Cellular Physiology and Biochemistry

Cell Physiol Biochem 2012;30:523-537
DOI: 10.1159/000341435
Published online: July 19, 2012
Accepted: June 05, 2012

1015-8987/12/0303-0523$38.00/0

Original Paper

In vivo Electrophysiological Characterization of TASK-1 Deficient Mice

Stella Petric[1]* Lukas Clasen[1]* Carina van Weßel[1] Nora Geduldig[1] Zhaoping Ding[2] Martina Schullenberg[1] Jan Mersmann[3] Kai Zacharowski[3] Maria I. Aller[4,#] Klaus G. Schmidt[1] Birgit C. Donner[1]

[1]Department of Pediatric Cardiology and Pneumology, University Children´s Hospital, Duesseldorf; [2]Department of Cardiovascular Physiology, Heinrich Heine University Duesseldorf; [3]Clinic of Anesthesiology, Intensive Care Medicine and Pain Therapy, University Hospital Frankfurt, Frankfurt; [4]Department of Clinical Neurobiology, University of Heidelberg, Heidelberg; *The first two authors contribute equally to the manuscript and therefore share first authorship; [#]Current address: Instituto de Neurosciencias, San Juan de Alicante, Alicante

Key Words
TASK-1 knock-out • *In vivo* electrophysiology • Cardiac repolarization • Prolonged QT interval • Long QT syndrome (LQTS) • Heart rate turbulence • Programmed electrical stimulation

Abstract
Background/Aims: TASK-1 is a potassium channel predominantly expressed in heart and brain. We have previously shown that anesthetized TASK-$1^{-/-}$ mice have prolonged QT intervals in surface electrocardiograms (ECGs). In addition, heart rate variability quantified by time and frequency domain parameters was significantly altered in TASK-$1^{-/-}$ mice with a sympathetic preponderance. Aims of the present study were the analysis of QT intervals by telemetric ECGs, to determine potential influences of anesthesia and β-adrenergic stimulation on repolarization in surface ECGs, to investigate *in vivo* electrophysiological parameters by intracardiac electrical stimulation and to quantify heart rate turbulence after ischemia/reperfusion or ventricular pacing in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice. ***Methods:*** Rate corrected QT intervals (QTc) were recorded in conscious mice by telemetry and in surface ECGs following administration of various anesthetics (tribromoethanol (Avertin®), pentobarbital and isoflurane). TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice were characterized by programmed electrical stimulation using an intracardiac octapolar catheter. The baroreceptor reflex was analyzed by heart rate turbulence (turbulence onset and slope) after ischemia/reperfusion and by stimulated premature ventricular contractions. **Results:** Telemetric and surface ECGs in mice sedated with Avertin® and pentobarbital, showed a significantly lengthened rate corrected QT interval in TASK-$1^{-/-}$ mice (telemetry: TASK-$1^{+/+}$ 43±3ms vs. TASK-$1^{-/-}$ 49±5ms, n=6, $p<0.05$; Avertin®: TASK-$1^{+/+}$ 36±8ms vs. TASK-$1^{-/-}$ 48±4ms, n=13/16, $p<0.0001$). The prolongation of the QT interval was most pronounced at lower heart rates. Isoflurane, known for its stimulatory effects on the TASK channel family, attenuated the

Birgit C. Donner, MD, PhD

Department of Pediatric Cardiology and Pneumology
University Children´s Hospital, Moorenstrasse 5, 40225 Duesseldorf (Germany)
Tel. +49 211 811 7671, Fax +49 211 811 6287
E-Mail Birgit.Donner@med.uni-duesseldorf.de

rate corrected QT interval prolongation in TASK-$1^{-/-}$ mice. Intracardiac electrical stimulation revealed normal values for electrical conduction and refractoriness. No significant arrhythmias after atrial and ventricular burst stimulation were induced before and after adrenergic challenge in both genotypes. Turbulence onset after premature ventricular contraction was significantly altered in TASK-$1^{-/-}$ mice. ***Conclusion:*** TASK-$1^{-/-}$ mice exhibit a phenotype of QT prolongation, which distinct relation to heart rate. TASK-1 deficiency does neither alter key electrophysiological parameters nor increases atrial/ventricular vulnerability after electrical stimulation. The heart rate response after premature ventricular contractions is significantly abolished indicating a diminished baroreceptor reflex in TASK-$1^{-/-}$ mice.

Introduction

Heart failure, myocardial infarction and arrhythmia are accompanied by alterations in the expression of cardiac ion channels. This arrhythmogenic remodeling constitutes the molecular determinant for significant cardiac morbidity and mortality [1]. In addition, there are rapidly increasing data on cardiac channelopathies (e.g. long QT syndrome (LQTS)) caused by mutations in cardiac ion channels which may lead to life-threatening arrhythmia and sudden cardiac death [2]. Potassium channels constitute the largest family of all cardiac ion channels. Of the three superfamilies of potassium channels, the K2P channels are the most recently identified group [3]. Their common structural motifs are four transmembrane domain proteins and two-pore-forming loops arranged in tandem [4]. Functionally they have characteristics of background K^+ currents being important for maintenance of the resting membrane potential [4]. TASK-1 (TWIK-related acid-sensitive K channel 1) is mainly expressed in the heart, the nervous system, the carotid body and the adrenal cortex [3]. There is increasing evidence that TASK-1 plays a functional role in cardiac physiology. Pharmacological inhibition of the TASK-1 channel by A293 and methoxamine results in a significant prolongation of the APD in Xenopus oocytes or isolated rat cardiac myocytes [5]. Likewise, in murine ventricular cardiomyocytes TASK-1 is inhibited by the inflammatory phospholipid platelet-activating factor via protein kinase Cε, which leads to a delay in repolarization and the occurrence of early afterdepolarizations [6, 7]. Recently we have shown, that TASK-$1^{-/-}$ mice have significantly prolonged action potential duration in Langendorff perfused hearts by recording monophasic action potentials [8]. This was in accordance with a prolongation of the rate corrected QT interval in surface ECGs of TASK-$1^{-/-}$ mice [8, 9]. Analyzing heart rate variability by calculating time and frequency domain parameters showed reduced total autonomic variability and a sympathetic preponderance in TASK-$1^{-/-}$ mice [8]. In human atria I_{TASK-1} contributes to the sustained outward current I_{Ksus} and is therefore a major component of the background conductance, which might have functional relevance in the pathogenesis of atrial fibrillation [10]. Therefore, TASK-1 seems to play a crucial role in cardiovascular function by influencing repolarization and autonomous cardiac reflexes.

In the present study we intended to analyze QT and rate corrected QT intervals in unrestrained mice by telemetric ECG analysis for 24 h. A focus was the comparison of QT intervals in relation to heart rate in the two genotypes, since this is a characteristic hallmark in some long QT syndromes. Potential influences of anesthesia and β-adrenergic stimulation on repolarization in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice were determined. In addition, we aimed to investigate *in vivo* electrophysiological parameters after programmed electrical stimulation to determine conduction and refractory parameters as well as electrical vulnerability in TASK-$1^{-/-}$ mice. Based on our previous findings of altered heart rate variability in TASK-$1^{-/-}$ mice, we also analyzed heart rate turbulence (HRT). The phenomenon of HRT refers to sinus rhythm cycle-length perturbations after isolated premature ventricular contracticons. The physiological pattern consists of brief heart rate acceleration (quantified by the turbulence onset) followed by more gradual heart rate deceleration (quantified by the turbulence

slope) before the rate returns to a pre-ectopic level [11]. HRT is thought to provide indirect assessment of the baroreflex. A pathological heart rate turbulence is a strong and independent predictor of death in conditions like congestive heart failure, myocardial infarction and structural heart disease [11].

Materials and Methods

TASK-1-deficient mice (C57/Bl6 x 129Sv) were provided from the Department of Clinical Neurobiology, University of Heidelberg [12]. All animal experiments were approved by the local animal care committee and were conducted in accordance with the convention of the National Institute of Health Guide for the care and use of laboratory animals (NIH Publication No. 85-23, revised 1996). All analysis were done in age- and sex-matched littermates of wild-type (TASK-$1^{+/+}$) or knockout mice (TASK-$1^{-/-}$).

