Combination of Sildenafil and Ba\(^{2+}\) at a Low Concentration Show a Significant Synergistic Inhibition of Inward Rectifier Potassium Current Resulting in Action Potential Prolongation

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Sildenafil (Viagra) is a vasodilator mainly used in the treatment of erectile dysfunction. Atrial or ventricular fibrillation may rarely occur as a side effect during sildenafil therapy. Although changes in inward rectifier potassium currents including \(I_{K1}\) are known to contribute to the pathogenesis of fibrillation, the effect of sildenafil on \(I_{K1}\) has not been studied. In experiments, Ba\(^{2+}\) is used as a specific inhibitor of \(I_{K1}\) at high concentrations (usually 100 µM). Being an environmental contaminant, it is also present in the human body; Ba\(^{2+}\) plasmatic concentrations up to 1.5 µM are usually reported in the general population. This study was primarily aimed to investigate changes of \(I_{K1}\) induced by sildenafil in a wide range of concentrations (0.1–100 µM). Additionally, the effect of combination of sildenafil and Ba\(^{2+}\) at selected clinically-relevant concentrations was tested, at 0.1 µM both on \(I_{K1}\) and on the action potential duration (APD). Experiments were performed by the whole-cell patch-clamp technique on enzymatically isolated rat ventricular cardiomyocytes, mostly at 23°C with the exception of APD measurements which were performed at 37°C as well. Sildenafil caused a significant, reversible, and concentration-dependent inhibition of \(I_{K1}\) that did not differ at −50 and −110 mV. Simultaneous application of sildenafil and Ba\(^{2+}\) at 0.1 µM revealed a massive inhibition of both inward and outward components of \(I_{K1}\) (this synergy was missing at other tested combinations). The combined effect at 0.1 µM (45.7 ± 5.7 and 43.0 ± 6.9% inhibition at −50 and −110 mV, respectively) was significantly higher than a simple sum of almost negligible effects of the individual substances and it led to a significant prolongation of APD at both 23 and 37°C. To our knowledge, similar potentiation of the drug-channel interaction has not been described. The observed massive inhibition of \(I_{K1}\) induced by a combined action of the vasodilator sildenafil and environmental contaminant Ba\(^{2+}\) at a low concentration and resulting in a significant APD prolongation may contribute to the genesis of arrhythmias observed in some patients treated with sildenafil.

Keywords: arrhythmia, barium, cardiomyocytes, inward rectifier potassium current, sildenafil, synergy
INTRODUCTION

Sildenafil (Viagra), a phosphodiesterase type 5 inhibitor, is a vasodilator used in the treatment of erectile dysfunction and pulmonary arterial hypertension (Galiè et al., 2009; Burks et al., 2018). The therapeutic plasma concentrations vary between 0.2 and 1.6 µM (Jackson et al., 1999; Croom and Curran, 2008; Gruenberg et al., 2009). Although the side effects of sildenafil are usually transient and mild (Croom and Curran, 2008), serious or even fatal arrhythmias may occur. Atrial fibrillation (AF) or less often ventricular tachycardia (VT)/ventricular fibrillation (VF) were reported after sildenafil administration, mostly in patients suffering from chronic heart diseases, however, usually without arrhythmias in anamnesis (Hayashi et al., 1999; Awan et al., 2000; Rasmussen et al., 2007; Varma et al., 2012). Episodes of AF after the use of sildenafil were reported even in healthy individuals (Rasmussen et al., 2007; Ruhela and Bagarhatta, 2018). Tracqui et al. (2002) published a case of sudden cardiac death closely connected to a massive intake of sildenafil (post-mortem plasmatic concentration reached 13.2 µM). Fatal arrhythmia was suggested as the cause of death of the patient. Since sildenafil is mostly used by older male patients with an increased cardiovascular risk (Kessler et al., 2019), arrhythmias occurring in them may be misattributed to their cardiovascular disease. Therefore, the true prevalence of sildenafil-related arrhythmias and deaths may be higher than that reported.

Changes in inward rectifier potassium currents including I_K1 are known to considerably contribute to the pathogenesis of both AF and VF (e.g., Dhamoon and Jalife, 2005; Atienza et al., 2006; Ehrlich, 2008; Heijman et al., 2014). The effect of sildenafil on I_K1 has not been studied so far.

