The stretch-activated potassium channel TREK-1 in rat cardiac ventricular muscle

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Abstract

Objective: The biophysical properties and the regulation of the two-pore-domain potassium channel TREK-1 were studied in rat cardiomyocytes.

Methods: RT-PCR, immunohistochemistry and patch-clamp recording were performed in isolated rat ventricular cardiomyocytes. In some whole-cell-clamp experiments the myocytes were mechanically stretched using a glass stylus.

Results: We found strong expression of a splice variant of TREK-1 in rat heart. Immunohistochemistry with antibodies against TREK-1 showed localization of the channel in longitudinal stripes at the external surface membrane of cardiomyocytes. When the cardiomyocytes were mechanically stretched, an outwardly rectifying K+ current component could be detected in whole-cell recordings. In single-channel recordings with symmetrical high K+ solution, two TREK-like channels with ‘flickery-burst’ kinetics were found: a ‘large conductance’ K+ channel (132±5 pS at positive potentials) and a novel ‘low-conductance’ channel (41±5 pS at positive potentials). The low-conductance channel could be activated by negative pressure in inside-out patches, positive pressure in outside-out patches, intracellular acidification and application of arachidonic acid. Its open probability was strongly increased by depolarization, due to decreased duration of gaps between bursts. The biophysical properties of the two cardiac TREK-like channels were similar to those of TREK-1 channels expressed in HEK293 cells, which both displayed low- and high-conductance modes.

Conclusions: Our results suggest that the two TREK-like channels found in rat cardiomyocytes may reflect two different operating modes of TREK-1. The novel low-conductance channels described here may represent the major operating mode of TREK-1. The current flowing through mechanogated TREK-1 channels may serve to counterbalance the inward current flowing through stretch-activated non-selective cation channels during the filling phase of the cardiac cycle and thus to prevent the occurrence of ventricular extrasystoles.

Keywords: K-channel; Arrhythmia (mechanisms); Stretch; Stretch-activated K-channels; K2P channels

See Editorial by Sung Joon Kim and Yung E. Earm (pages 13–14) in this issue.

1. Introduction

The electrical activity controls the mechanical activity of the heart. The time course and amplitude of the contraction of cardiac muscle depend on action potential duration, on frequency, and on the timing and pattern of preceding action potentials. Conversely, mechanical stimulation modulates the electrical activity of the heart in a complex manner, a phenomenon termed mechanoelectric feedback [1,2].
Mechanical stimulation activates a variety of ion channels in cardiac muscle including stretch-activated non-selective cation channels [3,4] and mechanosensitive potassium channels [5–7]. Mechanical stretch of isolated cardiomyocytes can give rise to changes in action potential configuration and to pacemaker-like afterdepolarizations [2,4,7]. Mechanical stretch of isolated hearts can initiate premature beats or ventricular fibrillation [8]. The arrhythmogenic effects of mechanoelectric feedback are of considerable pathophysiological interest [2], but the physiological function of stretch-activated channels in the heart is unknown. Here we describe the stretch-activated K+ channels in ventricular cardiomyocytes at the single-channel level and we propose a role for stretch-activated channels in mechanoelectric feedback.

Two-pore-domain potassium channels (K2P channels) are a family of potassium channels characterized by very complex regulation [9]. One subfamily of K2P channels, consisting of TREK-1, TREK-2 and TRAAK, is activated by polyunsaturated fatty acids, volatile anesthetics and mechanical stretch of the cell membrane [10]. The physiological role of mechanical activation of these channels, either in brain or in other tissues, has not been elucidated so far. Recently, evidence for the expression of TREK-1 in atrial [6,11] and cardiac ventricular myocytes [12,13] has been obtained. In the present study we have characterized a TREK-like potassium channel that has a much lower conductance than the ‘classical’ TREK-like channels described previously in cardiac cells [5,6,11,13]. Our experimental results suggest that both the classical high-conductance TREK-like channel [5,13] and the novel low-conductance TREK-like channel contribute to the whole-cell K+ current activated by stretch in ventricular myocytes and that the two native channels may represent different operating modes of TREK-1. We propose that K+ efflux through TREK-like channels may counterbalance the potentially arrhythmogenic cation influx through non-selective cation channels when the cardiomyocytes are stretched at the end of the filling phase of the cardiac cycle.

