Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2023**; 28(3): 51
https://doi.org/10.31083/j.fbl2803051

Original Research

The Effect of TWIK-1 Two Pore Potassium Channels on Cardiomyocytes in Low Extracellular Potassium Conditions

Ping Liu[1,2,3,†], Yawen Yang[3,†], Hengxi Zhang[3], Yingying Zhang[4], Jin Zeng[5,*], Dongchuan Zuo[1,2,3,*]

[1]National Traditional Chinese Medicine Clinical Research Base and Department of Cardiovascular Medicine of the Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, 646000 Luzhou, Sichuan, China
[2]Institute of Integrated Chinese and Western Medicine, Southwest Medical University, 646000 Luzhou, Sichuan, China
[3]Key Laboratory of Medical Electrophysiology, Ministry of Education & Medical Electrophysiological Key Laboratory of Sichuan Province (Collaborative Innovation Center for Prevention of Cardiovascular Diseases), Institute of Cardiovascular Research, Southwest Medical University, 646000 Luzhou, Sichuan, China
[4]Anesthesiology and Critical Care Medicine Key Laboratory of Luzhou, Department of Anesthesiology, The Affiliated Hospital of Southwest Medical University, 646000 Luzhou, Sichuan, China
[5]Department of Orthodontics, Orofacial Reconstruction and Regeneration Laboratory, The Affiliated Stomatology Hospital of Southwest Medical University, 646000 Luzhou, Sichuan, China
*Correspondence: zengjin@swmu.edu.cn (Jin Zeng); zuodongchuan@swmu.edu.cn (Dongchuan Zuo)
[†]These authors contributed equally.
Academic Editor: Daniela Valeria Miniero
Submitted: 31 August 2022 Revised: 28 November 2022 Accepted: 19 December 2022 Published: 15 March 2023

Abstract

Backgroound: At low extracellular potassium ($[K^+]_e$) conditions, human cardiomyocytes can depolarize to –40 mV. This is closely related to hypokalemia-induced fatal cardiac arrhythmia. The underlying mechanism, however, is still not well understood. TWIK-1 channels are background K^+ channels that are highly expressed in human cardiomyocytes. We previously reported that TWIK-1 channels changed ion selectivity and conducted leak Na^+ currents at low $[K^+]_e$. Moreover, a specific threonine residue (Thr118) within the ion selectivity filter was responsible for this altered ion selectivity. **Methods**: Patch clamp were used to investigate the effects of TWIK-1 channels on the membrane potentials of cardiomyocytes in response to low $[K^+]_e$. **Results**: At 2.7 mM $[K^+]_e$ and 1 mM $[K^+]_e$, both Chinese hamster ovary (CHO) cells and HL-1 cells ectopically expressed human TWIK-1 channels displayed inward leak Na^+ currents and reconstitute depolarization of membrane potential. In contrast, cells ectopically expressed human TWIK-1-T118I mutant channels that remain high selectivity to K^+ exhibited hyperpolarization of membrane potential. Furthermore, human iPSC-derived cardiomyocytes showed depolarization of membrane potential in response to 1 mM $[K^+]_e$, while the knockdown of TWIK-1 expression eliminated this phenomenon. **Conclusions**: These results demonstrate that leak Na^+ currents conducted by TWIK-1 channels contribute to the depolarization of membrane potential induced by low $[K^+]_e$ in human cardiomyocytes.

Keywords: hypokalemia; cardiomyocytes; membrane potential depolarization; TWIK-1 channels

1. Introduction

Hypokalemia is one of the common electrolyte disorders present in hospitalized patients. Moderate (blood K^+ 2.5–3.0 mM) and severe (blood K^+ <2.5 mM) hypokalemia can lead to life-threatening cardiac arrhythmia [1,2]. The membrane potential of cardiomyocytes in the resting state show hyperpolarization, which is essential for maintaining normal excitability of cardiomyocytes. Resting cardiomyocytes mainly open to background K^+ channels, and therefore the membrane potential of cardiomyocytes is close to the equilibrium potential of background K^+ channels (approximately –90 mV) [3]. According to Nernst equation theory, cardiomyocytes should be hyperpolarized when $[K^+]_e$ is low, as observed in mouse and rat cardiomyocytes [4,5]. However, previous studies showed that human, sheep and canine cardiomyocytes can paradoxically depolarize to around –40 mV when $[K^+]_e$ decreases [6–9]. Although this abnormal electrophysiological phenomenon is closely related to hypokalemia-induced fatal cardiac arrhythmia, the underlying mechanism is still not well understood.

K2P two-pore K^+ channels belonging to background K^+ channels help to maintain the membrane potential of many cell types [10–12]. TWIK-1 was the first subtype of K2P two-pore K^+ channels identified in human kidney tissues. Unlike other types of K^+ channels, TWIK-1 channels are dimers with two pore-forming loop domains in each subunit. The K^+ selectivity filter of TWIK-1 channels is a conserved signature sequence (TTGYG) in the P1 loop [13–15]. We previously reported that TWIK-1 channels can change ion selectivity and conduct leak Na^+ currents at sub-physiological $[K^+]_e$ [16,17]. Moreover, a specific threonine residue (Thr118) within the pore selectivity sequence TTGYG is responsible for the altered ion selectivity. This