Ba2+ is experimentally used as a potent concentration-dependent inhibitor of I_K1 (Alagem et al., 2001; Bhoelan et al., 2014). This metal is an environmental contaminant that can be identified in humans. It accumulates in the bones, teeth, heart, lungs, kidneys, and liver (Dallas and Williams, 2001; Kravchenko et al., 2014). Under standard exposure conditions, its plasmatic concentrations in the general population usually vary between 0.01 and 1.5 µM (Hung and Chung, 2004; Oskarsson and Reeves, 2007).

This study was aimed to investigate changes of the cardiac I_K1 induced by sildenafil in a wide range of concentrations including the clinically-relevant concentrations. Additionally, combined action of sildenafil and Ba2+ at selected concentrations was analysed.

MATERIALS AND METHODS

Ethical Approval

The experiments were carried out with respect to recommendations of the European Community Guide for the Care and Use of Laboratory Animals; the experimental protocol was approved by the Local Committee for Animal Treatment at Masaryk University, Faculty of Medicine, and by the Ministry of Education, Youth and Sports of the Czech Republic (MSMT-33846/2017-2).

Cell Isolation

All enzymes and chemicals used during the cell isolation and measurements were purchased from Sigma-Aldrich if not stated otherwise. Cardiomyocytes were isolated from right ventricles of adult male Wistar rats (290 ± 17 g, ~2.25 months old) anaesthetised by intramuscular administration of a mixture of tiletamine and zolazepam (65 mg/kg; Zoletil® 100 inj., Virbac, France) and xylazine (20 mg/kg; Xylapan® 20 mg/ml inj., Vetoquinol, Czech Republic). The dissociation procedure was previously described in detail (Bebarova et al., 2014). In short, the heart was retrogradely perfused via aorta with 0.9 mM CaCl2 Tyrode solution and then with nominally Ca2+-free Tyrode solution. During the first digestion step, the perfusion continued with nominally Ca2+-free Tyrode solution containing collagenase (type A, Roche Diagnostics GmbH, Germany, 1 mg ml−1) and protease (type XIV, 0.053 mg ml−1). In the second digestion step, protease was omitted. The enzyme solution was then washed out in two steps by perfusion with the low calcium Tyrode solutions (0.09 and 0.18 mM CaCl2). All solutions were oxygenated with 100% O2 at 37°C.

Solutions and Chemicals

Tyrode solution with the following composition was used both during the dissociation procedure and to perfuse myocytes during the measurements (in mM): NaCl 135, KCl 5.4, MgCl2 0.9, HEPES 10, NaH2PO4 0.33, CaCl2 0.9, glucose 10 (pH was adjusted to 7.4 with NaOH). To inhibit the calcium current ICa and the delayed rectifier potassium current IK, CoCl2 (2 mM) and tetraethylammonium chloride (TEA, 50 mM), respectively, were applied. Additionally, 1 µM atropine and 10 µM glybenclamide were administered to avoid a contribution of the acetylcholine-activated potassium current IK(Ach) and the ATP sensitive potassium current IK(ATP) to the observed IK1 changes despite it is unlikely under our experimental conditions (5 mM ATP in the pipette solution, isolated ventricular cells). Hence, the measured current should be mainly represented by IK1. During AP recordings, all specific inhibitors were omitted.

The patch electrode filling solution contained (in mM): L-aspartic acid 130, KCl 25, MgCl2 1, K2ATP 5, EGTA 1, HEPES 5, GTP 0.1, Na2-phosphocreatine 3 (pH 7.25 adjusted with KOH).

CoCl2 and atropine were prepared as 1 M and 1 mM stock solutions, respectively, in the deionized water. Glybenclamide was prepared as 100 mM stock solution in DMSO (DMSO below 0.01% in both control and test solution). To prepare the TEA-containing stock solution, NaCl in the used Tyrode solution (described above) was replaced equimolarly by TEA.

The sildenafil stock solution (10 mM; sildenafil was dissolved in DMSO) and the Ba2+ stock solution (10 mM; BaCl2 was dissolved in deionized water) were added to the Tyrode solution to obtain sildenafil concentrations between 0.1 and 100 µM and Ba2+ concentrations of 0.1, 0.3, 1, and 100 µM. During simultaneous application of both drugs, the following combinations of Ba2+ and sildenafil were used: 0.1 µM sildenafil + 0.1 µM Ba2+, 1 µM sildenafil + 0.1 µM Ba2+, 1 µM sildenafil + 1 µM Ba2+, and 0.1 µM sildenafil + 0.3 µM Ba2+.