2. Methods

2.1. Expression analysis

Isolation of cardiomyocytes and expression analysis of RNA from pure cardiomyocytes were performed as described previously [14]. Total RNA from isolated rat cardiomyocytes was extracted using HighPure RNA kit (Roche). Total heart RNA (1 μg) was reverse transcribed (RT) with random hexamers (Applied Biosystems) and Superscript II reverse transcriptase (Life Technologies). Qualitative RT-PCR expression analysis was performed with AmpliTaq Gold DNA polymerase (Applied Biosystems) using intron-spanning, gene-specific primers (listed in Supplementary Table 1) for TREK-1 splice forms and for cell-specific marker transcripts. Control experiments in the absence of RT were routinely performed and all PCR fragments were isolated and sequenced to verify correct amplification.

2.2. Electrophysiology

Single-channel measurements in freshly isolated cardiomyocytes, HEK293 cells or COS-7 cells were carried out as described previously [15,16] (sampling rate, 10 kHz; filter cutoff frequency 2 kHz). Pipette and bath solution contained (mM) 140 KCl, 1 MgCl2, 5 EGTA and 10 HEPES (pH, 7.3). For heterologous expression, the coding regions of rTREK-1a and rTREK-1b were amplified by PCR and cloned into the mammalian expression vector pcDNA3.1(+) (Invitrogen). Whole-cell clamp experiments investigating the effects of arachidonic acid were performed as described [17].

For whole-cell recordings during mechanical stretch, cardiomyocytes were prepared according to Isenberg and Klöckner [18]. The bath solution contained (mM) 150 NaCl, 5.4 KCl, 1.2 MgCl2, 20 glucose, and 10 HEPES/NaOH (pH, 7.4) and the pipette solution contained (mM) 140 KCl, 5 EGTA, 5.5 MgCl2, 5 Na2ATP, and 10 MOPS (pH, 6.0). Whole-cell currents were recorded with an RK-300 amplifier (Biologic). Voltage-clamp commands and mechanical stretch were controlled by custom-written software. Cardiomyocytes were continuously stimulated with 35 ms pulses from −45 to 0 mA at a rate of 1 Hz. To study the effect of cell deformation on net membrane currents, the membrane was clamped to +60 mV for 50 ms and then repolarised from +60 to −100 mA at a rate of −200 mA s−1. Curve fit and data evaluation were performed as described previously [7]. Mechanical stimulation was carried out with a glass stylus [4], the end of which was fire-polished to a semi-sphere with a diameter of ∼20 μm. Patch pipette and stylus were mounted on two piezo-driven micromanipulators (Burleigh PCS-5000) equipped with an analog input (npi, Heidelberg) for control of x and z axis positions. The distance between stylus and patch pipette was increased with a time constant of 20 ms. The extent of movement (micrometer per millivolt analog input) was calibrated in the microscope setup. Before attachment, stylus and patch electrode were separated by ∼50 μm (48±2 μm, n=41); in typical stretch experiments this distance was increased to 55 μm.

2.3. Immunofluorescence microscopy

Isolated myocytes were allowed to attach on acid-washed cover slips for 30 min. The cells were fixed with 4% PFA for 15 min, permeabilised with 0.1% Triton-X100 in PBS for 10 min and then blocked with 1% BSA and 5% horse serum for 30 min. The primary antibody for TREK-1, which detects both splice variants of the channel protein, was from Santa Cruz (TREK-1-E-19; dilution 1:100). The primary
Antibody for TASK-1 was from Alomone (dilution, 1:100). The Alexa Fluor 488 and 594 secondary antibodies were from Molecular Probes. The specificity of the antibodies was checked by pre-absorption with the blocking peptides supplied by the manufacturers. Wide field images of myocytes stained for TREK-1 were obtained with an inverted microscope (Olympus IX70) equipped with a ×60 objective (NA, 1.45) and a digital camera (PCO SensiCam, QE). Confocal images from 15 cardiomyocytes co-stained for TREK-1 and TASK-1 were obtained with a Zeiss LSM 510 Meta. Z-stacks were acquired at distances between 300 and 560 nm.

