TBAK-1 and TASK-1, two-pore \( \mathrm{K}^+ \) channel subunits: kinetic properties and expression in rat heart

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In this study, we examined in more detail the kinetic properties of mTASK-1 and mTBAK-1 under identical expression and experimental conditions and their modulation by both intracellular and extracellular pH. We studied their mRNA expression in different regions of the heart to determine whether the expression was homogeneous or localized in certain regions. Using isolated rat heart cells, we then examined whether a native cardiac \( \mathrm{K}^+ \) channel with properties similar to TASK-1 and TBAK-1 was present in certain regions of the heart. Our results showed that TBAK-1 and TASK-1 was present in membrane patches of rat heart cells.

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although the density and open probability of the native TBAK-1/TASK-1-like K\(^+\) channels were generally low.

**METHODS**

Expression of TBAK-1 and TASK-1 in COS-7 cells. mTBAK-1 was originally cloned from a mouse heart cDNA library and subcloned into pBluescript SK\(^{-}\) (8). mTASK-1 was also cloned from a mouse heart cDNA library using mTBAK-1 sequence as a probe. The coding regions of mTBAK-1 and mTASK-1 were subcloned into pcDNA3 (Invitrogen, Carlsbad, CA) for expression into COS-7 cells. Cells were transfected using LipofectAmine reagent (Life Technologies, Grand Island, NY). pGFP (Clontech, Palo Alto, CA) was cotransfected with pcDNA3-TBAK-1 or pcDNA3-TASK-1 to identify transfected cells. COS-7 cells were grown for several days on glass coverslips treated with polylysine before transfection and were used 2–4 days after transfection.

Northern blot analysis. Commercially prepared rat multiple-tissue Northern blot nitrocellulose membrane was purchased from Clontech Laboratories. Each lane was loaded with 2 \(\mu\)g poly(A)\(^+\) RNA. To determine mRNA expression within different regions of the heart, total RNA was isolated from the right atrium, left atrium, left ventricle (epicardium and endocardium separately), and right ventricle (RNeasy kit, Qiagen, Valencia, CA). Total RNA (20 \(\mu\)g/lane) was loaded onto a formaldehyde agarose gel (1.2%) and separated by electrophoresis. RNA was transferred to a nitrocellulose paper and then cross-linked by ultraviolet irradiation. A \(^32\)P-labeled fragment of rTASK-1 (690 bp) was used as a probe. This DNA fragment was obtained by RT-PCR using total RNA from the rat ventricle and contained two pore regions and most of the COOH terminus. Hybridization was performed according to the instructions using ExpressHybe solution (Clontech). The blot was probed again with human \(\beta\)-actin DNA, which cross-hybridizes with mouse and rat mRNA. After the film was exposed and developed, the relative expression was determined using a molecular imager (Bio-Rad GS-363 Imager and Gel Doc 1000).

RT-PCR. Total RNA from heart tissue prepared as described in Northern blot analysis was reverse transcribed using an oligo(dT) primer. PCR was carried out using two TBAK-1-specific primers (5'-ATCGTACGTCGAGACTTCCG and 5'-TGCACGACATGAGCAAC-3'). A plasmid containing TBAK-1 was used as control. Isolated heart cell preparation. Single atrial and ventricular cells of adult rat heart were prepared by enzymatic dissociation into single cells in the recording chamber. Bicarbonate-buffered solution containing (in mM) 118 NaCl, 4.7 KCl, 1 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 25 NaHCO\(_3\), 10 HEPES, 10 pyruvate, and 10 glucose.

