Knock-Out of the Potassium Channel TASK-1 Leads to a Prolonged QT Interval and a Disturbed QRS Complex

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Key Words

Prolonged QT interval • TASK-1 knock-out • Cardiac repolarization • *In vivo* electrophysiology • Ventricular arrhythmias

Abstract

Background/Aims: The aim of the study was to characterize the whole cell current of the two-pore domain potassium channel TASK-1 (K2P3) in mouse ventricular cardiomyocytes ($I_{\text{TASK-1}}$) and to analyze the cardiac phenotype of the TASK-1^{-/-} mice. **Methods and Results:** We have quantified the ventricular $I_{\text{TASK-1}}$ current using the blocker A293 and TASK-1^{-/-} mice. Surface electrocardiogram recordings of TASK-1^{-/-} mice showed a prolonged QTc interval and a broadened QRS complex. The differences in electrocardiograms between wild type and TASK-1^{-/-} mice disappeared during sympathetic stimulation of the animals. Quantitative RT-PCR, patch clamp recordings and measurements of hemodynamic performance of TASK-1^{-/-} mice revealed no major compensatory changes in ion channel transcription.

Action potential recordings of TASK-1^{-/-} mouse cardiomyocytes indicated that $I_{\text{TASK-1}}$ modulates action potential duration. Our *in vivo* electrophysiological studies showed that isoflurane, which activates TASK-1, slowed heart rate and atrioventricular conduction of wild-type but not of TASK-1^{-/-} mice. **Conclusion:** The results of an invasive electrophysiological catheter protocol in combination with the observed QRS time prolongation in the surface electrocardiogram point towards a regulatory role of TASK-1 in the cardiac conduction system.

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Introduction

Action potential duration of cardiomyocytes depends on the magnitude and kinetics of repolarizing potassium currents. Several potassium currents contributing to the repolarization phase of the action potential have been identified so far, including I_{K1} , I_{to} , I_{Ks} , I_{Kr} and I_{Kur} [1]. It has

been speculated that two-pore-domain K^+ (K_{2p}) channels may contribute to a 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_{ss}) [1, 2]. Cardiomyocytes express several K_{2p} channels including the stretch-activated K^+ channel TREK-1 (K2P2.1) [3], and the acid-sensitive K^+ channel TASK-1 (K2P3) [4-6]. The contribution of these channels to cardiac electrophysiology is still unknown. Recently, we provided a quantitative description of the TASK-1 current I_{TASK-1} in rat ventricular cardiomyocytes [6]. We used extracellular acidification, G_i -coupled current inhibition (by an α_1 -adrenergic agonist) and a novel TASK channel blocker, A293, as tools to separate I_{TASK-1} from other potassium currents [6]. The results of this study suggest that I_{TASK-1} shortens the action potential in rat ventricular cardiomyocytes.

In a recent study, Donner et al. have characterized some aspects of the phenotype of the TASK-1 knock-out mouse [7]. They found a significant prolongation of monophasic action potentials recorded extracellularly and a significant prolongation of the rate-corrected QT-interval in TASK-1 knock-out mice. In addition, the knock-out mice showed a higher heart rate than control animals [7]. In the present study, which was carried out independently and in parallel, we have also characterized TASK-1 knock-out mice, and our work partially confirms and extends the results of Donner et al. [7].

TASK-1 is predominantly expressed in ventricles of the developing heart and in the conduction system of adult mice [8]. We have characterized I_{TASK-1} in adult mouse ventricular cardiomyocytes and show that the TASK-1 knock-out results in a broadening of the QRS complex and a prolongation of the QT interval in the electrocardiogram. These changes in the electrocardiogram were not observed when the mice were treated with an intraperitoneal infusion of isoprenaline. In addition, we found that isoflurane, which activates TASK-1 channels [9, 10], slows heart rate and atrioventricular conduction in wild-type mice, whereas these effects are not present in TASK-1^{-/-} mice. Taken together, our results suggest that TASK-1 may be important for the conduction of cardiac excitation and for the ventricular repolarization under resting conditions of the heart.