2.4. Statistics and general conditions

Data are reported as means ± standard deviation. Statistical significance was calculated using Student’s t-test. All experiments were carried out at room temperature (22 °C). 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).

3. Results

3.1. Expression of TREK-1 channels in cardiomyocytes

The potassium channel TREK-1 (KCNK2) has at least two splice variants differing in exon 1 at their extreme N-terminus. The gene structure of human and rat TREK-1 is shown in Fig. 1A. The channel sequence originally described for the mouse [19] is denoted TREK-1a here. TREK-1a from rat (accession number AY727922) differs

![Gene structure of TREK-1](image)

Fig. 1. Gene structure of TREK-1. (A) Gene structure of hTREK-1 and rTREK-1 and the nucleotide sequences of the two variants of exon 1 (Exon 1a and Exon 1b). (B) N-terminal cDNA and amino acid sequences of human and rat TREK-1 splice variants. Accession numbers: hTREK-1b, AF004711; rTREK-1b, AF325671 and AF385402. The splice sites of the first intron are underlined and bold, the coding sequences are given in capital letters. (C) Alignment of the N-terminal region of the splice forms of TREK-1.
from its mouse ortholog in only six amino acids. In all orthologs of TREK-1a, exon 1 encodes only a methionine residue. In the other splice variant, denoted TREK-1b here, exon 1 encodes 16 amino acids [20] (Fig. 1B,C). RT-PCR experiments showed that both splice variants of TREK-1 are expressed in rat heart (Fig. 2A). Three sets of primers were used (Fig. 2C): (i) a primer pair recognizing all TREK-1 cDNAs (green arrows), (ii) a primer pair recognizing both TREK-1a and TREK-1b (red and black arrows), and (iii) a primer pair specifically recognizing TREK-1b (blue and black arrows). Note that two bands were obtained with the second primer pair, the smaller one of which was related to TREK-1a (Fig. 2A, arrow). The appearance of the double band is due to the fact that part of the non-coding nucleotide sequence is shared by the mRNA of TREK-1a and TREK-1b (Fig. 2C, grey shading upstream of exon 1). The other stretch-sensitive K2P channels, TREK-2 and TRAAK, could not be detected with specific primers (not illustrated). Using cell-specific expression analysis [14], we found robust expression of TREK-1b in rat cardiomyocytes (rCMC). Re-amplification of the PCR-products showed that TREK-1a is also expressed, albeit very weakly (Fig. 2B).

Immunohistochemistry with antibodies against TREK-1 showed a striking localization pattern. Wide field images of TREK-1 proteins, focused on the surface of rat cardiomyocytes, revealed distinct longitudinal stripes (Fig. 3A). The specificity of the TREK-1 antibody was checked by transfecting COS-7 cells with TREK-1a. Fig. 3B shows TREK-1 positive transfected cells; no staining was seen in non-transfected cells (Fig. 3C). After pre-absorption with the antigenic peptide only very weak punctuate staining was

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Fig. 2. RT-PCR analysis of TREK-1a and TREK-1b. (A) RT-PCR from rat heart. (B) Cell-specific RT-PCR of rat cardiomyocytes (rCMC). Troponin T (TropT) was used as a marker for cardiomyocytes, endothelin-1 (ET-1) for endothelial cells, and calponin-1 (hCalp) for vascular smooth muscle cells; GAPDH was used as housekeeping gene. Endothelin-1 and calponin-1 signals were negative in all myocyte preparations tested (n = 5). Robust expression of TREK-1b was detected with 35 PCR cycles. Re-amplification showed very weak expression of TREK-1a. (C) Schematic drawing of the mRNA of TREK-1a and TREK-1b. The primer pair recognizing all TREK-1 cDNAs (lane 2 in panel A) is indicated by green arrows. The forward primers for TREK-1a (red) and TREK-1b (blue) were used in combination with the same reverse primer (black).