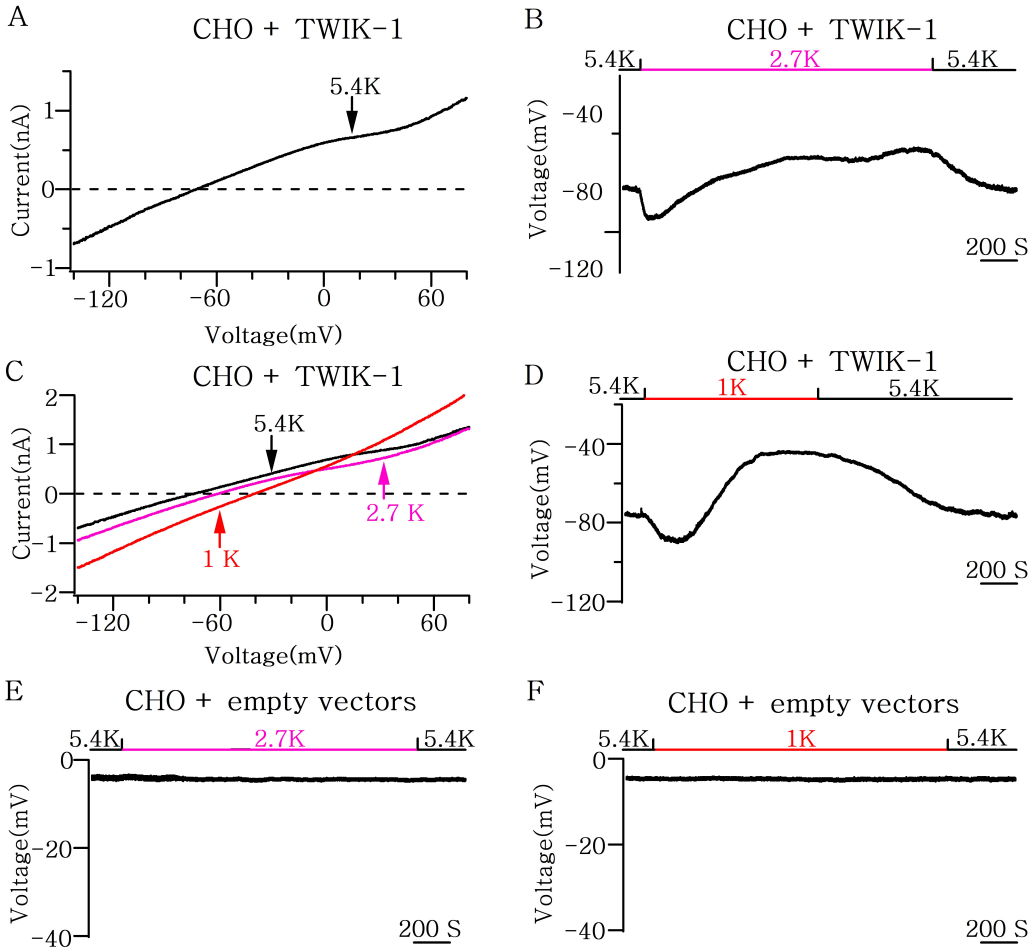

Fig. 1. CHO cells expressing human TWIK-1 channels show depolarization of membrane potential at low $[K]^+_e$. (A) showed whole-cell currents of human TWIK-1 channels at 5.4 mM $[K^+]_e$. (B,D) showed that CHO cells ectopically expressed human TWIK-1 channels were depolarized when $[K^+]_e$ were decreased from 5.4 mM to 2.7 mM or 1 mM, respectively. (C) showed whole-cell currents of human TWIK-1 channels at 5.4 mM $[K^+]_e$ (black lines), 2.7 mM (purple lines) and 1 mM $[K^+]_e$ (red lines), respectively. (E,F) showed that membrane potentials of CHO cells transduced with empty vectors remained unchanged when $[K^+]_e$ were decreased from 5.4 mM to 2.7 mM or 1 mM respectively.

finding indicates that TWIK-1 channels may have a depolarizing effect on cardiomyocytes at low $[K^+]_e$. The first aim of the present study was therefore to determine whether functional changes in TWIK-1 channels can induce depolarization of membrane potential in response to low $[K^+]_e$. This was studied in CHO cells using mammalian heterologous expression systems. The second aim was to determine whether TWIK-1 channels have a depolarizing effect on cardiomyocytes at low $[K^+]_e$. This was studied using HL-1 cells (a mouse cardiac myocytes cell line) and human iPSC-derived cardiomyocytes (hiPSC-CMs).

2. Materials and Methods

2.1 Cell Culture and Transduction

Recombinant lentiviral vectors carrying sequences encoding human TWIK-1, human TWIK-1 mutant channel (human TWIK-1-T118I) and human TWIK-1 specific shRNA (GCACATCATAGAGCATGACCAACTGTCCT) were constructed and packaged into the corresponding lentiviruses (Cyagen Biosciences, Suzhou, China). CHO cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) containing 1% penicillin-streptomycin and 10% FBS (fetal bovine serum) (Invigentech, Irvine, USA). HL-1 cells were cultured in Claycomb medium containing 10% FBS, 1% penicillin-streptomycin, 0.1 mM norepinephrine and 2 mM L-glutamate. Cells at 80% confluence were transduced with lentiviral-GFP (Green fluorescent protein) -TWIK-1 or lentiviral-GFP-TWIK-1-T118I at concentrations of 1.8–3 $\times$ 10^7 PFU mL^{-1}. After 48 hours transduction, the GFP positive cells were used for patch clamp recordings.

hiPSC-CMs were purchased from Help Therapeutics (Nanjing, China). CHO cells and HL-I cells were obtained