Electrophysiology. Gigaseals were formed using Sylgard-coated thin-walled borosilicate pipettes (Kmax) with \(-4\) M\(\Omega\) resistances. Channel currents were recorded with an Axopatch 200B patch-clamp amplifier (Axon Instruments, Foster City, CA), digitized with a digital data recorder (VR10, Instrutech, Elmont, NY), and stored on videotape using a videotape recorder. The recorded signal was filtered at 3 kHz using an eight-pole Bessel filter (\(-3\) dB; Frequency Devices, Haverhill, MA) and transferred to a computer (Dell) using the Digidata 1200 Interface (Axon Instruments) at a sampling rate of 20 kHz. The filter dead time was \(-100\) \(\mu\)s (0.3/cutoff frequency); therefore, events shorter than \(-50\) \(\mu\)s will be missed in our analysis. Continuous single-channel currents were analyzed with the pCLAMP program (version 6.0.3, Axon Instruments). Data were analyzed to obtain a duration histogram, amplitude histogram, and channel activity (NP\(_o\), where N is the number of channels in the patch and P\(_o\) is the probability of a channel being open. NP\(_o\) was determined from 1–2 min of current recording. The pipette and bath solutions contained 140 mM KCl, 2 mM MgCl\(_2\), 10 mM HEPES, and 5 mM EGTA (pH 7.2). To change solutions perfusing the cylindrical surface of inside-out patches, the pipette with the attached membrane was brought to the mouth of the polypropylene tubing through which the desired solution flowed at a rate of \(-1\) ml/min. Macroscopic membrane currents from COS-7 cells were recorded with the whole cell configuration. Membrane potential was held at 0 mV, and then a voltage step of 500 ms in duration was applied from \(-80\) mV to \(+80\) mV in 20-mV increments every 5 s. Leak current was not subtracted. Current tracings shown in Figs. 2–6, 8, and 9 were filtered at 1 kHz. Data are represented as means \(\pm\) SD. Student’s t-test was used to test for significance between two values at the level of 0.05.

**RESULTS**

Single-channel currents of mTBAK-1 and mTASK-1 expressed in COS-7 cells. In this study, we have used mouse dones of TBAK-1 and TASK-1 to examine and compare their channel properties. The amino acid sequence of mTASK-1 has not been reported previously, and therefore we have aligned it with that of mTBAK-1. Alignment of TBAK-1 and TASK-1 shows that TBAK-1 is identical to TASK-1 except for the presence of an additional nine amino acids in the NH\(_2\) terminus of TBAK-1, suggesting that they are splice variants from the same gene (Fig. 1). Despite their overall sequence identity, they have been reported to exhibit markedly different channel kinetics, suggesting that the NH\(_2\) terminus of TBAK-1 may play an important role in channel gating. To confirm such a difference, we examined the single-channel kinetic properties of both TBAK-1 and TASK-1 under identical expression and ionic conditions.

In COS-7 cells expressing TBAK-1, cell-attached patches generally showed multiple single-channel openings. On formation of inside-out patches, a gradual rundown of channel activity was present in many patches, and this could not be reversed by application of 4 mM ATP. Channel openings at different membrane potentials were obtained in inside-out patches as shown in Fig. 2A. An amplitude histogram shows the presence of two levels, and the higher level was not a multiple of the lower level (Fig. 2B). The duration histogram shows that the mean open times of the two levels were not significantly different and that the averaged value was \(0.8 \pm 0.1\) ms at \(-80\) mV (n = 7; Fig. 2C). The conductance of the main (lower) level was 14.6 \(\pm\) 1.0 pS (n = 7) at \(-80\) mV and 9.1 \(\pm\) 1.3 pS (n = 7) at \(+80\) mV. Thus the current-voltage relationship showed a weak inward rectification in the presence of 2 mM Mg\(^{2+}\) (Fig. 2D). Outward currents measured at \(+80\) mV were blocked by application of 3 mM Ba\(^{2+}\) to the bath solution in inside-out patches. Channel activity (NP\(_o\)) was not significantly affected by membrane potential.

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A change in intracellular K⁺ concentration ([K⁺]i) from 140 mM to 280 mM produced a \(-15 \pm 1\) mV (n = 3) shift of the reversal potential, as expected for K⁺-selective channels. These K⁺ channels were not present in nontransfected COS-7 cells or in COS-7 cells not expressing green fluorescent protein (GFP) (n = 10).

We also expressed mTASK-1 in COS-7 cells and studied the channels in inside-out patches under identical experimental conditions. Channel openings at +80 and -80 mV are shown in Fig. 3A. The amplitude and duration histograms indicated that the kinetics of TASK-1 were indistinguishable from those of mTBAK-1 (Fig. 3B). As with mTBAK-1, two conductance levels for which the higher level was not a multiple of the lower one were also present. The single-channel conductances of main levels were 13.8 ± 2.8 pS at -80 mV and 10.1 ± 2.1 pS at +80 mV (n = 6). The mean open time was 0.6 ± 0.1 ms (n = 6) at -80 mV. The current-voltage relationship obtained using the amplitudes of the main level for mTBAK-1 and mTASK-1 could be superimposed. Channel activity (NPₒ) was not significantly affected by membrane potential (Fig. 3E). Outward mTASK-1 current measured at +80 mV was also blocked by 3 mM Ba²⁺ in the bath.
solution in inside-out patches. Thus these results showed that the stretch of nine amino acids in the NH$_2$ terminus of mTBAK-1 had no effect on the gating kinetics of the K$^+$ channel.

