

Propofol-induced vasodilation of mesenteric arterioles via BK_{Ca} channel and gap junction

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Abstract. The present study aimed to investigate the role of propofol in mediating the vasomotor activity of the mesenteric arteriole (MA) of Sprague Dawley (SD) rats, and to elucidate the underlying mechanisms. The pressure myograph technique was used to examine the effect of different concentrations of propofol on the relaxation of blood vessels in the 2-3 mm MA segments freshly separated from the SD rats. The whole-cell patch-clamp technique was applied to observe the outward current of single vascular smooth muscle cells (VSMCs) obtained from the MAs of the SD rats. Furthermore, immunofluorescence was utilized to assess the expression of connexin (Cx) in the MAs of SD rats. The results indicated the following: i) Propofol relaxed the MA of SD rats in a concentration-dependent manner from 1×10^{-7} to 3×10^{-4} mol/l; ii) in the acutely dissociated VSMCs, propofol (1×10^{-7} to 3×10^{-4} mol/l) enhanced the outward current of VSMCs in a concentration-dependent manner; iii) the enhanced outward currents induced by propofol (1×10^{-5} mol/l) may be reversed by tetraethylammonium (TEA; 1 mmol/l), a calcium-activated K⁺ channel inhibitor; iv) the effect of propofol on the relaxation of the vasculature was reduced after perfusion with 1 mmol/l TEA; v) Cx40, Cx43 and Cx45 were expressed on the MA; 6) 18β-glycyrrhetinic acid and 2-aminoethoxydiphenyl borate, two types of gap junction blocker, inhibited the propofol-induced relaxation. The present study provides evidence that propofol relaxes the MA, which

may be associated with its effect of enhancing the channel current of large-conductance calcium voltage-activated potassium channels, contributing to the K⁺ outflow and leading to VSMC hyperpolarization; the gap junction may facilitate the hyperpolarization, which may lead to vascular synchronized relaxation and thereby reduce the blood pressure.

Introduction

Propofol is an intravenous anesthetic extensively used in clinical practice and is characterized by rapid induction of anesthesia, as well as prompt recovery from its effects. However, propofol has an obvious side effect of inducing hypotension, particularly in patients with high blood pressure or in elderly patients. Furthermore, patients with vena cava collapse are prone to develop significant hypotension when treated with propofol, which may be a direct result of vascular relaxation (1-4). Certain studies have indicated that propofol elicits vascular relaxation via the following mechanisms: i) Activation of large-conductance calcium voltage-activated potassium channels (BK_{Ca}) (2); ii) activation of ATP-sensitive K⁺ channels (K_{ATP}) (5); iii) inhibition of voltage-operated calcium channels and receptor-operated calcium channels (6); and iv) increases in the availability of nitric oxide (7,8). Propofol may cause vasodilation via four different pathways, however, to the best of our knowledge, no experimental study has assessed the mechanism of vasodilation induced by propofol on the rat mesenteric artery. The differences between these results may thus be due to different subjects, while propofol has no significant effect on the BK_{Ca} channel of mesenteric arteries in rats.

BK_{Ca} channels are abundant on vascular smooth muscle cells (VSMCs) and have a dominant role in the regulation of vascular tone. Peripheral resistance exerts a significant function in regulating blood pressure and blood flow distribution in tissues and organs. Peripheral vascular resistance is the basic condition for the generation of blood pressure, and the formation of peripheral resistance is mainly due to the myogenic tone of MSCs. The BK_{Ca} channel mediates 70-80% of the outward current of VSMCs, implying a close association between the BK_{Ca} channel and the myogenic tone of VSMCs (9). Of note, the BK_{Ca} channel is able to regulate the contraction and

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relaxation of the blood vessels by regulating the myogenic tone of VSMCs (9). Furthermore, the activation of BK_{Ca} channels leads to K⁺ efflux, contributing to membrane hyperpolarization. This membrane potential change leads to the closure of L-type voltage-gated Ca²⁺ channels, which in turn reduces [Ca²⁺] and induces vasorelaxation (10). A previous study indicated that inhibition of BK_{Ca} channels resulted in vasoconstriction (11). Therefore, BK_{Ca} channels have a pivotal role in the regulation of vascular tone and blood pressure (12,13).

Blood vessels are mainly composed of endothelial cells (ECs) and VSMCs, and numerous gap junctions exist among ECs, among VSMCs and between the layers of these two cell types (14). Gap junctions, which directly link the cytoplasm, are essential for coordinating tissue homeostasis and regulating vascular responses, which allows for conduction of intercellular signals between adjacent cells (15). This behavior enables the vasculature, which consists of numerous cell types, to behave as an integrated system (16). Therefore, gap junctions are of great importance to ensure the synchronization and coordination of vasomotor activity, and to maintain the stability and consistency of the physiological function of the vessel (17-19).

The present study aimed to observe the relaxation of propofol and to further clarify the roles of BK_{Ca} channels and gap junctions in the vasodilation effect of propofol.

Materials and methods

Animals. The present study was approved by the Animal Experimental Ethical Inspection Committee of the First Affiliated Hospital Shihezi University (Shihezi, China). A total of 80 Sprague Dawley (SD) rats (age, 8-12 weeks; weight, 250-300 g), both males and females, were obtained from the Center for Disease Control and Prevention of Xinjiang Uygur Autonomous Region [Urumchi, China; animal certificate of conformity no. SCXK (Xin) 2003-0001]. The rats were housed in separate cages in a specific pathogen-free environment (temperature, 24±3°C; humidity of ~49%) under a 12-h light-dark cycle, and were provided food and water *ad libitum*. All protocols were approved by the Animal Experimental Ethical Inspection of First Affiliated Hospital, Shihezi University School of Medicine (Shihezi, China) and were in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The SD rats were euthanized under deep general anesthesia by intraperitoneal injection of 350 mg/kg 10% chloral hydrate. No rats exhibited peritonitis at this dosage. Rats were then sacrificed via exsanguination and the MA and its branches were harvested from the upper ileum mesentery.