Investigation of body parameters

Body weight (BW), heart weight (HW), and tibia length (TL) in adult mice and the relation of HW/BW and HW/TL was not significantly different between the two genotypes as described previously [data not shown and 8].

Breeding of TASK-$1^{-/-}$ mice and genotyping

TASK-$1^{-/-}$ mice (C57/Bl6 x 129Sv, more than 10 back-crosses) appear healthy, have a typical life span and breed normally [data not shown and 12]. For genotyping DNA was isolated from tails (DNeasy fibrous tissue mini kit®, Qiagen, Hilden, Germany). Sequencing the *TASK-1* gene with the TAG_3IRESlacZpAneopA cassette introduced showed that a 72 bp deletion of exon 1 occurred [12]. Therefore, we used a forward primer that binds within the region which is deleted in the TASK-$1^{-/-}$ mouse (forward, 5´-aga atg tgc gca cgt tgg c – 3´, reverse, 5´- cgg cgc cca cca gca -3´) and a second primer pair that recognizes the *lacZ* domain (forward, 5´- ccc att acg gtc aat ccg c – 3´, reverse, 5´-aca acc cgt cgg att ctc c – 3´).

Surface ECG

Surface ECGs were performed as previously described [8]. Briefly, mice were sedated with tribromoethanol/2-methyl-2-butanol (2.5% Avertin®, 250 mg/kg), pentobarbital (30 mg/kg) or isoflurane (1.5 Vol%), respectively. ECGs were recorded using a PowerLab 8/30 supplemented with an animal BioAmp and analyzed by the LabChart software (all ADInstruments, Spechbach, Germany). Measurements encompassed heart rate (beats per minute) and cycle length (RR interval in ms), P-wave duration (ms), PR interval (ms), QRS interval (ms), (rate corrected) QT interval ($QTc=QT_0/(RR_0/100)^{1/2}$, in ms) [13]. This formula is a modified Bazett formula, which is adjusted for mice. For comparison, the rate corrected QT intervals recorded by telemetry were also calculated using a modified Framingham formula (QTc=QT+0.154(100-RR) [14]. The end of the T-wave was determined as the point at which the slow component of the biphasic T-wave returned to the isoelectric line. All data are the mean of at least 50 beats per mouse that were manually analyzed by two independent examiners who were blinded to the genotypes.

Telemetric ECG analysis

An ECG transmitter was implanted into the peritoneal cavity of adult mice (model TA10EA-F20, Data Sciences, Minneapolis, USA), with a position of the electrodes generating a lead II on ECG [8]. After a recovery period from surgery of 2 weeks, ECG was recorded with a sampling rate of 1 kHz. RR intervals, QT and rate corrected QT intervals were analyzed during day time (at least eight beats every 20 min, resulting in 288 single beats) by two independent examiners who were blinded to the genotype using the LabChart software (ADInstruments, Spechbach, Germany).

Intracardiac programmed electrical stimulation

In vivo transvenous electrophysiological experiments were performed in anesthetized (Avertin®) adult TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice as described [15]. A 2 F octapolar electrophysiology catheter was advanced via the jugular vein into the right atrium and ventricle. The octapolar catheter has eight 0.5 mm circular electrodes with an electrode-pair sparing of 0.5 mm (Ciber Mouse, NuMed Inc., NY, USA). The catheter was positioned

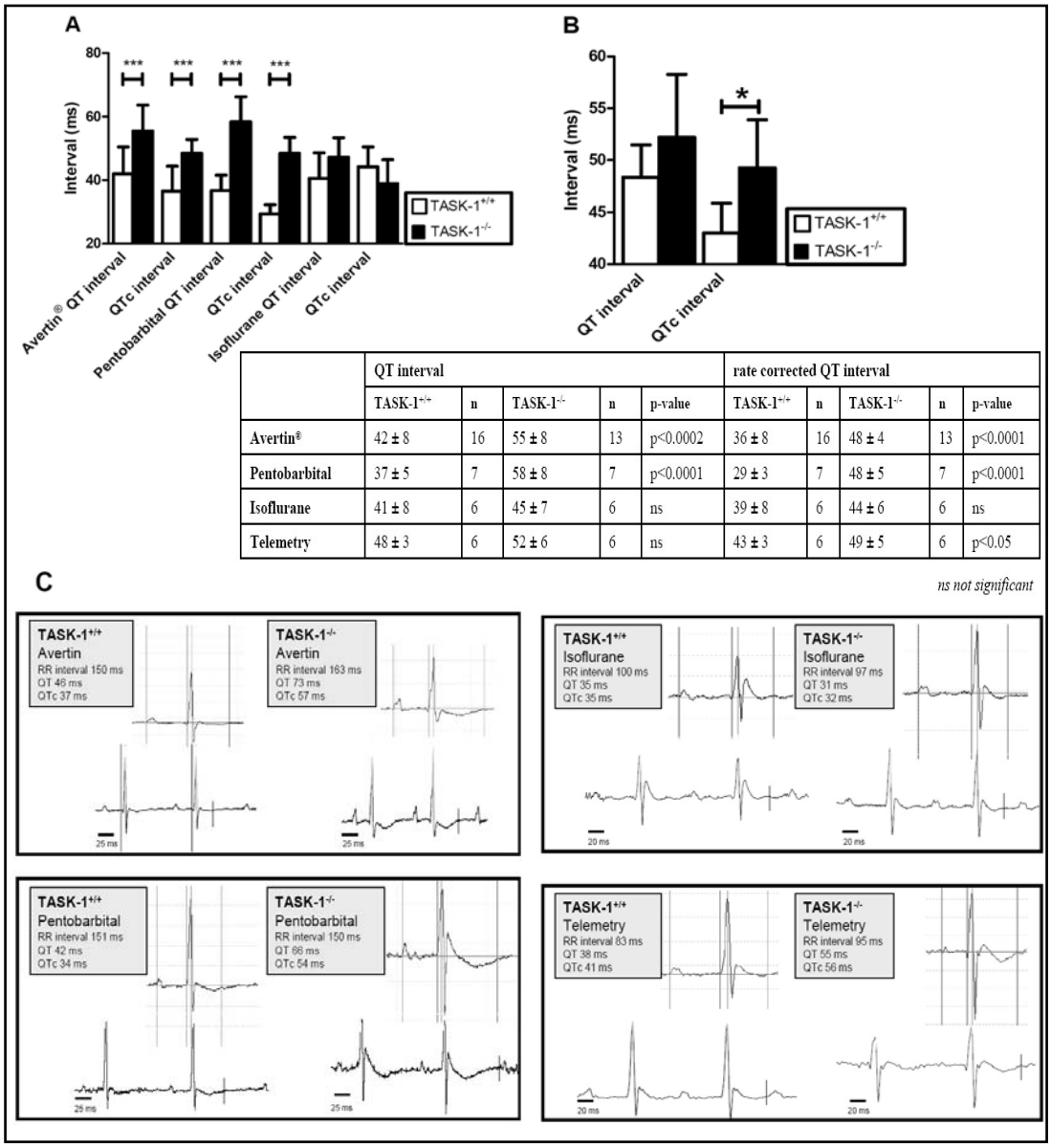
by electrocardiographic monitoring. The two most distal electrode pairs 1/2 and 3/4 sensed ventricular activities, the electrode pair 5/6 recorded on the level of the AV valve, and the electrode pairs 7/8 detected atrial activity. Atrial and ventricular stimulations was achieved by rectangular stimulus pulses and twofold intensity of the pacing threshold using an electrical stimulator and a PowerLab 8/30 (ADInstruments, Spechbach, Germany). Surface ECGs and intracardial electromyograms were recorded simultaneously and analyzed by the Lab Chart Software 7.0 (ADInstruments, Spechbach, Germany). Sinus node recovery time (SNRT) was measured twice after 30 atrial stimuli at 120 ms, 100 ms, and 80 ms. Rate corrected SNRT (SNRTc) was calculated by subtracting the steady-state cycle length from the SNRT. Atrial refractory periods (ARP), atrioventricular nodal refractory periods (AVNRP) and ventricular refractory periods (VRP) were evaluated by fixed rate and extrastimulus pacing. Wenckebach periodicity was determined by eight atrial stimuli starting with a cycle length that was 20 ms shorter than physiological cycle length and further shortening of 2-5 ms. Atrial vulnerability was tested by burst stimulation for 5 s starting with 50 ms cycle length up to 10 ms. Atrial fibrillation was defined as rapid and fragmented atrial electrograms with irregular AV-nodal conduction for > 1 s [16]. Ventricular vulnerability was tested by ventricular stimulation with eight stimuli at 110 ms, 100 ms and 80 ms followed by up to three extrastimuli. Extrastimuli were applied with decreasing cycle lengths up to the ventricular refractory period. In addition, ventricular burst stimulation was done for 5 s starting with 50 ms cycle length up to 10 ms. Ventricular tachycardia was defined as four or more ventricular ectopic beats. In order to accelerate conduction and provoke catecholamine-sensitive tachyarrhythmias, isoprenaline (1 mg/kg intraperitoneal) was given and the increase in heart rate was monitored. If there was no increase in heart rate of at least 20% or a decrease to less than 100 ms cycle length the half of the starting dose of isoprenaline was given additionally. QT and rate corrected QT intervals were analyzed directly before and after isoprenaline to compare the influence of adrenergic stimulation on these parameters. After two minutes atrial and ventricular burst stimulations were repeated.