**Effect of pH on mTBAK-1 and mTASK-1.** TASK-1 has been reported to be particularly sensitive to pH$_o$ (2, 10). Because TBAK-1 is essentially identical to TASK-1, TBAK-1 is likely to be pH$_o$-sensitive as well. We wanted to confirm this view and also to examine whether the effect of pH$_o$ on the current was due to the accompanying change in intracellular pH (pH$_i$). We also studied the effect of pH$_o$ on mTBAK-1 and mTASK-1 single-channel currents to determine whether pH affected the conductance and/or the frequency of opening. First, we studied the effect of pH$_o$ on the whole cell currents of mTBAK-1 expressed in COS-7 cells. Cell membrane potential was held at 0 mV and then stepped to potentials ranging from $-80$ mV to $+80$ mV in 20-mV increments for 500 ms. Figure 4A shows whole cell currents at three pH$_o$ values in the same cell and illustrates the marked sensitivity of the TBAK-1 current to pH$_o$, particularly in the acidic range. The effect of 3 mM Ba$^{2+}$ in the bath solution on the whole cell current at pH 7.2 in the same cell is also shown. Ba$^{2+}$-sensitive currents were then plotted as a function of voltage to better illustrate the effect of pH$_o$ (Fig. 4B). Averaged currents at different pH$_o$ values at $-80$ and $+80$ mV were determined from three experiments and plotted in Fig. 4C. The results showed that TBAK-1 had a high sensitivity to pH$_o$, particularly between 7.2 and 8.0.
6.4. Changing pHo from 7.2 to 6.4 resulted in a 73 ± 16% and 72 ± 17% reduction of the mTBAK-1 current for inward and outward currents, respectively.

To obtain the channel activity-pHo relationship, channel activities were determined at three pHo values from inside-out patches using pipettes of similar tip resistances. Channel openings at different pHo and their respective amplitude histograms are shown in Fig. 5A. Current-voltage relationships obtained from these channel currents showed that pHo decreases produced small but not significant decreases in the current amplitude and single-channel conductance (Fig. 5B). Analyses of the open time duration also indicated that pH did not significantly affect the mean open times (0.7 ± 0.1 ms, 0.7 ± 0.1 ms, and 0.7 ± 0.1 ms at pH 6.4, 7.2, and 8.0, respectively). The averaged activities from eight patches were plotted as a function of pHo in Fig. 5C. Channel activity (NPo) at pH 7.2 was 0.06 ± 0.02. The marked pHo sensitivity of TBAK-1 could be clearly demonstrated. A better way to study the effect of pHo would be to use outside-out patches and measure channel activity as the pH of the bath solution is changed. However, unstable baseline currents in nearly all outside-out patches prevented accurate analyses of channel activity. In three successful outside-out patches, we were able to show the pHo sensitivity of mTBAK-1 when pHo of the bath perfusion solution was changed from 8.0 to 7.2 and 6.4 and then back to 7.2 (Fig. 5D). In outside-out patches, relative channel activities at 8.0, 7.2, and 6.4 were 0.093 ± 0.026, 0.063 ± 0.021, and 0.012 ± 0.009, respectively.

An effect of the pHo on ion channel activity could be mediated via a change in pHi. Therefore, to determine the effect of pHi on TBAK-1 current, the cytoplasmic side of outside-out patches was perfused with solution at different pHi values. Figure 6A shows expanded current tracings from an inside-out patch at three pHi values and shows the corresponding amplitude histograms. The single-channel conductances at −80 and +80 mV were not significantly affected by changes in pHi (Fig. 6B). However, channel activity was reduced at pH 6.4 and augmented at pH 8.0 compared with that observed at pH 7.2. The reduction of channel activity by a change in pHi from 7.2 to 6.4 was 42 ± 10% compared with that produced by the same change in pHo (81 ± 14%, n = 4). Thus these results indicate that TBAK-1 is more sensitive to pHo than to pHi in the pH range from 7.2 to 6.4. In the pH range from 7.2 to 8.0, the effect of pHo and pHi on TBAK-1 activity was not significantly different (50 ± 15% vs. 44 ± 17%, P < 0.05). The results also show that the effect of pHo is not due to a change in pHi, because a decrease in pHo from 7.2 to 6.4 reduces pHi by <0.2 pH unit in cardiac cells (7).

