The acid-sensitive potassium channel TASK-1 in rat cardiac muscle

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Abstract

Objective: The outward current flowing through the two-pore domain acid-sensitive potassium channel TASK-1 (I_{TASK}) and its inhibition via α1-adrenergic receptors was studied in rat ventricular cardiomyocytes.

Methods: Quantitative RT-PCR experiments were carried out with mRNA from rat heart. Patch-clamp recordings were performed in isolated rat cardiomyocytes. TASK-1 and other K+ channels were expressed in Xenopus oocytes to study the pharmacological properties of a new TASK-1 channel blocker, A293.

Results: TASK-1 channels were found to be strongly expressed in rat heart. Analysis of the sensitivity of various K+ channels to A293 in Xenopus oocytes showed that at low concentrations A293 was a selective blocker of TASK-1 channels. I_{TASK} in rat cardiomyocytes was dissected by application of A293 and by extracellular acidification to pH 6.0; it had an amplitude of ~0.30 pA/pF at +30 mV. Application of 200 nM A293 increased action potential duration (APD_{50}) by 31±3% at a stimulation rate of 4 Hz. The plausibility of the effects of A293 on APD_{50} was checked with a mathematical action potential model. Application of the α1-adrenergic agonist methoxamine inhibited I_{TASK} in Xenopus oocytes co-injected with cRNA for TASK-1 and α1A-receptors. In cardiomyocytes, methoxamine inhibited an outward current with characteristics similar to I_{TASK}. This effect was abolished in the presence of the α1A-antagonist 5-methyl-urapidil.

Conclusions: Our results suggest that in rat cardiomyocytes I_{TASK} makes a substantial contribution to the outward current flowing in the plateau range of potentials and that this current component can be inhibited via α1A-adrenergic receptors.

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Keywords: membrane currents; K channel; adrenergic agonists; action potential duration; cardiac myocytes; alpha1A adrenergic receptors

1. Introduction

The duration of the cardiac action potential depends on the precise balance between inward currents, carried by Na+

and Ca^{2+} ions, and outward currents, carried mostly by K+ ions. Several potassium current components contributing to the repolarization phase of the action potential have been identified so far [1,2]: (i) the inward rectifier current (I_{K1}), (ii) the transient outward current (I_{Io}), (iii) the rapid component of the delayed rectifier current (I_{Kr}), (iv) the slow component of the delayed rectifier current (I_{Ks}) and (v) a very rapidly activating, non-inactivating K+ current component that is incompletely characterized in most mammalian species (sometimes referred to as I_{Kur}, I_{Kp} or I_{Io}). It has been speculated [1,3] that two-pore domain K+ (K_{2P}) channels may contribute to the latter current component.

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K$_{2P}$ channel subunits are expressed in brain and many other tissues [3–5]. They possess four transmembrane domains and two pore domains; functional K$_{2P}$ channels are probably dimers with an overall topology similar to that of inward rectifier channels, but their molecular structure is still unknown. Cardiomyocytes express several K$_{2P}$ channels including the stretch-activated K$^+$ channel TREK-1 [4,6] and the acid-sensitive K$^+$ channel TASK-1 [7,8]. However, the contribution of these channels to the electrical activity of the heart is still far from clear.

The present study provides a quantitative description of the current contributed by TASK-1 channels in rat ventricular cardiomyocytes at different potentials. We used extracellular acidification and a novel TASK channel blocker, A293, as tools to separate $I_{\text{TASK}}$ from other current components. To clarify the functional role of $I_{\text{TASK}}$, we studied the effect of A293 on action potential duration in isolated cardiomyocytes and tried to reproduce this effect in a mathematical action potential model. Our results suggest that outward current flow through TASK-1 channels shortens the action potential in rat cardiac ventricular muscle.

Activation of G-protein-coupled receptors that signal through Goq-subunits has been shown to inhibit TASK-1 channels in neurons [3,5]. Therefore, we also tested the effects of stimulation of $\alpha_1$-adrenergic receptors, which are also coupled to Goq-subunits, on the steady-state outward current in rat cardiomyocytes and in X. laevis oocytes co-injected with cRNA coding for TASK-1 and $\alpha_1\text{A}$-receptors. We found that $I_{\text{TASK}}$ is inhibited by application of the specific $\alpha_1$-adrenergic agonist methoxamine. These findings suggest that inhibition of TASK-1 may contribute to the prolongation of the cardiac action potential elicited by stimulation of $\alpha_1$-adrenergic receptors [9–11].