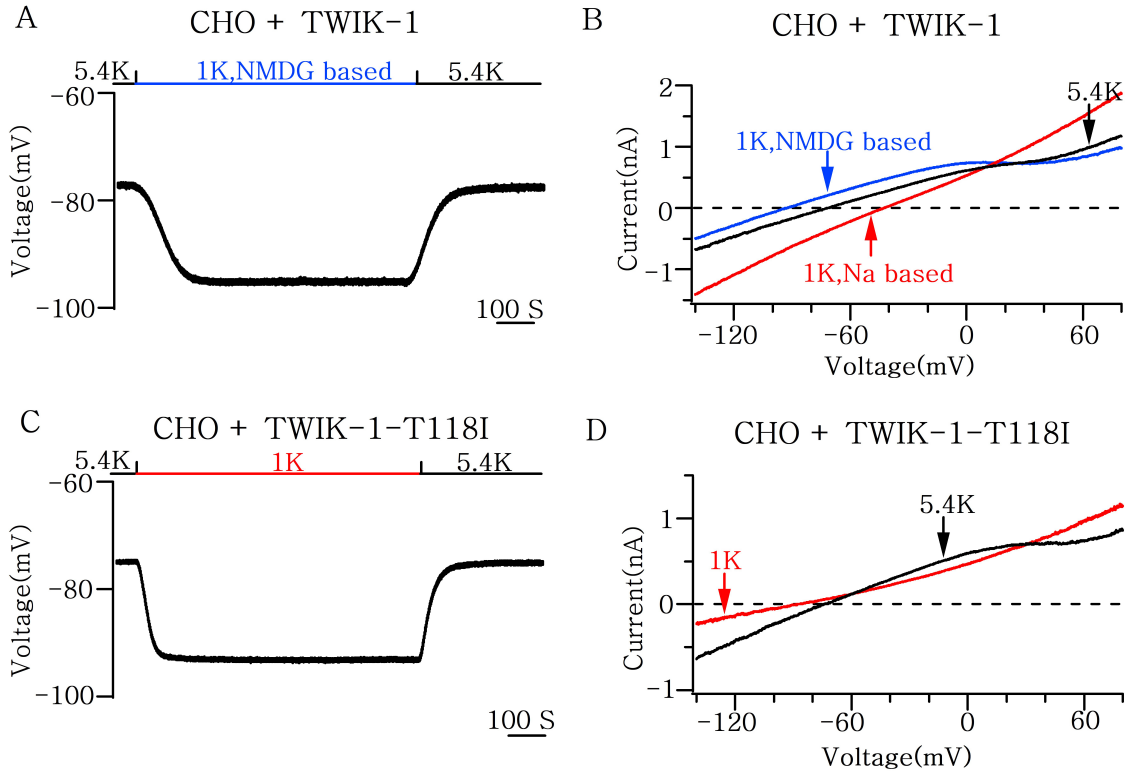

Fig. 2. Inward Na^+ currents conducted by TWIK1 channels induce the depolarization of membrane potentials in response to low $[K^+]_e$. (A) showed that CHO cells expressing human TWIK-1 channels were hyperpolarized when bath solutions containing 5.4 mM $[K^+]_e$ changed to NMDG-based bath solutions containing 1 mM $[K^+]_e$. (B) showed whole-cell currents of TWIK-1 channels at 5.4 mM $[K^+]_e$ (black lines), or 1 mM $[K^+]_e$ (red lines), and NMDG-based bath solutions containing 1 mM $[K^+]_e$ (blue lines). (C) showed that CHO cells expressing human TWIK-1-T118I channels were hyperpolarized when $[K^+]_e$ were decreased from 5.4 mM to 1 mM. (D) showed whole-cell currents of TWIK-1-T118I channels at 5.4 mM $[K^+]_e$ (black lines), or 1 mM $[K^+]_e$ (red lines).

from the Institute of Cardiovascular Research, Southwest Medical University (Luzhou, China). The cells were a mixture of atrial-, nodal- and ventricular-like myocytes. The cells were cultured with Cardiomyocyte Plating Medium, which was later replaced with Cardiomyocyte Maintenance Medium. After culture for 8–20 days, cells were transduced with lentiviral -GFP-TWIK-1 shRNA, and patch clamp recording was performed 2 days after viral transduction.

2.2 Electrophysiology

The HEKA patch clamp system (EPC10) was used to record membrane potentials and whole cell currents separately under current clamp mode and voltage clamp mode. A standard 2.2 second voltage ramp protocol from –140 mV to +80 mV every 15 seconds was performed at a sample rate of 2 KHz [16,17]. Patch pipettes with resistances of 3.0–5.0 M were used and data was analyzed by PatchMaster version 2x91 (HEKA Elektronik, Lambrecht, Germany) and IGOR pro version 6.05 (WaveMetrics, Portland, OR, USA) software. All data are reported as the mean ± SEM. Student's *t*-tests were used to evaluate the significance of differences between two groups.

Solutions for patch clamp recordings in CHO cells, pipette solution (mM): 140 KCl, 1 $MgCl_2$, 10 EGTA, 1 K_2-ATP and 5 Hepes (pH 7.4), bath solution (mM): 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 15 glucose and 10 Hepes (pH 7.4). Solutions for patch clamp recordings in HL-1 cells and human iPSC-derived cardiomyocytes, pipette solution (mM): 20 KCl, 120 K aspartate, 1 $MgCl_2$, 5 Na_2-ATP, 0.5 Na_2-GTP, 10 EGTA and 5 Hepes (pH 7.4), bath solution (mM): 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 Hepes (pH 7.4). To prepare the bath solutions containing low K^+, the $[K^+]_e$ was decreased and equimolar Na^+ was added to the bath solutions. $CoCl_2$ and TTX (tetrodotoxin) were added to bath solutions to block voltage-gated Ca^{2+} currents and voltage-gated Na^+ currents in cardiomyocytes.

3. Results

3.1 CHO Cells Expressing TWIK-1 Channels Exhibit Depolarization of Membrane Potential at Low $[K^+]_e$

CHO cells ectopically expressed human TWIK-1 channels were used in order to observe whether functional changes in these channels can have a depolarizing effect on the membrane potential in response to low $[K^+]_e$. Fig. 1A