Heart rate turbulence (HRT) in TASK-1$^{+/+}$ and TASK-1$^{-/-}$ mice after ischemia/reperfusion injury and after ventricular pacing

HRT reflects sinus rhythm cycle length variations after isolated premature ventricular contractions. The physiological pattern consists of a brief heart rate acceleration (quantified by the turbulence onset (TO)) followed by a heart rate deceleration (quantified by the turbulence slope (TS)) [11]. HRT was determined in TASK-1$^{+/+}$ and TASK-1$^{-/-}$ mice after ischemia/reperfusion and after programmed ventricular stimulation in sedated mice (pentobarbital 30 mg/kg), respectively. Spontaneous PVC were analyzed after induction of 40 min of ischemia followed by 6 h of reperfusion in TASK-1$^{+/+}$ and TASK-1$^{-/-}$ mice as described [17]. In addition, HRT was determined in stimulated ventricular beats with coupling intervals that were 20 ms, 40 ms and 60 ms shorter than their preceding cycle length. For calculation of the turbulence onset (TO) the following formula was used: $TO = (RR_1+RR_2)-(RR_{-2}+RR_{-1})/(RR_{-2}+RR_{-1})x100$ [in %]. RR_{-2} and RR_{-1} are the two RR intervals preceding the premature ventricular contraction. RR_1 und RR_2 are the two RR intervals following the compensatory pause. RR_{3-7} relates to the number of RR intervals following the premature ventricular contraction. Turbulence slope (TS) is defined as the maximum positive regression slope assessed over any five consecutive sinus rhythm RR intervals within the first 15 RR intervals after a premature ventricular contraction. Physiologically, the initial acceleration of the sinus rate after a premature ventricular contraction (PVC) is reflected by a negative TO and the rate deceleration is reflected by a positive TS [11]. PVCs were analyzed during reperfusion if prematurity was > 20% and a compensatory pause was detectable. Stimulated PVCs were evaluated if the pacing stimulus led to a ventricular beat with a broadened QRS complex followed by a compensatory pause. Measurements of at least ten ventricular contractions of three coupling intervals (50 ms, 60 ms and 70 ms) were averaged.

Statistical analysis

Data are presented as mean±SD from a certain number of mice as specified in the different experimental settings. Statistical significance was calculated using the unpaired t-test, one-way ANOVA and linear regression as indicated, with a p-value < 0.05 considered to indicate significant differences between groups. Statistical calculations were done using GraphPadPrism 5.0® (La Jolla, CA, USA).



	QT interval					rate corrected QT interval				
	TASK-1$^{+/+}$	n	TASK-1$^{-/-}$	n	p-value	TASK-1$^{+/+}$	n	TASK-1$^{-/-}$	n	p-value
Avertin®	42 ± 8	16	55 ± 8	13	p<0.0002	36 ± 8	16	48 ± 4	13	p<0.0001
Pentobarbital	37 ± 5	7	58 ± 8	7	p<0.0001	29 ± 3	7	48 ± 5	7	p<0.0001
Isoflurane	41 ± 8	6	45 ± 7	6	ns	39 ± 8	6	44 ± 6	6	ns
Telemetry	48 ± 3	6	52 ± 6	6	ns	43 ± 3	6	49 ± 5	6	p<0.05

ns not significant

Fig. 1. (A) QT and rate corrected QT intervals (QTc) in surface ECGs in mice after different anesthetics. TASK-1$^{-/-}$ showed a prolonged QT and QTc interval when sedated with Avertin® or pentobarbital, respectively. Isoflurane abolished the QT interval prolongation. At least 50 beats were analyzed per mouse. (B) Analysis of QT and QTc intervals in ECGs of unrestrained mice by telemetry for 24 h. There was a significant prolonged QTc interval in TASK-1$^{-/-}$ mice compared to the TASK-1$^{+/+}$ controls. At least 288 beats were analyzed per mouse. (C) ECG examples of surface ECGs and telemetric ECGs from TASK-1$^{+/+}$ and TASK-1$^{-/-}$ mice. Genotype, mode of anesthesia, cycle length, QT and rate corrected QT intervals (QTc) are indicated. The inlet above shows the measurements of a single interval, the inlet below depicts two beats.

Results

Comparison of surface ECG analysis in TASK-1$^{+/+}$ and TASK-1$^{-/-}$ mice after different modes of anesthesia (pentobarbital, Avertin® and isoflurane) and in untreated mice

Our previous results showed prolonged QT intervals in TASK-1$^{-/-}$ mice sedated with ketamine/xylazine [8]. In order to test, if this effect is due to the anesthetic, ECGs were analyzed from mice sedated with two other commonly used drugs. TASK-1$^{-/-}$ showed a

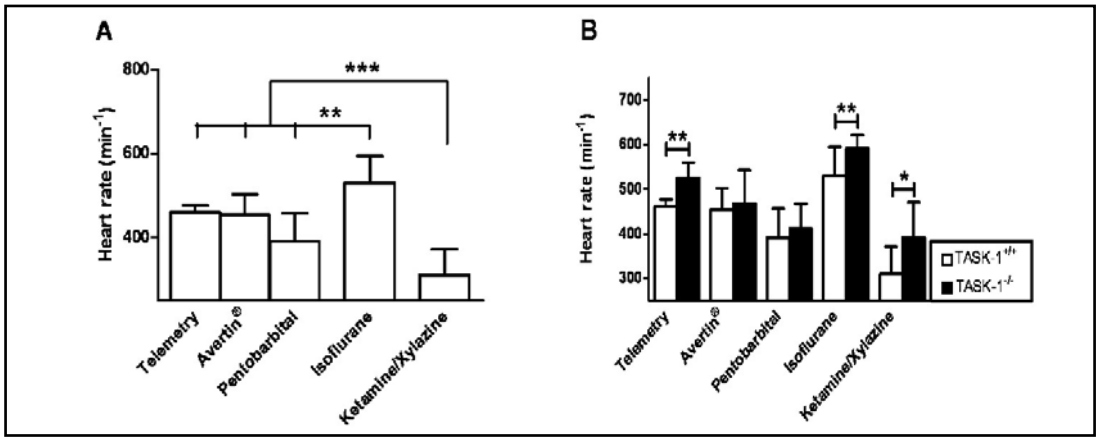


Fig. 2. Comparison of heart rates after different modes of anesthesia and between the two genotypes (A) TASK-$1^{+/+}$ mice sedated with Avertin® or pentobarbital, respectively, show no significant lower heart rates compared to the mean heart rates recorded by telemetry. Anesthesia with ketamine/xylazine resulted in significantly lower heart rates compared to heart rates in conscious TASK-$1^{+/+}$ mice or TASK-$1^{+/+}$ mice sedated with Avertin®, isoflurane or pentobarbital, respectively. Sedation with pentobarbital led to a significantly lower heart rate compared to anesthesia with isoflurane *(one-way ANOVA, ** p<0.005, ***p<0.001).* (B) Mean heart rate analyzed by telemetry or by surface ECGs using ketamine/xylazine or isoflurane revealed significantly higher heart rates in TASK-$1^{-/-}$ than in TASK-$1^{+/+}$ mice *(unpaired t-test, * p<0.05, ** p<0.005).*

significantly prolonged QT and rate corrected QT interval compared to the TASK-$1^{+/+}$ mice when sedated with Avertin® and pentobarbital (Fig. 1A). However, isoflurane, known for its stimulatory effect on K2P channels abolished the effect in TASK-$1^{-/-}$ mice (Fig. 1A, C). All other ECG parameters (P wave duration, PR interval, QRS duration) did not show any significant difference between TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice under all conditions (data not shown).