Fig. 3. Immunofluorescence microscopy of TREK-1 channels. (A) Immunostaining for TREK-1 proteins; the image was focused on the surface of the cell (scale bar, 5 µm). (B–E) Tests for the specificity of the TREK-1 antibodies. COS-7 cells were immunostained 48 h after transfection with TREK-1a (B) or at the same time point without transfection (C). (D) Control image taken after pre-absorption of the antibodies with the antigenic peptide. (E) Control image taken only with the secondary antibody. The scale bar for panels B–E is 1 µm.
observed (Fig. 3D). The secondary antibodies showed no unspecific staining (Fig. 3E). Confocal imaging of cardiomyocytes stained for TREK-1 channel proteins (n=15) demonstrated that the longitudinal stripes of TREK-1 channels (red) were restricted to the cell surface membrane (Fig. 4). The localization of TREK-1 fluorescence to the sarcolemma can also be seen in the z-stack presented online as Supplementary Fig. 1. The cardiomyocytes were co-stained with TASK-1 antibodies (green) to obtain a better overview of the three-dimensional structure of the cells. Staining of the T-tubular membrane with the rat TASK-1 antibody has been reported previously [21].

3.2. Changes in whole-cell current induced by arachidonic acid and axial stretch

Functional expression of the channels was tested by measuring the effect of arachidonic acid, a known activator of TREK-1 [10], on the steady-state current–voltage relation of ventricular cardiomyocytes. To eliminate any contribution of ATP-sensitive K+ channels and voltage-activated Ca2+ channels, the measurements were carried out in the presence of 1 μM glibenclamide and 100 μM CdCl2. Application of 10 μM arachidonic acid induced an additional steady-state outward current between −60 and +30 mV that increased with depolarization (Fig. 5). The difference current measured after application of arachidonic acid reversed near the calculated K+ equilibrium potential (−84 mV) and showed outward rectification. Under control conditions the current measured at +30 mV was 164±44 pA (n=26); 10 min after application of 10 μM arachidonic acid the current significantly increased to 266±46 pA (n=26; p<0.05). Since the other two arachidonic acid-sensitive K2p channels, TREK-2 and TRAAK, are not expressed in rat heart our findings suggest that TREK-1 can carry a substantial current component in the plateau range of potentials.

Another important characteristic of TREK-1 is activation by membrane stretch. Therefore we studied the changes in whole-cell current induced by local axial stretch of cardiomyocytes. Previous studies in mouse and guinea-pig cardiomyocytes had shown that axial stretch changed several current components including currents carried by stretch-activated non-selective cation channels (SACs), stretch-activated K+ channels (SAKs) and inward rectifier channels [4,7]. We have now confirmed these findings for rat cardiomyocytes (not illustrated). The stretch-activated current at positive potentials could be described by two components: a linear stretch-activated non-selective cation current with a reversal potential of −10 mV [4,6] and an outwardly rectifying stretch-activated K+ current. Application of 5 μm stretch increased the outward current at +60 mV from 950±283 to 1542±496 pA (n=10); this increase includes currents carried by both SAKs and SACs.

To study the stretch-activated K+ current in more detail, we carried out whole-cell measurements in the presence of a blocker cocktail that minimized L-type Ca2+ current, Na+/Ca2+ exchange current, inward rectifier current, ATP-sensitive K+ current and the effects of β-adrenergic receptors (which might inhibit TREK-1 channels via PKA [11]). We also tried to eliminate SACs with gadolinium (20 μM GdCl3), but this blocked the mechanosensitivity of the K+ current components as well. Since outward currents through SACs may superimpose on mechanosensitive K+ currents, we selected those experiments (5 out of 10) for analysis in which stretch produced only a small difference current at the K+ equilibrium potential (<50 pA between −87 and −91 mV).