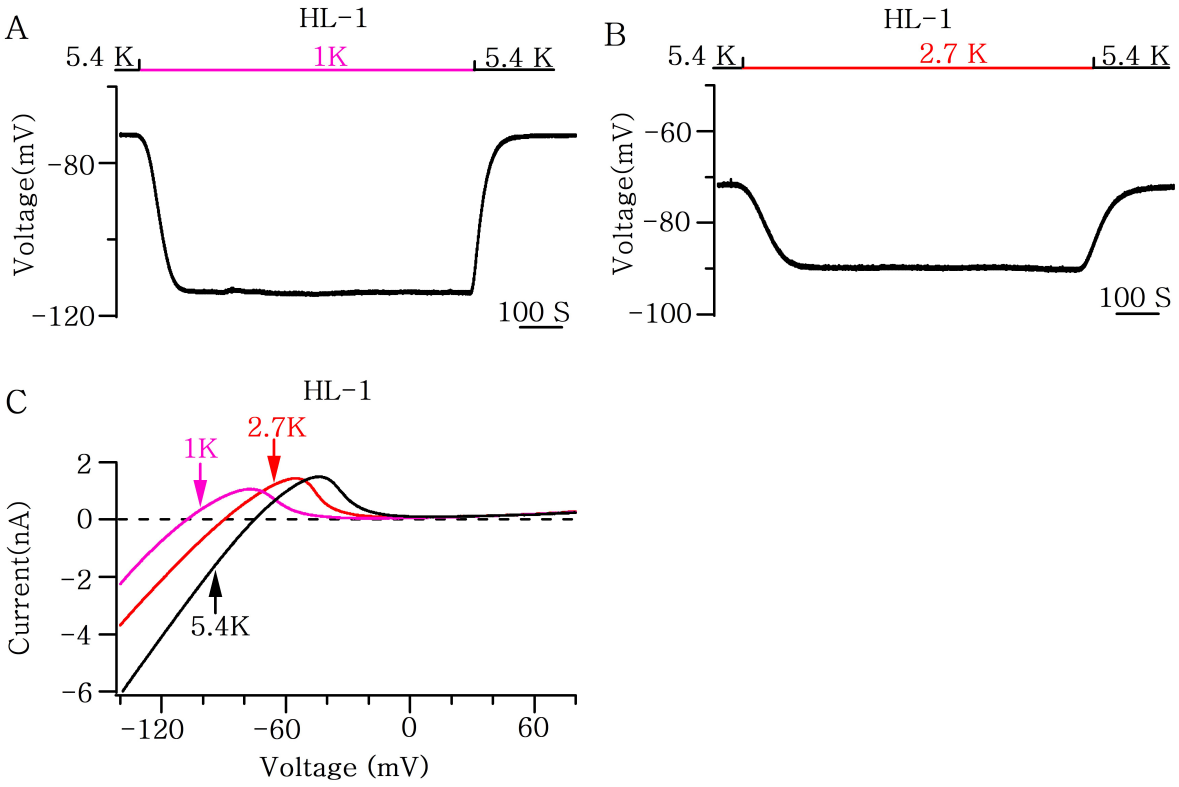

Fig. 3. HL-1 cells exhibited hyperpolarization of membrane potential at low $[K]^+_e$. (A,B) showed that HL-1 cells were hyperpolarized, when $[K^+]_e$ were decreased from 5.4 mM to 1 mM or 2.7 mM, respectively. (C) showed whole cell currents of HL-1 cells at 5.4 mM $[K^+]_e$ (black lines), 2.7 mM $[K^+]_e$ (red lines) or 1 mM $[K^+]_e$ (purple lines).

shows a typical TWIK-1 whole cell current at 5.4 mM $[K^+]_e$. We evaluated the effects of low $[K^+]_e$ on membrane potentials and whole cell currents in CHO cells expressing TWIK-1. When $[K^+]_e$ was lowered from 5.4 mM to 2.7 mM and to 1 mM, transduced CHO cells initially hyperpolarized, and then depolarized to –60 ± 3 mV and to –41 ± 2 mV, respectively (n = 16; Fig. 1B,D). The reversal potentials of TWIK-1 whole cell currents depolarized from –77 ± 0.9 mV to –58 ± 2 mV, and then to –40 ± 2 mV, respectively (n = 12; Fig. 1C). Fig. 1E,F show that 2.7 mM and 1 mM $[K^+]_e$ had no effects on the membrane potentials of CHO cells transduced with empty vectors (n = 8–11). Based on these results, we conclude that TWIK-1 channels cause depolarization of membrane potential at low $[K^+]_e$.

3.2 Na^+ Currents Mediated by TWIK-1 Channels Induce Depolarization of Membrane Potential in CHO Cells at Low $[K^+]_e$

We performed two sets of experiments to investigate whether Na^+ currents mediated by TWIK-1 channels can induce depolarization of membrane potential in CHO cells at low $[K^+]_e$. First, extracellular Na^+ was removed and changes in the membrane potential in response to low $[K^+]_e$ were monitored. When bath solution containing 5.4 mM $[K^+]_e$ was switched to an NMDG-based solution containing 1 mM $[K^+]_e$, the transduced CHO cells hyperpolarized to –92 ± 4 mV (n = 15; Fig. 2A) and the reversal potentials of TWIK-1 currents hyperpolarized from –77 ± 0.9 mV to –89 ± 3 mV (n = 13; Fig. 2B). Second, we transduced CHO cells with human TWIK-1-T118I channels that remain high selectivity to K^+ at low $[K^+]_e$. The CHO cells expressing human TWIK-1-T118I channels hyperpolarized to –90 ± 2 mV in response to 1 mM $[K^+]_e$ (n = 13; Fig. 2C), while the reversal potentials of TWIK-1-T118I currents hyperpolarized from –74 ± 3 mV to –87 ± 2 mV (n = 12; Fig. 2D).

3.3 HL-1 Cells Transduced with TWIK-1 Channels Exhibit Depolarization of Membrane Potential at Low $[K^+]_e$

The phenomenon of membrane potential depolarization induced by low $[K^+]_e$ was not found in mouse cardiomyocytes [5,18]. We therefore used HL-1 cells transduced with human TWIK-1 channels to observe whether functional changes to these channels have a depolarizing effect on cardiomyocytes at low $[K^+]_e$. Cells that were electrophysiologically quiescent were selected to investigate the effects of low $[K^+]_e$ on the membrane potential. When $[K^+]_e$ was lowered from 5.4 mM to 1 mM or 2.7 mM, HL-1 cells hyperpolarized to –108 ± 5 mV and –89 ± 2 mV, respectively (n = 17; Fig. 3A,B), while the reversal potentials of whole cell currents hyperpolarized from –78 ± 2 mV to –105 ± 3 mV and –90 ± 2 mV, respectively (n = 17; Fig. 3C). In contrast, HL-1 cells expressing human

