferred onto nylon membrane and probed with a 32P-labeled internal primer specific for TREK-1, 5′-CTAGGTGGTTCAAGCACATAG-3′. For control, actin was amplified and analyzed according to the same protocol.

**Membrane Preparations and Western Blot**

Membrane preparations and Western blot for fresh isolated adult rat atrial myocytes. Cells were plated on two-chamber Falcon culture slides and fixed with 4% paraformaldehyde in PBS. Cells were permeabilized in 0.3% Triton X-100 for 15 minutes and then incubated in blocking buffer (5% normal goat serum and 0.05% Triton X-100 in PBS) (3 hours at room temperature). Cells were hereafter incubated with TREK-1 antibodies (1:3000 to 1:5000) at 4°C overnight. Development was performed using either the biotinylated horseradish peroxidase–diaminobenzidine (DAB) method (Vectastain Elite ABC kit, Vector Laboratories) or the Alexa Fluor 488 goat anti-rabbit IgG antibody (Molecular Probes).

**Results**

**Voltage-Clamp Recording of a Potassium Current Activated by Chloroform**

To our knowledge, among all K+ channels that have been detected by molecular biology techniques in the heart, TREK-1 is the only one to be activated by chloroform. We used this property to investigate the possible presence of a TREK-1–like potassium current in adult rat atrial cells. Voltage-clamp experiments were performed using a protocol with two depolarizing steps (Figure 1A). The current recorded at −40 mV was rapidly increased by chloroform (0.18 mmol/L), whereas both peak and steady-state outward currents at +40 mV were inhibited. This initial inhibition at +40 mV is probably due to an effect on voltage-dependent potassium channels, as already reported for halothane in other preparations.28,29,39 When chloroform application was maintained, a nonactivating outward current finally developed at +40 mV. This current, not sensitive to 10 μmol/L glibenclamide, was mainly carried by potassium ions as indicated by the reversal potential (−80 mV in this example) of the current-voltage curve obtained under chloroform (Figure 1B). Chloroform effects were completely reversible and reproduced in five other cells. Isoflurane (0.69 mmol/L) also produced a strong increase of a similar nonactivating potassium current (n=3, not shown).

**Identification of $I_{KAA}$**

In inside-out patches, we first used negative pressure (stretch) and AA stimulation to confirm the presence of $I_{KAA}$26. In physiological K+ conditions, stretch (P≈12 mm Hg) instantaneously activated a channel (Figure 2A) characterized by a
flickering behavior and bursting openings, with a mean open time of 1.63±0.18 ms at +40 mV (n=4). This channel was not voltage-sensitive from −10 to +40 mV. In the particular recording shown in Figure 1, the maximum open probability (P_max) averaged over six different voltages was 0.36±0.01. Channel activity was not affected by 10 μmol/L glibenclamide or by 10 mmol/L TEA, a blocker for K_ATP and voltage-sensitive K⁺ channels, respectively, and it was not calcium-dependent, as we used calcium-free extracellular solution. AA (10 mmol/L) applied to the cytosolic side of the patch was able to induce bursting openings but only when the channel had been preactivated (P_max=0.13±0.02 versus P_max=0.03±0.01 in control conditions, at 0 mV, n=3). Isoflurane (0.69 mmol/L) activated the channel in the same way as chloroform (0.36±0.01 averaged over this voltage range. The current-voltage curve established under chloroform (Figure 3D) was perfectly superimposable on the one obtained under AA and stretch. Channel activity was significantly reduced after chloroform washout (Figures 3A and 3C), but it was then always possible to reactivate the channel with stretch. As illustrated in Figure 4A, halothane (0.11 mmol/L) was also able to induce bursting openings but only when the channel had been preactivated (P_max=0.13±0.02 versus P_max=0.03±0.01 in control conditions, at 0 mV, n=3, P<0.05). Isoflurane (0.69 mmol/L) activated the channel in the same way as chloroform (Figure 4B): P_max=0.26±0.04 versus P_max=0.01±0.01 in control conditions (at 0 mV, n=3, P<0.05). The current-voltage curves of isoflurane- and chloroform-induced currents were perfectly superimposable and also superimposable to the current-voltage curves obtained after activation by AA and stretch. Washout of the isoflurane-induced effects was rapid (Figure 4C), and the channel could then be reactivated by stretch.

