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Journal

Pflügers Archiv - European Journal of Physiology, 471(10)

ISSN

0031-6768

Authors

Kamatham, Samuel
Waters, Christopher M
Schwingshackl, Andreas
et al.

Publication Date

2019-10-01

DOI

10.1007/s00424-019-02306-y

Peer reviewed

HHS Public Access

Author manuscript

Pflugers Arch. Author manuscript; available in PMC 2022 April 11.

Published in final edited form as:

Pflugers Arch. 2019 October ; 471(10): 1263–1272. doi:10.1007/s00424-019-02306-y.

TREK-1 protects the heart against ischemia reperfusion-induced injury and from adverse remodeling after myocardial infarction

Samuel Kamatham[1], **Christopher M. Waters**[2], **Andreas Schwingshackl**[3], **Salvatore Mancarella**[1,*]

[1]Department of Physiology, University of Tennessee Health Sciences Center, Memphis, TN.

[2]Department of Physiology, University of Kentucky, Lexington, KY.

[3]Department of Pediatrics, University of California, Los Angeles, CA.

Abstract

The TWIK-related K+ channels (TREK-1) is a two-pore domain potassium channel that produces background leaky type potassium currents. TREK-1 has a protective role against ischemia-induced neuronal damage. TREK-1 is also expressed in the heart, but its role in myocardial ischemia-reperfusion (IR)-induced injury has not been examined. In the current study, we used a TREK-1 knockout (KO) mouse model to show that TREK-1 has a critical role in the cardiac I/R-induced injury and during remodeling after myocardial infarction (MI). At baseline, TREK-1 KO mice had similar blood pressure and heart rate as the wildtype (WT) mice. However, the lack of TREK-1 was associated with increased susceptibility to ischemic injury and compromised functional recovery following ex-vivo I/R-induced injury. TREK-1 deficiency increased infarct size following permanent coronary artery ligation, resulting in greater systolic dysfunction than the WT counterpart. Electrocardiographic (ECG) analysis revealed QT interval prolongation in TREK-1 KO mice, but normal heart rate (HR). Acutely isolated TREK-1 KO cardiomyocytes exhibited prolonged Ca^{2+} transients duration associated with action potential duration (APD) prolongation. Our data suggest that TREK-1 has a protective effect against I/R-induced injury and influences the post-MI remodeling processes by regulating membrane potential and maintaining intracellular Ca^{2+} homeostasis. These data suggest that TREK-1 activation could be an effective strategy to provide cardioprotection against ischemia-induced damage.

[*]**Correspondence to:** Salvatore Mancarella, Ph.D., Assistant Professor, Department of Physiology, University of Tennessee Health Science Center, 71 S. Manassas Street, Memphis, TN 38163, USA, Phone office: 901 448 7348, smancare@uthsc.edu.

Authors' Contributions.

SM was the principal investigator of the study, participated in the design of the study, carried out the experiments, performed the statistical analysis, and drafted the manuscript. SK Ca^{2+} transients measurements and analyzed the data, AS and CW provided the TREK-1 KO mice. All authors read and approved the final manuscript.

Disclosures.

There are no competing interests.

Compliance with Ethical Standards:

All animal experiments were performed according to protocols approved by the Institutional Animal Care and Use Committees and comply with USA regulations on animal experimentation.

Informed consent:

Informed consent was obtained from all individual participants included in the study.

Keywords

Potassium channel; ischemia-reperfusion; mice; telemetry; myocardial infarction; TREK-1

Introduction

Two-pore domain potassium channels (K2P channels) are a newly identified class of channels expressed throughout the animal kingdom and in plants (10, 12). K2P channels are encoded by 15 different genes classified in six clades. Assembled as functional dimers, as opposed to other potassium channels, which are tetramers, each subunit has four transmembrane domains (M1-M4) and two-pore domains (36). In contrast to the voltage-dependent channel, K2P channels lack an S4 voltage-sensing domain, and it is now clear that they are functionally important in the thalamus [6], immune cells [12], the heart [10,8], in the adrenal cortex [5] and the lung [28].

Evidence of a functional role for K2P channels in the heart is strongest for TWIK-related acid-sensitive K(+) channel 1 (TASK-1) and TWIK-related potassium channel-1 (TREK-1) channels [14]. The presence of TREK-1 mRNA was detected in cardiac tissue and rat cardiomyocytes (2, 27), functional TREK-1-like channels in ventricular myocytes was demonstrated with patch-clamp technique [34]. In the human heart, TREK-1 channels downregulation has been linked to heart failure and cardiac arrhythmogenesis (25). Although TREK-1 is the most studied among all the K2P members, the importance of TREK-1 in native cells has been difficult to characterize because of the lack of potent or selective inhibitors.

TREK-1 channel was initially regarded as a passive ‘leak channel’ that could influence both the resting membrane potential and the repolarization phase of the action potential along with the voltage-gated and Ca^{2+}-activated outward rectifiers [29]. TREK-1 channels can be activated by a variety of physical stimuli including membrane stretch, pH, temperature, and polyunsaturated fatty acids such as docosahexaenoic acid and arachidonic acid (AA) and by volatile anesthetic [17]. Conversely, TREK-1 channels are inhibited by cAMP and following Gαq and Gαs activation [16,22,26]. Interestingly, cardiac ischemia and ischemia followed by reperfusion are associated with accumulation of fatty acids, including AA and changes in pH that may regulate TREK-1 channels activity with profound effects on the progression of ischemic injury. Nonetheless, the overall contribution of TREK-1 in regulating cardiac excitability is unknown.

Given the potential importance of TREK-1 in cardiac pathophysiology, in this study, we used TREK-1 KO and WT mice to show that loss of TREK-1 exacerbated I/R-induced injury and impaired the recovery of contractile function after an ischemic event. We further demonstrated that absence of TREK-1 increase adverse ventricular remodeling after myocardial infarction. At cellular level, TREK-1 channel is involved in the regulation of Ca^{2+} transients and cardiac action potential duration (APD). We concluded that TREK-1 channel activity protects the ischemic myocardium and is beneficial during post-MI remodeling by regulating APD and preventing intracellular Ca^{2+} overload. New

pharmacological approaches targeting this channel represents a new class of potential therapeutic agents.