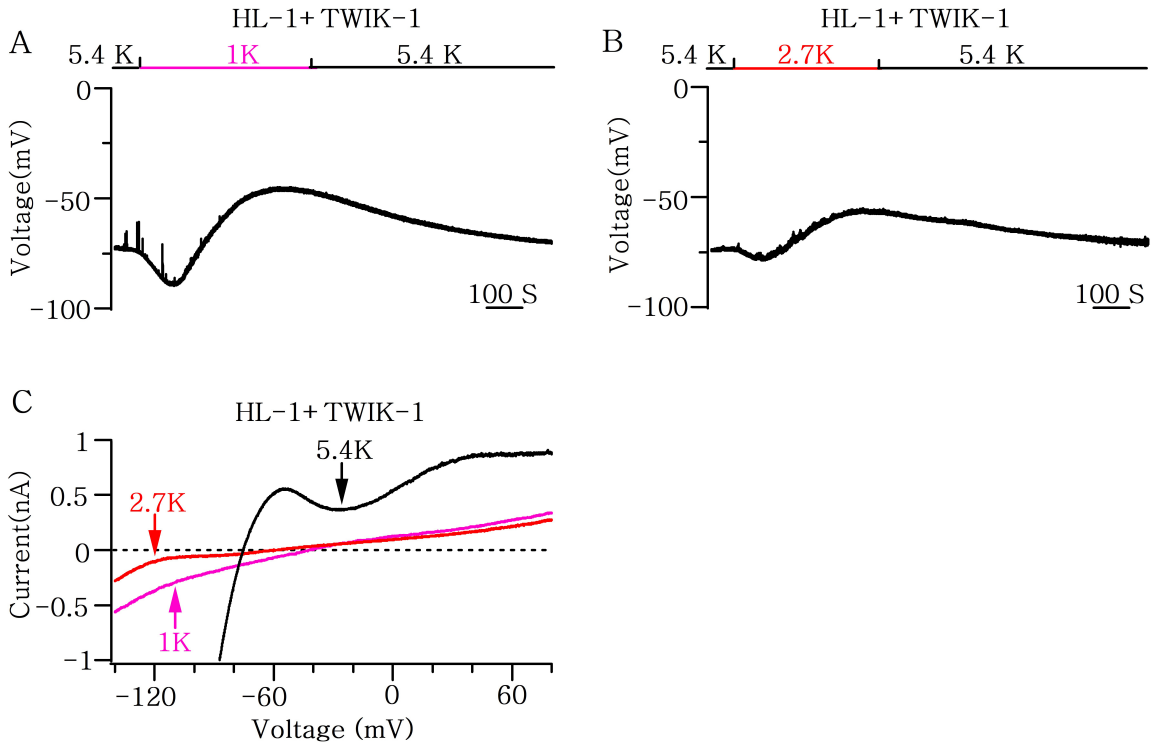

Fig. 4. HL-1 cells ectopically expressed human TWIK1 channels show depolarization of membrane potential at low $[K]^+_e$. (A,B) showed that HL-1 cells ectopically expressed human TWIK1 channels were depolarized, when $[K^+]_e$ were decreased from 5.4 mM to 1 mM or 2.7 mM, respectively. (C) showed whole cell currents of HL-1 cells ectopically expressed human TWIK1 channels at 5.4 mM $[K^+]_e$ (black lines), 2.7 mM $[K^+]_e$ (red lines) or 1 mM $[K^+]_e$ (purple lines).

TWIK-1 channels initially hyperpolarized, and then depolarized to –41 ± 3 mV and –58 ± 4 mV, respectively (n = 10; Fig. 4A,B), while the reversal potentials of whole cell currents depolarized from –82 ± 2 mV to –39 ± 2 mV and –60 ± 3 mV, respectively (n = 17; Fig. 4C). To confirm that TWIK-1 channels induce depolarization of membrane potential in HL-1 cells by conducting Na^+ currents in response to low $[K^+]_e$, we transduced these cells with human TWIK-1-T118I channels. When $[K^+]_e$ was lowered from 5.4 mM to 2.7 mM or 1 mM, the cells remained hyperpolarized (n = 12; Fig. 5A,B), while the reversal potentials of whole cell currents hyperpolarized from –76 ± 2 mV to –87 ± 2 mV and –107 ± 4 mV, respectively (n = 12; Fig. 5C).

3.4 TWIK-1 Channels Contribute to the Low $[K^+]_e$-Induced Depolarization of Membrane Potential in hiPSC-CMs

hiPSC-CMs were used to investigate whether TWIK-1 channels contribute to low $[K^+]_e$-induced depolarization of membrane potential in human cardiomyocytes. When $[K^+]_e$ was lowered from 5.4 mM to 1 mM, the membrane potential in 35 cells out of 113 depolarized to –42 ± 3 mV (Fig. 6A). These cells had inward leak Na^+ currents with a reversal potential of –40 ± 2 mV (Fig. 6C). When extracellular Na^+ was removed, the 1 mM $[K^+]_e$-induced membrane potential depolarization was eliminated, and the inward Na^+ currents were abolished (n = 10; Fig. 6B,C).

To evaluate the contribution of TWIK-1 channels to low $[K^+]_e$-induced depolarization of membrane potential in hiPSC-CMs, we first verified the quinine-sensitive Na^+ currents at 0 mM $[K^+]_e$ to demonstrate TWIK-1 expression. Among the 35 cells that showed depolarization of membrane potential in response to 1 mM $[K^+]_e$, we recorded quinine-sensitive TWIK-1-like inward Na^+ currents in 10 cells at 0 mM $[K^+]_e$ (Fig. 6D). Next, we used previously validated TWIK-1-specific shRNA to knockdown TWIK-1 channels in hiPSC-CMs. Less than 10% of cells that expressed TWIK-1-specific shRNA showed depolarization of membrane potential at 1 mM $[K^+]_e$, whereas 31% of control cells showed this phenomenon (Fig. 6E).

4. Discussion

TWIK-1 channels play important physiological and pathophysiological roles and are expressed in brain, heart, and kidney [19]. Mice that are deficient in TWIK-1 show defective phosphate transport in the proximal tubule and defective water transport in the medullary collecting duct of the kidney, as well as aberrant membrane potential in pancreatic β cells [20,21]. TWIK-1 also contributes to the intrinsic excitability of dentate granule cells in mouse hippocampal brain tissue [22,23]. Silencing TWIK-1 in zebrafish showed cardiac bradycardia and atrial dilation of