2. Methods

2.1. Expression analysis

Isolation of cardiomyocytes, reverse transcription and polymerase chain reaction (RT-PCR) were performed as described previously [6,12]; the primers are listed online in Supplementary Table 1. Total rat brain and heart RNA was purchased from BD Biosciences-Clontech. Control experiments in the absence of RT were routinely performed, and all PCR products were sequenced to verify correct amplification. Cell-specific RT-PCR with RNA from selected cardiomyocytes was performed as described [12]. Troponin T, SUR2A and SUR1 were used as markers for cardiomyocytes [13], endothelin-1 for endothelial cells, calponin-1 and SUR2B for vascular smooth muscle cells; GAPDH was used as housekeeping gene. Endothelin-1, calponin-1 and SUR2B signals were negative in all myocyte preparations tested ($n=5$). Quantitative RT-PCR was performed with a different set of primers using an MX3000P real-time PCR system (Stratagene) as detailed in Supplementary Table 2.

2.2. Patch-clamp experiments with isolated rat cardiomyocytes

Myocytes from either ventricle were superfused with solution containing (mM) 140 NaCl, 5.4 KCl, 1 CaCl$_2$, 1 MgCl$_2$, 0.33 Na$_2$HPO$_4$, 10 glucose and 5 HEPES (pH 7.4 with NaOH) at room temperature. Patch-clamp experiments were performed in the whole-cell configuration using pipettes pulled from borosilicate glass capillaries. The pipette resistance was $\sim 4$ M$\Omega$; the pipette solution contained the following (in mM): 60 KCl, 65 K-glutamate, 5 EGTA, 2 MgCl$_2$, 3 K$_2$ATP, 0.2 Na$_2$GTP and 5 HEPES (pH 7.2 with KOH). Steady-state current voltage relations were obtained by applying voltage ramps (15 mV s$^{-1}$) between $+40$ and $-100$ mV. The ramps were preceded by a test pulse (duration, 1.5 s) from a holding potential of $-30$ mV to $+40$ mV. Action potentials were elicited by injection of brief current pulses (1 ms, 4 nA) in the current-clamp mode. For evaluation of action potential duration and for preparation of the figures, the stimulus artifact was subtracted digitally. Data acquisition was performed with an Axopatch 200B amplifier (Axon Instruments, Burlingame, CA), an A/D converter (PCI-MIO 16-XE-10, National Instruments) and data acquisition software developed in our laboratory (PC.DAQ1.1).

2.3. Action potential modelling

A mathematical model of rat left ventricular myocytes [14] was used to simulate the effects of currents through TASK-1 channels on action potentials and calcium handling. $I_{\text{TASK}}$ was described by the Goldman–Hodgkin–Katz equation [15]. The potassium permeability ($P_K$) related to TASK-1 was adjusted to reproduce the patch-clamp experiments with isolated rat cardiomyocytes. In the simulation studies, the same stimulus (1 ms, 4 nA) was used as in the experiments. The simulations were carried out with the Euler method for numerical solution of ordinary differential equations. The temporal discretisation for solving the equations was chosen heterogeneously for various components of the model. The model was used to analyze the changes in action potential duration after removal of $I_{\text{TASK}}$. A steady state was reached after the 50th repetitive stimulation.

2.4. Heterologous expression of K$_{2P}$ channels in Xenopus oocytes

Cardiac ion channels, $\beta$-subunits and the $\alpha_1\text{A}$-adrenoceptor were subcloned into oocyte expression vectors (pSGEM, pSP64T or pBluescript II KS$^{(+)}$). Quality and quantity of the injected cRNA were controlled by gel electrophoresis and UV spectroscopy. X. laevis oocytes were obtained from tricaine-anesthetized animals as described previously [16]. Oocytes were individually injected with 0.05–10 ng cRNA encoding the different channels. Standard two-
electrode voltage-clamp recordings were carried out with a Turbo Tec 10CD amplifier (npi, Tamm, Germany), a Digi-data 1200 A/D converter and PClamp7 software. Macroscopic currents were recorded 2–4 days after injection. Currents were measured in ND96 solution containing (in mM) 96 NaCl, 2 KCl, 1.8 CaCl2, 1 MgCl2, 5 HEPES (pH 7.4 with NaOH), except for experiments with the α1A-adrenoceptor, in which a "Cl⁻-free ND96 solution" was used (in mM): 96 NaMeSO₃, 2 KCl, 1 CaCl2, 1 MgCl2, 5 HEPES (pH 7.6 with NaOH). Cl⁻-free solution was required to avoid artifacts related to the opening of Ca²⁺-activated Cl⁻ channels. The effect of A293 on TASK-1 was studied by measuring steady-state currents at potentials ranging from −60 to +60 mV, applied in 10-mV increments from a holding potential of −80 mV. The effects of A293 on other ion channels were studied using standard pulse protocols. The interpulse interval for all protocols was 10 s. The concentration required for 50% block of current (IC₅₀) was estimated from Hill plots using 3–5 drug concentrations for each mutant (3–10 oocytes/point).