ECGs obtained by telemetry in both genotypes showed significantly prolonged rate corrected QT intervals in TASK-$1^{-/-}$ compared to TASK-$1^{+/+}$ mice (Fig. 1B, C).

Influence of anesthesia and genotype on heart rate

Mean heart rate in TASK-$1^{+/+}$ mice sedated with Avertin®, isoflurane, or pentobarbital, respectively, was not significantly different compared to the heart rate in conscious mice (Fig. 2A). Comparing the heart rate of mice after pentobarbital with the heart rate of mice after isoflurane showed a higher heart rate in the isoflurane group (Fig. 2A). The use of ketamine/xylazine resulted in significantly lower heart rates compared to all other sedating medication used (Fig. 2A). There was a significantly higher heart rate in conscious TASK-$1^{-/-}$ mice and in TASK-$1^{-/-}$ mice after sedation with ketamine/xylazine or isoflurane compared to the TASK-$1^{+/+}$ mice, respectively (Fig. 2B).

QT and rate corrected QT intervals in relation to β-adrenergic stimulation by isoprenaline

Since TASK-$1^{-/-}$ mice have a phenotype resembling a long QT syndrome, we tested the influence of β-adrenergic stimulation with isoprenaline on the duration of repolarisation in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice. The RR intervals decreased significantly in both genotypes after isoprenaline (Fig. 3A). QT and rate corrected QT intervals remained significantly prolonged in TASK-$1^{-/-}$ mice compared to TASK-$1^{+/+}$ mice after isoprenaline. However, whereas TASK-$1^{-/-}$ mice showed a significant shortening of their QT and rate corrected QT intervals after isoprenaline, no significant difference was seen in the TASK-$1^{+/+}$ mice (Fig. 3B). Plotting the rate corrected QT intervals in relation to the RR intervals showed a trend that after isoprenaline the rate corrected QT intervals align between the two genotypes (Fig. 3C).

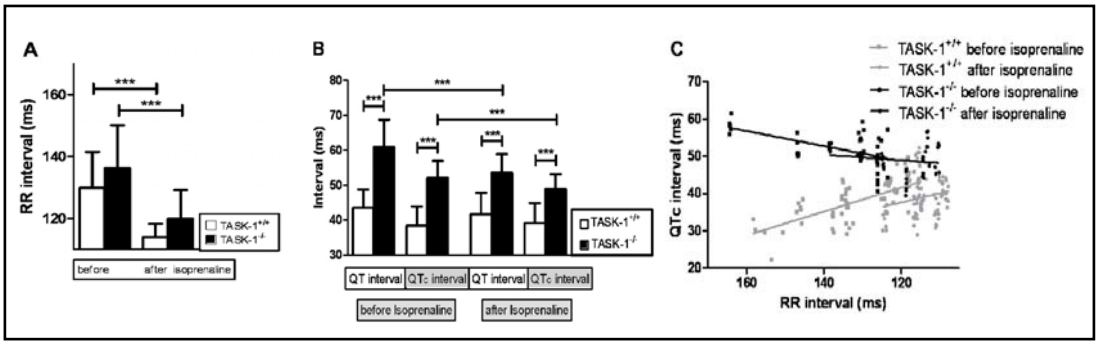


Fig. 3. QT and rate corrected QT intervals in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice before and after isoprenaline (A) Isoprenaline ip resulted in a significant shortening of RR intervals in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice sedated with avertin *(p<0.001, n=6).* (B) There was a significant prolongation of the QT and rate corrected QT (QTc) interval in the TASK-$1^{-/-}$ mice compared to TASK-$1^{+/+}$ mice before and after isoprenaline *(p<0.001, t-test).* After isoprenaline TASK-$1^{-/-}$ showed a significant shortening of their QT and QTc interval, respectively *(p<0.001, paired t-test).* However, no significant changes in QT and QTc interval after isoprenaline were seen in the TASK-$1^{+/+}$ mice. (C) Scatter plot of rate corrected QT intervals in relation to their RR interval. With lower RR intervals QTc intervals of TASK-$1^{-/-}$ and TASK-$1^{+/+}$ tend to align to each other. *TASK-$1^{+/+}$ before isoprenaline: y=-0.3x + 80, TASK-$1^{+/+}$ after isoprenaline: y=-0.2x + 67, n=72. TASK-$1^{-/-}$ before isoprenaline: y=0.2x + 25, TASK-$1^{-/-}$ after isoprenaline:y=0.08x +40, n=40*

QT and rate corrected QT intervals in relation to spontaneous variation in cycle lengths in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice

A characteristic feature of long QT syndrome 1 (loss of function mutations in *KCNQ1*, reduced I_{Ks}) is a paradoxic lengthening of the QT interval at higher heart rates. We therefore tested, how QT and rate corrected QT intervals in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice differ in relation to their RR intervals during 24 h ECG recordings by telemetry. All QT and rate corrected QT intervals of both genotypes were plotted against the corresponding RR interval and analyzed by linear regression.

The slope of the straight line, which illustrates how QT and rate corrected QT intervals change at different RR intervals, indicated that QT and rate corrected QT intervals between the two genotypes vary at most at lower heart rates (Fig. 4A, B). The observation that rate corrected QT intervals prolong with increasing heart rates (shorter RR intervals) is a known phenomenon of the correction formula [18].

To exclude that the observation of increasing differences in rate corrected QT intervals between the two genotypes at lower heart rates was due to the correction formula, we compared the rate corrected QT intervals calculated by the Mitchell formula with another commonly used correction formula in humans adapted to the mouse model. Linear regression analysis showed that in TASK-$1^{+/+}$ mice at lower heart rates the QTc intervals based on the Mitchell formula are shorter than those obtained by the Framingham calculation ($p<0.001$). In contrast, at shorter RR intervals this relation is inverted (Fig. 4C). This observation is even more pronounced in the TASK-$1^{-/-}$ mice (Fig. 4D). Comparing the two correction formula with TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice by plotting the RR interval against the QTc/QT relation showed that this relation was minimal in TASK-$1^{-/-}$ mice at low heart rates when rate corrected QT intervals were calculated by the Mitchell formula (Fig. 4E). Therefore, the even stronger increase in rate corrected QT interval in TASK-$1^{-/-}$ at lower heart rates is not an effect of the correction formula but rather an intrinsic phenotype due to the lack of TASK-1.