Volatile Anesthetics Activate I_KAA
The 41-pS conductance of I_KAA in physiological K⁺ conditions is close to the 48-pS conductance previously measured for TREK-1 expressed in COS cells.²⁴ To further demonstrate that TREK-1 generates I_KAA, we first examined the effect of three volatile anesthetics that are known to activate TREK-1.²⁷ (chloroform, halothane, and isoflurane) on the native cardiac channel in the inside-out configuration. Chloroform (0.18 mmol/L), applied to the cytosolic side of the patch, activated a channel (Figure 3A) with a P_max (calculated from amplitude histograms in Figure 3B) of 0.33±0.04 (at 0 mV, n=3) (Figure 3C). Chloroform-induced openings occurred in bursts (mean open time of 1.6 ms in this example) and were highly flickering as shown in Figure 3D (inset). This chloroform-activated current did not display voltage sensitivity from −10 to +40 mV, as indicated by the P_max of 0.36±0.01 averaged over this voltage range. The current-voltage curve established under chloroform (Figure 3D) was perfectly superimposable on the one obtained under AA and stretch. Channel activity was significantly reduced after chloroform washout (Figures 3A and 3C), but it was then always possible to reactivate the channel with stretch. As illustrated in Figure 4A, halothane (0.11 mmol/L) was also able to induce bursting openings but only when the channel had been preactivated (P_max=0.13±0.02 versus P_max=0.03±0.01 in control conditions, at 0 mV, n=3, P<0.05). Isoflurane (0.69 mmol/L) activated the channel in the same way as chloroform (Figure 4B): P_max=0.26±0.04 versus P_max=0.01±0.01 in control conditions (at 0 mV, n=3, P<0.05). The current-voltage curves of isoflurane- and chloroform-induced currents were perfectly superimposable and also superimposable to the current-voltage curves obtained after activation by AA and stretch. Washout of the isoflurane-induced effects was rapid (Figure 4C), and the channel could then be reactivated by stretch.

Figure 1. Chloroform effects on whole-cell outward current of an atrial myocyte. A, Top, Superimposed original currents recorded after stimulation with a 600-ms voltage step to −40 mV (from a holding potential of −80 mV) followed by a 10-second voltage step to +40 mV, every 15 seconds. Shown is outward current at +40 mV in control conditions (middle trace) and during initial (CHCl₃low, lower trace) and late (CHCl₃high, upper trace) application of chloroform (0.18 mmol/L). Bottom, Time course of steady-state current (I ss) measured at −80 mV (●) and at end of voltage steps to −40 mV (○) and +40 mV (●) during chloroform application (CHCl₃, 0.18 mmol/L, horizontal bar). Labeled arrows indicate recording time at which traces shown in the top panel were obtained. B, Current-voltage curves of I ss (measured at the end of voltage steps) in control conditions (●) and during chloroform application (CHCl₃low, ○), using 1-second voltage steps from −120 to +40 mV (20-mV increments) from a holding potential of −80 mV, every 6 seconds. Inset, Current-voltage curve of chloroform-induced I ss.

Figure 2. I_KAA recording in physiological K⁺ conditions. Inside-out patch of rat atrial myocyte held at +40 mV in physiological K⁺ conditions. I_KAA was activated by applying either a negative pressure of −12 mm Hg (stretch, A) or 10 μmol/L AA to the patch (B). One channel was present in this patch. C, Corresponding current-voltage curves of I_KAA obtained in physiological K⁺ conditions (●, average of four patches) or in symmetrical K⁺ conditions (○, average of three patches).
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stimulation, we tested whether the inhibition of $I_{\text{KAA}}$ by 0.18 mmol/L chloroform (CHCl$_3$) in bath solution. B, Amplitude histograms realized from recording illustrated in panel A. White columns correspond to control recording (a in panel A), and black columns to chloroform application (b in panel A). C, Time course of the single-channel maximum open probability ($P_{\text{Omax}}$). Labeled arrows indicate $P_{\text{Omax}}$ corresponding to the recording traces shown in panel A. Horizontal bar represents chloroform application (CHCl$_3$). D, Current-voltage curve of chloroform-induced current. Inset, Current trace recorded at +40 mV showing flickering openings of the channel. Dotted line indicates 0 current.