Methods

Mouse experiments.

All mice experiments were performed according to protocols approved by the Institutional Animal Care and Use Committees (IACUC) and comply with USA regulations on animal experimentation. TREK-1 KO and WT mice were bred on the same C57BL/6 genetic background and were a kind gift from Dr. Robert Bryan (Baylor Houston). Mice were housed in a facility maintained at 22°C with an alternating 12-h light/dark cycle and had free access to food and water ad libitum. All mice used in these experiments were between 8-14 weeks of age, weighing between 20-30 gr, and gender-matched.

Telemetric blood pressure and ECG measurements.

Implantable radio transmitters were used to assessECG and blood pressure (BP), the full procedure has been reported previously [4]. Briefly, mice were anesthetized with isoflurane and implanted with radio-transmitters (model HD-X11, Data Sciences International). The catheter was placed near the ascending aorta via the right carotid artery and the emitter inserted subcutaneously in the right dorsal flank. The two ECG leads were implanted subcutaneously, with one lead toward the right upper chest and the other near the left lower chest. Mice were allowed to recover from surgery for 5-7 days before baseline recording started. ECG, heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were collected using Dataquest ART data acquisition system (Data Sciences International, Inc.). Upon activation of the transmitter by a magnet, the electrical signals were transmitted wirelessly to a nearby receiver, and amplified signals were sent to a computer system. Data were sampled continuously and stored on a hard disc for offline analysis with LabChart Pro8 (ADInstruments). For analysis purposes, 20-40 second intervals (every 5 minutes) of recording were analyzed and averaged. In another group of animals, ECG activity was monitored during anesthesia as follows: single mice was housed in an anesthesia induction chamber (25 × 10 × 12 cm) containing a mixture of 1.2% isoflurane and room air at a rate of 0.5 l/min (SamnoSuite, Kent Scientific Corporation, Connecticut, USA). The anesthesia was maintained for a 45-min period (anesthetic maintenance state). In preliminary experiments, 3% isoflurane anesthesia often resulted in sick sinus syndrome and these experiments were terminated. All experiments were conducted between 1:00pm and 4:00pm.

Ischemia-reperfusion in the isolated heart

Mice were sacrificed, hearts were rapidly excised and retrogradely perfused via the aorta with modified Krebs– Henseleit solution (in mmol/l: NaCl 118.5, $CaCl_2$ 1.85, KCl 4.5, glucose 11.1, $NaHCO_3$ 25, $MgSO_4$ 2.5, NaH_2PO_4 1.4) gassed with a mixture of 95% O_2 and 5% CO_2 at 37 °C ± 0.5 °C, pH 7.35 ± 0.05. Perfusion flow through the aorta was constant and perfusion pressure was maintained between 70 and 90 mmHg. Left ventricular developed pressure (LVDP) was measured by a polyethylene tube with a cling film balloon tip filled with degassed water and inserted into the left ventricle through a small incision in

the left atrium. The other end of the tube was connected to a pressure transducer. After an equilibration period (15-20 min), hearts were paced electrically paced (6 Hz) and subjected to global no-flow ischemia (20-min), followed by reperfusion (40 min). Data were discarded if one of the following criteria appeared: i) hearts with irreversible arrhythmia during equilibration or after ischemia, and/or ii) absence of recovery after the reperfusion phase.

Assay of myocardial infarct area.

After reperfusion, the heart was arrested in diastole by adding 2,3-butanedione monoxime (10 mM) to the perfusion buffer. Next, the heart was quickly removed and placed at −20°C for a few minutes to harden. The heart was then sliced transversally at a thickness of 1 mm and immersed in neutral 2,3,5-triphenyltetrazolium (TTC) solution for 15 min to 20 min at 37°C, and then placed in 4% formaldehyde solution overnight. The viable myocardium was stained as the water-soluble compound TTC was converted by active mitochondrial dehydrogenases into an insoluble red precipitate. The extent of staining correlates with the number of viable mitochondria and differentiated viable and non-viable tissue [13]. TTC stained area (red staining, ischemic area) and non-TTC stained area (white, infarct area) were analyzed with a digital imaging system by computer, and myocardial infarct area was calculated [36].

Chronic myocardial infarction model.

Two to three-month-old male mice were anesthetized with isoflurane. Mice were intubated and attached to a ventilator. After surgical preparation, a 1 cm vertical incision was performed in the midclavicular line for a lateral thoracotomy. Mice were subjected to myocardial infarction by permanent ligation (8/0 nylon suture) of the left anterior descending coronary artery at about 1 mm below the edge of the left auricle, or to sham operation. The chest was closed, and the pneumothorax reduced. Mice were monitored continuously during recovery until the righting reflex was regained. Post-operative analgesia (buprenorphine, 0.075 mg/kg) was administered subcutaneously twice daily for three days. Non-invasive echocardiographic evaluation was performed to monitor cardiac function. At the end of the fourth week post-MI period, mice were sacrificed, and hearts were collected for further analysis.

Echocardiography.

Mice from both groups were anesthetized with isoflurane and placed on the warming pad of a recording stage of a Vevo 2100 ultrasound machine. The anterior chest was shaved, ultrasound coupling gel was applied, electrodes were connected to each limb, and an electrocardiogram was simultaneously recorded while body temperature was monitored. Two-dimensional (short axis-guided) M-mode measurements (at the level of the papillary muscles) were taken using an 18–32 MHz MS400 transducer as previously described [19,25]. Images were also recorded in the parasternal long-axis. For analysis purposes, three or more beats were averaged; measurements within the same HR interval (450 ± 50 bpm) were used for analysis.