Invasive electrophysiological studies in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice

Although TASK-1 mRNA was mainly detectable in ventricular and to a lesser extent in murine atrial cardiomyocytes [8], the cellular localization of TASK-1 is still not fully characterized. In particular, the question if TASK-1 is predominantly expressed in the

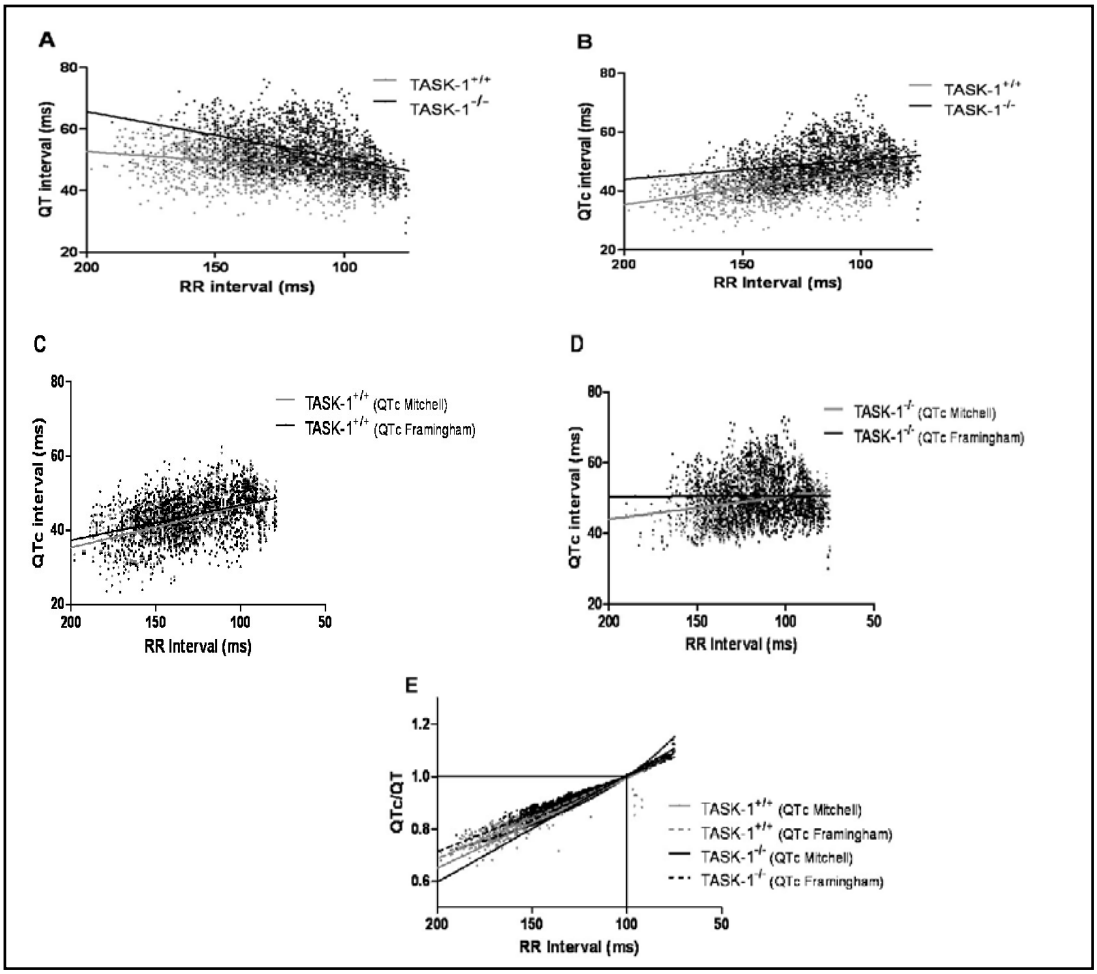


Fig. 4. Comparison of QT and rate corrected QT (QTc) intervals in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice in relation to their cycle lengths (RR intervals) obtained by telemetry during a 24 h measurement. (A, B) Scatter plots of RR intervals versus QT and QTc intervals, respectively, in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice and their corresponding regression lines. (A) QT values had a negative linear trend with decreasing RR intervals. (B) QTc intervals had a positive linear trend with decreasing RR intervals. (A,B) The prolongation of the QT and QTc interval in TASK-$1^{-/-}$ mice were more pronounced at longer RR intervals *(A: TASK-$1^{+/+}$: y=0.06x + 41, n=1627, TASK-$1^{-/-}$: y=0.15x+35, n=1821, p<0.0001, B: TASK-$1^{+/+}$: y=-0.11x + 58, n=1627, TASK-$1^{-/-}$: y=-0.06x + 57, n=1821, p<0.0001).* (C, D) Comparison of two correction formulas for rate correction of QT intervals. (C) The calculation of the rate corrected QT interval by the equation of Mitchell, that bases on the Bazett formula showed in TASK-$1^{+/+}$ mice shorter QTc intervals at higher RR intervals (lower heart rates) compared to the correction of the modified Framingham formula. (D) These differences were even more pronounced when the two formulas were compared in the TASK-$1^{-/-}$ mice. (E) Linear regression lines of two correction formulas in both genotypes were analyzed in respect of the the QTc/QT relation to the RR interval. At a cycle length of 100 ms, QT and QTc interval are of the same value. These analyses showed that the Mitchell formula resulted in lower values of QTc/QT and therefore lower QTc intervals at higher RR intervals (lower frequencies) in both genotypes when compared to the Framingham formula.

conduction system is still not proven yet. We therefore did an *in vivo* electrophysiological study using a protocol that was adapted from the clinical use in order to characterize TASK-$1^{-/-}$ mice in respect of *in vivo* electrophysiological parameters (Table 1). Firstly, sinus node function was analyzed by determining the sinus node recovery time (SNRT) at different time intervals. No significant difference was detected in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice for the SNRT

Table 1. *In vivo* electrophysiological data after programmed electrical stimulation.

	TASK-$1^{+/+}$	n	TASK-$1^{-/-}$	n	p-value
Sinus node function					
SNRT 120 ms	180 ± 29	14	174± 33	9	n.s.
SNRT 100 ms	179 ± 33	15	156 ± 23	12	< 0.05
SNRT 80 ms	168 ± 37	7	153 ± 25	11	n.s.
SNRTc (@100 ms)	44 ± 22	15	40 ± 14	10	n.s.
SNRT/SCL*100 (@100 ms)	133 ± 14	15	122 ± 17	12	n.s.
Atrioventricular conduction					
AV interval	39 ± 6	5	41 ± 1	5	n.s.
AH interval	26 ± 7	5	29 ± 3	5	n.s.
HV interval	12 ± 2	5	12 ± 3	5	n.s.
Wenckebach point	81 ± 7	13	81 ± 6	12	n.s.
Refractory periods					
AV-nodal RP 120 ms	57 ± 11	9	55 ± 7	7	n.s.
AV-nodal RP 110 ms	56 ± 10	14	55 ± 8	11	n.s.
AV-nodal RP 100 ms	62 ± 9	14	57 ± 10	11	n.s.
ARP 120 ms	50 ± 12	10	54 ± 6	5	n.s.
ARP 110 ms	50 ± 9	14	51 ± 9	10	n.s.
ARP 100 ms	54 ± 10	14	51 ± 9	10	n.s.
VRP 120 ms	62 ± 17	9	55 ± 7	5	n.s.
VRP 110 ms	57 ± 15	12	59 ± 7	6	n.s.
VRP 100 ms	58 ± 16	12	58 ± 7	8	n.s.

SNRT sinus node recovery time, SNRTc corrected SNRT, SNRT/SCL SNRT/sinus cycle length, AV interval atrioventricular interval, ARP atrial refractory period, VRP ventricular refractory period

Table 2. Arrhythmia after atrial and ventricular burst stimulation before and after isoprenaline bolus.

	TASK-$1^{+/+}$ [n=16]	TASK-$1^{-/-}$ [n=12]
	Absolute number of events / number of animals with event	
Premature ventricular contraction	10/4	11/4
Couplet, Triplet	6/3	7/3
Ventricular tachycardia	3/2	4/3
Atrial fibrillation	3/3	0/0

at 80 ms and 120 ms. Although SNRT at 100 ms was significantly shorter in the TASK-$1^{-/-}$ mice, this was abolished when the SNRT was related to the steady state cycle length (SNRTc and SNRT/SCL*100). Secondly, atrioventricular conduction was characterized by the AV interval and the Wenckebach point. Whenever His signals were detectable, AH and HV intervals were measured. There was no significant difference between the two genotypes studied. Thirdly, refractory periods in the atria, the AV-node and the ventricle were determined by extrastimuli at different cycle lengths. No significant differences were detected when comparing TASK-$1^{+/+}$ with TASK-$1^{-/-}$ mice. Finally, repeated atrial and ventricular burst stimulation with different cycle lengths before and after injection of isoprenaline were applied to provoke arrhythmia. There were no significant differences in the occurrence of arrhythmia in both genotypes (Table 2).