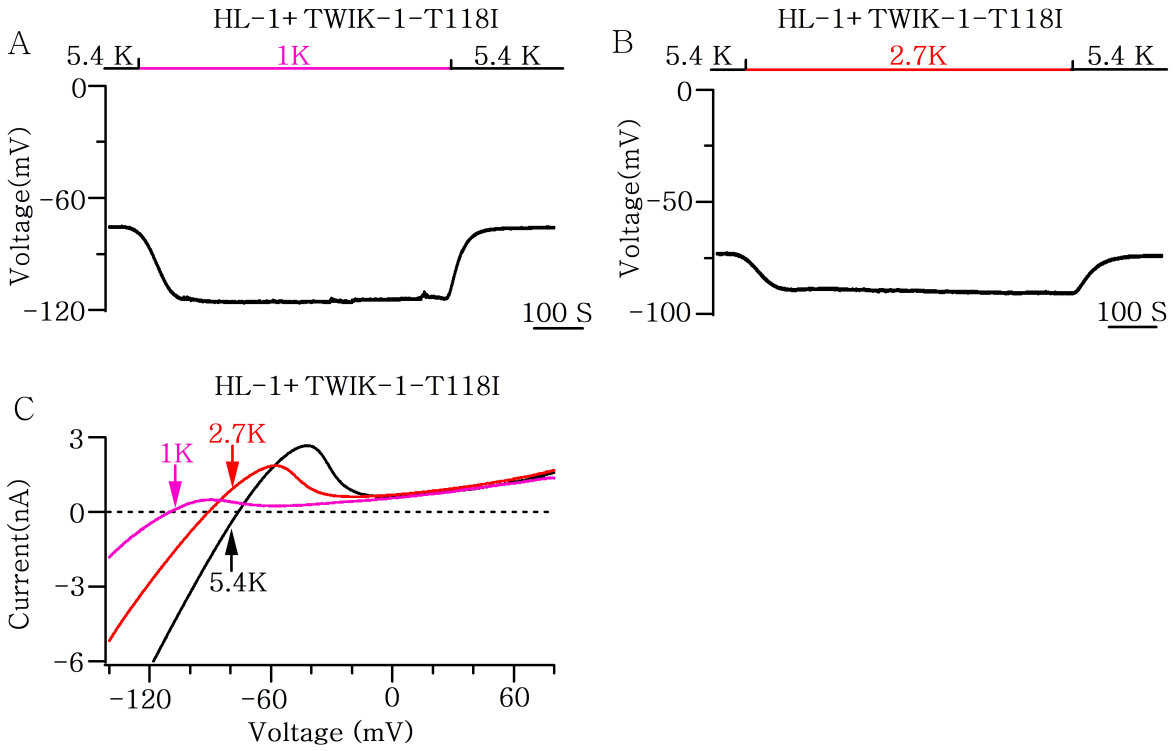

Fig. 5. HL-1 cells transduced with human TWIK-1-T118I channels exhibited hyperpolarization of membrane potential at low $[K]^+_e$. (A,B) showed that HL-1 cells ectopically expressed human TWIK1-T118I channels were hyperpolarized, when $[K^+]_e$ were decreased from 5.4 mM to 1 mM or 2.7 mM, respectively. (C) showed whole cell currents of HL-1 cells ectopically expressed human TWIK1-T118I channels at 5.4 mM $[K^+]_e$ (black lines), 2.7 mM $[K^+]_e$ (red lines) or 1 mM $[K^+]_e$ (purple lines).

the heart [24]. In transgenic mice that conditionally express TWIK-1 specifically in the heart, we recently reported that TWIK-1 induces ventricular ectopic beats and sudden death due to hypokalemia [25].

K2P channels are responsible for setting the membrane potential [26]. We previously demonstrated that TWIK-1 channels conducted leak Na^+ currents at low $[K^+]_e$ [16]. This led us to hypothesize that TWIK-1 channels have a depolarizing effect on the membrane potential. We first investigated the effect of TWIK-1 channels on the membrane potential by employing a mammalian heterologous expression system in CHO cells. CHO cells ectopically expressed of human TWIK-1 channels was found to be depolarized at 1 mM or 2.7 mM $[K^+]_e$. This result demonstrates that TWIK-1 channels have a depolarizing effect on the membrane potential under both moderate and severe hypokalemia conditions.

The membrane potential of cardiomyocytes is mostly maintained by IK1 currents that are primarily mediated by Kir2.x channels. Under hypokalemia conditions, the membrane potential of mouse or rat cardiomyocytes was hyperpolarized due to the activity of Kir2.x channels [4,5]. TWIK-1 channels are not expressed in mouse cardiomyocytes [27]. Therefore, we used HL-1 cells to evaluate whether TWIK-1 channels could have a depolarizing effect on cardiomyocytes at low $[K^+]_e$. Control HL-1 cells and HL-1 cells transduced with human TWIK-1-T118I mutant channels was found to be hyperpolarized, whereas that of HL-1 cells transduced with TWIK-1 channels was depolarized at low $[K^+]_e$. These results are consistent with our previous report that TWIK-1-T118I mutant channels remain high selectivity to K^+ at low $[K^+]_e$ [16]. Moreover, they demonstrate that TWIK-1 channels can cause depolarization of membrane potential in cardiomyocytes by mediating leak Na^+ currents in response to low $[K^+]_e$.

Depolarized membrane potential of cardiomyocytes is the main electrophysiological basis for hypokalemia-induced fetal cardiac arrhythmia. A study on sheep cardiomyocytes showed that depolarized membrane potential induced by low $[K^+]_e$ was abolished when extracellular Na^+ was removed, suggesting that Na^+ influx is essential for this phenomenon [28]. In the present study, we provide evidence to support the hypothesis that TWIK-1 leak Na^+ currents contribute to hypokalemia-induced depolarization of membrane potential in human cardiomyocytes. We first showed that hiPSC-CMs exhibited depolarization of membrane potential in response to low $[K^+]_e$, consistent with previous studies in human adult cardiomyocytes [6,9]. Furthermore, this phenomenon could be eliminated by the removal of external Na^+. Overall, these results imply that Na^+ currents are responsible for the depolarization of membrane potential induced by low $[K^+]_e$ in human car-