Histological and morphometric analysis.

For morphometric studies, the heart was arrested in diastole by KCl injection and was fixed in 4% paraformaldehyde for 20 minutes under pressure (diastolic arterial pressure). The heart was excised, weighed, and stored for 24-48 hours in 4% paraformaldehyde at 4°C. Picrosirius Red and Masson's trichrome staining were performed on paraffin-embedded tissue sections from four weeks infarcted hearts, fibrosis and the infarcted area was measured [36].

Cardiomyocytes isolation and intracellular Ca^{2+} measurements.

Intracellular Ca^{2+} activity was measured using methods described previously [21,18]. Hearts were Langendorff-perfused with nominally Ca^{2+}-free Tyrode's solution containing the following (in mM): 137 NaCl, 5.4 KCl, 1 $MgCl_2$, 0.33 NaH_2PO_4, 10 HEPES, and 10 glucose (pH 7.4) equilibrated with 100% O_2 (35 ± 1°C). After the blood was washed out, collagenase type-2 (1 mg/ml; Worthington, Biochemical), protease type XIV (0.02 mg/ml; Sigma), and elastase (0.2 mg/ml; Worthington, Biochemical) were added to the perfusion solution. When the heart became dilated, it was detached from the system, both atria were removed and myocytes from the left ventricle were dispersed in the solution. Ventricular myocytes were subjected to Ca^{2+} re-adaptation and finally re-suspended in Tyrode's solution (0.5 mM $CaCl_2$). Cells were used within six hours from the isolation. For intracellular Ca^{2+} measurements, myocytes were incubated with fura-2 for 30 min at room temperature (22–24°C). Excess fura-2 was washed out and the cells were re-suspended in physiological buffer containing 0.5 mM $CaCl_2$. Cells were placed on a stage of an inverted microscope and continuously perfused with Tyrode's solution (1 mM $CaCl_2$). Cells were electrically paced at 0.5-Hz to accurately measure resting diastolic intracellular Ca^{2+} ($[Ca^{2+}]_i$). Fura-2 Ca^{2+} transients were captured using excitation wavelength set at 340 and 380nm and emission was detected at 510 nm with a dual-excitation fluorescence multiplier system (IonOptics).

Optical membrane potential measurements.

Experiments using FluoVolt-AM ester (ThermoFisher) at 1/1000 dilution was used according to manufacturer's instructions. FluoVolt loaded cells were allowed to settle at the bottom of a perfusion chamber with a coverslip base, which was mounted on an inverted fluorescence microscope mounting a 40X silicone oil immersion objective. Cells were perfused with a buffer containing 1mM $CaCl_2$, and electrically paced at 1-Hz. Fluorescence emission between 520 and 535 nm was detected by a photomultiplier tube (IonOptics).

Data analysis.

All results are expressed as mean ± S.E.M., and N = sample size (N represents the number of cells, animals or independent experiments as reported in the text). Outlier values were identified as values outside of the mean ± 2 SD interval. Statistical analysis was performed by using a paired *t*-test or, where appropriate, one-way ANOVA followed by Bonferroni correction, which was calculated with GraphPad Prism, version 7 (GraphPad Software; San Diego, CA, USA). For all statistical comparisons, statistical significance was set at $P < 0.05$.

Results

TREK-1 KO hearts show increased susceptibility to myocardial I/R-induced injury.

Myocardial ischemia followed by reperfusion has detrimental effects on the heart, often resulting in injured myocardium. TREK-1 channels protect neurons against I/R-induced injury [15]. Therefore, we investigated whether TREK-1 channel activity preserve cardiac function during I/R-induced damage. We studied left ventricular (LV) function in Langendorff-perfused isolated hearts subjected to 20 min of global ischemia followed by 40 min of reperfusion. During equilibration time (pre-ischemia), LVDP was similar between the TREK-1 KO and WT hearts suggesting that at baseline TREK-1 deletion does not alter the mechanical properties of the cardiac muscle (figure 1A). Recovery of LVDP after ischemia was significantly impaired in the TREK-1 KO hearts at each time point examined during the reperfusion period (figure 1A). At the end of the reperfusion phase, TTC staining revealed that the partial LVDP recovery in TREK-1 KO hearts was associated with larger infarct size when compared to the infarct size from the control hearts (figures 1B and C). Thus, disruption of TREK-1 severely compromises recovery of cardiac contractility in TREK-1 KO hearts and results in greater I/R-induced cardiac damage.

Absence of TREK-1 accelerates heart failure post-MI.

To determine whether TREK-1 is a critical determinant of the cardiac response to in-vivo chronic ischemic injury, we subjected TREK-1 KO and littermate WT mice to permanent coronary artery ligation or sham surgery. Four weeks post-surgery, TREK-1 KO hearts exhibited a much larger infarct size than their WT counterpart as demonstrated by Sirius Red staining (figures 2A and B). Quantitative estimation of LV function by echocardiography showed that cardiac contractility as indicated by fractional shortening (%FS) and left ventricular end-systolic volume (LVESV) were similar between WT and TREK-1 KO sham-operated mice. However, infarcted hearts showed deteriorated cardiac function in both groups as indicated by a drop %FS, which was significantly greater in the TREK-1 KO mice (figure 2C). TREK-1 KO mice post-MI showed a significant increase in LVESV when compared to the WT post-MI group (figure 2D). No difference in heart weight/body weight ratio was observed (figure 2E).

Functional alteration is paralleled by structural changes, detailed structural measurements performed on the sham-operated and post-MI mice are included in Table 1. At baseline TREK-1 mice did not ehibit and structural abnormality, however, analysis post-MI mice revealed greater LV diameter in TREK-1 KO hearts associated with thinner posterior wall thickness, which are indicative of accelerated maladaptive cardiac remodeling. These data suggest that TREK-1 deletion does not alter cardiac function and structure at baseline, but accelerates adverse post-MI ventricular remodeling.