During the experiment, the cardiomyocytes were transfected with an expression vector pcDNA3-AChRα2 (GeneCopoeia, Rockville, MD) using the Polyplus-transfection reagent Polyjet® Max (Polyplus Transfection, Illkirch, France) following the manufacturer’s instructions. The transfected cells were maintained in Tyrode solution until the time of experiment. The AChRα2 channel was detected by the intracellular calcium increase using Fluo-4/AM (ThermoFisher Scientific, Waltham, MA) as described previously (Dhamoon and Jalife, 2005).
The concentration of DMSO in the final solution was kept below 0.01% in all experiments, thus, it should not cause any changes of the cardiac $I_{K1}$ by itself as mentioned above (Ogura et al., 1995; Bosch et al., 1999).

The solutions were applied into close vicinity of the measured cell through a cannula directed on the cell and distant from it by about 100–150 µm via a gravity-operated perfusion system; the time to change the solution in the surroundings of the measured cells was approximately 2 s.

**Electrophysiological Measurements and Evaluation**

Single rod-shaped cells with well visible striations were used for the membrane current and voltage recordings applying the whole-cell patch-clamp technique in the voltage-clamp and current-clamp modes, respectively. The patch pipettes were pulled from borosilicate glass capillary tubes and heat polished on a programmable horizontal puller (Zeitz-Instrumente, Germany). The resistance of the filled glass electrodes was below 1.5 MΩ to keep the access resistance as low as possible. For the generation of experimental protocols and data acquisition, the Axopatch 200B amplifier, Digidata 1322A, and pCLAMP 9.2 software (Molecular Devices, United States) were used. The series resistance was compensated up to 75%. The measured ionic currents and APs were digitally sampled at 10 kHz and stored on the hard disc. Experiments were performed mostly at room temperature (23 ± 1°C), just some AP recordings were performed at 37°C. The holding potential was $-85$ mV, and the stimulation frequency was 0.2 Hz in all experiments on $I_{K1}$. During AP recordings, the measured cell was stimulated at 1 Hz. $I_{K1}$ was evaluated as the current sensitive to 100 µM Ba$^{2+}$ at the end of 500-ms pulse, both to $-50$ mV (the sodium current $I_{Na}$ was inactivated at the beginning of this pulse) and to $-110$ mV to check the outward and inward current component, respectively. Additional recordings during a 3-s voltage ramp from $-110$ to $-10$ mV were performed as well.

**Statistical Analysis**

The results are presented as arithmetic means ± S.E.M. (normality of the data distribution tested by the Kolmogorov-Smirnov test, K-S test) from $n$ cells (Origin, version 8.5.1, Origin Lab Corporation). The curve fitting paired and unpaired t-test, and ANOVA test (with the Tuckey’s post-test) were performed.
using the GraphPad Prism, version 6.05 (GraphPad Software, Inc.); p < 0.05 was considered statistically significant.

RESULTS

Sildenafil at a representative therapeutic concentration of 1 µM partially inhibited $I_{K1}$ at both −50 and −110 mV (Figure 1A; for the used experimental protocol, see the upper panel). The inhibition proceeded fast (the steady-state effect was reached within 7.5 ± 2.5 s on average; $n = 15/8$ and 16/8 at −50 and −110 mV, respectively) and was fully reversible during the subsequent wash-out (Figure 1B, left panel). A similar time course of the inhibition and wash-out was observed in other tested concentrations (for an example at the highest tested sildenafil concentration, see Figure 1B, right panel). Figure 1C shows the average current-voltage relationship in control and under the effect of 1 µM sildenafil ($n = 5/3$). The magnitude of control $I_{K1}$ and the cell membrane capacitance significantly correlated (not illustrated; the Pearson correlation coefficient was 0.50 and −0.39 at −50 and −110 mV, respectively, $p < 0.05$). The evaluated current is therefore further expressed as the current density (in pA/pF) to reduce differences among cells caused by their varying size.