Reagents. Propofol was purchased from AstraZeneca (Cambridge, UK; production lot no. GF357). EDTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), tetraethylammonium (TEA), collagenase A, papain, bovine serum albumin (BSA), dithiothreitol (DTT), Na-hydroxyethyl piperazine ethanesulfonic acid (HEPES), 2-aminoethoxydiphenyl borate (2-APB), 18β-glycyrrhetic acid (18β-GA), TritonX-100, propidium iodide (PI) were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Connexin (Cx)40 antibody was purchased from

Abcam (Cambridge, MA, USA; cat. no. ab213688), Cx43 antibody was purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA; cat. no. 3512) and Cx45 antibody was purchased from Abcam (Cambridge, MA, USA; cat. no. ab78408). KCl and all other reagents were acquired locally. All solutions used in the pressure myograph system and whole-cell patch-clamp technique were prepared using physiological saline solution (PSS). Extracellular solution was a stock sample prepared prior to further dilution with external solution to achieve the final concentration. The formulations of PSS/saline solution with high potassium and the external solution were in accordance with the literature (17).

Instruments. The following instruments were used in the present study: Pressure myograph system (110P; Danish Myo Technology A/S, Aarhus, Denmark), MyoVIEW software (Danish Myo Technology A/S, version 2.0), Axon MultiClamp 700B patch-clamp amplifier (Axon; Molecular Devices LLC, Sunnyvale, CA, USA), micromanipulator (PCS5001; Siskiyou Design, Grants Pass, OR, USA), P-97 microelectrode pullers (Sutter Instrument Co., Novato, CA, USA), heated water bath (HSS-1B; Chengdu Science Instrument Factory, Chengdu, China), multiple perfusion administration system (Huazhong University of Science and Technology, Wuhan, China) and a laser scanning confocal microscope (Zeiss LSA 510 META; Zeiss AG, Oberkochen, Germany).

Pressure myograph measurement. The MA was freed from surrounding fat and connective tissues, and placed in normal PSS supplemented with the following (in mM): NaCl, 118.9; KCl, 4.69; MgSO₄·7H₂O, 1.17; KH₂PO₄, 1.18; CaCl₂, 2.5; NaHCO₃, 25; EDTA, 0.026; and glucose, 5.5 (pH 7.4; osmolarity, 300 mOsm/l). A short segment of the vessel (with the diameter of 2-3 mm) was attached to a bath glass microelectrode. Nylon wire (10-0) was used to fix both ends of blood vessels to prevent air leakage and was connected to the experimental device, and maintained at a constant saturation of 95% O₂ and a constant temperature of 37°C, and the vascular cavity pressure was maintained at 60 mmHg. After the sample had been equilibrated in PSS for 30 min, the experiment was started. After addition of 60 mmol/l KCl, the vascular contraction reached a steady state, and subsequently, portions of propofol were added to reach concentrations of 1×10⁻⁷-3×10⁻⁴ mol/l. When the maximum relaxation effect was reached at a high concentration of propofol, the changes in blood vessel diameter were observed and recorded. The diameter was continuously determined and recorded via a video dimension analyzer and the DMT Vessel Acquisition Suite comprising a Pressure myograph system (110P; Danish Myo Technology A/S, Aarhus, Denmark) and MyoVIEW software version 2.0 (Danish Myo Technology A/S). Diameter changes between contraction and relaxation (D; in μm) were calculated via the formula D=Dx-Dp, where Dx is the value of the vasodilation stability after propofol administration and Dp is the diameter of the vessel in the KCl solution. The MyoVIEW software (Danish Myo Technology A/S) was used to control the blood vessel pressure and record the experimental data.

Whole-cell patch-clamp recording. The arterioles were detached in a buffer solution [NaCl, 142 nM; KCl, 5.0 nM;

CaCl₂, 0.05 nM; MgCl₂, 1.0 nM; Na-HEPES, 4.0 nM; HEPES, 5.0 nM; and glucose, 7.5 nM (pH 7.3)] containing 1.0 mg/ml BSA, 0.5 mg/ml collagenase A, 1.0 mg/ml papain and 1 mg/ml DTT for 10 min at 37°C. After replacing the supernatant with normal extracellular solution [in mM: NaCl, 142; KCl, 5.0; CaCl₂, 0.05; MgCl₂, 1.0; Na-HEPES, 4.0; HEPES, 5.0; and glucose, 7.5 (pH 7.3)], the single MA SMCs were obtained, and the cells were transferred to a Petri dish containing poly-L-lysine-coated coverslips at the bottom. Samples were then incubated for 10 min at 37°C. Once the dispersed cells were attached to the surface of the cover slips, they were mounted on an inverted microscope and perfused with normal extracellular solution for whole-cell recording. The specimen was continuously superfused in normal external solution (0.2 ml/min) at room temperature (22-25°C). Conventional whole-cell recordings were performed using an Axon 700B amplifier (Axon; Molecular Devices LLC). Recording pipettes were fabricated from borosilicate glass capillaries and filament with a P-97 microelectrode puller. Typically, the pipette had a tip with an outer diameter of 1 μm and a resistance of 6-9 MΩ after being filled with normal internal solution (NIS), which was composed of the following (in mM): K-gluconate, 130; NaCl, 10; CaCl₂, 2.0; MgCl₂, 1.2; HEPES, 10; EGTA, 5; and glucose, 7.5; adjusted to pH 7.2 and an osmolarity of 290 mOsm/l. The pipette capacitance was well-compensated when a GΩ seal with the cell was achieved. The membrane current or voltage signal was low-pass filtered at 5 kHz (-3 dB). Data were recorded on a personal computer equipped with a Digidata 1440A AD-interface and pClamp 10.2 software (Axon; Molecular Devices LLC). A Minidigi digitizer and Axoscope 10.2 software (Axon; Molecular Devices LLC) were used to perform a gap-free recording at a sampling interval of 50 msec throughout the experiment.