Materials and Methods

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) and has been approved by local authorities.

TASK-1^{-/-} mice and genotyping

TASK-1 deficient mice (C57/Bl6x129Sv, more than 10 backcrosses) were obtained from the Department of Clinical Neurobiology, University of Heidelberg [11]. For all experiments TASK-1 knock-out mice were compared to wild-type littermates. For genotyping, DNA was isolated from mouse tails (DNeasy Blood&Tissue kit, Qiagen, Hilden, Germany). Genotyping has been performed as previously described [11].

Expression analysis

Reverse transcription and polymerase chain reaction (RT-PCR) were performed as described previously [6]. Quantitative RT-PCR was performed using an MX3000P real-time PCR system (Stratagene, Santa Clara, United States). Control experiments in the absence of RT were routinely performed, and all PCR products were sequenced to verify correct amplification. Reverse transcription and polymerase chain reaction (RT-PCR) were performed as described previously. Quantitative RT-PCR was performed using an MX3000P real-time PCR system (Stratagene, Santa Clara, United States). Control experiments in the absence of RT were routinely performed, and all PCR products were sequenced to verify correct amplification. Primers used in quantitative RT-PCR expression analysis: mGAPDH for 5'-ACT TCA ACA GCA ACT CCC ACT CT-3', rev 5'-GCT GTA GCC GTA TTC ATT GTC ATA-3'; mTASK-1 for 5'-TAT TAC TAC TGC TTC ATC ACC CTC A-3', rev 5'-ACG AAG CTG AAG GCC ACA TAC T-3'; mTASK-2 for 5'-CTA TTC CTT CAT CAC CAT CTC CAC-3', rev 5'-AGA TCC AAA GCT CCA CAA AGT ATC-3'; mTASK-3 for 5'-CTT CTG TAT GTT CTA CGC TGT GCT-3', rev 5'-TTC TTG ATA CGT TTC AGC AGG TAG-3'; mTREK-1 for 5'-GAC GAA CTC ATC CAG CAA ATA GTG-3', rev 5'-AAA GAA GAA AGA GCT TCC GAG GTC-3'; mKv1.5 for 5'-CTT CAT CGG AGT CAT CCT CTT CT-3', rev 5'-ATA GTG ACT ACT GCC CAC CAG AAT-3'; mKv2.1 for 5'-CTG TGC TGA GAA GAG GAA GAA ACT-3', rev 5'-AAT GGT GGA GAG GAC AAT GAA C-3'; mKv4.3 for 5'-AGA CCA CTG GGT TAT CCT ATC TTG-3', rev 5'-CAT GCA GTT CTG CTC AAA CAT CT-3'; mKir2.1 for 5'-TTT GTA GTG CCA GAG ACT TAG CAG-3', rev 5'-TCA CTA TCC TCC TCT TCC TCT TTG-3'; mERG for 5'-GCT TAC TGC CCT CTA CTT CAT CTC-3', rev 5'-TAT AGG TTC AGA GGC TCT CCA AAG-3'; mKCNQ for 5'-TGT TCC TCA CAT CAC TTA TGA TCC-3', rev 5'-TGT GCT TAC TTC CAG CAG TGT AG-3'.

Patch-clamp experiments with isolated mouse cardiomyocytes

Ventricular myocytes were isolated from anesthetized (5% isoflurane/ 95% O₂, per inhalation) adult animals, as described previously [12, 13]. Myocytes from either ventricle were superfused with solution containing (in mM): 140 NaCl, 5.4 KCl, 1 CaCl₂, 1 MgCl₂, 0.33 NaH₂PO₄, 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 ~4 MΩ. The pipette solution contained (in mM):

60 KCl, 65 K-glutamate, 5 EGTA, 2 MgCl₂, 3 K₂ATP, 0.2 Na₂GTP and 5 HEPES (pH 7.2 with KOH). Data acquisition was performed with an EPC10 amplifier and Patchmaster Software (HEKA Instruments). Data were analyzed using Fitmaster or Origin Software (Origin Labs).