The average effects of sildenafil at concentrations between 0.1 and 100 µM on $I_{K1}$ density are shown in Figure 2. Sildenafil elicited a statistically significant decrease of $I_{K1}$ in all used concentrations ($p < 0.05$). The subclinical 0.1 µM sildenafil evoked a mild inhibition of $I_{K1}$ by $6.0 ± 0.6\%$ ($n = 6/6$) and $6.4 ± 0.7\%$ ($n = 10/8$) at −50 and −110 mV, respectively. The therapeutic 1 µM sildenafil inhibited $I_{K1}$ by $7.3 ± 0.6 (n = 15/8)$ and $8.1 ± 0.8 (n = 16/8)$ at −50 and −110 mV, respectively. The supratherapeutic concentrations of sildenafil gave rise to a more potent decrease of $I_{K1}$ (14.1 ± 2.1 and 17.9 ± 3.0% inhibition by 10 µM sildenafil, $n = 6/6$, and 20.6 ± 2.4 and 19.5 ± 4.8% inhibition by 100 µM sildenafil, $n = 5/5$, at −50 and −110 mV, respectively, Figure 2A). The reduction of $I_{K1}$ was significantly more profound when the higher sildenafil concentrations were applied, both at −50 mV and −110 mV (Figure 2B; $p < 0.05$). No statistically significant difference was found between the sildenafil effects at −50 and −110 mV.

Subsequently, changes of $I_{K1}$ after both separate and combined application of 0.1 µM sildenafil and 0.1 µM Ba$^{2+}$ were analysed (Figure 3). As described above, the inhibition of $I_{K1}$ by the subclinical 0.1 µM sildenafil was only mild, but significant at both voltages. The inhibitory effect of Ba$^{2+}$ at a clinically-relevant concentration of 0.1 µM was statistically significant ($p < 0.05$) both at −50 mV (10.9 ± 0.9, $n = 16/5$) and at −110 mV (9.8 ± 0.9, $n = 15/6$). Combined application of sildenafil and Ba$^{2+}$ significantly and massively reduced $I_{K1}$ by 45.7 ± 5.7 ($n = 12/3$) and 43.0 ± 6.9% ($n = 14/5$) at −50 and −110 mV, respectively, (Figure 3A; $p < 0.05$). The effect of the combined solution was significantly more potent than a simple sum of effects of separate solutions (Figure 3B; $p < 0.05$). No statistically significant difference between the effects at the tested voltages was observed. The high variability of the combined action of sildenafil and Ba$^{2+}$ (Figure 3B) is likely related to the varying composition and, thus, properties of $I_{K1}$ channel heterotetramers in individual measured cells (see Discussion).

Several combinations of sildenafil and Ba$^{2+}$ at various concentrations were then tested to elucidate concentration dependence of the synergistic effect described above. As apparent, the combination of 0.1 µM sildenafil + 0.1 µM Ba$^{2+}$ seems to be unique because it was the only one causing the synergistic effect (Figure 4A, left panel). If 0.1 µM Ba$^{2+}$ was combined with 1 µM sildenafil (Figure 4A, middle panel) or if 1 µM Ba$^{2+}$ was combined with 1 µM sildenafil (Figure 4A, right panel), no significant increase of the effect was present in comparison with the effect of 0.1 and 1 µM Ba$^{2+}$ and 1 µM sildenafil alone, respectively. We also investigated a paired comparison of two combinations of the substances, the originally tested combination of 0.1 µM sildenafil + 0.1 µM Ba$^{2+}$ and a new combination of 0.1 µM sildenafil + 0.3 µM Ba$^{2+}$. Figure 4B demonstrates that no synergistic effect under the combined action of 0.1 µM sildenafil + 0.3 µM Ba$^{2+}$ could be revealed, despite the...
originally tested combination of 0.1 \( \mu \text{M} \) sildenafil + 0.1 \( \mu \text{M} \) Ba\textsuperscript{2+} induced a clear synergistic effect in the same cell (\( n = 4/1 \), \( p < 0.05 \)).