2.5. Statistics and general conditions

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Data are reported as means±SEM, n indicates the number of cardiomyocytes or oocytes. Statistical significance was calculated using Student’s t-test. All experiments were carried out at room temperature (22–24 °C).

Fig. 1. Expression analysis of K₂P channels. (A) RT-PCR analysis of K₂P channels in rat brain tissue, heart tissue and isolated pure cardiomyocytes. (B) Control experiments for cell specific expression analysis in rat cardiomyocytes. (C) RT-PCR of SUR1, SUR2A and SUR2B in rat heart tissue. The two alternative splicing products SUR2A and SUR2B were amplified with a forward primer located in exon 37 and two splice variant-specific reverse primers located in exon 39 (SUR2A) or exon 40 (SUR2B). Note that two bands were obtained with the second primer pair, the smaller one of which was related to SUR2B. (D) Quantitative PCR of TASK-1, TASK-3, Kv2.1 and Kir2.1 with cDNA from rat heart. (E) Summary of qPCR results (n=7 qPCR runs for each channel). Relative expression was quantified as 2⁻ΔCt, where ΔCt is Ct(GAPDH) – Ct(K⁺ channel).
3. Results

3.1. Expression of K\textsubscript{2P} channels in rat cardiomyocytes

Expression of the K\textsubscript{2P} channels TASK-1 and TREK-1 in the heart has been shown previously [8,17]. Using RT-PCR with RNA isolated from rat heart, we confirmed robust expression of TASK-1 and TREK-1 (Fig. 1A). In addition, we found robust expression of THIK-1, TREK-2, TWIK-1 and TWIK-2. However, since the heart is composed of many different cell types, this does not necessarily indicate that these channels are also present in cardiomyocytes. Cell-specific RT-PCR experiments [12] with isolated cardiomyocytes showed expression of TASK-1, TASK-3 and TREK-1, but not of THIK-1, TREK-2, TWIK-1 or TWIK-2. Control experiments showed that troponin T, SUR2A and SUR1, but not endothelin-1, H1-calponin or SUR2B were expressed in rat cardiomyocytes (Fig. 1B). SUR2B was found only in RNA from whole heart (Fig. 1C). Quantitative RT-PCR using cDNA from rat heart (pooled from 200 hearts) showed that the mRNA copy number of TASK-1 was about 1000-fold higher than that of TASK-3 and about three-fold higher than that of Kir2.1 (Fig. 1D, E).

In outside-out patches of cardiac ventricular muscle, single channels with the properties of TASK-1 [18] were regularly observed, as presented online in Supplementary Fig. 1. At \(-80\) mV, the mean single-channel conductance was \(17.1\pm2.4\) pA (\(n=5\)) and the mean open time was \(0.33\pm0.03\) ms (\(n=3\)). In contrast, ion channels with the properties of TASK-3 (or TASK-3/TASK-1 heterodimers, which have the same conductance as TASK-3 homodimers [19]) were never observed in cell attached, inside–out or outside–out patches (\(n>100\)) from rat cardiomyocytes.

3.2. Effects of extracellular acidification on the steady-state outward current

We first tried to quantify the current flowing through TASK-1 channels in rat ventricular cardiomyocytes by analyzing the effects of extracellular pH on the steady-state current–voltage relation. The voltage protocol was designed to inactivate voltage-dependent ion channels (see Section 2.2). In addition, we used a blocker cocktail designed to eliminate (i) the rapid voltage-activated K\textsuperscript{+} current, \(I_{\text{K1}}\) (5 \(\mu\)M E-4031); (ii) the slow voltage-activated K\textsuperscript{+} current, \(I_{\text{Ks}}\) (2 \(\mu\)M HMR1556); (iii) the transient outward current, \(I_{\text{to}}\) (2 mM 4-aminopyridine); (iv) the ATP-sensitive K\textsuperscript{+} current, \(I_{\text{KATP}}\) (2 \(\mu\)M glibenclamide); and (iv) the voltage-activated Ca\textsuperscript{2+} current, \(I_{\text{Ca}}\) (10 \(\mu\)M nifedipine). In the presence of the blocker cocktail no residual transient outward current was observed during the initial voltage clamp step to +40 mV. Control experiments showed that the blocker cocktail had no effect on TASK-1 channels expressed in \textit{Xenopus} oocytes (Supplementary Fig. 2).