Fig. 6A shows a representative example of the outward currents recorded in the presence of the blocker cocktail. Application of stretch, i.e. increasing the distance between stylus and patch pipette by 5 μm, increased the stretch-activated K+ current at +60 mV (olive line in Fig. 6B) by ≈200 pA. On average, 5 μm stretch increased the current at +60 mV from 1120±90 to 1320±40 pA (n=5). This increment in outward current was much larger than the calculated linear current component related to opening of SACs (light blue line). The voltage dependence of the difference current (Fig. 6C) showed outward rectification.

![Fig. 4. Subcellular localization of TREK-1 and TASK-1 in cardiomyocytes. Confocal image of a rat cardiomyocyte co-stained for TREK-1 (red) and TASK-1 (green); XY (bottom), XZ (top) and YZ (right) plane. Sarcomere length was 1.75 μm.](image-url)
3.3. Native TREK-like K+ channels in rat cardiomyocytes

To get more information on the elementary events underlying the stretch-activated K+ current we then turned to single-channel recording. In outside-out patches from ventricular myocytes we found two K+ channels that could be activated by stretch of the patch membrane and displayed the ‘flickery burst’ kinetics typical for K2P channels: bursts of brief openings interrupted by extremely short closures and relatively long closures in between bursts (Fig. 7AB). Typical all-point histograms of the two channels measured at ~40 mV are shown in Fig. 7CD.

The channel with the larger conductance (Fig. 7B), found in ~15% of outside-out patches, showed outward rectification and had a conductance of 132.3 ± 5.1 pS (n = 4) at positive potentials (Fig. 7F, ■). Its biophysical properties were similar to those of the ‘classical’ TREK-1 channel [5,11,13,22] and were therefore not studied further. The channel with the smaller conductance (Fig. 7A) has not been described previously in cardiac muscle. It showed slight inward rectification and had a conductance of 41.4 ± 4.8 pS (n = 10) at positive potentials (Fig. 7E, ■). This channel was found in ~60% of outside-out patches. The filled circles in Fig. 7E and F show the single-channel current–voltage relations measured after reduction of extracellular K+ from 140 to 32 mM. The extrapolated reversal potential (×) of both channels was shifted by about ~34 mV, as predicted by the Nernst equation, indicating that the channels were K+ selective. The conductance of both TREK-like channels depended on the presence of extracellular divalent cations. Removal of external Mg2+ induced a large increase of the conductance in the inward direction. In the low-conductance channel, removal of external Mg2+ increased the slope conductance at +60 mV from 60.7 ± 6.0 (n = 10) to 155.7 ± 14.8 pS (n = 4; Fig. 7E, triangles). In the high-conductance channel removal of external Mg2+ increased the slope conductance at ~60 mV from 52.5 ± 4.5 (n = 5) to 209.3 ± 12.0 pS (n = 3; Fig. 7F, triangles), resulting in inward rectification.

In another series of experiments we tested the effects of K+ channel blockers on the smaller TREK-like channel. The current amplitude at +40 mV in outside-out patches was unchanged by application of 1 mM TEA (2.10 ± 0.29 vs. 2.07 ± 0.34 pA; n = 4), 1 mM 4-amino-pyridine (2.03 ± 0.30 vs. 2.02 ± 0.33 pA; n = 3) or 1 mM BaCl2 (1.97 ± 0.36 vs. 1.94 ± 0.31 pA; n = 3). The value of NPo at +40 mV was not affected by any of the three drugs.