TREK-1 KO mice display prolonged QT-interval

Potassium channels play a vital role in the control of the electrical activity of the heart. Whether TREK-1 is involved in the propagation of electric current through the myocardium during each heartbeat is still undetermined. Non-invasive unrestrained telemetry was used to investigate the cardiac electrical conduction in TREK-1 KO and WT mice.

ECG recordings revealed similar R-R, P-R and QRS intervals between TREK-1 KO and WT mice (figure 3A). ECG traces were evaluated for disturbances in rhythm and waveform morphology, and traces from TREK-1 KO mice displayed prolonged QT intervals associated with relatively wide dispersion, averaging 60.1 ± 5.6 ms in TREK-1 KO mice vs. 49.4 ± 7.3 ms in the control group ($P<0.05$) (figures 3B and C). All mice analyzed maintained a normal sinus rhythm during the recording despite the prolonged QT interval (figure 3D). Previous reports have described the presence of TREK-1 in smooth muscle cells [20,7], therefore, knockout of TREK-1 in smooth muscle could be a significant contribution to the observed mouse cardiac phenotype. Hemodynamic parameters including mean arterial pressure (MAP), systolic and diastolic blood pressure parameters were measured simultaneously to the ECG we found that all hemodynamic parameters measured were within a normal range between the two groups of animals (Table 2).

TREK-1 KO exaggerates isoflurane-induced QT prolongation.

The ability of volatile anesthetics to activate TREK-1 channels is well documented [29]. Clinically relevant concentration of isoflurane is known to prolong the QT interval duration without significant changes in QRS or PR duration in both laboratory animals [30], and in healthy human hearts [33,37,27]. Hence, we examined the effect of isoflurane on TREK-1 KO and WT mice while recording ECG. We reasoned that activation of TREK-1 by isoflurane would result in attenuated isoflurane-induced QT interval prolongation.

Mice carrying telemetric transmitters were singly housed in an anesthesia induction chamber while ECG recording was capturing the heart electrical behavior. At baseline, in the absence of isoflurane, the heart rate was not significantly different between the two groups (WT, 774 ± 55 *vs.* TREK-1 KO, 753 ± 68, $P<0.05$). The heart rate was slightly higher than what is usually observed in this mouse strain. Given that mice that are placed in their cages did not show any difference in heart rate we conclude that the higher heart rate is a result of the mice being placed into the induction chamber. When isoflurane was introduced in the chamber a steady drop of HR was observed in both TREK-1 KO and WT mice. Although HR decrease was similar between the two groups, after prolonged exposure to the anesthetic, WT mice displayed a lower HR than the TREK-1 KO mice (figure 4A). At baseline, QT interval duration was higher in the TREK-1 KO mice as compared to the WT mice. In presence of isoflurane, QT interval was similar in the two groups and only after prolonged exposure to isoflurane TREK-1 KO mice exhibited a greater QT interval duration than the WT counterpart (figure 4B). These results suggest that TREK-1 activation by isoflurane protects the heart against excessive QT interval prolongation.

Loss of TREK-1 delay intracellular Ca^{2+} clearance and prolongs action potential duration in isolated cardiomyocytes.

Changes in intracellular Ca^{2+} dynamics and APD prolongation in cardiomyocytes often precede ischemic damage and maladaptive post-MI remodeling. Intracelluar Ca^{2+} and membrane potential play critical roles in determining the extent of tissue injury. We measured Ca^{2+} transients in freshly isolated ventricular cardiomyocytes from TREK-1 KO and WT hearts. Cardiomyocytes isolated from both groups and subjected to electrical field stimulation showed no difference in Ca^{2+} transient amplitude at steady state (figures 5A

and B). Cells exhibited similar decay constant of the Ca^{2+} transient at 10% decay time (figure 5C). In contrast, time to 50% and 90% Ca^{2+}decay was prolonged in TREK-1 KO cells when compared to WT cells. Interestingly, when cells were paced at higher frequency resting intracellular Ca^{2+} concentration was higher in TREK-1 KO cells (figures 6A and B). Analysis of optically recorded action potential waveforms from isolated adult ventricular myocytes revealed that action potential duration was significantly prolonged in TREK-1 KO cells as compared to WT cells (figures 6C and D). This is consistent with the prolonged QT interval and the long-lasting Ca^{2+} decay observed in TREK-1 KO mice. Our results suggest that TREK-1 channels reduce cell repolarization time facilitating intracellular Ca^{2+} clearance and that TREK-1 deletion can lead to Ca^{2+} overload and have a harmful effect during I/R and post-MI remodeling.

Discussion.

The presence of TREK-1 channels in the cardiomyocytes and the likelihood that these channels regulate cardiac function has been reported [1,3,8]. However, it is not clear whether TREK-1 activity has any role during pathological conditions such as ischemia or during the progression of post-MI injury. Pharmacological approaches that attempt to manipulate TREK-1 function are difficult to interpret because TREK-1 is insensitive to most of the classical K^+ channel blockers. Using a TREK-1 KO mouse model, we showed that loss of TREK-1 exacerbated myocardial IR-injury resulting in increased myocardial damage and worsening functional recovery during reperfusion. We also provided evidence that TREK-1 could be protective against post-MI maladaptive remodeling. We further demonstrated that TREK-1 actively regulated cardiac repolarization and intracellular Ca^{2+} clearance by shortening the APD. Overall, our data show a critical role of TREK-1 in the healthy heart and during pathological conditions.