In rat cardiomyocytes, the steady-state outward current before application of the blocker cocktail was \(1.50\pm0.08\) pA/pF at +30 mV (\(n=32\)). Application of the blocker cocktail reduced the outward current measured at +30 mV by \(0.31\pm0.03\) pA/pF (\(n=32\); not shown). In the presence of the blocker cocktail, extracellular acidification to pH 6.0 reduced the outward current measured at potentials positive...
to −40 mV (Fig. 2A). The inset shows the averaged difference current at higher magnification. The difference current showed slight outward rectification between −40 and +40 mV; its amplitude was 0.33±0.05 pA/pF at +30 mV (n=9). These findings are consistent with blockage of TASK-1 channels by extracellular acidification. At potentials negative to −80 mV, acidification also inhibited a fraction of the inward rectifier current (<30%). Since at potentials positive to −40 mV the residual inward rectifier current should be close to zero, the current blocked by extracellular acidification between −40 and +40 mV should give a good approximation of the amplitude of \( I_{\text{TASK}} \).

To test whether TASK-3 channels might generate a measurable whole-cell current we applied ruthenium red, a specific blocker of TASK-3 channels [20,21]. We found that 2 \( \mu \)M ruthenium red, which should block TASK-3 completely [20,21], had virtually no effect on the current–voltage relation of rat cardiomyocytes; the difference current at +30 mV was 0.04±0.01 pA (n=4). Collectively, our single-channel and whole-cell patch-clamp measurements and our quantitative RT-PCR data suggest that TASK-3 channels make only a minor contribution, if any, to the acid-sensitive current in rat cardiac ventricular muscle.

### 3.3. Effects of the TASK channel blocker A293 on steady-state outward current

To further characterize \( I_{\text{TASK}} \), we studied the effect of a novel TASK-1 blocker, the aromatic carbonamide A293 (supplied by Aventis, Frankfurt, Germany), on the current–voltage relation of rat cardiomyocytes (Fig. 2B, C). As a first step, we analyzed the pharmacological properties of A293 by measuring its effects on human TASK-1 (and other \( K^+ \) channels expressed in the heart) in Xenopus oocytes. The typical recording presented in Fig. 3A illustrates that TASK-1 currents activate and deactivate in two phases, an instantaneous phase and a time dependent phase that reaches a steady state within 200 ms, as described previously [15,22]. Application of 200 nM A293 inhibited both phases by approximately 50%. The concentration–effect curve for hTASK-1 could be fitted with an IC\(_{50}\) of 222±38 nM and a Hill coefficient of 1.10±0.09 (Fig. 3B). The IC\(_{50}\) for hTASK-3 was 0.95±0.10 \( \mu \)M, and the sensitivity to A293 of the other \( K^+ \) channels tested was much lower (Table 1). Thus, at low concentrations A293 appears to be a selective blocker of TASK-1 and TASK-3. In the presence of 1 \( \mu \)M A293 the outward currents through TASK-2, TASK-4, TREK-1, Kv1.1, Kv1.3, Kv1.4, Kv1.5, Kv4.3, HCN1, HCN2, HCN4, Kv11.1 (hERG) and Kv7.1 (KCNQ1)/KCNE1 were inhibited by less than 10% (Fig. 3C). Kir2.1 was inhibited by 12±4%.

The effects of A293 on the current–voltage relation of cardiomyocytes in the presence of the blocker cocktail are shown in Fig. 2B. At potentials positive to −40 mV, application of 2 \( \mu \)M A293 inhibited an outward current that was similar to the pH-sensitive outward current (Fig. 2A). The current blocked by the drug was 0.28±0.06 pA/pF at +30 mV (n=6). Next, we tried to selectively inhibit \( I_{\text{TASK}} \) by using a low concentration of A293 in the absence of any other ion channel blockers (Fig. 2C). The difference current obtained