Immunofluorescence technique. For the purpose of identifying the expression of Cx40, Cx43 and Cx45 on the MA, immunostaining was performed. The MA samples were randomly assigned to two groups, namely the experimental and control groups. After harvesting, the MAs were placed in 4% paraformaldehyde for 2 h at room temperature. After rinsing thrice with PBS, the MAs were incubated in immunostaining blocking liquid (5% BSA) for 1 h. Subsequently, the MA was rinsed thrice with PBS and incubated with 5% BSA for 1 h at room temperature. Each sample was then rinsed with PBS and treated with 1:200 dilutions of anti-Cx40, anti-Cx43 and anti-Cx45 and incubated for 1 h at room temperature, separately. Finally, each sample was placed in a wet box at 4°C for 12 h for maintenance. Subsequently, the samples were brought to 37°C over 1 h. The MAs were transferred to Eppendorf tubes and rinsed thrice in PBS, and any remaining liquid was absorbed with filter paper strips. The samples were treated with the secondary antibody (fluorescein isothiocyanate conjugated goat anti-rabbit immunoglobulin G; 1:200 dilution; OriGene Technologies, Inc., Rockville, MD, USA; cat. no. ZF-0311) and then placed in a wet box for 1 h of incubation. Each sample was rinsed for 20 sec prior to staining with 1:200 PI and incubated for 20 sec at room temperature. Finally, each MA was rinsed thrice with PBS again, and was then transferred onto a microscope slide. The extra PBS was absorbed with filter papers before the slide was sealed with 85% glycerinum

for fluorescence quenching. The fluorescence was observed and recorded with a laser scanning confocal microscope. The MA in the control group underwent the same treatment, apart from the anti-Cx40, anti-Cx43 and anti-Cx45 antibody being replaced by PBS.

Statistical analysis. Values are expressed as the mean ± standard error. Statistical analysis was performed using the SPSS statistical software package, version 17.0 (SPSS Inc., Chicago, IL, USA). A homogeneity test for variance was performed, followed by one-way analysis of variance, and comparisons between two groups were assessed using the paired t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Propofol relaxes rat MAs in a concentration-dependent manner. After the diameter of the MAs was stable in the presence of 60 mmol/l KCl, the isolated vasculature exhibited a concentration-induced response, namely a gradual increase of the diameter when propofol was added to reach final concentrations of 1x10⁻⁷, 3x10⁻⁷, 1x10⁻⁶, 3x10⁻⁶, 1x10⁻⁵, 3x10⁻⁵, 1x10⁻⁴ and 3x10⁻⁴ mol/l (Fig. 1A). The respective increase in MA diameter was by 4.01±0.10, 10.06±2.45, 17.81±3.06, 32.67±4.79, 49.43±6.93, 75.71±8.24, 161.24±11.43 and 195.88±11.04 μm. The relaxation rate of the MAs is presented in Fig. 1B, which also indicated that propofol caused concentration-dependent increases in the relaxation of MAs. All of the results indicated that propofol increased the vascular diameter in a concentration-dependent manner.

Propofol enhances the outward current of VSMCs from MAs in a concentration-dependent manner. Whole-cell voltage-clamp experiments were performed on dispersed VSMCs obtained from MAs. Application of different concentrations of propofol increased the outward current induced by voltage steps from the holding potential (HP) of -40 mV in the VSMCs (Fig. 2A). The results in Fig. 2B indicated that the whole-cell current/voltage (I/V) curve slope, following stimulus (-80 to 60 mV), was increased in the entire voltage range, and the 3x10⁻⁵ mol/l propofol-induced net current exhibited a significant enhancement. The cells displayed a concentration-dependent response after treatment with 1x10⁻⁷, 3x10⁻⁷, 1x10⁻⁶, 3x10⁻⁶, 1x10⁻⁵, 3x10⁻⁵, 1x10⁻⁴ and 3x10⁻⁴ mol/l propofol. The outward current was increased from 185.33±33.32 pA (when cells were stable) to 247.72±37.54, 325.76±35.12, 454.90±29.23, 628.28±64.68, 975.39±56.06 and 1,451.9±31.67 pA, respectively (Fig. 2C). These data suggest that the outward current was enhanced by propofol.

Propofol-induced increases in the outward current are blocked by TEA. The cells were maintained at -40 mV and then subjected to a series of test potentials ranging from -80 to +60 mV (At a holding potential of -40 mV, the stimulation voltage was increased from -80 to 60 mV with a 20 mV a ladder; Fig. 3). Taking the data obtained at +60 mV as an example, the outward current was initially 259.89±24.11 pA, and after addition of 1x10⁻⁵ mol/l propofol, the outward current was enhanced to 727.11±39.95 pA. However, supplementation

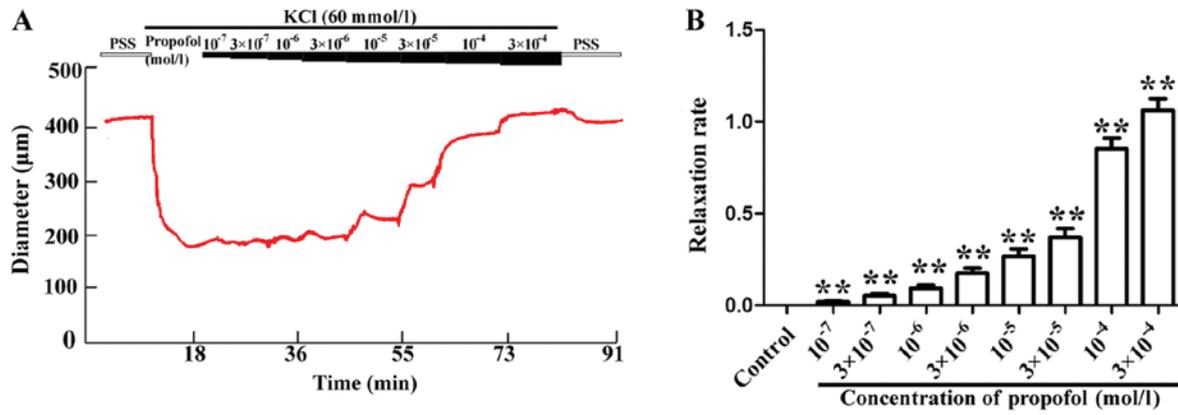


Figure 1. Different concentrations of propofol induce the relaxation of MA. (A) Using representative video photos, an isolated MA that was equilibrated, pressurized, stimulated with propofol and then exposed to different concentrations of propofol was traced. The diameter of vessels was increased when the propofol concentration was increased from 1x10⁻⁷-3x10⁻⁴ mol/l. (B) The normalized vascular diameter was markedly increased when MA was treated with propofol (1x10⁻⁷-3x10⁻⁴ mol/l). **P<0.01 vs. control (n=8). MA, mesenteric arteriole; PSS, physiological saline solution.