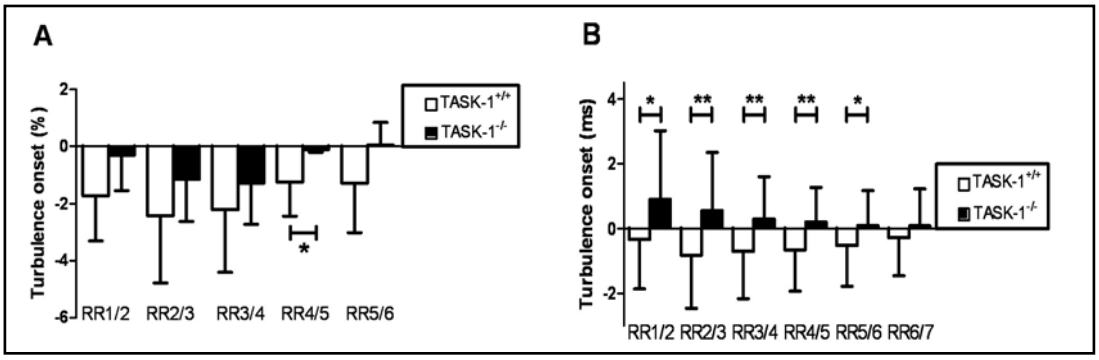


Fig. 5. Turbulence onset (TO) in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice in two different experimental settings. (A) The TO after spontaneous premature ventricular contractions during reperfusion was reduced in TASK-$1^{-/-}$ mice and reached significance at RR4/5 (TASK-$1^{+/+}$: 8±4 PVC, TASK-$1^{-/-}$: 5±4 PVC during 6 h of reperfusion in n=5 mice of each genotype). (B) After pacing induced premature ventricular contractions (n=30 PVC analyzed in n=9 mice of each genotype) there was a statistically significant reduced turbulence onset in the first six RR intervals in the TASK-$1^{-/-}$ mice. (* $p<0.05$, ** $p<0.005$).

Heart rate turbulence in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice after ischemia/reperfusion and after single paced ventricular premature contraction

Our previous data showed that the heart rate variability analyzed by time domain and frequency domain parameters is significantly reduced in TASK-$1^{-/-}$ mice [8]. Here, we examined if heart rate turbulence is also altered in TASK-$1^{-/-}$ mice. We therefore chose two different experimental approaches to calculate turbulence onset and turbulence slope. HRT after 40 min of ischemia during reperfusion did not show a significant difference in the number of PVC during six hours of reperfusion analyzed by telemetry (TASK-$1^{+/+}$ 8±4 PVC vs. TASK-$1^{-/-}$ 5±4 PVC, each n=5). There was a tendency of reduced TO in TASK-$1^{-/-}$ mice. However, significant differences were only seen at one time point (Fig. 5 A). However, TO was significantly reduced in five of the six RR intervals after paced PVC (Fig. 5 B). No significant difference was seen in turbulence slope in both experimental settings (data not shown).

Discussion

In the present report we further characterized the cardiac phenotype of TASK-$1^{-/-}$ mice by *in vivo* electrophysiological studies. The main findings in the current study are that TASK-1 deficiency results in a phenotype resembling long QT syndrome. TASK-$1^{-/-}$ mice show a characteristic increase in QT and QTc prolongation at lower heart rates. Conduction properties, refractoriness or susceptibility for arrhythmia after burst stimulation are not altered in TASK-$1^{-/-}$ mice. However, TASK-1 deficiency affects heart rate turbulence and therefore impairs baroreceptor sensitivity.

QT prolongation in TASK-$1^{-/-}$ mice

We previously demonstrated a rate corrected QT prolongation in the surface ECG of TASK-$1^{-/-}$ mice anesthetized with ketamine/xylazine [8]. Since ketamine/xylazine results in significant bradycardia, we analyzed QT and rate corrected QT intervals in conscious TASK-$1^{-/-}$ mice and after different anesthetics [19] to evaluate QT and rate corrected QT intervals at physiological heart rates. Although heart rates in mice sedated with ketamine/xylazine were significantly lower compared to conscious mice, no difference was seen in the mean heart rate of conscious mice and mice sedated with Avertin®, pentobarbital or isoflurane, respectively. This means that the heart rate of the sedated mice was still in the lower physiological range. The mean heart rate of conscious mice was in the range of previously published data

obtained by telemetry on mice with a C57/Bl6 background [20]. Therefore, differences in QT and rate corrected QT interval in conscious and sedated mice (Avertin®, pentobarbital and isoflurane) can not be explained by differences in heart rate. Pentobarbital, isoflurane and Avertin® in the concentrations we used did not affect ECG parameters like P duration, PR and QRS interval in TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice when compared to the data obtained in the conscious mice of both genotypes. A study in mice showing that pentobarbital prolongs PQ and QT intervals used twice the dosage we used and a different mouse strain [21]. However, since pentobarbital reduces transmural dispersion of repolarization and prevents torsades de pointes tachycardias in models of acquired and congenital long QT syndrome [22], the programmed electrical stimulation was done with Avertin®, which is often used for studying cardiac function in mice [19]. During anesthesia with isoflurane no difference in QT interval or rate corrected QT interval could be detected between TASK-$1^{+/+}$ and TASK-$1^{-/-}$ mice. It is known that isoflurane activates TASK-1, TREK-1, TASK-3 and other K2P channels [4, 23]. We have shown previously that - besides TASK-1 - TREK-1 and to a lesser extent TASK-3 is expressed in murine heart. Although we could exclude a compensatory up-regulation of these channels in the TASK-$1^{-/-}$ mice by real-time PCR and western blotting [8], we hypothesize that isoflurane abolishes the prolongation of repolarization due to TASK-1 deficiency by activating other K2P channels expressed in cardiac ventricular myocytes. Therefore, irrespective of the different anesthetics used apart from isoflurane, a significant QT and rate corrected QT interval prolongation was detected in TASK-$1^{-/-}$ mice. Rate corrected QT prolongation was also recorded in ECGs of unrestrained TASK-$1^{-/-}$ mice by telemetry.

We saw in both genotypes the characteristic increase in the QTc/QT relation after isoprenaline as a hallmark of the Bazett formula, which means that at more rapid heart rates the rate corrected QT interval increases relatively to the measured QT interval (data not shown) [18]. Since the QT and rate corrected QT interval prolongation in TASK-$1^{-/-}$ mice was most striking at the lowest heart rates, we wanted to exclude overestimation due to the correction formula. We therefore used a modified Framingham equation for comparison. Calculation of the rate corrected QT intervals by this approach even augmented the rate corrected QT interval prolongation in TASK-$1^{-/-}$ mice at lower heart rates. Therefore, the comparison of the QTc intervals calculated by the modified Bazett formula (according to Mitchell) with those calculated by the Framingham formula further support the validity and comparability of the calculated QTc intervals in mice and men.

Higher heart rates reduced the difference in QT and rate corrected QT interval prolongation in TASK-$1^{-/-}$ compared to TASK-$1^{+/+}$ mice. These findings are in accordance with our previous data, demonstrating that the prolongation of the monophasic action potential in Langendorff perfused TASK-$1^{-/-}$ hearts declined at higher heart rates [8] and correspond to the data of Decher et al. [9] who showed that isoprenaline abolished the differences in the QT, rate corrected QT and QRS intervals they had recorded in surface ECGs of TASK-$1^{-/-}$ mice. Provocative testing using catecholamine infusion or exercise can be used clinically to unmask in particular concealed LQTS1 and was used for diagnostics before genetic testing was available [24, 25]. Under physiological conditions β-adrenergic stimulation leads to an increase in net outward repolarizing current by directly activating the Ca^{2+} activated slow component of the delayed rectifier potassium current $I_{ks.}$ Mutations in the corresponding gene *KCNQ1* result in a paradoxical increase in QT intervals in LQTS1 in more than 90%. This relation could not be fount in LQTS2 and LQTS3 [24, 25]. Although we have shown a significant prolongation of the QT and the rate corrected QT interval in different experimental settings, this paradoxical response could also not be observed in TASK-$1^{-/-}$ mice. In contrast, the QT and QTc prolongation in TASK-$1^{-/-}$ mice is most pronounced at lower heart rates. This finding might reflect that I_{TASK-1} gets more relevant for repolarization under resting conditions when I_{Ks} is not activated. In addition, the direct relation of QT and QTc prolongation to lower heart rates in TASK-$1^{-/-}$ mice, might explain the lack of arrhythmia or even fatal events during physical exercise like swimming and treadmill challenge followed by pharmacological adrenergic stimulation as shown recently [8]. The strong relation of QT interval to heart rate

might rather resemble the characteristic clinical finding in patients with LQTS3 who exhibit their fatal arrhythmia at sleep.