<table>
<thead>
<tr>
<th>Channel</th>
<th>IC(_{50}) (( \mu )M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hTASK-1</td>
<td>0.222±0.038</td>
</tr>
<tr>
<td>mTASK-1</td>
<td>0.195±0.018</td>
</tr>
<tr>
<td>hTASK-3</td>
<td>0.95±0.1</td>
</tr>
<tr>
<td>rTREK-1a</td>
<td>9.8±1.7</td>
</tr>
<tr>
<td>hTASK-2</td>
<td>8.1±1.9</td>
</tr>
<tr>
<td>hTASK-4</td>
<td>18.1±0.9</td>
</tr>
<tr>
<td>hKv1.1</td>
<td>10.8±1.5</td>
</tr>
<tr>
<td>hKv1.3</td>
<td>8.1±1.6</td>
</tr>
<tr>
<td>hKv4.3</td>
<td>10.5±0.9</td>
</tr>
</tbody>
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**Table 1** IC\(_{50}\) values for A293 (\( \mu \)M)

Fig. 3. Characterization of the TASK blocker A293 in Xenopus oocytes. (A) Effect of A293 (200 nM) on whole-cell current in an oocyte expressing hTASK-1 channels. (B) Dose–response curve of the effects of A293 on TASK-1 currents. (C) Effects of 1 \( \mu \)M A293 on various \( K^+ \) channels heterologously expressed in Xenopus oocytes.
after application of 200 nM A293 was 0.22±0.06 pA/pF at +30 mV (n=9). This value is about 20% smaller than the difference current observed with 2 μM A293 in the presence of the blocker cocktail, which suggests that native TASK-1 in isolated cardiomyocytes may have an IC_{50} for A293 of ≤ 100 nM. This interpretation is consistent with an estimate for the IC_{50} derived from experiments with CHO cells transfected with hTASK-1 (~100 nM, data not shown). Taken together, the data described above support the idea that the pH-sensitive current measured in the presence of the blocker cocktail (Fig. 2A) consisted mainly of TASK-1, and that in the absence of the blocker cocktail selective, although not complete inhibition of I_{TASK} can be achieved with 200 nM A293 (Fig. 2C).

3.4. The contribution of TASK-1 to the action potential in rat cardiomyocytes

To assess the functional relevance of I_{TASK}, we studied the effects of A293 on action potential duration. The upper part of Fig. 4A shows typical ventricular action potentials recorded before and 3 min after addition of 200 nM A293. At a stimulation rate of 4 Hz, the range of APD_{50} was 11 to 134 ms and the range of APD_{90} was 36 to 186 ms, which is consistent with previous reports [23,24]. In our mixed transmural sample of ventricular cardiomyocytes application of 200 nM A293 increased APD_{50} by 31±3% and APD_{90} by 21±2% (n=6). The change in APD started shortly after the

![Fig. 4. Effects of A293 on action potentials. (A) Action potentials from rat cardiomyocytes recorded in the steady state before (thin lines) and after application of A293 (stimulation rate, 4 Hz). Lower traces: first derivative of the action potentials shown in the upper traces. The left ordinate was calculated from the equation $I_{\text{net}} = C_m \times -dV/dt$ (where $C_m$ is the membrane capacitance of the cardiomyocyte). (B) Time course of the change of action potential duration during and after application of 200 nM A293; stimulation rate, 4 Hz (filled circles, APD_{50}, open circles, APD_{90}). (C) The action potential calculated for a subepicardial rat cardiomyocyte at 4 Hz using the model of Pandit et al. [14] with minor modifications. The bold line indicates the action potential calculated in the steady state after removal of I_{TASK}.](image-url)
solution change and reached a steady state within 3–10 s (Fig. 4B). The effect of A293 on APD was reversible within 1–2 min after wash-out of the drug.

The lower part of Fig. 4A shows the first derivative (dV/dt) of the action potential (right-hand ordinate), which is a measure of the net current flow across the cell membrane. The corresponding net current (\(I_{\text{net}} = C_m \times \frac{dV}{dt}\)) is shown on the left-hand ordinate (outward current upward). It can be seen that the initial surge of net inward current (cut off) was followed by a transient net outward current, which was largely due to activation of \(I_o\). In most cells, the net outward current then settled to a “plateau”, the amplitude and duration of which were quite variable and depended on APD. Under control conditions (before application of A293) the mean current flowing at 0 mV ranged from 50 to 600 pA (median, 190 pA; \(n=9\)). The mean TASK-1 current measured in our whole-cell clamp experiments in cardiomyocytes (Fig. 2) was \(\sim 0.15\) pA/pF at 0 mV, which corresponds to \(\sim 20\) pA. Thus, \(I_{\text{TASK}}\) comprised between 3% and 40% (median, 10%) of the total net outward current at 0 mV, depending on the duration of the action potential.