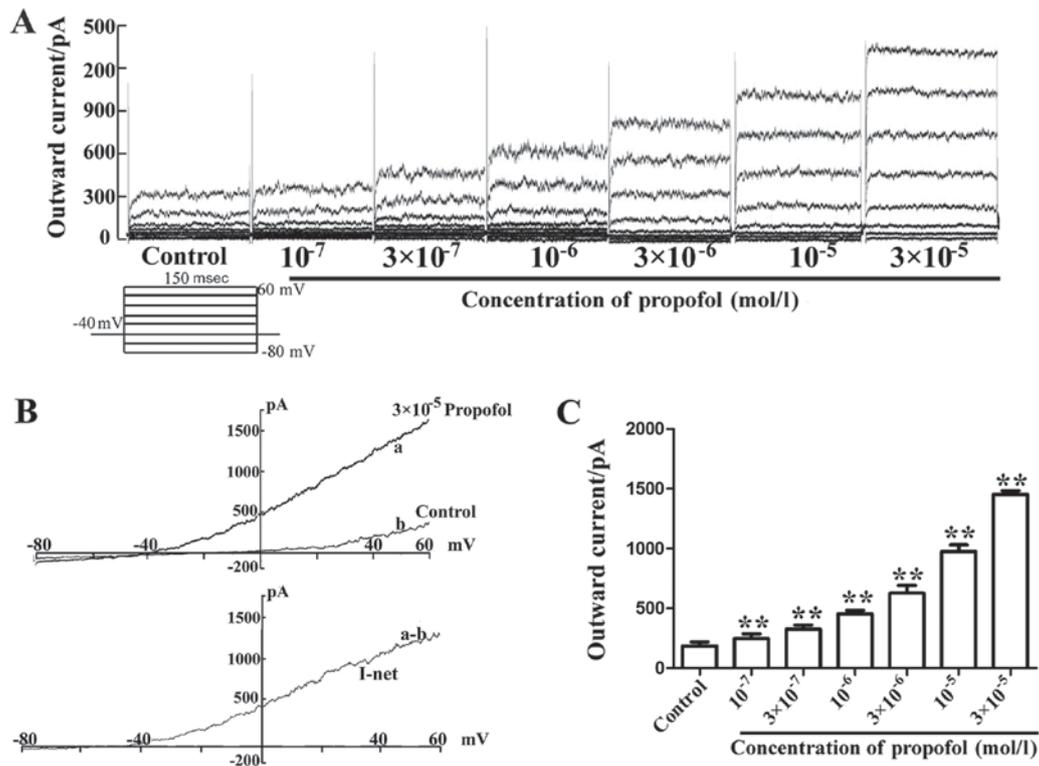


Figure 2. Propofol enhances the outward current of vascular smooth muscle cells in MAs in a concentration-dependent manner. (A) Whole-cell current traces induced by voltage steps prior to and during application of 1x10⁻⁷, 3x10⁻⁷, 1x10⁻⁶, 3x10⁻⁶, 1x10⁻⁵ and 3x10⁻⁵ mol/l propofol. (B) Whole-cell I/V curves in the absence and presence of 3x10⁻⁵ mol/l propofol in cells of MA (top panel) and the respective propofol-sensitive net current I/V curves (lower panels). Note that propofol caused an increase of the slope conductance of single cells. (a) Application of 3x10⁻⁵ mol/l propofol; (b) ramp trace in the absence of 3x10⁻⁵ mol/l propofol; (a-b) net current induced by 3x10⁻⁵ mol/l propofol. (C) Mean outward current induced by 1x10⁻⁷, 3x10⁻⁷, 1x10⁻⁶, 3x10⁻⁶, 1x10⁻⁵ and 3x10⁻⁵ mol/l propofol in comparison to the control. **P<0.01 vs. control (n=6). MA, mesenteric arteriole; I/V, current/voltage.

with 1 mmol/l TEA (BK_{Ca} channel blocker) significantly reduced the outward current to 150.14±14.43 pA, while the inhibition was recovered to 280.78±35.86 pA after washing with drug-free normal external solution (Fig. 3A and E). The data obtained from 6 similar experiments focusing on the effect of TEA on the mean current density are summarized in Fig. 3D. The results demonstrated that TEA reduced the current density from 109.23±9.65 to 25.66±3.91. In addition,

the whole-cell I/V curve slope exhibited an increasing trend in the entire voltage range and the 1x10⁻⁵ mol/l propofol-induced net current was significantly enhanced (Fig. 3B). Furthermore, as displayed in Fig. 3C, it was indicated that after perfusion with 1 mmol/l TEA, no significant increase in the current was obtained following addition of 1x10⁻⁵ mol/l propofol. All of the results indicated that propofol enhanced the outward current, which was mediated via the BK_{Ca} channel.