Electrocardiograms in vivo

Electrophysiological studies *in vivo* were performed essentially as described previously [14]. Animals were placed on a thermo regulated plate (37°C) and anesthetized using a single intraperitoneal injection of 2.5% Avertin® (tribromoethanol, 250-350 mg/kg body weight) while breathing spontaneously. Electrocardiographic recordings were performed at 10 kHz with subcutaneous 27-gauge needles placed in all limbs. After stabilization of the preparation, a control recording period (2 min) was followed by intraperitoneal injection of isoprenaline (2 mg/kg), and electrocardiograms were recorded for an additional time period. Electrocardiograms were analyzed for RR interval, heart rate, QT, QT_c, QRS width, and PR interval. QT_c was calculated after Bazett's formula: QT_c = QT/RR^{0.5} (QT in msec, RR in sec) [15].

In vivo electrophysiological analyses

In vivo electrophysiological analyses of TASK-1^{-/-} hearts were performed as described before [16]. Animals were anesthetized by inhalation of isoflurane (0.8-2.5%) diluted in oxygen. An octapolar catheter was advanced from the right internal jugular vein through the right atrium to the right ventricle. The distal electrodes were used to pace and record from the right ventricle, while the proximal electrodes were used to pace and record from the right atrium. Recordings were displayed and simultaneously stored by a computer via an analog-to-digital converter. Standard clinical pacing protocols [16] were used to determine basic electrophysiological parameters, including atrio-ventricular nodal conduction properties and refractory periods.

Hemodynamic measurements

Hemodynamic measurements were carried out as previously described [17]. Briefly, mice were anesthetized by inhalation of isoflurane (5% with 95% O₂) while they were placed on a heated board to maintain body temperature at 37°C, intubated, and connected with a rodent respirator (Harvard Apparatus). A Millar® catheter (1.4 F, model SPR671) was inserted into the right carotid artery for hemodynamic measurements. The catheter was placed into the aorta and left ventricle for respective measurements under light anesthesia.

Drugs

A293 (2-(Butane-1-sulfonyl-amino)-N-[1-(R)-(6-methoxypyridin-3-yl)-propyl]-benz-amide) was obtained from Sanofi Aventis GmbH Germany. E-4031, HMR-1556, 4-AP, glibenclamide and nifedipine (Sigma) were stored as DMSO stocks and final DMSO concentration did not exceed 0.1%.

Statistics and general conditions

Data are reported as means ± S.E.M.. Statistical significance was calculated using Student's t-test. All experiments

Fig. 1. Quantitative RT-PCR analysis of K⁺ channel expression in mouse ventricular myocytes (n = 6). The inset shows RT-PCR data from hearts of wild-type and TASK-1^{-/-} animals, confirming the lack of TASK-1 mRNA transcripts after KCNK3 knock-out.

were performed at room temperature (22 - 24 °C). *, indicates *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

Results

Expression of K_{2p} channels in mouse cardiomyocytes

The K_{2p} channels TASK-1 and TREK-1 are expressed in mouse heart [4, 18, 19]. Using quantitative PCR experiments from isolated mouse heart ventricles, we analyzed the relative expression of TASK-1 in comparison to other potassium channels (Fig. 1). TASK-1 showed a robust transcription similar to that of Kir2.1 (Fig. 1); TASK-1 mRNA levels were higher than those for ERG, KCNQ1 and Kv1.5 channels (Fig. 1). We also found transcripts of TREK-1, albeit not at the same expression level as for TASK-1. Next, we studied TASK-1^{-/-} mice, which were generated and obtained by Aller et al. and bred as previously described [11]. Control experiments with hearts from TASK-1^{-/-} mice show lack of TASK-1 mRNA transcripts (Fig. 1, inset).