$I_{\text{KAA}}$ Is Regulated by the PKA-Dependent Pathway

TREK-1 is potently inhibited by intracellular increases of cAMP via a PKA activation that leads to a phosphorylation of a well-identified serine residue situated in the cytoplasmic C-terminal region. We investigated whether $I_{\text{KAA}}$ could also be regulated by cAMP. Spontaneous channel activity was never recorded in cell-attached experiments. Therefore, the analysis of cAMP effects was first carried out after application of AA. When $I_{\text{KAA}}$ was activated by AA (10 $\mu$mol/L) (Figure 5), additional application of 500 $\mu$mol/L CPT-cAMP, a permeant analog of cAMP, fully inhibited channel activity within 1.5 to 2 minutes ($n=3$). We then examined whether CPT-cAMP could regulate $I_{\text{KAA}}$ when it had been induced by chloroform. Extracellular application of 0.18 mmol/L chloroform activated the same flickering current ($P_{\text{Omax}}=0.14\pm0.01$ at 0 mV, $n=4$) as AA, an effect that was again inhibited (Figure 6A) after a 1.5- to 2-minute application of CPT-cAMP (Figure 6B). We also recorded the CPT-cAMP inhibition of $I_{\text{KAA}}$ after activation of this current by stretch ($P=12$ mm Hg, $n=2$, not shown).

Because in cardiac cells cAMP is produced by adenylate cyclase from cytosolic ATP, as a result of $\beta$-adrenergic stimulation, we tested whether the inhibition of $I_{\text{KAA}}$ could be produced by stimulation with isoproterenol, a specific agonist of $\beta$-adrenergic receptors. In cell-attached experiments, extracellular application of isoproterenol (1 $\mu$mol/L) strongly inhibited the AA-induced current within 14.2±0.7 seconds ($n=4$) (Figures 7A and 7B).

TREK-1 Is Expressed in Cardiac Preparations

RT-PCR techniques indicate that the mRNA for TREK-1 was present in atria and ventricles as well as in the septum of adult rat heart (Figure 8A). To determine whether TREK-1 proteins could also be detected, rat ventricular and atrial microsomes were separated on SDS polyacrylamide gels and immunoblotted with affinity-purified polyclonal anti–TREK-1 antibodies. Figure 8B shows that the anti–TREK-1 antibody recognizes a single band at ~45 kDa, which is the same size as that obtained in membranes from TREK-1–transfected COS cells that express this channel at a high level in their plasma membrane. The presence of TREK-1 in the plasma membrane was confirmed in a second set of experiments in which isolated atrial myocytes, the same preparation used in parallel for electrophysiology, were fixed and immunostained with anti–TREK-1 antibodies. Figure 8C illustrates typical experiments, showing the staining of atrial cells (a and c). No labeling was seen in atrial cells subjected to the same staining protocols, except that the primary antibody was omitted (b).

Discussion

This work provides biophysical and pharmacological evidence indicating that $I_{\text{KAA}}$, the atrial background potassium current activated by membrane tension (stretch) and by AA, is probably generated by the TREK-1 channel or a very closely related member of the $K_p$ channel family, as follows.

(1) $I_{\text{KAA}}$ in symmetrical K$^+$ conditions has a single-channel
conductance of 118 pS, close to the 101 pS measured for TREK-1 in COS cells.\(^{(2)}\) (2) Both the native channel that generates \(I_{\text{KAA}}\) and the TREK-1 channel are activated by stretch and by AA\(^ {15,24,26}\). Moreover, in physiological K\(^+\) conditions, the resulting current is outwardly rectifying in both cases (Figure 1C and Patel et al\(^ {24}\)), and conductances are also very similar, 41 pS for \(I_{\text{KAA}}\) and 48 pS for TREK-1 (in COS cells\(^ {24}\)). (3) Neither \(I_{\text{KAA}}\) nor the current generated by TREK-1 in a heterologous system is sensitive to voltage in physiological K\(^+\) conditions. Both the native channel and TREK-1 display flickering openings occurring in bursts as long as the stimulus is maintained. (4) Both the native currents corresponding to \(I_{\text{KAA}}\) and to TREK-1 are sensitive to intracellular acidification\(^ {25,26}\), and in both cases decreasing pH leads to an increase in stretch sensitivity. (5) TREK-1 is