3.4. Modulation of open probability by pH, stretch and arachidonic acid

The open probability (P0) of the novel cardiac TREK-like K+ channel increased with intracellular acidification (Fig. 8A). Compared to control (pH 7.3), NPo (number of channels in the patch × P0) increased by a factor of 5.7 at pH 6.8 and by a factor of 136 at pH 6.3 (Fig. 8D). NPo could also be increased by changes in hydrostatic pressure applied to the patch pipette. In inside-out patches, application of negative pressure (~40 cm H2O; Fig. 8B) increased NPo by a factor of ~27, whereas application of positive pressure had no effect. In outside-out patches,
positive pressure (+80 cm H₂O; Fig. 8B) increased NP₀ by a factor of ~3, whereas negative pressure had no effect. Thus, in both inside-out and outside-out patches, distension of the cell membrane appeared to increase P₀ (Fig. 8D).

Arachidonic acid activated the novel TREK-like channel in inside-out patches (Fig. 8C). On average, NP₀ increased by a factor of ~18 during application of 10 μM arachidonic acid (n=4; Fig. 8D). Thus, the novel channel shared many
of the characteristic properties of classical TREK-1 channels [10].

3.5. Heterologous expression of TREK-1a and TREK-1b

To test whether one of the native cardiac TREK-like K+ channels might correspond to TREK-1 we expressed TREK-1a and TREK1b in HEK293 cells. The biophysical properties of the two splice variants were virtually identical (Supplementary Fig. 2). Surprisingly, transfection of either splice variant in HEK293 cells produced two populations of channels with different conductances. In all inside-out patches (18/18) of TREK-1a we found a low-conductance channel very similar to that observed in cardiomyocytes, with a conductance of 65.8 ± 4.6 pS (n = 12) at negative potentials and 37.4 ± 5.9 pS at positive potentials; the corresponding values for TREK-1b (n = 7) were 65.9 ± 4.9 and 38.0 ± 4.1 pS. In some of the inside-out patches of TREK-1a channels (n = 7/18) we found, in addition, a high-conductance channel that opened and closed independently (Supplementary Fig. 3). This channel was reminiscent of classical TREK-1; at positive potentials it had a mean conductance of 105.4 ± 24.4 pS in the case of TREK-1a (n = 4) and 96.6 ± 27.8 pS in the case of TREK-1b (n = 3). In cell-attached patches, both channel types were also regularly observed. Expression of the TREK-1 splice variants in COS-7 cells gave similar results as in HEK293 cells (Supplementary Fig. 4). In a few cases, the channel changed from the high- to the low-conductance mode or vice versa (Supplementary Fig. 3). In conclusion, the biophysical properties of the two splice variants were identical in all patch-clamp configurations examined. Two different modes of TREK-1a or TREK-1b channels were observed, but the majority of patches showed only the low-conductance mode.

3.6. Characterization of the low-conductance TREK-1b channel

We then compared biophysical properties of the low-conductance cardiac TREK-like channel (Fig. 9A) with those of the low-conductance mode of TREK-1b expressed in HEK293 cells (Fig. 9B). Their single-channel current–voltage relations were indistinguishable (Fig. 9C, Δ, Δ). The conductance of the cardiac channel was 67.2 ± 1.9 pS (n = 4) at negative potentials and the conductance of TREK-1b was 65.9 ± 4.9 pS (n = 7). The kinetics of channel opening was also very similar. Both the native and the cloned channel opened in bursts interrupted by very short closures. At +40 mV, the smaller TREK-like channel in cardiomyocytes had a mean open time of 0.29 ± 0.03 ms (n = 3) and TREK-1b had a mean open time of 0.28 ± 0.03 ms (n = 3).
Fig. 9. Comparison of cardiac TREK-like channels with cloned TREK-1b channels. (A) Inside-out recording of the low-conductance channel in rat cardiomyocytes. (B) Inside-out recording of TREK-1b expressed in HEK cells. The recordings were performed in symmetrical high-K⁺ solution. Note that the gaps between bursts are shorter at positive potentials. (C) Current–voltage relations of transfected TREK-1b channels in HEK293 cells (△) and of the low-conductance TREK-like channel in cardiomyocytes (●). (D) Whole-cell current–voltage relation of HEK293 cells transfected with TREK-1b (○; n = 6) and of mock-transfected cells (■; n = 18), recorded in high-K solution. The insets show whole-cell current traces obtained from transfected (lower right) and mock-transfected cells (upper left). (E) All-point amplitude histograms measured at +40 mV (upper panel) and at −40 mV (lower panel) in an inside-out patch from a transfected HEK293 cell. (F) Dependence of NPo of TREK-1b channels on membrane potential.
like the cardiac TREK-like channels, could be activated by stretch and by intracellular acidification (not illustrated).