Fig. 2. Characterization of I_{TASK-1} in mouse ventricular myocytes. Knock-out of TASK-1 leads to a loss of the methoxamine- and A293-sensitive I_{TASK-1} . Steady-state currents were recorded using voltage steps of 500 ms duration to voltages in the range of -90 to +40 mV, in 10 mV increments. Holding potential was 0 mV to inactivate voltage dependent potassium channels. Sweep time interval was 10 s. For all experiments $n = 8$. (A) Steady-state current-voltage relations for wild-type mice before (black circles) and after application of 10 μ M methoxamine (open circles). The difference curve representing I_{TASK-1} is plotted as open squares. (B) The same experiments as in (A) using methoxamine were performed for $TASK-1^{-/-}$ mice. (C) I_{TASK-1} density from (A) and (B) plotted as methoxamine-sensitive current at +30 mV in wild-type and $TASK-1^{-/-}$ mice. (D) Steady-state current voltage relations for wild-type mice before (black circles) and after application of 200 nM A293 (open circles). The difference curve representing I_{TASK-1} is plotted as open squares. The same voltage protocol was used as described above. (E) The same experiments with A293 performed with myocytes from $TASK-1^{-/-}$ mice. (F) I_{TASK-1} density plotted as A293-sensitive current at +30 mV in wild-type and $TASK-1^{-/-}$ mice.

TASK-1 knock-out mice have a complete loss of methoxamine- and A293-sensitive current

In order to quantify the current flowing through TASK-1 channels in mouse ventricular cardiomyocytes, we analyzed the effects of the selective α_1 -adrenergic agonist methoxamine and the TASK-1 blocker A293 on the steady-state current-voltage relation. In these experiments, the holding potential applied prior to the voltage steps was 0 mV to inactivate voltage dependent potassium channels, and a blocker mixture containing 2 μ M HMR1556, 2 mM 4-aminopyridine, 2 μ M glibenclamide, 10 μ M nifedipine and 5 μ M E-4031 to block I_{K_S} , I_{to} , $I_{K_{ATP}}$, I_{Ca} and I_{K_r} was used [6]. In this way, current components other than I_{TASK-1} were minimized or abolished (see also [6]).

In the presence of the blocker mixture, application of 10 μ M methoxamine reduced the steady-state outward current (Fig. 2A). The methoxamine-sensitive outward current, analyzed at +30 mV, was 0.39 ± 0.04 pA/pF ($n = 8$) (Fig. 2A, C). In contrast, no significant methoxamine-sensitive current was observed in cardiomyocytes from $TASK-1^{-/-}$ mice (Fig. 2B, C). These findings are consistent with the idea that in mouse cardiac muscle, like in rat cardiomyocytes [6], activation of α_1 -adrenoceptors leads to inhibition of TASK-1 channels.

To further characterize I_{TASK-1} , we studied the effect of the blocker A293 on the current-voltage relation of mouse cardiomyocytes. We have previously shown, that at low concentrations A293 is a selective blocker of

Fig. 3. Cardiac phenotype of TASK-1^{-/-} mice. (A) Representative electrocardiogram of a wild-type (black) and a TASK-1^{-/-} mouse (grey) under resting conditions. (B-E) Statistics of electrocardiogram parameters recorded from wild-type (n = 8) and TASK-1^{-/-} animals (n = 8). Data for wild-type animals are represented by black bars. For TASK-1^{-/-} data are shown in white. In (B) heart rate (HR) and RR interval, in (C) PR interval and P duration (P_{dur}), in (D) QT time, QTc time and in (E) QRS interval are presented. (F) Representative electrocardiogram recordings of either wild-type or TASK-1^{-/-} animals after intraperitoneal application of isoprenaline. In (G) QT, QTc and QRS interval times of either wild-type (n = 8) or TASK-1^{-/-} animals (n = 8) are presented. The first 2 min are always analyzed under resting conditions, isoprenaline was injected i.p. after 2 min.