![Figure 5](image-url)

**Figure 5.** Regulation of \(I_{\text{KAA}}\) by cAMP. Continuous cell-attached recording (indicated by arrows) in which atrial cell membrane was held at 0 mV. Extracellularly applied AA (10 \(\mu\)mol/L, gray bar) induced \(I_{\text{KAA}}\) openings within 5 minutes. Addition of 500 \(\mu\)mol/L CPT-cAMP (white bar), the permeant analog of cAMP, to bath solution completely inhibited channel activity within 2 minutes.

![Figure 6](image-url)

**Figure 6.** Regulation of chloroform-induced \(I_{\text{KAA}}\) by cAMP. A, Cell-attached recording of \(I_{\text{KAA}}\) activated by extracellularly applied chloroform (\(\text{CHCl}_3\), 0.18 mmol/L) in atrial patch held at 0 mV. Channel activity was fully inhibited after 2-minute application of 500 \(\mu\)mol/L CPT-cAMP to bath solution. B, Time course of single-channel maximum open probability \((P_{\text{Omax}})\) of current illustrated in panel A. Labeled arrows indicate \(P_{\text{Omax}}\) corresponding to recording traces shown in panel A. Chloroform and CPT-cAMP applications are indicated by horizontal bars.

![Figure 7](image-url)

**Figure 7.** Stimulation of \(\beta\)-adrenergic receptors inhibits \(I_{\text{KAA}}\). A, Recording of \(I_{\text{KAA}}\) in a cell-attached experiment in which atrial membrane was held at +20 mV. AA (10 \(\mu\)mol/L)-induced current (b) was strongly inhibited by extracellular application of 1 \(\mu\)mol/L isoproterenol (c), and a complete inhibition was observed within 2 minutes (d). B, Time course of the single-channel maximum open probability \((P_{\text{Omax}})\) measured in the experiment illustrated in panel A. Reactivation of channel by AA after isoproterenol treatment was observed in two patches. AA and isoproterenol applications are indicated by horizontal bars. Labeled arrows indicate \(P_{\text{Omax}}\) corresponding to recording traces shown in panel A.
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I TREK-1 activity is increased by chloroform, halothane, and isoflurane.27 TWIK-2 is inhibited by be present in the heart is modified by volatile anesthetics but in

m cellular application of 10

m mol/L LPC (n = 3, not shown). (6) Neither I_{KAA} nor TREK-115 is inhibited by glibenclamide or TEA or is Ca^{2+}-sensitive.

None of the other cloned K_{sp} channels, demonstrated to be present in the heart and analyzed for their biophysical and pharmacological properties,12,14,16,20- 23 display characteristics as close to I_{KAA} as do those of TREK-1.24 The only difference is the stretch sensitivity (P_{1/2} = −12 mm Hg for I_{KAA}26 and P_{1/2} = −23 mm Hg for I_{TREK-1}). The most probable interpretation of this difference between the native and the cloned channel is that there are additional subunits that associate with the TREK-1 protein to modify its sensitivity to pressure just as β-type subunits change the voltage sensitivity (and/or kinetics) of voltage-sensitive K^{+} channels and the Ca^{2+} sensitivity of K_{Ca,2} channels.