To find out the impact of the observed \( I_{K1} \) changes on cardiac cell repolarization, action potentials (APs) were recorded at separate and combined application of sildenafil and Ba\textsuperscript{2+}, both at 0.1 \( \mu \text{M} \). In contrast to \( I_{K1} \) recordings, these measurements were performed in the absence of any specific inhibitors. As clearly demonstrated using the representative AP recordings in Figure 5A, the action potential duration (APD) was markedly prolonged in presence of both sildenafil and Ba\textsuperscript{2+}, but not if each of the drugs was applied alone; changes of other parameters of APs were not apparent. APD evaluated at 90\% repolarization (\( APD_{90} \); Figure 5B, \( n = 7/3 \)) was not significantly changed if sildenafil and Ba\textsuperscript{2+} were applied separately, but it was significantly higher in their combined presence (\( p < 0.05 \)). The combined effect was significantly higher than the effect of sildenafil and Ba\textsuperscript{2+} alone (Figure 5B; \( p < 0.05 \)) which is in a good agreement with \( I_{K1} \) data presented in Figure 3. The synergistic effect of sildenafil and Ba\textsuperscript{2+} on APD was also preserved, even up to a comparable extent, at physiological temperature (Figures 5C, D, \( n = 4/2 \)).

**DISCUSSION**

This is the first study reporting the effect of sildenafil on the cardiac Kir channels. Sildenafil caused a significant and reversible concentration-dependent inhibition of \( I_{K1} \) at \(-110 \text{ mV} \) and \(-50 \text{ mV} \), even at clinically-relevant concentrations. Surprisingly, simultaneous application of subclinical concentration of sildenafil (0.1 \( \mu \text{M} \)) and low clinically-relevant concentration of Ba\textsuperscript{2+} (0.1 \( \mu \text{M} \)) massively decreased both inward and outward components of \( I_{K1} \) and resulted in a significant action potential prolongation, even at physiological temperature. The combined effect was significantly higher than a simple sum of effects of the individual substances at both tested voltages. To our best knowledge, similar synergistic effect of a drug and Ba\textsuperscript{2+} (or other ions) has not been described.

\( I_{K1} \) channels are homo- and heterotetramers formed by individual Kir2x subunits, namely Kir2.1, Kir2.2, and Kir2.3 in mammalian hearts. The channels formed by various combinations of these subunits may exert different properties including different sensitivity to drugs. Even the inhibitory effect of Ba\textsuperscript{2+} differs in the individual Kir2x subunits and their various
combinations (Schram et al., 2003). The expression profile for Kir2x isoforms in the human cardiac right ventricle is Kir2.1 > Kir2.2 > Kir2.3 (Reilly and Eckhardt, 2021). Similar pattern can be seen in rodent ventricles (Panama et al., 2007). In general, Kir2x expression patterns display very little variance among species investigated thus far (De Boer et al., 2010). Hence, we expect that changes of $I_{K1}$ observed in this study on isolated rat cardiomyocytes should be applicable in human. We plan to verify this in our future study.

Changes of $I_{K1}$ are known to affect the action potential duration (APD). As well known, $I_{K1}$ inhibition may prolong APD, which may lead to the occurrence of early afterdepolarizations (EADs) in cardiomyocytes. In atria, EADs may trigger ectopic beats in pulmonary veins or other foci, which may initiate AF (Tse, 2016). In ventricles, prolonged APD may result in the long QT syndrome with a high risk of fatal arrhythmias such as polymorphic VT, including torsades de pointes. Hence, the combined action of sildenafil and Ba$^{2+}$ observed in this study, inhibiting almost half of $I_{K1}$ (Figure 3), may contribute to arrhythmogenesis.

No changes of APD in isolated cardiomyocytes have been observed at therapeutic sildenafil concentrations in previous animal studies (Geelen et al., 2000; Chiang et al., 2002). Beside the potential role of Ba$^{2+}$ (which may be absent in laboratory animals), it might reflect effect of sildenafil on more cardiac ionic channels which can mutually compensate for their effect on APD. Chiang et al. (2002) discovered that sildenafil dose-dependently inhibited the depolarizing L-type calcium current