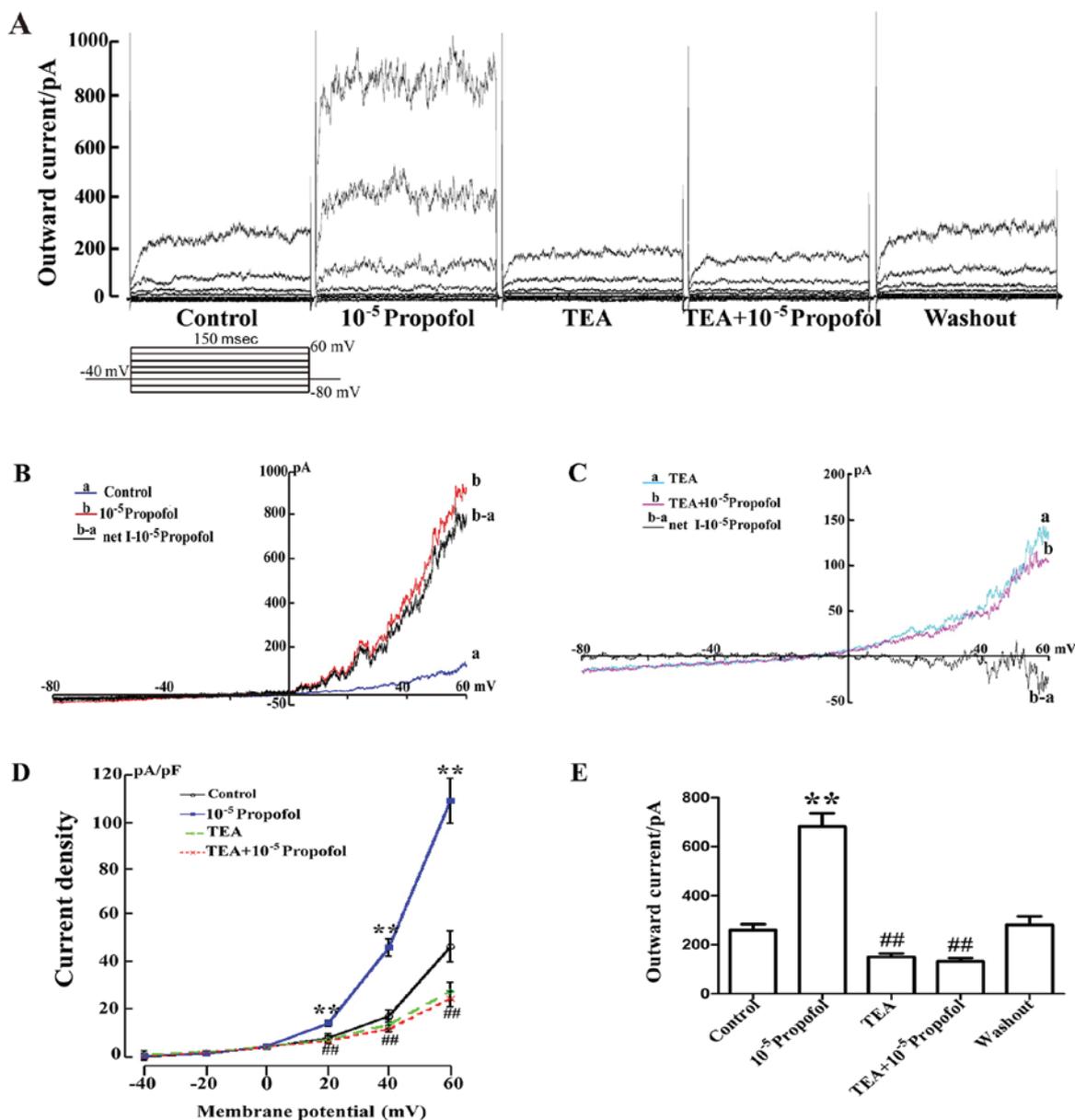


Figure 3. TEA inhibits the outward current induced by propofol. (A) Representative recordings of whole-cell currents elicited by a stimulus ladder of -80 to 60 mV, demonstrating that propofol does not increase the current in the presence of TEA. (B) I/V plots indicating that propofol enhanced the outward current at depolarizations beyond -20 mV. (C) Ramp-constructed I/V curves depicting that propofol induces only a minimal net current in the presence of TEA. (D) Current density of vascular smooth muscle cells. The plots are based on data after leak subtraction and normalized against the control value of each cell. (E) Quantification of the results in A indicated that the mean outward current was increased by 1×10^{-5} mol/l propofol, which was inhibited by TEA. ** $P < 0.01$ vs. control, ## $P < 0.01$ vs. propofol ($n=6$). TEA, tetraethylammonium; I/V, current/voltage.

Inhibitory effect of TEA on the relaxation elicited by propofol.

The vessel diameter was in a stable, constricted state in the presence of 60 mmol/l KCl, and the increases in diameter obtained by addition of propofol (1×10^{-7} , 3×10^{-7} , 1×10^{-6} , 3×10^{-6} , 1×10^{-5} , 3×10^{-5} , 1×10^{-4} and 3×10^{-4} mol/l) were significantly inhibited by pre-treatment with 1 mmol/l TEA (a BK_{Ca} inhibitor) for 20 min, resulting in increases in diameter by 2.45 ± 0.90 , 5.28 ± 1.17 , 10.46 ± 1.46 , 15.95 ± 1.55 , 21.93 ± 1.96 , 31.98 ± 3.10 , 59.17 ± 4.45 and 102.85 ± 5.91 μ m, respectively. Furthermore, the vasodilator effect of propofol attenuated significantly in the presence of TEA (Fig. 4B). As indicated in Fig. 4B, the propofol-induced increase in diameter/vessel relaxation was inhibited in the presence of TEA. In addition, in the absence and presence of the BK_{Ca} inhibitor TEA, a

significant difference was obtained with different concentrations of propofol, indicating a regulatory role for BK_{Ca} in vascular vasodilation induced by propofol. However, propofol induced vasodilation is not entirely mediated by BK_{Ca}.

MAs possess gap junctions. Immunofluorescence microscopy was used to observe the expression of Cx in the MAs. The MAs were labelled with polyclonal antibody to Cx40, Cx43 or Cx45, and the nuclei were stained with PI. The results indicated that the MAs contained Cx40, Cx43 and Cx45 on their inner surface, indicating the presence of gap junctions (Fig. 5).