Programmed electrophysiological stimulation in TASK-1$^{+/+}$ and TASK-1$^{-/-}$ mice

For programmed intracardiac stimulation we applied Avertin® for anesthesia, which is used frequently since it has minor cardiac side effects [26]. We avoided the use of pentobarbital for these experiments because pentobarbital can prevent torsades de pointes tachycardia in murine models of the long QT syndrome by reducing transmural dispersion, albeit prolonging the QT interval [22]. When using isoflurane, activating effects on TASK-1, TASK-3 and TREK-1 are described [23]. Therefore, we wanted to avoid that activation of other K2P channels except TASK-1 affect the electrophysiological properties in TASK-1$^{-/-}$ mice.

We could not find any differences in sinus node function, conduction time (AV interval, Wenckebach point) and refractoriness (ARP, VRP, AV-nodal RP) between TASK-1$^{+/+}$ and TASK-1$^{-/-}$ mice. The lack of differences in the sinus node recovery time between the two genotypes is in accordance with the finding that TASK-1 mRNA barely exists in the sinus node [27]. There was no significant difference in the PQ interval in ECGs recorded by telemetry and in surface ECGs using Avertin®, pentobarbital or isoflurane. The lack of difference in the atrioventricular conduction was confirmed by intracardiac measurements of the AV-interval. Since we could not detect any differences in the conduction properties between TASK-1$^{+/+}$ and TASK-1$^{-/-}$ mice, we hypothesize that TASK-1 is not expressed preferentially in the conduction system. In addition, we and others have shown previously that TASK-1 mRNA is detectable in significant amounts in atria and ventricles of the murine heart [8, 28], which makes an exclusive expression of TASK-1 protein in the conduction system rather unlikely. However, the cellular and spatial distribution of TASK-1 protein in the murine heart is still not fully understood. To our knowledge none of the TASK-1 antibodies used for cellular localization of the TASK-1 protein in the heart was proven for specificity in the TASK-1$^{-/-}$ mouse [8, 12, 29]. For comparison, Decher et al. demonstrated recently that anesthesia with isoflurane shifted the Wenckebach point towards shorter cycle lengths in the TASK-1$^{-/-}$ mice [9]. They hypothesize that isoflurane induced activation of I_{TASK-1} hyperpolarizes cells of the conduction system, thus slowing down impulse propagation. Although we have recorded significantly longer monophasic action potential duration in TASK-1$^{-/-}$ Langendorff perfused hearts [8], we could not identify differences in refractoriness in both genotypes. TASK-1 mRNA is abundantly expressed in human atria [10] and even up-regulated in atrial fibrillation [30]. Therefore, TASK-1 is thought to play a role in electrical remodelling in atrial fibrillation by shortening the action potential. If this hypothesis is true, TASK-1$^{-/-}$ mice might be even more resistant to atrial fibrillation. Atrial burst stimulation before and after adrenergic stimulation with isoprenaline injection did not show an increased risk for atrial arrhythmia in both genotypes. Of note is the observation, that the same programmed atrial burst stimulation induced atrial fibrillation in another transgenic mouse model [Donner et al, unpublished data]. Therefore, atrial fibrillation in wildtype mice after burst stimulation occurs so rarely, that the question, whether TASK-1$^{-/-}$ mice are less prone to atrial fibrillation could not be evaluated. Since TASK-1 is expressed in murine ventricular cardiomyocytes [5, 8] and TASK-1$^{-/-}$ mice have a significant QT prolongation, we tested ventricular vulnerability and the risk for ventricular tachycardia by ventricular burst stimulation. However, no ventricular tachycardia could be electrically induced before and after isoprenaline challenge. The prolonged repolarization in TASK-1$^{-/-}$ mice does not result in a higher incidence of ventricular arrhythmia.

Heart rate turbulence in TASK-1$^{-/-}$ mice

Our previously reported analysis on time and frequency domain showed reduced overall heart rate variability and a sympathetic preponderance [8]. In addition, epinephrine challenge supported the hypothesis of a blunted baroreceptor reflex in TASK-1$^{-/-}$ mice [8]. To further analyze the autonomic regulation in TASK-1$^{-/-}$ mice, the heart rate turbulence pattern was studied in both genotypes. Whereas for time and frequency domain analysis ECGs should

be free of artefacts and arrhythmia, HRT parameters reflect the heart rate response after premature ventricular contractions. In HRT, the initial heart rate acceleration is triggered by transient vagal inhibition in response to the missed baroreflex afferent input caused by hemodynamically inefficient ventricular contraction and concomitant hypotension [11]. The HRT pattern is blunted in patients with reduced baroreflex sensitivity for example after myocardial infarction or in the course of heart failure [11].

The calculations of turbulence onset in TASK-$1^{-/-}$ mice after I/R reached significance at one interval only. The reason may be due to the relatively low number of premature ventricular contractions in the six hours of reperfusion that were analyzed by telemetry in both genotypes. In addition, it is well known that after singular PVC the HRT pattern is often masked by heart rate variability of other origins. Thus, a number of PVC has to be averaged to characterize the pattern accurately [11]. Therefore, we used the analysis of HRT after programmed ventricular stimulation as a second experimental approach, since it has been shown previously that heart rate turbulence can also be induced by ventricular pacing in an electrophysiological study or by a pacemaker [31, 32]. Turbulence onset was significantly altered in TASK-$1^{-/-}$ mice after paced premature ventricular contractions. TO and TS can be used as independent clinical variables. For example, an altered TO, but not TS is associated with a higher risk for ventricular tachyarrhythmia in patients with myotonic dystrophy type 1 [33]. Therefore, the reduced TO in TASK-$1^{-/-}$ mice might reflect either the sympathetic predominance and/or an impaired cardiac parasympathetic activity that leads to an altered baroreceptor reflex.

In summary, the present study further elucidates the electrophysiological findings of TASK-1 deficiency and the dual functions of this ion channel in the cardiovascular system. TASK-$1^{-/-}$ mice show a clinical phenotype of QT prolongation with a characteristic increase in QT prolongation at rest. The altered heart rate variability is most likely due to a blunted baroreceptor reflex.

Acknowledgements

The TASK-$1^{-/-}$ mouse was supplied by Dr. W. Wisden from the Department of Clinical Neurobiology, University of Heidelberg. Dr. Sager and Dr. Engelhardt from the animal care facility in Duesseldorf are greatly acknowledged for their continuous support with animal care and surgery. The work was supported by grants from the University of Duesseldorf and by the German Society for Pediatric Cardiology.

References

1 Nattel S, Maguy A, Le Bouter S, Yeh YH: Arrhythmogenic ion-channel remodelling in the heart: heart failure, myocardial infarction, and atrial fibrillation. Physiol Rev 2007;87:425-456.

2 Priori SG: The fifteen years of discoveries that shaped molecular electrophysiology. Circ Res 2010;107:451-456.

3 Bayliss DA, Sirois JE, Talley EM: The TASK family: two-pore domain background K^+ channels. Mol Interv 2003;3:205-219.

4 Lesage F, Lazdunski M: Molecular and functional properties of two-pore-domain potassium channels. Am J Physiol Renal Physiol 2000;279:F793-F801.