To check the plausibility of the observed changes in action potential configuration we used the mathematical model of Pandit et al. [14], which gives a fairly complete description of the electrophysiological behavior of rat ventricular myocytes. We modelled \(I_{\text{TASK}}\) on the basis of our experimental results and subtracted it from the total transmembrane current calculated at each point in time. For simplicity, \(I_{\text{TASK}}\) was assumed to rise instantaneously and its voltage dependence was assumed to follow the Goldman–Hodgkin–Katz current equation [15]. When \(I_{\text{TASK}}\) was subtracted from the total transmembrane current, APD\(_{50}\) of subepicardial myocytes at 4 Hz increased by 13.8% and APD\(_{90}\) at 4 Hz increased by 11.8% (Fig. 4C). These calculations support the idea that inhibition of TASK-1 may be responsible for the major part of the prolongation of the action potential induced by application of A293. Furthermore, the simulations indicated smaller, but still significant increases in subepicardial APD for stimulus frequencies of 1 and 2 Hz. The model of Pandit et al. [14] did not allow reconstruction of subendocardial action potentials for 3 and 4 Hz.

3.5. Coupling of \(\alpha_1\)-adrenergic receptors to TASK-1

Next, we addressed the question whether activation of cardiac \(\alpha_1\)-adrenergic receptors, which are coupled to G\(_{q}\)-subunits, may inhibit TASK-1 channels. We first expressed TASK-1 channels alone in Xenopus oocytes and found that application of the \(\alpha_1\)-adrenergic agonist methoxamine (1 \(\mu\)M) had no effect (Fig. 5A, upper panels). We then co-expressed the three K\(_{2P}\) channels transcribed in rat cardiomyocytes, TASK-1, TASK-3 or TREK-1, with \(\alpha_1A\)-adrenoceptors in Xenopus oocytes. Under these conditions, application of 1 \(\mu\)M methoxamine produced almost complete inhibition of TASK-1 currents (90.8\(\pm\)2.7%; \(n=7\); Fig. 5A–D). The inhibition of TASK-1 was sustained as long as the agonist was present (Fig. 5B). In oocytes co-expressing \(\alpha_1A\)-receptors and TASK-3 channels, 1 \(\mu\)M methoxamine produced a transient inhibition of the acid-sensitive outward current (57.6\(\pm\)3.7%; \(n=5\)), followed by a much weaker steady-state inhibition (Fig. 5B). These data are in line with a recent study of the inhibition of TASK-1 and TASK-3 via...
M1-muscarinergic receptors [25]. TREK-1 was relatively insensitive to inhibition via \( \alpha_{1A} \)-adrenergic receptors; application of 1 \( \mu \)M methoxamine caused a transient inhibition of TREK-1 current amplitude by 7.8 ± 5.1\% (\( n=7 \); Fig. 5B, C). Thus, of the three \( K_{2P} \) channels expressed in rat cardiomyocytes, TASK-1 was most sensitive to inhibition via \( \alpha_{1} \)-adrenergic receptors. In the heterologous expression system, the IC\(_{50} \) for methoxamine was 20.2 ± 0.4 nM and the Hill coefficient was 0.63 (Fig. 5D).

3.6. Effects of methoxamine on \( I_{\text{TASK}} \) in rat cardiomyocytes

Superfusion of rat cardiomyocytes with the selective \( \alpha_{1} \)-adrenergic agonist methoxamine (Fig. 6A) in the presence of the blocker cocktail reduced the outward current at potentials positive to −40 mV in a similar way as extracellular acidification or application of A293 (Fig. 2). The difference current produced by 2 \( \mu \)M methoxamine was 0.32 ± 0.08 pA/pF at +30 mV (\( n=6 \)). Application of higher concentrations of methoxamine produced similar changes in the current–voltage relation. At +30 mV the difference current was 0.34 ± 0.03 pA/pF with 10 \( \mu \)M methoxamine (\( n=5 \)) and 0.36 ± 0.05 pA/pF with 100 \( \mu \)M methoxamine (\( n=7 \)), indicating that 2 \( \mu \)M methoxamine caused maximal activation of cardiac \( \alpha_{1} \)-adrenoceptors. These findings are consistent with the idea that in cardiac muscle, like in neurons, activation of \( \alpha_{1} \)-adrenoceptors can inhibit TASK-1 channels.