Another key element indicating the TREK-1 nature of I_{KAA} is provided by its unique sensitivity to volatile anesthetics. The activity of several of the K_{sp} channels that have been shown to be present in the heart is modified by volatile anesthetics but in

a different way. TASK-1 is not sensitive to chloroform but is activated by halothane and isoflurane.27 TWIK-2 is inhibited by chloroform and by halothane.14 THIK-1 is not sensitive to chloroform and is inhibited by halothane.22 TALK-2 is inhibited by chloroform and by halothane but is slightly activated by isoflurane.23 Among the K_{sp} channels present in the heart, only TREK-1 activity is increased by chloroform, halothane, and isoflurane.27 In inside-out experiments, we have found that I_{KAA}

was also reversibly activated by clinical concentrations of

(in mmol/L) chloroform 0.18, halothane 0.11, and isoflurane 0.69. This indicates, as also previously observed for TREK-1,27 that these anesthetics do not exert their effect via second-messenger molecules but rather directly on the channel macromolecule.

In addition to its sensitivity to volatile anesthetics, TREK-1 is inhibited by cAMP via PKA phosphorylation of Ser333 in the C-terminal part of the channel structure.24 The native cardiac channel generating I_{KAA} can also be regulated by cAMP. In cell-attached experiments, CPT-cAMP, the permeant analog of cAMP, produced a complete inhibition of I_{KAA} when the current had been activated by either AA (Figure 4), chloroform (Figure 5), or stretch. Interestingly, the same inhibitory effect was observed after stimulation of the atrial-cell β-adrenergic receptors with the specific agonist isoproterenol, a treatment that is known to increase the intracellular cAMP content.

All of these results taken together provide very strong evidence that I_{KAA} is the endogenous correlate of TREK-1 in adult rat atrial myocytes. In addition, RT-PCR, Western blot, and immunohistochemistry experiments independently demonstrate the presence of TREK-1 in adult rat heart. The mechanosensitive I_{KAA} channel is also present in ventricular cells with a single-channel conductance of 106±12 pS at +60 mV and 72±10 pS at −60 mV and the same pressure and pH sensitivity as the atrial channel.26 The mechanism of regulation of this ventricular I_{KAA} channel via AA production was recently analyzed.41 In the adult human heart, the expression of TREK-1 is much less important than in rodent heart but it could be significantly upregulated during development or in aging and/or heart diseases.

In the working atrial and ventricular myocytes, the main background K^{+} current is generally considered to be carried by the inwardly rectifying current I_{K1}. Under physiological conditions, it stabilizes the resting membrane potential near the K^{+} equilibrium and participates in the final repolarization phase of the AP. I_{TREK-1}, as an outwardly rectifying current, will balance any membrane depolarization and will contribute to the regulation of the AP duration. Because of its stretch sensitivity, we suggest that this current could be a negative feedback after stretch activation of nonselective cationic channels.52 TREK-1 would then have a beat-to-beat regulation of atrial function. It is particularly interesting to note that both I_{Kc}43 and I_{TREK-1} (the present study) are inhibited by the specific β-adrenergic agonist isoproterenol via a phosphorylation process involving cAMP-dependent kinase. Then, the positive inotropic effect exerted by β-adrenergic agonists on the heart will not only be produced by activation of voltage-dependent Ca^{2+} channels but will also be the result of prolonged Ca^{2+} entry into the cell by inhibition of I_{K1} and I_{TREK-1} activities.

Because I_{TREK-1} is present in atrial cell and is activated by physiological levels of stretch, it might be involved in the regulation of atrial natriuretic peptide (ANP) secretion. Stretch is known to be the main stimulus eliciting this secretion (for a review, see Ruskoaho44) which plays an important role in the control of blood pressure. The initial depolarization process associated with the intracellular Ca^{2+} increase that is necessary for ANP secretion is expected to be triggered by stretch activation of nonselective stretch-activated cationic channels.42 Half-maximal activation of these cationic channels occurs for a pressure of 1.5 mm Hg, whereas it occurs ≈12 mm Hg for the
K⁺ channel \( I_{\text{TREK}} \) by stretch can only occur secondary to the activation of these cationic channels. The function of TREK-1 could then be to act as a negative feedback for ANP secretion, and a β-adrenergic stimulation inhibiting TREK-1 could then be expected to enhance ANP release from atrial myocytes, an effect that has indeed been observed.\(^{44}\)

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A TREK-1–Like Potassium Channel in Atrial Cells Inhibited by β-Adrenergic Stimulation and Activated by Volatile Anesthetics