TREK-1 contribute to the cardiac electrical signal. Telemetry recordings from awake unanesthetized mice revealed a significant increase of QT intervals at baseline condition. Our data are supported by similar results reported earlier using a cardiac-specific TREK-1 KO mouse model and a mouse model lacking TASK-1 channels, another member of the K2P channel [31,11]. Furthermore, exposure to clinically relevant concentrations of isoflurane is known to prolong the QT-interval in the rodents and the human heart mainly by suppression of I_{to} current. Indeed, isoflurane significantly increased QT interval duration in TREK-1 KO mice, suggesting that the absence of TREK-1 current delays ventricular repolarization. Although TREK-1 KO mice showed a normal cardiac rhythm at rest, the HR in TREK-1 KO mice exposed to isoflurane was higher than the HR in WT mice; this suggest a functional role of TREK-1 in the sino-atrial nodal cell. These findings are supported by earlier reports which identified functional TREK-1 currents during sinoatrial node excitation [31]. The potential role of TREK-1 on these cells need to be further explored.

The most striking finding was that TREK-1 KO hearts were more susceptible to I/R-induced cardiac damage than the control counterpart. This is somewhat different from a previous finding showing that TREK-1 knockdown in neonatal rat cardiomyocytes is protective against ischemic damage in vitro [35]. Nevertheless, neonatal rat cardiomyocytes are not fully differentiated characterized by an hypometabolic status and are more tolerant to

ischemia than the adult mouse hearts [2,23]. Our results are more in line to those studies that utilize in vivo models, for instance, it has been shown that TREK-1 KO adult mice are susceptible to brain ischemia [15]. Mechanistically, TREK-1 channel may be activated by intracellular acidification and AA accumulation known to occur during ischemia [32,34,9]. TREK-1 activation could facilitate membrane repolarization and shorten the APD protecting the cells from Ca^{2+} overload, hence, preventing cell death and tissue damage.

Studies on computational models have shown that subtraction of TREK-1 currents from rodent action potential results in about 12 % increase in APD [31]. Here we have employed cell-permeable fluorophores for a rapid and convenient measure of the cardiomyocyte membrane potential and intracellular Ca^{2+} changes. These experiments demonstrate that TREK-1 is active during membrane repolarization and its absence results in a significantly prolonged APD in TREK-1 KO cardiomyocytes, which is associated with a delay in Ca^{2+} transient decay. Although these fluorophores are not suitable for assessing resting membrane potential, previous studies have shown that TREK-1 contributes to the setting of resting membrane potential [38]. Prolongation of APD, coupled with prolonged Ca^{2+} clearance time, suggests that TREK-1 KO cells are prone to Ca^{2+} overload during ischemic conditions. Disturbance of the Ca^{2+} clearance and, therefore, increasing intracellular Ca^{2+} concentration became more evident when cells were stimulated at higher frequency, again suggesting that these cells are prone to Ca^{2+} overload and explains TREK-1 KO cells vulnerability to ischemic injury. Because these cells are mechanically unoaded they cannot reach the same frequency of the mouse heart rate. Further investigation into TREK-1 role in the regulation of intracellular Ca^{2+} and ADP during ischemic conditions may provide new important regulatory mechanisms involving TREK-1 in the protection against IR -induced damage and post-MI cardiac remodeling. Prolongation of both action potential and Ca^{2+} transients can have inotropic effects due to an increase in sarcoplasmic reticulum Ca^{2+} uptake. We did not observe an increase in contractility in TREK-1 KO hearts, probably due to the permanent deletion of TREK-1 and potential remodeling of the excitation contraction-coupling key components to compensate for the loss of TREK-1 channel.

TREK-1 global KO show worsening of post-MI remodeling when compared to the WT group, however, it is difficult to pinpoint which cell population is contributing to the MI injury. Careful interpretation of the results of these experiments is critically important. For instance, it could be argued that since TREK-1 has been reported in smooth muscle, changes in coronary artery function may be responsible for worsening of the cardiac function after injury. Earlier reports have clearly shown that absence of TREK-1 does not affect the vascular system [24]. This accords with our findings where basic hemodynamic and blood pressure is not changed in the TREK-1 KO mice when compared to data from the WT mice. Moreover, during the IR experiments, perfusion pressure and flow rate of the coronary were not different between WT and TREK-1 KO hearts. We did not attempt a detailed analysis of cardiac remodeling post-MI because of the involvement of immnune-response and cardiac fibroblast during this process which can also be affected by TREK-1 deletion [1]. Our studies in acutely isolated cardiomyocytes suggest a functional role of TREK-1 in the myocardium. Further studies in this direction could benefit from tissue-specific and inducible TREK-1 KO models. Overall, the data presented here suggest the involvement of TREK-1 channels in regulating cardiac excitability. The possibility that TREK-1 channel

openers can be cardio-protective without causing adverse effects on the vasculature deserves further investigation.

Acknowledgments.

We would like to thank Mr. Jesse Gammons for his help in finalizing this manuscript. This work was supported by intramural department funds and by National Heart, Lung and Blood Institute (grant number HL114869, to Dr. S.M. and HL131526 and HL123540 to CMW).