Despite the inward rectification of the single-channel current–voltage relation, the whole-cell current in HEK293 cells transfected with TREK-1b showed slight outward rectification (Fig. 9D; ○). This apparent discrepancy was probably due to an increase in open probability with depolarization [20]. Typical amplitude histograms of a single TREK-1b channel at −40 and +40 mV are shown in Fig. 9E. \( N_p \) was estimated by dividing the amplitude histogram at the minimum between the two peaks (dotted line) and calculating the ratio of data points corresponding to the open and to the closed state, respectively. Fig. 9F shows that \( N_p \) strongly increased with depolarization in the voltage range −80 to +80 mV. Analysis of single-channel kinetics in outside-out patches from transfected HEK293 cells showed that the higher open probability of TREK-1b channels at more positive potentials was mainly attributable to shorter gaps between bursts (Supplementary Fig. 5).

Taken together, our kinetic measurements suggest that under physiological conditions the whole-cell current flowing through low-conductance TREK-1 channels should be outwardly rectifying, despite the fact that, with symmetrical K⁺ concentrations, the single-channel current–voltage relation showed inward rectification (Fig. 9C). The outward rectification is due, firstly, to the voltage dependence of \( N_p \) and, secondly, to the asymmetric K⁺ concentrations across the sarcolemma.

4. Discussion

4.1. TREK-like channels in cardiomyocytes

The first TREK-like K⁺ channels in rat ventricular myocytes were recorded by Donghee Kim [25,22,23]. Although K₂P channels were not known at the time, the channel described by Kim resembles TREK-1 in many respects: it showed the typical ‘flickery bursts’ and was activated by arachidonic acid, stretch and intracellular acidification. With symmetrical K⁺ concentrations, the channel displayed outward rectification with a slope conductance of 106 pS at +60 mV [5]. A similar ventricular stretch-activated, pH-sensitive channel was later found by Tan et al. [13]. This channel displayed a nearly linear current–voltage relation with a slope conductance of 111 pS at +60 mV. The high-conductance cardiac channel described here is clearly different: it showed inward rectification with a mean conductance of 66 pS at negative and 41 pS at positive potentials. It was found in ~60% of outside-out patches, about four times more frequently than the high-conductance TREK-like channel. The following considerations suggest that this novel channel was also encoded by TREK-1: (i) its single-channel current–voltage relation was indistinguishable from that of the low-conductance mode TREK-1a or TREK-1b expressed in HEK cells; (ii) its kinetics was very similar to those of the low-conductance mode of TREK-1b; (iii) both channels were activated by stretch and intracellular acidification; and (iv) TREK-1b was robustly expressed in cardiomyocytes, whereas the two other stretch-sensitive K⁺ channels, TREK-2 and TRAAK, could not be detected. Since TREK-1a was barely detectable by cell-specific RT-PCR, most of the cardiac low- and high-conductance TREK-like channels were probably encoded by TREK-1b.

The properties of TREK-1a and TREK-1b channels were virtually identical. Both splice variants showed a low- and a high-conductance mode and both modes were regulated by physical and chemical stimuli in a very similar way. The simplest explanation for this surprising observation is that covalent modification of the channel or interaction with an accessory intracellular protein may induce a second mode of TREK-1 channels with a different conductance. However, it is also possible that the two conductance modes are related to mechanical tension of the membrane or to interaction of the channel protein with phospholipids [30].