Expression of TBAK-1/TASK-1 in heart tissues. In mouse tissues, TASK-1 was expressed abundantly in the heart and at low levels in the lung (2, 8). Our Northern blot analysis using the TBAK/TASK probe also showed a strong ~4.5-kb band in the heart
and a weak band in the lung of the adult rat (Fig. 7A). TBAK-1/TASK-1 distribution within the heart tissue was then examined to determine whether it was expressed in any specific region of the rat heart. As shown in Fig. 7B, TBAK-1/TASK-1 mRNA was expressed in all five regions studied. Relative expression of TBAK-1/TASK-1 was assessed as a fraction of β-actin expression in the same blot using a densitometer. If the expression in the right atrium is taken as 1.0, the expression in the left atrium, right ventricle, and left ventricle (epicardium and endocardium) was 0.8 ± 0.2, 0.7 ± 0.1, 0.9 ± 0.2, and 0.7 ± 0.2, respectively (n = 3 each). The results show that the expression of TBAK-1/TASK-1 in different regions of the heart is relatively even. To help distinguish the expressions of TBAK-1 and TASK-1, we performed RT-PCR using primers specific for TBAK-1. No PCR product (112 bases) for TBAK-1 was detected in any of the five heart regions, whereas PCR product for TASK-1/TBAK-1 was clearly present (n = 3 each). The PCR product was sequenced to confirm that it was the correct DNA fragment. A typical result with right atrial tissue is shown in Fig. 7C. Therefore, these results indicate that in the rat heart, TASK-1 is the predominant form.

A native K⁺ channel with kinetic properties similar to TBAK-1/TASK-1. The relatively high expression of TBAK-1/TASK-1 mRNA in the heart tissue suggested that the small-conductance (14 pS) K⁺ channel observed in transfected COS-7 cells may be present in rat heart cells. To identify such a K⁺ channel, we prepared single dissociated cells from the right atrium and from the left ventricular epicardium of rat heart. In cell-attached patches, we looked for a channel that has kinetics of opening similar to that of TBAK-1 expressed in COS-7 cells. Once such a channel was considered to be present, inside-out patches were formed and currents recorded at different membrane potentials in symmetric 140 mM KCl. Figure 8 shows current recordings from inside-out patches of a right atrial cell and a COS-7 cell expressing TBAK-1, respectively. For the atrial K⁺ channel, the open time duration histogram could be fitted with a single-exponential function with a time constant of 0.8 ± 0.1 ms (n = 3). The mean current amplitudes of the main open level at −80 and +80 mV were 1.08 ± 0.04 and 0.77 ± 0.02 pA, respectively, similar to the values for mTBAK-1/mTASK-1 in COS-7 cells. The single-channel conductances at −80 and +80 mV were 13.5 ± 0.5 and 9.6 ± 0.3 pS, respectively (n = 3). The current-voltage relationship obtained using the amplitudes of the main open level is shown in Fig. 8E. These channels were K⁺ selective, as judged by a −31.6 ± 2.8-mV shift after switch of the KCl in the pipette solution from 140 mM to 35 mM (expected shift from Nernst equation is −36 mV) and by the absence of these K⁺ channel openings (inward current) when 3 mM Ba²⁺ was present in the pipette solution (n = 5).

Although nearly all patches examined showed channel openings with similar kinetics, the open probability was always very low. In right atrial cells, only ~20% of the patches studied showed channel activity such as that shown in Fig. 8A (P < 0.012; n = 3). In ventricular epicardial cells, nearly all patches we studied showed the presence of a channel with similar opening kinetics, although the open probability was also very low (P < 0.01). In a few patches, we were able to record long enough to estimate their single-channel conductances and mean open times. Figure 9A shows current recordings from a cell-attached patch formed on an epicardial ventricular cell at two membrane potentials. The single-channel conductances were 14.1 ±
1.1 and 11.2 ± 1.6 pS at +80 and ~80 mV, respectively (n = 3). The mean open time averaged from ~100 openings was 0.8 ± 0.1 ms. For comparison, channel openings in COS-7 cells transfected with TBAK-1 are also shown (Fig. 9B). Figure 9C shows an inward current recording from an inside-out patch in which opening of ATP-sensitive K⁺ channels (72 ± 2 pS; n = 3) was present. After rundown of the ATP-sensitive K⁺ channel, we could see openings of the small 14-pS channel, as indicated by the arrows in Fig. 9C. Similarly, Fig. 9D shows a recording from an inside-out patch in which one open background K⁺ channel (IKH) with a single-channel conductance of 24 ± 2 pS (n = 3) was present. We could see the small ~14-pS channel on top of IKH, as indicated by the arrows in Fig. 9D. Thus, despite their low open probability, TBAK-1-like channels were observed in both atrial and ventricular cells. However, because of the extremely low open probability, we were unsuccessful in studying the effect of pH₀ on these native K⁺ channels. Nevertheless, our results show that the K⁺ channel with kinetic properties nearly identical to those of TBAK-1 is present in heart cells.