5 Putzke C, Wemhöner K, Sachse FB, Rinné S, Schlichthörl G, Li XT, Jae L, Eckhardt I, Wischmeyer E, Wulf H, Preisig-Müller R, Daut J, Decher N: The acid-sensitive potassium channel TASK-1 in rat cardiac muscle. Cardiovasc Res 2007;75:59-68.

6 Barbuti A, Ishii S, Shimizu T, Robinson RB, Feinmark SJ: Block of the background K^+ channel TASK-1 contributes to arrhythmogenic effects of platelet-activating factor. Am J Physiol Heart Circ Physiol 2002; 282:H2024-H2030.

7 Besana A, Barbuti A, Tateyama MA, Symes AJ, Robinson RB, Feinmark SJ: Activation of protein kinase Cε inhibits the two-pore domain K^+ channel, TASK-1, inducing repolarization abnormalities in cardiac ventricular myocytes. J Biol Chem 2004;279:33154-33160.
8 Donner BC, Schullenberg M, Geduldig N, Hüning A, Mersmann J, Zacharowski K, Kovacevic A, Decking U, Aller MI, Schmidt KG: Functional role of TASK-1 in the heart: studies in TASK-1 deficient mice show prolonged cardiac repolarization and reduced heart rate variability. Basic Res Cardiol 2011;106:75-87.
9 Decher N, Wemhöner K, Rinné S, Netter MF, Zuzarte M, Aller MI, Kaufmann SG, TL Xian, Meuth SG, Daut J, Sachse FB, Maier SKG: Knock-out of the potassium channel TASK-1 leads to a prolonged QT interval and a disturbed QRS complex. Cell Physiol Biochem 2011;28:77-86.
10 Limberg SH, Netter MF, Rolfes C, Rinné S, Schlichthörl G, Zuzarte M, Vassiliou T, Moosdorf R, Wulf H, Daut J, Sachse FB, Decher N: TASK-1 channels may modulate action potential duration of human atrial cardiomyocytes. Cell Physiol Biochem 2011;28:613-624.
11 Bauer A, Malik M, Schmidt G, Barthel P, Bonnemeier H, Cygankiewicz I, Guzik P, Lombardi F, Müller A, Oto A, Schneider R, Watanabe M, Wichterle D, Zareba W: Heart rate turbulence: standards of measurement, physiological interpretation, and clinical use. J Am Coll Cardiol 2008;52:1353-1365.
12 Aller MI, Veale EL, Linden A-M, Sandu C, Schwaninger M, Evans LJ, Korpi ER, Mathie A, Wisden W, Brickley SG: Modifying the subunit composition of TASK-1 channels alters the modulation of a leak conductance in cerebellar granule neurons. J Neurosci 2005;25:11455-11467.
13 Mitchell GF, Jeron A, Koren G: Measurement of heart rate and Q-T interval in the conscious mouse. Am J Physiol 1998;274:H747-H751.
14 Sagie A, Larson MG, Goldberg RJ, Bengston JR, Levy D: An improved method for adjusting the QT interval for heart rate (the Framingham heart study). Am J Cardiol 1992;70:797-801.
15 Berul CI: Electrophysiological phenotyping in genetically engineered mice. Physiol Genomics 2003;13:207-216.
16 Schrickel JW, Brixius K, Herr C, Clemen CS, Sasse P, Reetz K, Grohé C, Meyer R, Tiemann K, Schröder R, Bloch W, Nickenig G, Fleischmann BK, Noegel AA, Schwinger RHG, Lewalter T: Enhanced heterogeneity of myocardial conduction and severe cardiac electrical instability in annexin A7-deficient mice. Cardiovasc Res 2007;76:257-268.
17 Petzelbauer P, Zacharowski PA, Miyazaki Y, Friedl P, Wickenhauser G, Castellino FJ, Gröger M, Wolff K, Zacharowski K: The fibrin-derived peptide Bβ15-42 protects the myocardium against ischemia-reperfusion injury. Nat Med 2005;11:298-304.
18 Luo S, Michler K, Johnston P, Macfarlane PW: A comparison of commonly used QT correction formulae: the effect of heart rate on the QTc of normal ECGs. J Electrocardiology 2004;37 Suppl:81-90.
19 Hart CYT, Burnett JC, Redfield MM: Effects of avertin versus xylazine-ketamine anesthesia on cardiac function in normal mice. Am J Physiol Heart Circ Physiol 2001;281:H1938-1945.
20 Weiergräber M, Henry M, Südkamp M, de Vivie E-R, Hescheler J, Schneider T: Ablation of Cav2.3/E-type voltage-gated calcium channel results in cardiac arrhythmia and altered autonomic control within the murine cardiovascular system. Basic Res Cardiol 2005;100:1-13.
21 Zeller A, Arras M, Jurd R, Rudolph U: Identification of a molecular target mediating the general anesthetic actions of pentobarbital. Mol Pharmacol 2006;71:852-859.
22 Shimizu W, McMahon B, Antzelevitch C: Sodium pentobarbital reduces transmural dispersion of repolarization and prevents torsades de pointes in models of acquired and congenital long QT syndrome. J Cardiovasc Electrophysiol 1999;10:154-164.
23 Patel AJ, Honoré E, Lesage F, Fink M, Romey G, Lazdunski M: Inhalational anesthetics activate two-pore-domain background K^+ channels. Nat Neurosci 1999;2:422-426.
24 Ackerman MJ, Khositseth A, Tester DJ, Hejlik JB, Shen WK, Porter CB: Epinephrine-induced QT interval prolongation: A gene-specific paradoxical response in congenital long QT syndrome. Mayo Clin Proc 2002;77:413-421.
25 Shimizu W, Antzelevitch C: Differential response to beta-adrenergic agonists and antagonists in LQT1, LQT2 and LQT3 models of the long QT syndrome. J Am Coll Cardiol 2000;35:778-786.
26 Roth DM, Swaney JS, Dalton ND, Gilpin ES, Ross J: Impact of anesthesia on cardiac function during echocardiography in mice. Am J Physiol Heart Circ Physiol 2002;282:H2134-2140.

27 Chandler NJ, Greener ID, Tellez JO, Inada S, Musa H, Molenaar P, DiFrancesco D, Baruscotti M, Longhi R, Anderson RH, Billeter R, Sharma V, Sigg DC, Boyett MR, Dobrzynski H: Molecular architecture of the human sinus node. Insights into the function of the cardiac pacemaker. Circulation 2009;119:1562-1575.

28 Kim D, Fujita A, Horio Y, Kurachi Y: Cloning and functional expression of a novel cardiac two-pore background K^+ channel (cTBAK-1). Circ Res 1998;82:513-518.

29 Graham V, Zhang H, Willis S, Creazzo TL: Expression of a two-pore domain K^+ channel (TASK-1) in developing avian and mouse ventricular conduction systems. Dev Dyn 2006;235:143-151.

30 Barth AS, Merk S, Arnoldi E, Zwermann L, Kloos P, Gebauer M, Steinmeyer K, Bleich M, Kääb S, Hinterseer M, Kartmann H, Kreuzer E, Dugas M, Steinbeck G, Näbauer M: Reprogramming of the human atrial transcriptome in permanent atrial fibrillation: expression of a ventricular-like genomic signature. Circ Res 2005;96:1022-1029.

31 Raj SR, Sheldon RS, Koshman M, Roach DE: Role of hypotension in heart rate turbulence physiology. Heart Rhythm 2005;2:820-827.

32 Roach D, Koshman ML, Duff H, Sheldon R: Induction of heart rate and blood pressure turbulence in the electrophysiologic laboratory. Am J Cardiol 2002;90:1098-1102.

33 Casella M, Russo AD, Pace M, Pelargonio G, Ierardi C, Sanna T, Messano L, Bencardino G, Valsecchi S, Mangiola F, Lanza GA, Zecchi P, Crea F, Bellocci F: Heart rate turbulence as a noninvasive risk predictor of ventricular tachyarrhythmias in myotonic dystrophy type 1. J Cardiovasc Electrophysiol 2006;17:871-876.