**FIGURE 5** | Changes of action potential duration (APD) under the effect of 0.1 µM sildenafil alone and in combination with Ba$^{2+}$ (0.1 µM); APD was evaluated at 90% repolarization ($APD_{90}$). (A) Representative AP waveforms in control and under the effect of the drugs alone and in combination at 23°C. (B) Average $APD_{90}$ changes at 23°C ($n = 7/3$). (C) Values of $APD_{90}$ and resting membrane potential (RMP) in a representative cell measured both at 23 and 37°C. (D) Comparison of the average relative $APD_{90}$ changes under the effect of sildenafil in the absence and presence of Ba$^{2+}$ at 23 and 37°C ($n = 4/2$); * and ** — statistical significance at $p < 0.05$ and 0.01, respectively.
ICa in guinea-pig ventricular myocytes, at least slightly even at the therapeutic concentration of 1 µM (which might compensate the inhibitory effect of sildenafil on IK1 observed in our study). A significant shortening of APD (presumably caused by ICa inhibition) was observed in supratherapeutic sildenafil concentrations above 10 µM (Chiang et al., 2002). Sildenafil was also shown to inhibit delayed rectifier potassium current (IKr; the human hERG channels expressed in a cell line), but only at supratherapeutic concentrations (a half inhibition at 33.3 µM, Duslan Sarazan et al., 2004). In agreement, Geelen et al. (2000) pointed out that sildenafil significantly inhibited the human hERG channels expressed in a cell line and prolonged APD in guinea-pig isolated hearts at supratherapeutic concentrations above 30 µM.

Anyway, our experiments proved a significant AP prolongation during combined application of 0.1 µM sildenafil and 0.1 µM Ba2+ by about 20% both at the room temperature of 23°C and at the physiological temperature of 37°C (Figure 5). Hence, proarrhythmic changes under the effect of sildenafil should be considered. It might explain occurrence of arrhythmias in some patients treated with sildenafil, namely those with an accumulation of Ba2+.

The therapeutic plasma concentration of sildenafil (1 µM) elicited only a mild inhibitory effect on IK1 (Figures 1, 2). However, little is known about precise sildenafil concentration in the human heart in vivo. Sildenafil is a lipophilic agent with high distribution volume (105 L; Chaumais et al., 2013; Nichols et al., 2002; Langtry and Markham, 1999), that much exceeds the high distribution volume (105 L; Chaumais et al., 2013; Nichols et al., 2002) of BA, but only at supratherapeutic concentrations (33.3 µM, Duslan Sarazan et al., 2004).

Regarding the mechanism of synergistic action of sildenafil and Ba2+ on IK1 and its unique presence only at a low concentration of the substances (Figures 3, 4), we can just speculate. Considering the size and character of the sildenafil molecule which is relatively big and lipophilic, we expect its binding either on the outer channel pore or on the cytoplasmatic portion of the channel. This might cause an allosteric conformational change (modulation) of the channel structure, thus, tighter binding of Ba2+ within the channel pore. Two main residues were shown to affect the inhibitory effect of Ba2+ on IK1, E125 located at the outer channel vestibule and T141 located close to the selectivity filter (Alagem et al., 2001). These lie close to the presumed aforementioned allosteric modulation sites. Other Kir2.1 channel residues may also affect the sensitivity of the channel to Ba2+-induced inhibition, for example the residue at the position 121 (Zhou et al., 1996). Absence of the synergy at higher concentrations might be theoretically related to a competitive inhibition of the channel by sildenafil and Ba2+ which would disable binding of another molecule if the site was already occupied by the other one. To reveal the specific binding site of sildenafil on IK1 channel and explain mechanism of the synergistic effect of sildenafil and Ba2+, a separate study is planned in the future, encompassing several techniques, namely the structural modelling, site directed mutagenesis, and patch-clamp technique.

We conclude that sildenafil caused a significant, reversible, and concentration-dependent inhibition of both inward and outward components of IK1 even at therapeutic concentrations. The observed massive inhibition of IK1 induced by simultaneous application of sildenafil and Ba2+ at a low concentration and resulting in a significant AP prolongation is unique, not described in the literature so far according to our knowledge. Since both sildenafil and Ba2+ can accumulate in the human body tissues, we presume that, when
certain conditions are met, the use of sildenafil and the related changes in $I_{K1}$ might result in arrhythmia.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The animal study was reviewed and approved by Local Committee for Animal Treatment at Masaryk University, Faculty of Medicine, and the Ministry of Education, Youth and Sports of the Czech Republic (MSMT-33846/2017-2).

**AUTHOR CONTRIBUTIONS**

MM—patch-clamp measurements, data analysis including statistical analysis and graphical processing, writing of the paper. OŠ—patch-clamp measurements, cell isolation, technical assistance during experiments. MB—patch-clamp measurements, study design, cell isolation, data analysis including statistical analysis and graphical processing, writing of the paper.

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