Inhibitory effect of 18 β -GA on the vasorelaxation induced by propofol. The MA was pre-treated with gap junction inhibitor

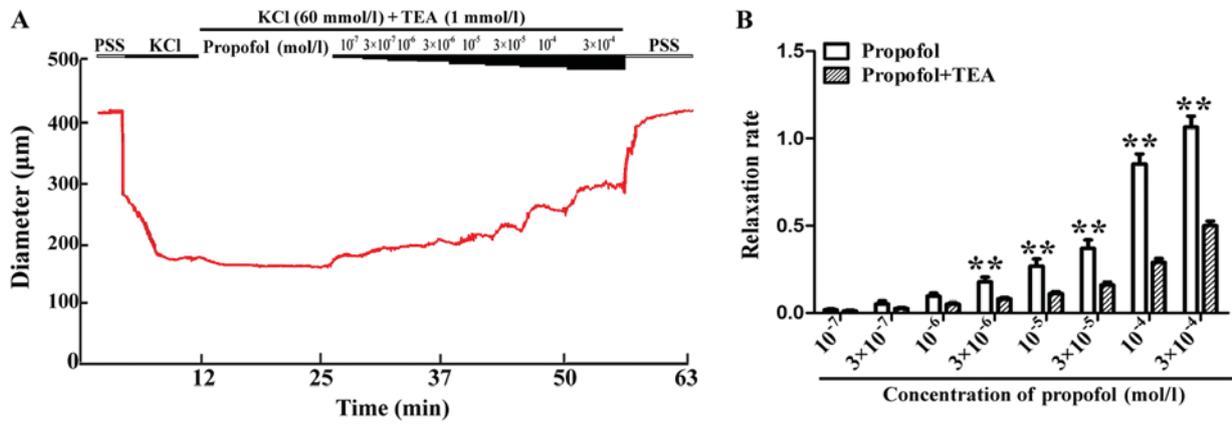


Figure 4. Pre-treatment with TEA, a calcium-activated K^+ channel inhibitor, decreases the relaxation elicited by propofol. (A) Using representative video photos, isolated mesenteric arteriole pre-treated with TEA (1 mmol/l) were traced following 20 min. The vessel diameter was decreased after TEA was added, and was increased in a dose-dependent manner when propofol was increased from 1×10^{-7} to 3×10^{-4} mol/l. (B) The relaxation elicited by propofol to increase the normalized vascular diameter was markedly inhibited in the presence of TEA. $^{**}P < 0.01$ vs. propofol ($n=8$). TEA, tetraethylammonium; PSS, physiological saline solution.

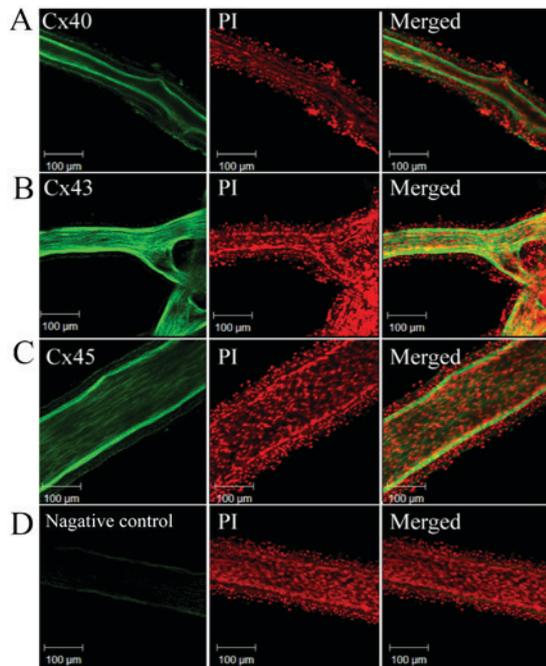


Figure 5. Cx₄₀, Cx₄₃ and Cx₄₅ expressed in MAs. Left panel, representative images indicating that (A) Cx₄₀, (B) Cx₄₃ and (C) Cx₄₅ were expressed on the surface of MAs. (D) A negative control was prepared with omission of the primary antibody. Middle panel, the nuclei of the vascular smooth muscle cells of the MAs were labeled with PI. Right panel, merged images of the left and middle panels (scale bars, 100 μ m). Cx, connexin; MA, mesenteric arteriole; PI, propidium iodide.

18β -GA (100 μ mmol/l) for 20 min, and 1×10^{-4} mol/l propofol was added after stable constriction of the MA with 60 mmol/l KCl. In the presence of 18β -GA, the vasodilation effect of propofol was decreased. The increase in diameter induced by 1×10^{-4} mol/l propofol was 161.24 ± 11.43 and 130.63 ± 8.80 μ m in the absence and presence of 18β -GA, respectively. 18β -GA reduced the diameter increment induced by 1×10^{-4} mol/l propofol by 34.03 ± 3.43 μ m, and its inhibition rate was $26.19 \pm 3.61\%$ ($P < 0.01$, $n=8$; Fig. 6A and B). The diastolic diameter was not marked in the picture; however, the relaxation rate

of blood vessels was measured in Figs. 6B and 7B. From the results of relaxation rate, it was determined that vasodilatation was weakened.

Inhibitory effect of 2-APB on the relaxation induced by propofol. In another experiment, the MAs were pre-treated with the gap junction inhibitor 2-APB (100 μ mmol/l) for 20 min, and after stable vasoconstriction was achieved with 60 mmol/l KCl, 1×10^{-4} mol/l propofol was added. The vasodilation effect of propofol was decreased in the presence of 2-APB. The increase in diameter induced by 1×10^{-4} mol/l propofol was 161.24 ± 11.43 and 143.15 ± 4.69 μ m, respectively, in the absence and presence of 2-APB. 2-APB reduced the diameter increment induced by 1×10^{-4} mol/l propofol by 19.16 ± 3.67 μ m, and its inhibition rate was $20.52 \pm 4.54\%$ ($P < 0.01$, $n=8$; Fig. 7A and B).