TASK-1 and TASK-3 (K2P9.1) [6]. Given the poor expression of TASK-3 in the mouse heart, A293, at low concentrations, was used to specifically isolate $I_{\text{TASK-1}}$. The effects of A293 on the current-voltage relation of cardiomyocytes in the presence of the blocker mixture are shown in Fig. 2D-F. The A293-sensitive outward current analyzed at +30 mV was 0.45 ± 0.03 pA/pF (n = 8) (Fig. 2D, F). The A293-sensitive current was abolished in cardiomyocytes from TASK-1^{-/-} mice (Fig. 2E, F).

In summary, these experiments show that $I_{\text{TASK-1}}$ in mouse ventricular cardiomyocytes has a current density of about 0.4 pA/pF similar as previously described for rat ventricular myocytes [6].

TASK-1 knock-out mice have a prolonged QT interval and a broadened QRS complex

Representative electrocardiographic recordings from wild-type and TASK-1^{-/-} mice are shown in Fig. 3A. The

heart rate (HR) (and the RR interval) of the TASK-1 knock-out mice was found to be unchanged in comparison to wild-type mice (~450 bpm; Fig. 3B). The PR interval and duration of the P-wave (P_{dur}) were also found to be unchanged in TASK-1^{-/-} mice (Fig. 3C), indicating that the spread of excitation across the atria and the conduction within the atrioventricular node were not impaired. In contrast, the QT interval, the heart-rate corrected QT time (QTc) (Fig. 3D) and the QRS complex (Fig. 3E) were significantly prolonged in TASK-1^{-/-} mice.

In order to assess whether stimulation of the adrenergic system can induce arrhythmias or alterations of the electrocardiogram, we infused isoprenaline intraperitoneally while recording electrocardiograms. Fig. 3F shows representative electrocardiograms from wild-type and TASK-1^{-/-} mice. Note that during the application of isoprenaline the electrocardiograms of wild-type and TASK-1^{-/-} mice differ much less than under resting conditions. This impression is underlined by the statistics given

in Fig. 3G. The first two minutes show mean results under resting conditions. Subsequently, isoprenaline was infused; the mean values under stimulation with isoprenaline (4 to 6 min after the start of the electrocardiogram recordings) are plotted. While under resting conditions QT, QTc and QRS time significantly differed between wild-type and knock-out animals, these parameters converged under adrenergic stimulation and no significant differences remained (Fig. 3G). These findings suggest that TASK-1 currents modulate conduction and repolarization of the action potential predominantly under resting conditions of the heart.

The relative heart weight (compared to body weight) of the whole heart (Fig. 4A) as well as the relative weight of the left (LV) and right (RV) ventricles were similar for wild-type and TASK-1^{-/-} mice (Fig. 4B).

Hemodynamic measurements in TASK-1^{-/-} mice

A previous study reported an increased blood pressure for female and a tendency to increased blood pressure in male TASK-1^{-/-} mice, using tail cuff measurements [20]. In contrast, another study reported a decreased blood pressure for male TASK-1^{-/-} mice, measuring the mean arterial blood pressure via the femoral artery [21]. We also carried out hemodynamic recordings in both male and female mice, but we only observed a weak tendency for an increase in blood pressure. The average maximum and minimum blood pressure recorded in the aorta were not significantly different for wild-type and TASK-1^{-/-} mice (Fig. 4C). In addition, the measurements of the maximum rate of change of left ventricular pressure ($dp/dt_{\max}$) showed no difference between wild-type and TASK-1 knock-out mice, which suggests that the contractility of TASK-1^{-/-} hearts was unchanged (Fig. 4D). Left ventricular volume, stroke volume and ejection fraction were also not significantly altered by the impairment of the TASK-1 gene (Fig. 4E). These findings make it unlikely that heart failure, structural abnormalities or increased blood pressure were the primary cause for the observed alterations in the electrocardiogram.