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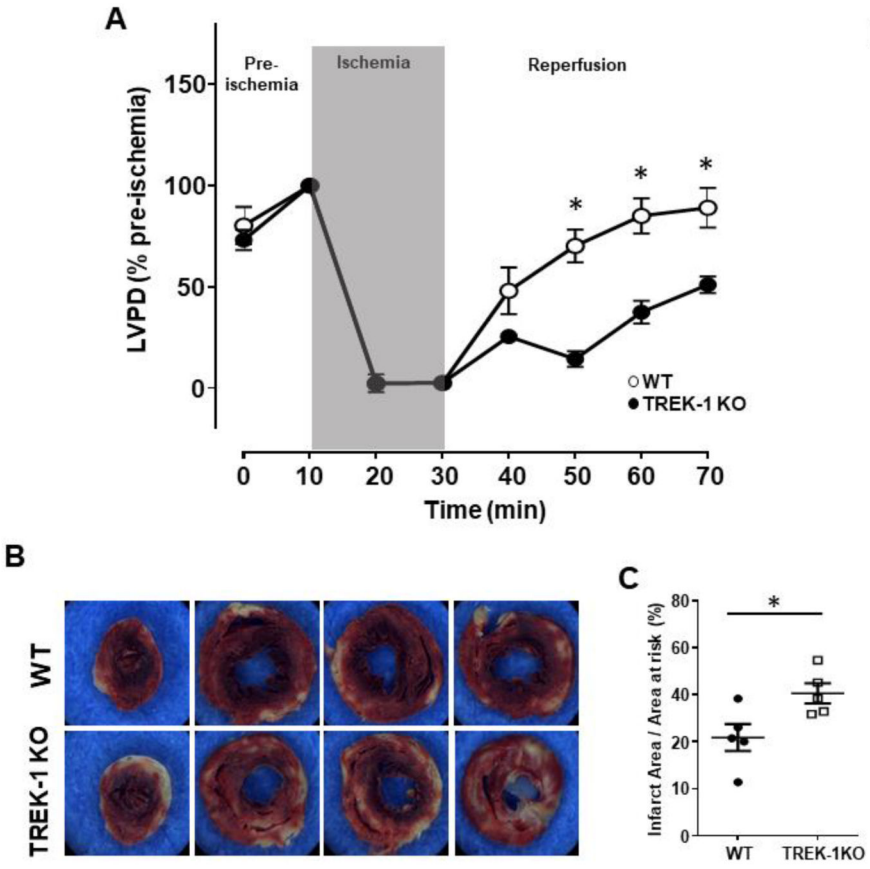

Figure 1.

TREK-1 deficiency impairs cardiac recovery after global IR. A) Time course representing changes in LVDP over time. The first LVDP value is obtained from spontaneously beating hearts; thereafter the organ was electrically paced (6Hz). The equilibration phase (10 min) is followed by 20 min of ischemia, where LVDP drops to near zero. Slow, but gradual LVDP recovery can be observed during the reperfusion phase (40 min). Overall, WT hearts showed better functional recovery during reperfusion than TREK-1 KO hearts. LVDP is expressed as % of baseline with data points measured at 10 min intervals. N = 8 hearts each group, *P < 0.05. B) Representative TTC stained ventricular sections from the apex (left picture) to the base (right picture) from WT and TREK-1 KO hearts. Myocardial infarct size was determined by TTC staining, and myocardial infarct size normalized to the area at risk. Unaffected myocardium is stained red; necrotic areas are white. Scale bar = 1mm. C) Quantification of the infarct size, (N = 5, mean ± S.E.), *P < 0.05 vs. WT controls.

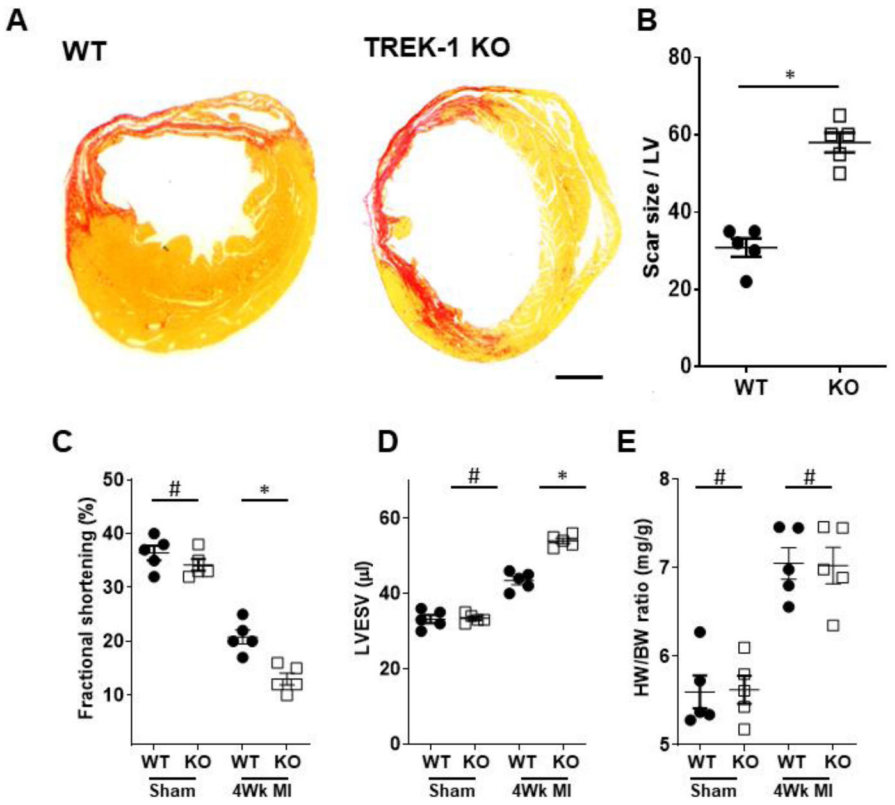

Figure 2.

Adverse effect of TREK-1 deletion during post-MI remodeling. A) Transverse ventricular sections from hearts after four weeks of permanent coronary artery ligation stained with Sirius Red. B) The percentage infarct segment in the left ventricle. C) Echocardiographic assessment of cardiac function by fractional shortening (FS) and D) left ventricular end-diastolic volume (LVEDV). E) Heart weight (HW)/body weight (BW) ratio. Data are extracted from WT (N = 5) and TREK-1 KO (N = 5) mice and are expressed as mean ± S.E. #P> 0.5; *P < 0.05 vs. WT controls. Statistical significance was evaluated using one-way ANOVA testing for the difference between multiple groups followed by Bonferroni's post-hoc comparisons tests. Scale bar = 1mm.