Comparison between the inhibitory effects of 18β -GA and 2-APB on the relaxation induced by propofol. Next, the vasodilation response to 1×10^{-4} mol/l propofol in the presence of 18β -GA or 2-APB was compared. The relaxation rate of MAs induced by 1×10^{-4} mol/l propofol was $85.26 \pm 5.83\%$, but the relaxation rate was reduced to 57.73 ± 2.69 and $62.27 \pm 2.73\%$, respectively, in the presence of 18β -GA and 2-APB. The relaxation induced by propofol was inhibited by 18β -GA and 2-APB, but no significant difference between the inhibitory effect of 18β -GA and 2-APB was identified ($P < 0.01$, $n=8$; Fig. 8).

Discussion

Propofol is widely applied in the clinic as an intravenous anesthetic; however, it frequently causes hypotension at the time of induction of anesthesia (20), particularly in elderly and hypertension patients, whose cardiac cycle fluctuations are more obvious, and which may contribute to various conditions of the cardiovascular system, including arrhythmias, myocardial ischemia and myocardial infarction (21). The blood pressure in the circulatory system is dependent on blood volume, cardiac ejection and peripheral resistance. The peripheral resistance of the circulatory system is a prerequisite for the generation

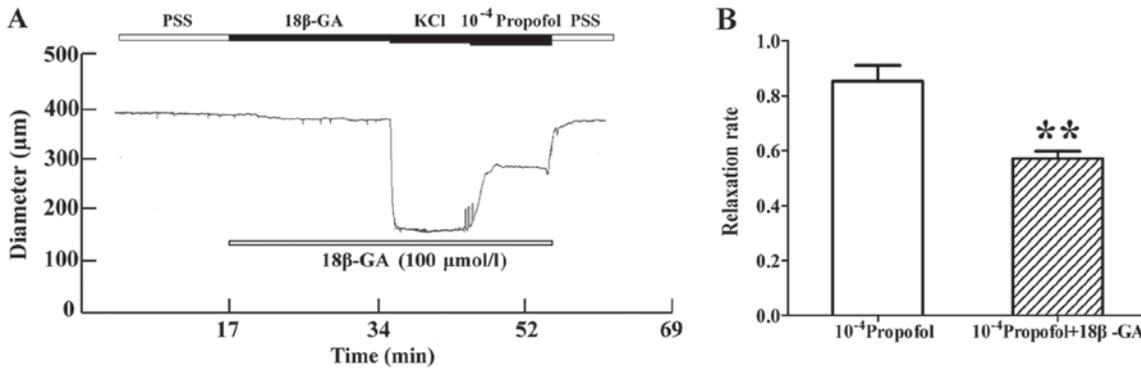


Figure 6. Effects of gap junction inhibitor 18β-GA on the vasodilation induced by propofol. (A) The relaxation of mesenteric arterioles induced by 1x10⁻⁴ mol/l propofol was inhibited by 18β-GA, a gap junction blocker. (B) Bar graph presenting the relaxation rate. **P<0.01 vs. 10⁻⁴ propofol (n=8). PSS, physiological saline solution; 18β-GA, 18β-glycyrrhetinic acid.

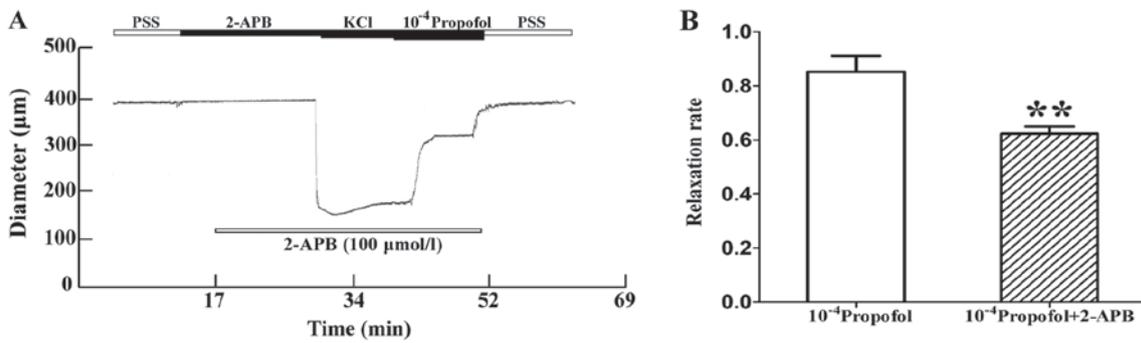


Figure 7. Effects of gap junction inhibitor 2-APB on vasodilation induced by propofol. (A) The relaxation of MA induced by 1x10⁻⁴ mol/l propofol was inhibited by 2-APB, a gap junction blocker. (B) Bar graph presenting the relaxation rate. **P<0.01 vs. 10⁻⁴ propofol (n=8). PSS, physiological saline solution; 2-APB, 2-aminoethoxydiphenyl borate.

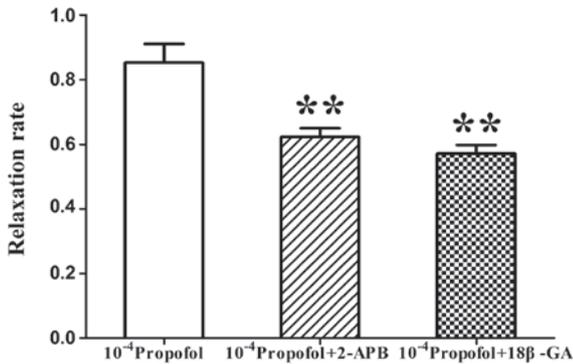


Figure 8. Inhibitory effect of 18β-GA and 2-APB on propofol-induced relaxation of MA. The relaxation induced by 1x10⁻⁴ mol/l propofol on the MA was restrained by 100 μmol/l 18-GA and 100 μmol/l 2-APB, but no significant difference was identified between the two treatments. **P<0.01 vs. 10⁻⁴ propofol (n=8). PSS, physiological saline solution; 2-APB, 2-aminoethoxydiphenyl borate; 18β-GA, 18β-glycyrrhetinic acid; MA, mesenteric arteriole.

of blood pressure, and this resistance refers to micro/small arterial resistance to blood flow. It has been suggested that the increased peripheral resistance often increases the diastolic and systolic blood pressure (22). The blood flow and vascular resistance are inversely proportional to the fourth power of the radius (23), which mainly depends on the caliber of blood vessels that may be modified by VSM contraction. Therefore,

the present study aimed to investigate these types of blood vessel based on an experiment in separated 2-3 mm segments from MAs, which may be categorized as small arteries, are resistance vessels and also have a role in blood pressure regulation.