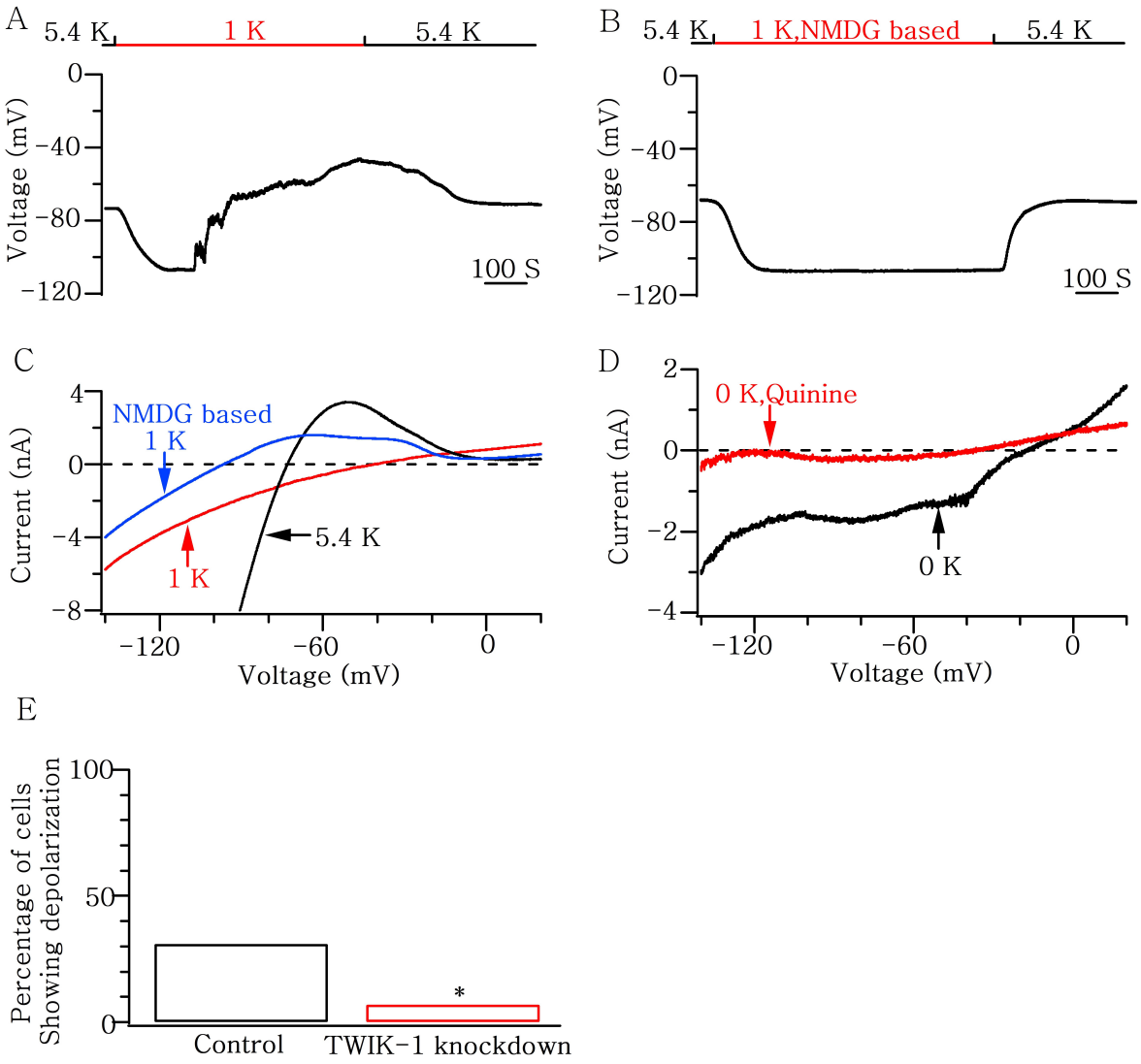

Fig. 6. TWIK-1 channels are responsible for the depolarization of membrane potential induced by low $[K]^+_e$ in hiPSC-CMs. (A) showed that hiPSC-CMs were depolarized when $[K^+]_e$ were decreased from 5.4 mM to 1 mM. (B) showed that hiPSC-CMs were hyperpolarized when bath solutions containing 5.4 mM $[K^+]_e$ changed to NMDG-based bath solutions containing 1 mM $[K^+]_e$. (C) showed whole-cell currents of hiPSC-CMs at 5.4 mM $[K^+]_e$ (black lines), or 1 mM $[K^+]_e$ (red lines), and NMDG-based bath solutions containing 1 mM $[K^+]_e$ (blue lines). (D) showed whole cell currents of hiPSC-CMs at 0 mM $[K^+]_e$ in the absence (black line) or presence (red line) of 1 mM quinine. (E) percentage of hiPSC-CMs (with and without shRNA interference) showing depolarization of membrane potential in 1 mM $[K^+]_e$. **p* = 0.0001, compare with scrambled shRNA; n = 113 to 120 cells for each group.

diomyocytes. Second, we demonstrated that TWIK-1 are the major channels responsible for Na^+ currents. We found that 31% of control hiPSC-CMs showed depolarization of membrane potential in response to low $[K^+]_e$, compared to <10% of cells that expressed TWIK-1-specific shRNA. Other non-selective cation ion channels may be responsible for the depolarized membrane potential in the remaining cells. A previous study reported that inward Ca^{2+} flux may also contribute to the depolarization of membrane potential induced by low $[K^+]_e$ in cardiac Purkinje fibres [29].

There are several limitations with the present study. First, the effect of TWIK-1 on membrane potential was studied only in cell lines, which may not be the best model to test our hypothesis. Therefore, the results require further confirmation using primary cultures of cardiomyocytes. Second, the difficulty in obtaining primary cultures of human cardiomyocytes meant that the effect of TWIK-1 was tested only in hiPSC-CMs. Future studies should therefore aim to determine the effects of TWIK-1 using clinical samples of hypokalemic hearts. Third, our experiments were performed *in vitro*, and hence the functional role of TWIK-1 in the pathophysiology of hypokalemia remains to be confirmed *in vivo*.

5. Conclusions

TWIK-1 channels contribute to the depolarization of membrane potentials induced by low $[K^+]_e$ in human cardiomyocytes by conducting inward leak Na^+ currents.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

PL, YY, HZ, YZ and JZ performed the experiments. DZ participated in the design of the experiments, analysis of data, and writing of the paper. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Ethical approval to conduct the in vitro studies was granted by the ethics committee of the Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University (20210801).

Acknowledgment

We thank Professor Haijun Chen (University at Albany, State University of New York, Albany, NY, USA) for providing the plasmids and the technical assistance.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (81800303), the Luzhou-Southwest Medical University Joint Project (2019LZXNYDJ01, 2020LZXNYDJ05), the National TCM Clinical Research Base construction unit scientific research project (the Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, [2020]33), Major project of Sichuan Provincial Administration of Traditional Chinese Medicine (2021ZD015), and the project from Science and Technology Department of Sichuan Province (2022YFS0618).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Lippi G, Favaloro EJ, Montagnana M, Guidi GC. Prevalence of hypokalaemia: the experience of a large academic hospital. Internal Medicine Journal. 2010; 40: 315–316.

[2] Schaefer TJ, Wolford RW. Disorders of potassium. Emergency Medicine Clinics of North America. 2005; 23: 723–747, viii–ix.