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Expanded Materials and Methods

Cell isolation

Male Wistar rats (200-300 g) were anesthetized by intraperitoneal injection of sodium pentobarbital (90 mg·kg\(^{-1}\)). The hearts were rapidly removed and placed in a cold (4°C) oxygenated Bicarbonate-buffered solution (in mM): NaCl 125; KCl 5; MgSO\(_4\) 2; KH\(_2\)PO\(_4\) 2; NaHCO\(_3\) 25; glucose 10. They were cannulated through the aorta on a Langendorff column and retrogradely perfused at a constant flow and at 37°C, with the following solutions: (1) well-oxygenated Bicarbonate-buffered solution for 1-2 minutes in order to wash out the blood, (2) same solution containing 0.06% collagenase (type II, Worthington) and 0.03% hyaluronidase (type IV-S, Sigma) for 20-45 minutes, (3) oxygenated modified KB medium for 2 minutes to stop the dissociation (in mM): K-glutamate 90; KH\(_2\)PO\(_4\) 30; MgCl\(_2\) 1; pyruvic acid 5; taurine 20; glucose 20; HEPES 10 (pH adjusted to 7.2 with KOH). The hearts were then removed from the column, and atria were separated from ventricles. Atria and ventricles were cut into small pieces that were mechanically dissociated into single cells. Isolated myocytes were stored at 4°C in modified KB medium until use (within 8 hours).

Electrophysiological experiments

Atrial cells were continuously superfused (0.2 ml/min) with a local perfusion system. Whole-cell, inside-out and cell-attached configurations of the patch-clamp technique were performed at room temperature (20-22°C) with electrode resistance ranging from 3 to 5 MΩ. Pipettes were sylgard-coated when used for single-channel experiments. The standard intracellular solution was (in mM): KCl 150; MgCl\(_2\) 2; EGTA 5; HEPES 10 (pH adjusted to 7.2 with KOH). For cell-attached experiments, the
same solution without EGTA was used as bathing solution. The basic extracellular solution contained (in mM): NaCl 145; KCl 5; MgSO₄ 2; HEPES 10 (pH adjusted to 7.2 with NaOH). Arachidonic acid (10 µM, from a 10⁻¹ M stock solution in ethanol), L-α-lysophosphatidylcholine myristoyl (C14:0) (10 µM, from a 10⁻¹ M stock solution in ethanol), 8-(4-chlorophenylthio)-adenosine–cyclic monophosphate (CPT-cAMP, 500 µM), glibenclamide (10 µM, from a 10⁻² M stock solution in DMSO), tetraethylammonium (TEA, 10 mM) were freshly prepared before each experiment. All chemicals were purchased from Sigma-Aldrich Chimie (France). Negative pressure was applied to the pipette via a calibrated syringe. A RK300 patch-clamp amplifier (Bio-Logic, Claix, France) was used for whole-cell and single-channel recordings. Two stimulation protocols were applied in whole-cell experiments: 1) a 600 ms-voltage step to -40 mV (from a holding potential of -80 mV) followed by a 10 s-voltage step to +40 mV, every 15 s; 2) 1 s-voltage steps from -120 mV to +40 mV (from a holding potential of -80 mV) in 20 mV-increments, every 6 s. Recordings were digitized and analyzed with pClamp software. The steady-state current (Iₛₛ) was routinely measured at the end of each voltage step of both protocols. Single-channel recordings, filtered at 3 kHz, were digitized using a DAT recorder (Bio-Logic, Claix, France) and further analyzed with Biopatch software (Bio-Logic, Claix, France). Maximum open probabilities of a single channel (Pₒₘₐₓ, measured over 5 s every 20 or 30 s, during a burst of openings) and amplitude histograms were determined using filtered signals at 1 kHz. Results, expressed as mean±S.E.M, were considered as significant when P<0.05 with the t-Test Students’.