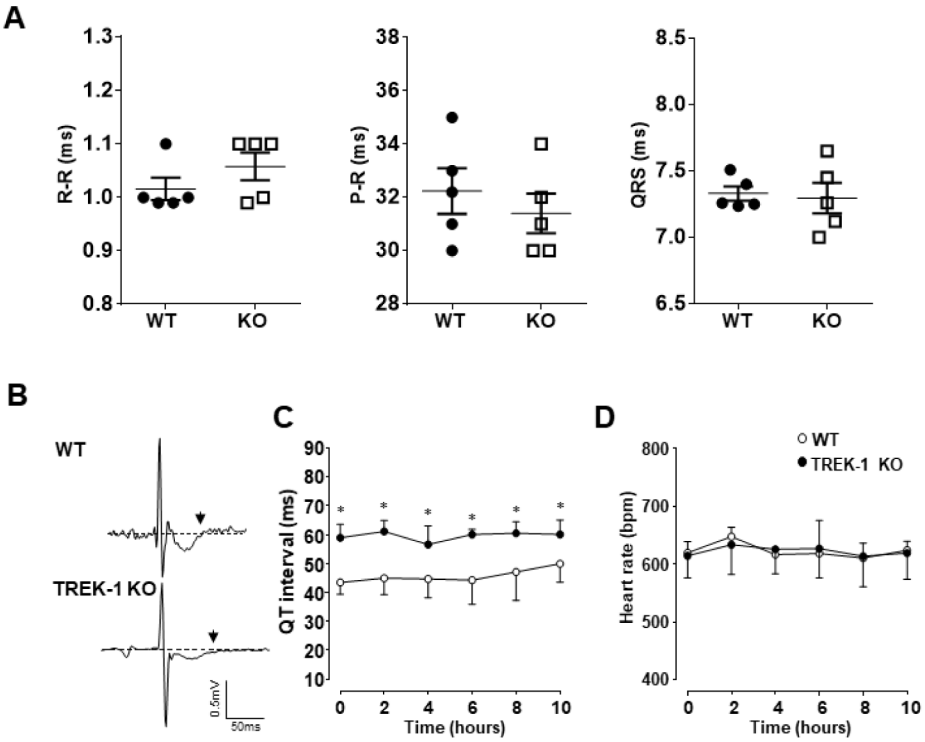

Figure 3.

Analysis of ECG subintervals recorded using a telemetric system. A) Averages of R-R, P-R, and QRS intervals are provided. All intervals are given in milliseconds (ms). B) Representative ECG traces from 2-lead recordings; waveforms were synchronized on the "R" peak. Top, an example of a recording from a WT mouse presenting a normal QT interval. Bottom, an example of a recording from a TREK-1 KO mouse showing prolonged QT dispersion. The end of the T-wave is indicated by the back arrow. C) Changes in QT intervals over a 10-hour period of continuous recording. D) Heart rate changes over the same period showed in (C). $n = 5$ per group; *$P < 0.05$ versus WT mice.

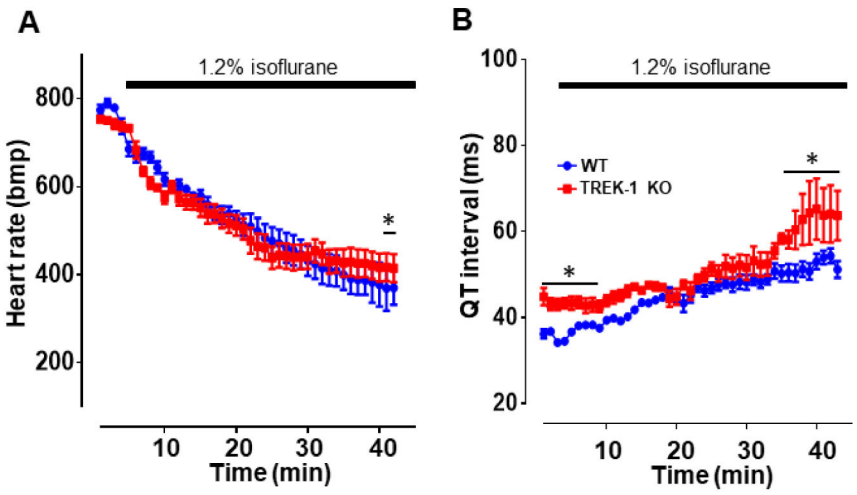

Figure 4.

Time course of the ECG changes during isoflurane administration in TREK-1 KO and WT mice. A) Heart rate recorded for 30 min in the presence of 1.2% isoflurane. B) QT interval duration extracted from the same traces used in A. Data are presented as mean ± S.E., n=5, *P < 0.05 vs. WT controls.

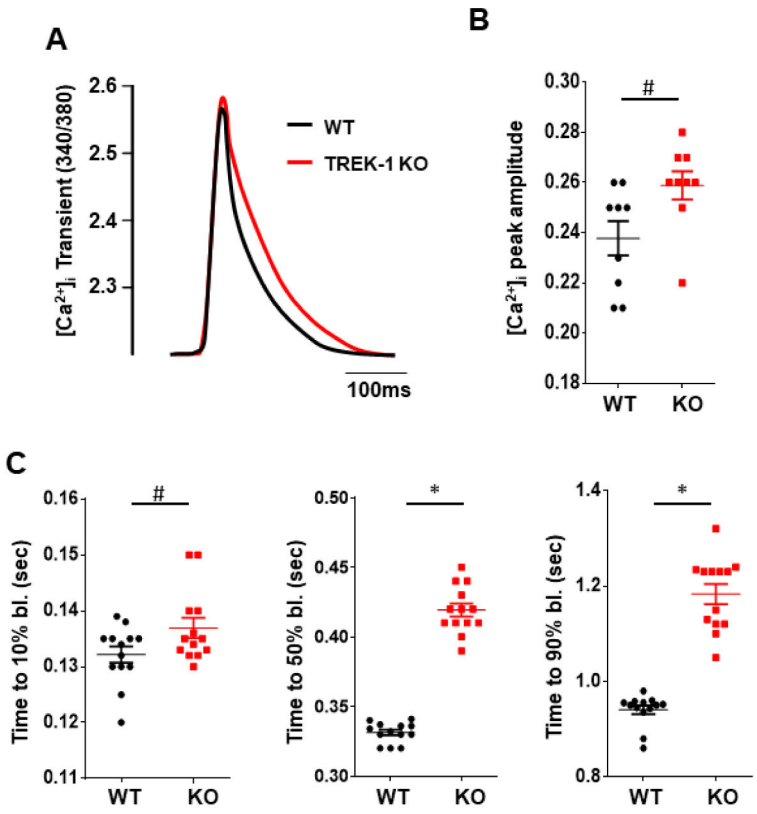

Figure 5.