Ion channel expression in ventricular myocytes isolated from control and TASK-1^{-/-} mice

To evaluate some potential compensatory mechanisms contributing to the electrophysiological phenotype, we analyzed the mRNA expression levels of a variety of potassium channel genes in ventricular cardiomyocytes of wild-type and TASK-1^{-/-} mice. The data shown in Fig. 5A suggest that the knock-out of TASK-1 channels did not give rise to any significant up- or down regulation of

Fig. 4. Hemodynamic measurements of the TASK-1^{-/-} mice. (A) Relative heart weight compared to body weight for wild-type (n = 6) and TASK-1^{-/-} mice (n = 5). (B) Relative weight of the left and right ventricle for wild-type (n = 6) and TASK-1^{-/-} (n = 5). (C-E) Parameters of hemodynamic recordings for wild-type and TASK-1^{-/-} animals. (C) Mean aortic pressure of wild-type (n = 7) and TASK-1^{-/-} mice (n = 6). (D) Data for contractility, end-systolic and end-diastolic pressure for wild-type (n = 14) and TASK-1^{-/-} animals (n = 13). (E) Left ventricular volume, stroke volume and ejection fraction of TASK-1^{-/-} mice (n = 14) and wild-type littermates (n = 13).

Fig. 5. Ion channel expression analysis and tests for electrophysiological remodelling in ventricular myocytes from TASK-1^{-/-} mice. (A) Quantitative RT-PCR analysis of K_{2P} and other cardiac potassium channels in mouse ventricular cells. Values for TASK-1^{-/-} animals (grey outline) and wild-type littermates (black) were normalized to GAPDH expression. Note that there is no significant change of ion channel expression. (B, C) Representative whole cell patch clamp recordings of I_{K1} currents (B) and I_{to} currents (C) from wild-type (black) and TASK-1^{-/-} mice (grey). I_{K1} currents were recorded using voltage steps of 500 ms duration to voltages in the range of -110 to -20 mV, in 10 mV increments, from a holding potential of -80 mV. Sweep time interval was 10 s. I_{to} currents were recorded using voltage steps of 1.5 s duration to voltages in the range of -50 to +50 mV, in 10 mV increments, from an holding potential of -60 mV, to inactivate sodium channels. Sweep time interval was 10 s. (D, E) Current-voltage relationship of I_{K1} currents (D) and I_{to} currents (E) from wild-type (black) and TASK-1^{-/-} mice (grey). For all experiments (n = 8). (F) Action holding potential recordings from wild-type (black) and TASK-1^{-/-} mice (grey). Action potentials were elicited by injection of a brief current pulse (1 ms, 2 nA) in the current clamp mode. The stimulation rate was 5 Hz. After action potentials reached a steady-state, an average of the last ten action potentials was analyzed for APD₉₀. Action potentials were recorded from 7 wild-type and 7 TASK-1^{-/-} mice.

the transcription of other cardiac potassium channels, including other cardiac K_{2P} channels. As these experiments do not rule out an electrical remodelling of cardiac potassium channels at the protein level, we recorded I_{K1} and I_{to} currents from mouse ventricular myocytes of wild-type and TASK-1^{-/-} mice, which represent the major determinants of the rate of repolarization in mouse ventricular cardiomyocytes (Fig. 5B-E). However, we did not observe any significant changes in the current voltage relationship of the I_{K1} and I_{to} currents (Fig. 5D, E).

Thus, the hemodynamic measurements, the qPCR data together with the patch clamp recordings of the I_{K1} and I_{to} currents suggest, that it is unlikely that the phenotype of the TASK-1^{-/-} mouse is caused by compensatory mechanisms such as electrical remodelling.

Next, we recorded action potentials from cardiomyocytes of wild-type and TASK-1^{-/-} mice (Fig. 5F). Action potential duration (APD) of TASK-1^{-/-} mice was prolonged; the APD at 90% repolarization (APD₉₀) increased from 35 ± 3 ms (n = 7) to 41 ± 3 ms (n = 7). This

action potential prolongation of 17% can, however, not fully explain the QT and QTc time prolongation observed in the surface electrocardiogram, which were 29% and 33%, respectively (Fig. 3D).