Three \( \alpha_{1} \)-receptor subtypes, \( \alpha_{1A} \), \( \alpha_{1B} \) and \( \alpha_{1D} \), are expressed in rat heart [26]. Therefore, we addressed the question which \( \alpha_{1} \) adrenergic receptor subtype was responsible for the effects of methoxamine. When we applied methoxamine (2 \( \mu \)M) in the presence of the specific \( \alpha_{1A} \) antagonist 5-methyl-urapidil (0.5 \( \mu \)M, ≥ 15 min pre-incubation) the difference current at positive potentials was almost completely abolished (Fig. 6A, lower insert); the mean current at +30 mV was 0.07 ± 0.04 pA/pF (\( n=7 \)). The effects with and without 5-methyl-urapidil were significantly different (\( p<0.05 \)) in the voltage range 0 to +30 mV. These findings suggest that methoxamine inhibited TASK-1 via \( \alpha_{1A} \)-adrenergic receptors.

Finally, we tested the effects of methoxamine at pH 6.0, where TASK-1 channels should be blocked. The cardiomyocytes were pre-incubated at pH 6.0 for ~3 min. When methoxamine was applied at pH 6.0 it produced no significant change in the current voltage relation (Fig. 6B). The mean difference current produced by application of 2 \( \mu \)M methoxamine at pH 6.0 was 0.08 ± 0.6 pA/pF at +30 mV (\( n=7 \)). These findings confirm the hypothesis that the current change produced by methoxamine was mainly due to inhibition of \( I_{\text{TASK}} \). The amplitude of the outward current inhibited by application of 2 \( \mu \)M methoxamine (Fig. 6A) was not significantly different (\( p>0.05 \)) from that produced by extracellular acidification (Fig. 2A) or application of A293 (Fig. 2B), which suggests that 2 \( \mu \)M methoxamine induced a complete block of TASK-1 channels.

4. Discussion

4.1. Quantitative description of \( I_{\text{TASK}} \) and its inhibition by A293

Quantitative analysis of individual potassium current components in cardiomyocytes is complicated by the fact that these cells express a multitude of different \( K^{+} \) channels [1]. We have characterized the current carried by TASK-1 channels (\( I_{\text{TASK}} \)) in rat cardiomyocytes by measuring the pH-sensitive current and by applying a novel TASK-1 channel blocker, A293. To separate \( I_{\text{TASK}} \) from the other \( K^{+} \) currents, we incubated the cardiomyocytes in a blocker cocktail that inhibited all other \( K^{+} \) channels in the relevant voltage range. Under these conditions, both extracellular acidification to pH 6.0 and application of a maximally effective concentration of A293 (2 \( \mu \)M) revealed an outwardly rectifying current component in the voltage range −40 to +30 mV. The mean difference current was ~0.3 pA/pF at +30 mV. The good agreement of the difference current obtained with either method suggests that this approach indeed allows dissection of \( I_{\text{TASK}} \). Our recordings also show that a small fraction of the inward rectifier current was inhibited by extracellular acidification and A293 (Fig. 2), but this did not interfere with
our analysis of $I_{\text{TASK}}$ because inward rectifier channels are closed in the voltage range ~40 to +30 mV.

Our analysis of the effects of A293 on various ion channels in *Xenopus* oocytes showed that TASK-1 was much more sensitive to inhibition by A293 than other cardiac ion channels. In oocytes, the IC$_{50}$ for inhibition of TASK-1 was 222 nM, in CHO cells it was ~100 nM. In cardiomyocytes, application of 200 nM A293 in the absence of the blocker cocktail produced a current change of 0.22 pA/pF at +30 mV, consistent with the estimate of the IC$_{50}$ obtained in CHO cells and a high selectivity of A293 for TASK-1.

4.2. The effect of the TASK channel blocker A293 on action potential duration

In our action potential measurements, the cardiomyocytes were usually stimulated at a rate of 4 Hz, which is still below the physiological heart rate in vivo (6–8 Hz). Our analysis of $dV/dt$ shows that, under these conditions, $I_{\text{TASK}}$ probably contributed between 3% and 40% (median, 10%) to the net current in the plateau range. Since we did not selectively isolate subepi- and subendocardial myocytes, our sample of cells probably included both cell types. Subendocardial action potentials are much longer than subepicardial ones, which is mainly due to the smaller amplitude and slower recovery from inactivation of the transient outward current ($I_{\text{T}}$) in subendocardial myocytes [23,24]. Thus, the action potentials shown in Fig. 4A might have been from a subendocardial (left) and a subepicardial ventricular myocyte (right), respectively. Furthermore, in rat ventricular muscle APD increases with increasing frequency, and this may also be due to a reduction in $I_{\text{Na}}$ [23]. Thus, in subendocardial myocytes and at higher frequencies the relative amplitude of $I_{\text{TASK}}$ compared to $I_{\text{Na}}$ (and the effect of A293 on APD) is expected to be much larger.