**DISCUSSION**

Among cloned mammalian genes that encode K⁺ channel subunits possessing two pore-forming domains and four transmembrane segments, only TBAK-1/TASK-1 have been found to be expressed primarily in the heart tissue (2, 8). In this study, we characterized the kinetic behavior of TASK-1 and TBAK-1 to identify a native K⁺ channel with similar kinetic properties. Our study showed that TBAK-1 and TASK-1 have identical single-channel kinetics and pH sensitivity. TBAK-1/TASK-1 mRNA was found to be relatively evenly expressed throughout the heart. We have identified a K⁺ channel in rat atrial and ventricular cells with
Single-channel kinetics of TBAK-1 and TASK-1. The single-channel TBAK-1/TASK-1 currents showed at least two levels of openings in which the channel with higher conductance (22 pS) was not a simple multiple of that with a smaller conductance (14 pS). The amplitude histograms for each level showed a broad peak, indicating the large fluctuation of current amplitudes. This could be due to channel openings whose short durations (<100 µs) are not fully detected by the limited resolution of our recording system (rise time 100 µs). At a low level of expression, when the patch showed one or two channel openings, the 14-pS channel was always predominant. Therefore, we used the current amplitudes of this level to plot all current-voltage relationships for comparison among two-pore K_1 channels studied here.

Our results showed that the kinetic properties of mTASK-1 are indistinguishable from those of mTBAK-1. This was surprising because of the earlier report that rTASK-1 showed long open bursts that lasted for seconds, interrupted by closings that also lasted for seconds (10). The amino acid sequence of mTASK-1 is 99% identical to that of rTASK-1, and no differences are found within the pore and membrane-spanning regions. Furthermore, the same channel homologs from different species normally show identical behavior when expressed heterologously, despite small differences in the amino acid sequence. Therefore, it is highly unlikely that the channel kinetics of mTASK-1 and rTASK-1 would be different. In any case, our results showed clearly that mTASK-1 and mTBAK-1 have identical channel kinetics, indicating that the short stretch of nine amino acids in the NH_2 terminus of TBAK-1 does not affect channel gating.

Sensitivity of TBAK-1 to pH_o and pHi. TASK-1 has been shown to be very sensitive to pH_o, particularly in the 6.4–7.4 range. This could be due to an effect from the intracellular side, because a change in pH_o causes a change in pH_i. Our results showed that TBAK-1 is sensitive to both pH_o and pH_i in a qualitatively similar way. Quantitatively, the same change in pH from 7.2 to 6.4 was twice as effective in blocking the current from the extracellular as that from the intracellular side. Therefore, the region of TBAK-1/TASK-1 that confers sensitivity to pH_o is on the extracellular side. The pH_o effect on the channel current showed a very weak voltage dependence, suggesting that H^+ probably acts at sites peripheral to the mouth of the pore. Our calculation of the electric distance (8) in the membrane at which H^+ interacts with the channel protein from the external side was 0.05.