Intracellular Ca^{2+} transients analysis in single cardiomyocytes isolated from TREK-1 KO and WT hearts. A) Representative traces of Ca^{2+} transient from TREK-1 KO and WT cells, traces are normalized and superimposed to facilitate comparison. B) Quantitative analysis showed no difference in the amplitude of Ca^{2+} transients between the two groups. C) Time to 10%, 50% and 90% decay of Ca^{2+} transient signal. N = 9 to 14cells/5 hearts. Values are presented as mean ± S.E. *P < 0.05 vs. WT.

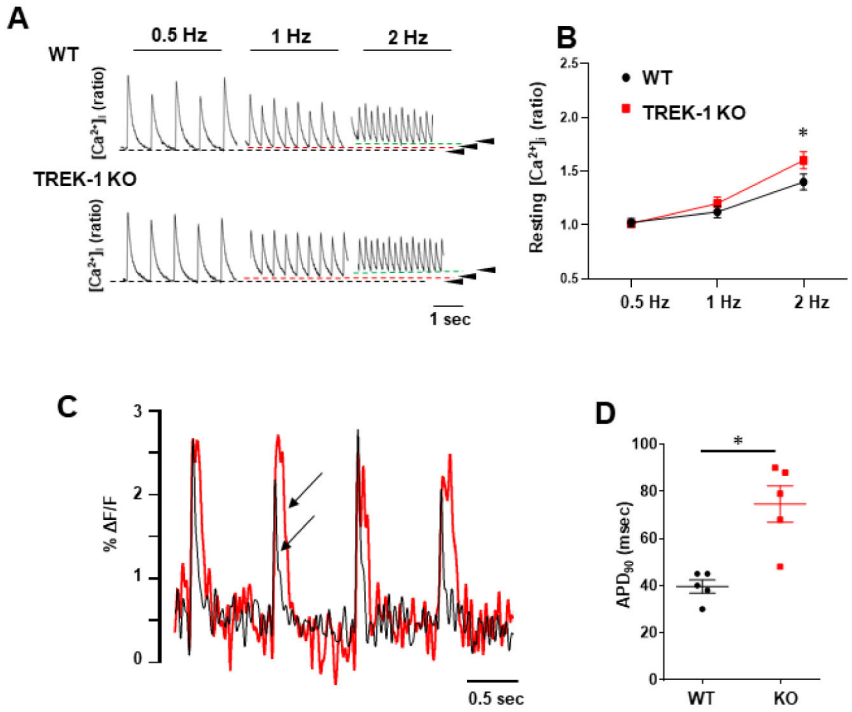

Figure 6.

Intracellular Ca^{2+} transients in response to increase in frequency and membrane potential measurements. Stimulation from 0.5 to 4 Hz. A) representative traces of intracellular Ca^{2+} in myocytes from adult Wt (n = 35) and TREK-1 KO (n = 27) myocytes (isolated from 4 mice each group). The numbers on the traces indicate the frequency of stimulation and transients evoked at 0.5, 1, and 2 Hz. B) Intracellular Ca^{2+} at resting level while stimulated at the correspondent frequency, values measured at the resting level indicated by the arrow-heads in panel A. C) Optical measurements of action potentials duration (AP) by using a voltage-sensitive dye (FluoVolt). Comparison of action potential measured using FluoVolt loaded cardiomyocyte isolated from TREK-1 KO and WT hearts. Cells were paced electrically at 1-Hz and AP waveform was recorded as a FluoVolt fluorescence signal. The figure shows a representative trace from each group containing five peaks superimposed to emphasize action potential prolongation in TREK-1 KO cardiomyocytes (arrow-heads, red trace). B) Peaks were evaluated for time to baseline (APD90%), five peaks from each cell were averaged, each point represents APD averaged from each heart 15 cells/5 hearts. Values are presented as mean ± S.E.*P < 0.05 compared to the control group.

Table 1.

Blood pressure values in WT controls and TREK-1 KO mice recorded by telemeters. N = 5 each group. Values are presented as mean ± S.E.

	Sham		MI		
	Control	**TREK-1**	**Control**	**TREK1**	**P<0.05**
N	7	7	9	7	
HR	450 ± 20	425 ± 18	435 ± 24	415 ± 13	n.s.
LVIDd (mm)	3.86 ± 0.06	3.80 ± 0.05	4.56 ± 0.05	5.82 ± 0.07*	P<0.05
LVIDs (mm)	3.08 ± 0.06	3.12 ± 0.05	3.85 ± 0.04	5.23 ± 0.04*	P<0.05
LVPWd (mm)	0.92 ± 0.06	0.96 ± 0.05	1.85 ± 0.08	1.44 ± 0.04*	P<0.05
LVPWs (mm)	1.48 ± 0.07	1.42 ± 0.04	2.34 ± 0.05	1.86 ± 0.05*	P<0.05

Table 2.

Echocardiographic results from sham-operated WT and TREK-1 KO mice and after four weeks post-myocardial infarction. N: number of animals; HR: heart rate; LVID: left ventricular internal diameter; LVPW: left ventricular diastolic posterior wall thickness; Data are expressed as mean ± S.E. *P<0.05 vs. WT four-week postinfarction group.

	Control	TREK-1
MAP (mmHg)	96.93 ± 2.63	96.64 ± 5.26
Systolic pressure (mmHg)	113.56 ± 3.73	114 ± 4.93
Diastolic pressure (mmHg)	81.01 ± 6.23	79.32 ± 5.89