Isoflurane modulates heart rate and atrioventricular conduction in wild-type but not in TASK-1^{-/-} mice (in vivo electrophysiological analysis)

Activation of TASK-1 channels by volatile anesthetics like isoflurane [9, 10] provides a putative mechanism of action for general anesthesia [10]. Isoflurane exerts cardiac side effects via activation of TASK-1 channels, at concentrations which are in a clinically relevant range [9]. In previous work, general anesthesia with 1% isoflurane was found to reduce the heart rate of wild-type C57BL6 mice from 615 to 468 beats per minute (bpm) [22], whereas an anesthesia with Avertin® did not bring about a reduction of heart rate [23]. We studied the influence of isoflurane on wild-type and TASK-1^{-/-} hearts by the means of *in vivo* electrophysiological experiments. During isoflurane anesthesia wild-type littermates showed a reduced heart rate in comparison to TASK-1^{-/-} mice (Fig. 6A). This effect might be explained by an activation of TASK-1 channels which are only present in wild-type mice, resulting in a stabilization of the membrane potential of sinoatrial node cells. In addition, we observed that under isoflurane anesthesia wild-type mice showed a prolonged PR-interval in comparison to TASK-1^{-/-} mice (Fig. 6B). This effect might be caused by slowing of atrioventricular conduction mediated by activation of TASK-1 channels.

Next, we tested for direct changes in atrioventricular conduction. To achieve this, we paced the atrium with an electrode at increasing frequencies while simultaneously recording the electrical activity of the ventricles. The highest pacing frequency (lowest cycle length, CL) at which a regular 1:1 conduction is possible is called the Wenckebach point. In wild-type mice a regular 1:1 conduction was observed up to 706 bpm (CL, 85 ms), while knock-out animals could be paced up to 845 bpm (CL, 71 ms) (Fig. 6C). The fact that wild-type mice showed a less efficient atrioventricular conduction might be explained by an activation of TASK-1 channels in the atrioventricular node by isoflurane. It is noteworthy, that even in the presence of the TASK-1 activator isoflurane the ventricular parameters for repolarization were not changed. For example, we did not observe changes in the effective refractory period for wild-type and TASK-1^{-/-} mice (Table 1). At cycle lengths of 75 and 85 ms the effective refractory period did not differ significantly

Fig. 6. Effects of isoflurane on heart rate and atrioventricular conduction in wild-type and TASK-1^{-/-} mice. (A) Heart rate and (B) PR-time during isoflurane anesthesia. (C) Wenckebach point under isoflurane anesthesia. For all experiments (A-C) wild-type mice (n = 6) were compared to TASK-1^{-/-} animals (n = 5).

	CL ¹	Wild-type	TASK-1 ^{-/-}
Effective refractory period	85 ms	50.0±6.9 ms	43.8±1.3 ms ^{ns}
	75 ms	47.5±6.6 ms	48.8±1.3 ms ^{ns}

Table 1. *In vivo* electrophysiological analysis. Effective refractory period of wild-type and TASK-1^{-/-} knock-out at CL of 75 and 85 ms, analyzed with an extra stimulus protocol. ¹CL = cycle length.

cantly between wild-type and knock-out animals (Table 1) and no arrhythmias were observed.

Discussion

We have characterized the phenotype of the TASK-1 knock-out mouse. The most striking findings were a prolongation of the QRS complex and an increase in the corrected QT-interval of the murine electrocardiogram by 30%. Moreover, we found that the action potential duration (APD₉₀) measured in isolated cardiomyocytes of TASK-1^{-/-} mice was increased by, on average, 17%. The latter result is in reasonable agreement with our previous study in rat cardiomyocytes [6], where we found that block of TASK-1 by A293 prolonged APD₉₀ by about 21%. A recent study on the same strain of knock-out mice by Donner et al. [7] also reported a QT prolongation and an increase in the duration of monophasic action potentials. Our hemodynamic measurements, together with the echocardiographic data of

Donner et al. [7], make it unlikely that heart failure, structural abnormalities or high blood pressure were the primary cause for the changes in the electrocardiogram. Since we did not find any significant change in the expression of other ion channels (nor did Donner et al. [7]) it is likely that the change in action potential duration in TASK-1^{-/-} mice was mainly due to the absence of TASK-1 channels.