A rat cardiac K_1 channel with kinetic behavior similar to that of TBAK-1/TASK-1. The presence of TBAK-1/TASK-1 mRNA in single rat ventricular and atrial cells has recently been confirmed by RT-PCR (8). Thus K_1 channels encoded by TBAK-1/TASK-1 would be expected to be present in the cardiac cell membrane, because they can form functional channels in COS-7 cells. To our knowledge, a cardiac K_1 channel with kinetic properties similar to those of TBAK-1/TASK-1 has not been previously reported in any species.
Voltage-dependent K^+ channel with 14-pS conductance in physiological solution has been reported in guinea pig ventricular cells (21). However, long openings that occur only at depolarized potentials indicate that this K^+ channel is not TBAK-1/TASK-1. Therefore, this is the first study to identify and report a TBAK-1/TASK-1-like K^+ channel in the rat heart. One reason why such a channel was not detected previously may be that the open probability was too low for the channel to be recorded in any consistent manner, as our study indicates. Another reason may be that the channel conductance was small relative to other inwardly rectifying K^+ channels that have higher conductances and open at rest with much greater open probability, thus overshadowing the small-amplitude channels. The estimated macroscopic current of the 14-pS K^+ channel at -80 mV would be -13 pA in an atrial cell with a capacitance of ~50 pF, assuming that cell capacitance is ~50 pF and that there are ~1,000 channels in the membrane. This is probably too small to be of physiological significance.

An important question is, why is the number of TBAK/TASK channels in the membrane patches low when mRNA expression is relatively abundant in the heart? It is possible that the major fractions of TBAK channels are located in the membrane of intracellular organelles. However, this seems unlikely because the channel density of TBAK-1/TASK-1 in the plasma membrane of transfected COS-7 cells was high. A functional K^+ channel is believed to consist of four pore-forming domains (12, 20). This would indicate that two TASK-1 or TBAK-1 subunits presumably assemble to form a functional K^+ channel. Several cardiac K^+ channels are now known to be formed by heteromeric assembly of different subunits (5, 9, 16). These include the G protein-gated K^+ channel (GIRK1/GIRK4; GIRK1/GIRK2), ATP-sensitive K^+ channel (Kir6.1/SUR), and delayed rectifier K^+ channel (Kv1.2-LQT1/mink). Therefore, it is possible that TASK-1 or TBAK-1 associates with another as yet unidentified two-pore K^+ channel subunit to form a heteromeric K^+ channel in the cardiac cell membrane. The kinetic properties of such heterodimers could be quite different from those of homodimers. Therefore, what we have identified in the cardiac membrane as TBAK-1/TASK-1-like channel could be the homodimers that may be present at a low density. An example of coexisting homomeric and heteromeric K^+ channel is the G protein-gated K^+ channel in atrial cells. It has been shown recently that native atrial membrane contains both GIRK1/GIRK4 and GIRK4/GIRK4 complexes (1). Atrial G protein-gated K^+ channels show the typically reported GIRK1/GIRK4 channel type (35 pS), but GIRK4/GIRK4 channel type (10–20 pS) can be observed at a lower frequency (15, 17). The TBAK-like K^+ channel described in this study is not the G protein-gated ACh-activated K^+ channel, because the current-voltage relationship of the TBAK-like channel is relatively linear compared with the strong inward rectification of the G protein-gated K^+ channel.

To further characterize and identify the newly discovered native K^+ channels as TBAK-1/TASK-1 channels, we have tried to study the effect of pH_0 on the K^+ channel in atrial cells. However, due to the extremely low pH_0 in nearly all patches, the rundown of channel activity with time in excised patches, and the difficulty in obtaining outside-out patches with only the TBAK-like channels, we were unable to evaluate the effect of pH_0. Nevertheless, the native K^+ channel that we describe here and TBAK-1/TASK-1 expressed in COS-7 cells are strikingly similar with respect to single-channel conductances and mean open times, current voltage-relationships, and the opening behavior at negative and positive voltages. This strongly suggests that the 14-pS rat K^+ channel is most likely encoded by TBAK/TASK. No other identifiable K^+ channels in the rat ventricular and atrial cells had any similarity to TBAK/TASK.

Biophysical properties of TBAK-1/TASK-1 indicate that it behaves like a background K^+ channel, because it lacks voltage and time dependence (2, 8). Because the K^+ channels are open at rest and active at all membrane potentials, they would be expected to contribute to the resting membrane potential as well as the action potential duration and repolarization. In working atrial and ventricular cells in which I_K1 is present, TBAK-1/TASK-1-like K^+ channels may not play a significant role in setting the resting membrane potential.
role in the control of resting membrane potential. However, in atrial cells that do not have $I_{K1}$ and have high input resistances such as those near the pacemaker region of the right atrium (19), even small-conductance K⁺ channels could, in principle, have a significant effect on the resting potential. According to our electrophysiological studies, the expression of 14-pS K⁺ channels does not appear to be uniform at the protein level even within the right atrium. Further studies are clearly needed to investigate the density and the role of the 14-pS K⁺ channels in different atrial cells.

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REFERENCES