[3] Aziz Q, Li Y, Tinker A. Potassium channels in the sinoatrial node and their role in heart rate control. Channels. 2018; 12: 356–366.

[4] Bouchard R, Clark RB, Juhasz AE, Giles WR. Changes in extracellular K+ concentration modulate contractility of rat and rabbit cardiac myocytes via the inward rectifier K+ current IK1. The Journal of Physiology. 2004; 556: 773–790.

[5] Oshita K, Kozasa Y, Nakagawa Y, Kuwabara Y, Kuwahara K, Nakagawa T, *et al*. Overexpression of the HCN2 channel increases the arrhythmogenicity induced by hypokalemia. The Journal of Physiological Sciences. 2019; 69: 653–660.

[6] Christe, G. Effects of low [K+]o on the electrical activity of human cardiac ventricular and Purkinje cells. Cardiovascular Research. 1983; 17: 243–250.

[7] Ellis D. The effects of external cations and ouabain on the intracellular sodium activity of sheep heart Purkinje fibres. The Journal of Physiology. 1977; 273: 211–240.

[8] Miura DS, Hoffman BF, Rosen MR. The effect of extracellular potassium on the intracellular potassium ion activity and transmembrane potentials of beating canine cardiac Purkinje fibers. The Journal of General Physiology. 1977; 69: 463–474.

[9] McCullough JR, Chua WT, Rasmussen HH, Ten Eick RE, Singer DH. Two stable levels of diastolic potential at physiological K+ concentrations in human ventricular myocardial cells. Circulation Research. 1990; 66: 191–201.

[10] Kim D. Physiology and pharmacology of two-pore domain potassium channels. Current Pharmaceutical Design. 2005; 11: 2717–2736.

[11] Goldstein SAN, Bayliss DA, Kim D, Lesage F, Plant LD, Rajan S. International Union of Pharmacology. LV. Nomenclature and molecular relationships of two-P potassium channels. Pharmacological Reviews. 2005; 57: 527–540.

[12] Xian Tao Li, Dyachenko V, Zuzarte M, Putzke C, Preisig-Müller R, Isenberg G, *et al*. The stretch-activated potassium channel TREK-1 in rat cardiac ventricular muscle. Cardiovascular Research. 2006; 69: 86–97.

[13] Miller AN, Long SB. Crystal structure of the human two-pore domain potassium channel K2P1. Science. 2012; 335: 432–436.

[14] Nematian-Ardestani E, Abd-Wahab F, Chatelain FC, Sun H, Schewe M, Baukrowitz T, *et al*. Selectivity filter instability dominates the low intrinsic activity of the TWIK-1 K2P K^+ channel. The Journal of Biological Chemistry. 2020; 295: 610–618.

[15] Aryal P, Abd-Wahab F, Bucci G, Sansom MSP, Tucker SJ. A hydrophobic barrier deep within the inner pore of the TWIK-1 K2P potassium channel. Nature Communications. 2014; 5: 4377.

[16] Ma L, Zhang X, Chen H. TWIK-1 two-pore domain potassium channels change ion selectivity and conduct inward leak sodium currents in hypokalemia. Science Signaling. 2011; 4: ra37.

[17] Zuo D, Chen K, Zhou M, Liu Z, Chen H. Kir2.1 and K2P1 channels reconstitute two levels of resting membrane potential in cardiomyocytes. The Journal of Physiology. 2017; 595: 5129–5142.

[18] Aronsen JM, Skogestad J, Lewalle A, Louch WE, Hougen K, Stokke MK, *et al*. Hypokalaemia induces Ca^{2+} overload and Ca^{2+} waves in ventricular myocytes by reducing Na^+,K^+-ATPase α_2 activity. The Journal of Physiology. 2015; 593: 1509–1521.

[19] Lesage F, Guillemare E, Fink M, Duprat F, Lazdunski M, Romey G, *et al*. TWIK-1, a ubiquitous human weakly inward rectifying K+ channel with a novel structure. The EMBO Journal. 1996; 15: 1004–1011.

[20] Nie X, Arrighi I, Kaissling B, Pfaff I, Mann J, Barhanin J, *et al*. Expression and insights on function of potassium channel TWIK-1 in mouse kidney. Pflugers Archiv: European Journal of Physiology. 2005; 451: 479–488.

[21] Chatelain FC, Bichet D, Douguet D, Feliciangeli S, Bendahhou S, Reichold M, *et al*. TWIK1, a unique background channel with variable ion selectivity. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109: 5499–5504.

[22] Yarishkin O, Lee DY, Kim E, Cho C, Choi JH, Lee CJ, *et al*. TWIK-1 contributes to the intrinsic excitability of dentate granule cells in mouse hippocampus. Molecular Brain. 2014; 7: 80.

[23] Choi JH, Yarishkin O, Kim E, Bae Y, Kim A, Kim S, *et al*. TWIK-1/TASK-3 heterodimeric channels contribute to the neurotensin-mediated excitation of hippocampal dentate gyrus granule cells. Experimental & Molecular Medicine. 2018; 50: 1–13.

[24] Christensen AH, Chatelain FC, Huttner IG, Olesen MS, Soka M, Feliciangeli S, *et al*. The two-pore domain potassium channel, TWIK-1, has a role in the regulation of heart rate and atrial size. Journal of Molecular and Cellular Cardiology. 2016; 97: 24–35.

[25] Shen R, Zuo D, Chen K, Yin Y, Tang K, Hou S, *et al*. K2P1 leak cation channels contribute to ventricular ectopic beats and sudden death under hypokalemia. FASEB Journal. 2022; 36: e22455.

[26] Enyedi P, Czirják G. Molecular background of leak K+ currents: two-pore domain potassium channels. Physiological Reviews. 2010; 90: 559–605.

[27] Lesage F, Lauritzen I, Duprat F, Reyes R, Fink M, Heurteaux C, *et al*. The structure, function and distribution of the mouse TWIK-1 K+ channel. FEBS Letters. 1997; 402: 28–32.

[28] Sheu SS, Korth M, Lathrop DA, Fozzard HA. Intra- and extracellular K+ and Na+ activities and resting membrane potential in sheep cardiac purkinje strands. Circulation Research. 1980; 47: 692–700.

[29] Wiggins JR, Cranefield PF. Two levels of resting potential in canine cardiac Purkinje fibers exposed to sodium-free solutions. Circulation Research. 1976; 39: 466–474.