The biophysical characteristics of two modes of TREK-1 channel activity described here are quite different from the two open states of TREK-1 channels observed by Bockenhauer et al. [20] in the presence of Mg²⁺, which were “consistent with unblocked and blocked current levels at negative potentials” [20]. On the other hand, our findings are in line with the results of Han et al. [28] who found four different arachidonic acid-activated TREK-like K⁺ channels in magnocellular hypothalamic neurons. One of these channels (denoted \( i_{\text{K,AA3}} \)), which was observed more frequently than the others, had properties almost identical to the low-conductance cardiac channel described here, whereas the other endogenous K⁺ channels showed the properties of classical TREK-1, TREK-2 and TRAAK. Thus, neuronal TREK-1 channels may also have two conductance modes.

4.2. Stretch-activated whole-cell currents in cardiac muscle

Confocal imaging showed that TREK-1 channel proteins are arranged in longitudinal stripes at the surface of
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cardiomyocytes. This pattern appears to be suitable for sensing longitudinal stretch of the cells. There is no obvious co-localization with membrane-associated cytoskeletal proteins, which are mostly arranged as “costamers” [31]. In cells superfused with physiological salt solution, stretch activated a large outward current at positive potentials that was probably attributable to activation of both SACs and SAKs [4,7]. We succeed in isolating the current component flowing through SAKs by applying only moderate stretch, by selecting cells that showed only little current through SACs and by inhibiting other current components with a blocker cocktail. The remaining stretch-activated whole-cell current showed outward rectification and was probably carried by both the high-conductance and the low-conductance mode of TREK-1 channels. It should be noted, however, that we studied only steady-state current changes. Single-channel recordings of stretch-activated TREK-like K+ channels in rat atrium [6] showed substantial inactivation (or adaptation) with a time constant of ~1 s. Thus, the phasic stretch-activated K+ current flowing during myocardial stretch in vivo, for example at the end of diastole during high preload, may be much larger than the steady-state outward current shown here. Furthermore, TREK-1 currents are expected to be much larger at 37 °C [29].

4.3. The possible functional role of TREK-1 channels in the heart

In the beating heart, stretch may activate both SACs and SAKs. The net effect of stretch applied during diastole is usually a depolarization that may initiate ventricular premature beats or even fibrillation when it reaches threshold [2,32–34]. However, arrhythmogenesis may not be the only function of SACs and SAKs; it is conceivable that they may also play a role in the length-dependent regulation of contractile force of cardiomyocytes. Rapid stretch of cardiac muscle produces an instantaneous change in isometric force of contraction, which is followed by slow increase in force over several minutes. This slow force response (also denoted the Anrep effect) is associated with a slow increase in the magnitude of the intracellular calcium transient [35,36]. It has been proposed that opening of SACs contributes to the slow length-dependent changes in the force of contraction [37–39]. At diastolic negative potentials currents through SACs are predominantly carried by Na+ ions [7], i.e. stretch of the myocytes during the ventricular filling phase is thought to increase the submembrane Na+ concentration, which in turn augments the cellular Ca2+ load via Na+/Ca2+ exchange [38,40]. The resulting increased filling of the intracellular Ca2+ stores [37–40] might contribute to the slow force response observed after an increase in preload.

These considerations suggest that Na+ influx through SACs during the late filling phase of the cardiac cycle may make a major contribution to the slow force response [37–39] and the same mechanisms may also be responsible for arrhythmic afterpotentials [38]. The simultaneous opening of SAKs might serve to prevent excessive diastolic depolarization under conditions of high preload. Thus, one of the major functions of TREK-1 channels in cardiomyocytes may be to counterbalance the inward current produced by activation of SACs and to prevent the occurrence of ventricular extrasystoles.

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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.2005.08.018.

References