At a stimulation rate of 4 Hz, application of 200 nM A293 increased APD$_{50}$ and APD$_{90}$ of rat cardiomyocytes by 31±3% and 21±2%, respectively. In the calculations based on the model of Pandit et al. [14] for subepicardial myocytes, APD$_{50}$ and APD$_{90}$ at 4 Hz were increased by 14% and 12%, respectively. Action potentials of subendocardial myocytes at 4 Hz could not be reproduced with the model of Pandit. Considering that our cell population included subendocardial cells with long action potentials, the agreement between the model and the experimental measurements was satisfactory. We conclude from these findings that inhibition of $I_{\text{TASK}}$ was responsible for the major part of the increase of APD produced by application of 200 nM A293. TASK-1 channels are also expressed in human atrium and ventricle [3,15], but their contribution to the electrical activity of the human heart remains to be determined.

4.3. Inhibition of $I_{\text{TASK}}$ via $\alpha_1$-adrenergic receptors

It has long been known that stimulation of $\alpha_1$-adrenergic receptors prolongs the duration of the ventricular action potential in various mammalian species [9–11,27–29]. This effect has been attributed to inhibition of a steady-state outward current [9], the transient outward current ($I_{\text{to}}$) [28] or the inward rectifier current [30]. In agreement with Ravens et al. [9], we have now obtained evidence that the $\alpha_1$-adrenergic agonist methoxamine blocks a steady-state outward current component in rat cardiomyocytes. Furthermore, we have shown that, in the presence of the K$^+$ channel blocker cocktail, application of 2 μM methoxamine inhibited an outward current component that was virtually identical to the pH- and A293-sensitive K$^+$ current. At pH 6, where TASK-1 channels are closed, methoxamine had no effect. These findings corroborate our identification of $I_{\text{TASK}}$ and suggest that stimulation of $\alpha_1$-adrenergic receptors can induce complete blockage of TASK-1 in cardiac ventricular muscle.

This conclusion was supported by heterologous expression of $\alpha_{1A}$-adrenergic receptors and TASK-1 channels in *Xenopus* oocytes. In this system, methoxamine inhibited the TASK-1 current completely with an IC$_{50}$ of 20 nM. In native cardiomyocytes, the IC$_{50}$ is probably different since it depends on the density and subcellular localization of receptors and channels. In the presence of the specific $\alpha_{1A}$-receptor blocker 5-methyl-urapidil, the effect of methoxamine on cardiac steady-state outward currents in the plateau range was abolished. This observation suggests that the effects of methoxamine on TASK-1 in cardiomyocytes were mainly mediated by $\alpha_{1A}$-adrenergic receptors.

Inhibition of TASK-1 is likely to contribute to the increase in APD observed after stimulation of $\alpha_1$-receptors in cardiac muscle, but the relative magnitude of this current change in comparison to inhibition of $I_{\text{to}}$ [28,29] and inhibition of inward rectifier channels [30] is still unclear. The signal transduction pathway between $\alpha_1$-receptors and TASK-1 is still controversial. It has been reported that in murine cardiomyocytes platelet-activating factor inhibits $I_{\text{TASK}}$ via activation of PKCε [31]. Inhibition of TASK-1 channels via Gqα-containing heterotrimeric G proteins has also been found in neurons [5], and the downstream mechanisms have been suggested to involve depletion of membrane-associated PI(4,5)P$_2$ [25,32] or a direct effect of Gqα-subunits on the channel [33].

4.4. Conclusions

We have given a quantitative description of the current flowing through TASK-1 channels in rat cardiomyocytes and found that $I_{\text{TASK}}$ can be completely inhibited by activation of $\alpha_{1A}$-adrenergic receptors. Application of the TASK-1 blocker A293 caused an increase in action potential duration and these results could be reproduced in a mathematical model. Our findings suggest that, despite its small amplitude, inhibition of $I_{\text{TASK}}$ may induce a substantial prolongation of the action potential in rat ventricular muscle.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cardiores.2007.02.025.

References