The principal findings of the present study may be summarized as follows: i) Propofol relaxes the MA in a concentration-dependent manner; ii) propofol enhances the outward current of VSMCs; iii) propofol enhances the outward current mediated via the BK_{Ca} channel, as the propofol-induced increases in the outward current and relaxation of MAs were blocked by the BK_{Ca} channel inhibitor TEA; iv) MAs contain gap junctions; v) propofol relaxes the vasculature via gap junctions, as the as the propofol-induced relaxation of MAs were blocked by two different gap junction inhibitors. These results suggest that the relaxation effect of propofol on MAs may be mediated via BK_{Ca} channels and gap junctions.

The experimental design of the present study was divided into two parts. In the first part, the MAs of experimental SD rats were used as the experimental models. The pressure myograph technique was applied to examine the effect of different concentrations of propofol on the relaxation of blood vessels, and the whole-cell patch clamp technique was employed to observe the outward currents of VSMCs induced by different concentrations of propofol. The experimental results obtained from the pressure myograph technique revealed that propofol was capable of relaxing MAs, and the whole-cell patch

clamp assay indicated that propofol enhanced the outward current of VSMCs. To further investigate the mechanisms of propofol-induced vasodilation and the increased outward current and identify the channels involved, various channel inhibitors were applied. The effect of propofol on the relaxation of blood vessels was decreased after treatment with TEA (a BK_{Ca} channel blocker), as indicated in the pressure myograph experiment, and the whole-cell patch clamp assay also demonstrated that pre-treatment with TEA inhibits the increase of the outward current induced by propofol. According to these experimental results, it may be concluded that the relaxation effect of propofol on the MA may exerted via enhancement of the BK_{Ca} current. The whole-cell patch clamp assay is a technique that is only performed on single SMCs, but in theory, the propofol-induced activation of the BK_{Ca} channel may lead to transfer of the hyperpolarization information, which further results in the fast and synchronized relaxation of the MA.

Due to the long artery span and alterations in blood flow intensity, regulation of the blood pressure by the microcirculation is required along the full length of the blood vessels. In order to achieve this equilibration, the cells in the blood vessels form a coordinated response (24), i.e. they constitute a network of coupled cells to facilitate a coordinated response. Gap junctions provide a pathway for the formation of intercellular junctions, which have a key role in cell communication and conduction of vasodilation (19).

The possible implication of gap junctions in the vasodilation effect of propofol was then investigated. In this second part of the present study, the expression of Cx40, Cx43 and Cx45 was verified by immunofluorescence microscopy, which was consistent with the results of previous studies (25,26), and indicated that gap junctions were present on MAs. In addition, it was demonstrated that pre-treatment with gap junction blockers, namely 18 β -GA and 2-APB, dampened the relaxation effect of propofol, indicating that gap junction communication has a role in propofol-induced vasodilation.

Klockgether-Radke *et al* (27) suggested that activation of the BK_{Ca} channel may contribute to the vasodilating effect of propofol on coronary arteries, and Sinha *et al* (28) indicated that propofol-induced vasodilation is mediated by transient receptor potential A1 ion channels and includes the activation BK_{Ca} channels. These studies provide compelling evidence that BK_{Ca} channels are important effectors in mediating VSM hyperpolarization and relaxation of numerous vessel types. Hyperpolarization is a highly efficient means of synchronizing cells, as it may exert an electric strain along a variety of cells that are coupled to each other. In addition, hyperpolarization has an important role in coordinating the behavior of the entire vasculature. The activation of BK_{Ca} and K⁺ efflux leads to cell membrane hyperpolarization, which contributes to the closure of voltage-dependent Ca²⁺ channels to block the influx of extracellular Ca²⁺ and thereby induce vasorelaxation (29,30). The membrane potential is one of the major factors that regulate the contractile activity of SMCs. Since the coordination of contraction or dilatation of SMCs is required to exert full control over the local circulation, synchronous changes in membrane potential in regions of neighboring SMCs are indispensable (24). Due to the low impedance of gap junctions and the high electrical conductivity, cells tend to transform into syncytium. The gap junction provides a

good platform for the rapid conduction of hyperpolarization along the blood vessels. Furthermore, the hyperpolarization mediated by gap junctions is able to ensure the synchronous change in membrane potential. The flow of K⁺ may result in the hyperpolarization of the membrane. Activation of the BK_{Ca} channel may cause membrane hyperpolarization, which leads to a corresponding hyperpolarization of the cell membrane potential due to the electrical communication between the gap junctions (31). Therefore, propofol-induced activation of the BK_{Ca} channel causes hyperpolarization, which may further affect the SMC potential via gap junction communication, and it is well recognized as a potential mechanism of vascular relaxation.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

HJW participated in designing and performing the experiments, analyzed the data, and wrote and revised the article. YW assisted in the experimental process, designed the immunofluorescence experiment, and contributed in data analysis and writing and revising the article. JQS participated in the conceptual design of the experiments and provided funding for research projects. LL participated in the study and design experimental design, assisted in performing the experiments, and provided funding for research projects.

Ethical approval and consent to participate

The use of animals was approved by the Ethical Inspection of the First Affiliated Hospital, Shihezi University School of Medicine (Shihexi, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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