**Anesthetics preparation**

For experiments using anesthetics, the final solutions were prepared from saturated solutions (15.3 mM isoflurane, 17.5 mM halothane, 66.6 mM chloroform) in saline at room temperature.¹ To determine the real concentration of anesthetics applied to the
recorded patch (0.69 mM Isoflurane, 0.11 mM Halothane, 0.18 mM Chloroform), samples of bath solution were collected at the mouth of the perfusion system and were analyzed by gas chromatography (Gc 6000 Vega Série 2 Carlo Erba). Chloroform, Halothane and Isoflurane were purchased from SDS (France), Laboratoires Belamont (France) and Pharmacia and Upjohn (France), respectively.

RT-PCR

Adult rat hearts were dissected and total RNAs from 4 different tissues (atrium, septum, left ventricle, right ventricle) were extracted by the guanidinium isothiocyanate method. 5 µg of RNA were reverse transcripted in a final volume of 40 µl. In a first step, these cDNAs were used as template for PCR by using primers deduced from the mouse TREK-1 sequence, sens primer 5’–TCAAGCACA TAGAAGGCTGG-3’ and reverse primer 5’-TCAGGTGGTTCACA GACAGG-3’. The amplified DNA corresponding to rat TREK-1 DNA was subcloned then sequenced. The deduced proteic sequence presents 100% of identity with mouse TREK-1 over 145 amino acids. In a second step, two rat specific primers were deduced from this sequence, sens primer 5’-GCCCTGGACG CCGCATCTAC-3’ and reverse primer 5’-GTTACCCGCCCAGCTCTGCA-3’ and used for PCR amplification. 1 µl of each sample was used as template. PCR conditions were 32 cycles of 30 s at 94°C, 30 s at 60°C and 30 s at 72°C. The amplified fragments were transferred onto nylon membrane and probed with a 32P-labelled internal primer specific of TREK-1, 5’-GGATTTGGCGATTATGTGGCA-3’. For control, actin was amplified and analyzed according to the same protocol.

Membrane preparations and Western blot

Rat ventricular and atrial microsomes were prepared from adult rats.2,3 After removal of ventricles and atria, tissues were placed on ice in Hepses buffered saline containing
a battery of protease inhibitors (Boehringer Ingelheim). Tissues were minced and homogenized with a Polytron (Brinkman), and the nuclei and debris were pelleted by centrifugation at 1000 g for 10 minutes. The supernatant was then centrifuged at 8000 g for 20 minutes and again the supernatant was recovered and then treated with 0.5 M KCl at 4°C during 30 minutes in order to solubilize myofibrils. Microsomes were recovered after centrifugation at 60000 g during 30 minutes. Homogenates of COS-7 transfected cells were prepared as described. Aliquots of solubilized ventricular and atrial microsomes (25 µg) and COS-7 transfected cell homogenates (5 µg) were fractionated on 10% SDS-PAGE gels and subjected to western blotting. Blots were then blocked in low-fat 4% milk and 0.1% Tween 20 in PBS for 1 hour, and exposed to affinity-purified anti-TREK-1 (1:1000) in 0.4% milk and 0.1% Tween 20 in PBS overnight at 4°C. After several washes, blots were incubated with a peroxidase conjugated goat anti-rabbit antibody (1:15000, Jackson). The immuno-complexes were revealed by using an enhanced chemiluminiscence method (Super Signal, Pierce).

**Immunohistochemistry**

Immunohistochemical studies were performed on freshly isolated adult rat atrial myocytes. Cells were plated on 2 chambers Falcon culture slides and fixed with 4% paraformaldehyde in PBS during 20 minutes at room temperature. Cells were then washed twice in PBS and left overnight in 70% ethanol at 4°C. After washing with PBS, cells were permeabilized in 0.3% Triton X-100 during 15 minutes and then incubated in blocking buffer (5% normal goat serum and 0.05% Triton X-100 in PBS) during 3 hours at room temperature. Cells were hereafter incubated with rabbit polyclonal antibodies directed against TREK-1 (1:3000-1:5000) in blocking buffer at 4°C overnight. Development was performed using either the biotinylated horse
radish peroxidase-DAB method (Vectastain Elite ABC kit, Vector Labs) or using the Alexa Fluor™ 488 goat anti-rabbit IgG antibody (Molecular probes, USA).

REFERENCES