The substantial increase in the QT-interval that we observed in TASK-1^{-/-} mice cannot be fully explained by the change in the action potential duration. Since in birds and mice TASK-1 is predominantly expressed in the conduction system [8], we hypothesize that, in addition to a prolongation of action potential duration in the ventricles, the knock-out of TASK-1 may also lead to a dispersion of the spread of excitation to different regions of the ventricular myocardium, and that this dispersion might be the major cause of the observed prolongation of the QRS complex and the QT-interval. It should be noted that in the mouse electrocardiogram no clear ST-segment can be distinguished and the T-wave merges with the final part of the QRS complex [24]. This is probably related to the fact that depolarization in some parts of the heart occurs simultaneously with repolarization in others. Thus, the observed high-amplitude QRS complex in the mouse electrocardiogram, which lasts ~20 ms, might reflect both, the spread of depolarization across the ventricles and the early phase of repolarization [25] and, in addition, the temporal dispersion of the conduction from the atrioventricular node to the ventricles.

The difference between the electrocardiograms of wild-type and TASK-1^{-/-} mice disappeared during sympathetic stimulation with isoprenaline. This finding is consistent with the idea that the presence of TASK-1 channels has a substantial influence on the ECG mainly under resting conditions. During sympathetic stimulation (and in the TASK-1 knock-out mice) other channels might dominate the adaptation of the electrical activity of cardiac cells to increased heart rates.

We and others [21] only observed non-significant changes in the heart rate of TASK-1^{-/-} mice as compared to wild-type. In contrast, Donner et al. observed an increase in mean heart rate and in heart-rate variability in TASK-1^{-/-} mice. At present, we have no explanation for this difference. Donner et al. attribute the increase in heart rate to the expression of TASK-1 in cardiorespiratory neuronal cells. Alternatively, the increase in heart rate of TASK-1 knock-out mice might be explained by a direct effect of TASK-1 channels on sinoatrial node cells, although expression of TASK-1 in the sinoatrial node has

so far been shown only for human heart [26]. Interestingly, we found that isoflurane anesthesia gave rise to a reduction in heart rate in wild-type mice but not in TASK-1^{-/-} mice. This observation might also be attributable to the lack of TASK-1 channel activation in the sinoatrial node by isoflurane in the knock-out animals. Interestingly, in *Drosophila* the genetic deletion of the two-pore-domain K⁺ channel ORK, which is predominantly expressed in spontaneously contracting myocytes, also resulted in an increased heart rate [27].

In electrocardiograms recorded under anesthesia with isoflurane, we observed significant prolongation of the PR interval in wild-type mice, but not in TASK-1 knock-out mice. Thus, under these conditions the spread of excitation across the atrial tissue, the atrioventricular node and subsequent conduction pathways were probably slower in wild-type mice. Consistent with this interpretation, in wild-type mice but not in TASK-1 knock-out mice, the Wenckebach point was shifted towards lower frequencies during isoflurane anesthesia. These observations suggest that the atrioventricular node of wild-type mice conducted impulses with reduced efficiency, perhaps due to a hyperpolarization caused by activation of TASK-1 currents by isoflurane.

Genetic defects in ion channels involved in repolarization of the action potential are the primary cause for inherited arrhythmias. Although a major focus of current research, the relative importance of the different ion channels involved in repolarization is still not fully known. Particularly little is known about ion channels regulating the membrane potential and the repolarization of cells in the conduction system. So far, only a few mutations in ion channel genes have been identified to cause changes in the impulse generation and conduction [28, 29]. The differences in PR interval and in the Wenckebach point between wild-type and TASK-1^{-/-} mice suggest that TASK-1, which is transcribed in human sinoatrial node, atrium and Purkinje fibres [26, 30], may also be a candidate gene for inherited forms of conduction disorders.

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