SPANDIDOS PUBLICATIONS

MOLECULAR MEDICINE REPORTS 16: 2620-2626, 2017

Effects of RMF on BKCa and Kv channels in basilar arterial smooth-muscle cells of SHR

YAN-FEI QIAN[1-3*], YANG WANG[1,2,4*], WEI-WEI TIAN[1,2], SHENG WANG[1,5], LEI ZHAO[1,2], LI LI[1,2], KE-TAO MA[1,2] and JUN-QIANG SI[1,2,4,6]

[1]Department of Physiology; [2]The Key Laboratory of Xinjiang Endemic and Ethnic Diseases, Shihezi University Medical College, Shihezi, Xinjiang 832002; [3]Department of Anesthesiology, Jiaxing First Hospital, Jiaxing, Zhejiang 314000; [4]Department of Physiology, Wuhan University School of Basic Medical Sciences, Wuhan, Hubei 430070; [5]Department of Anesthesiology, The First Affiliated Hospital of Shihezi University, Shihezi, Xinjiang 832002; [6]Department of Neurobiology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, P.R. China

Received June 11, 2016; Accepted April 19, 2017

DOI: 10.3892/mmr.2017.6881

Abstract. The current study observed the effects and investigated the mechanism of remifentanil (RMF) on the isolated cerebral basilar arteries of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. A pressure myograph system was used to observe and compare the effects of different concentrations of RMF (10^{-10}-10^{-5} mol/l) on the diameter changes of freshly isolated cerebral basilar arteries, which have been pre-shrunk by phenylephrine (PE), an endothelium-independent vasoconstrictor. Vascular smooth-muscle cells of the cerebral basilar artery (BASMCs) were freshly obtained via enzymolysis. BKCa (large-conductance calcium-activated potassium channels) current (I_{BKCa}) and Kv (voltage-gated potassium channels) current (I_{Kv}) were recorded using a whole-cell patch-clamp technique. The changes in I_{BKCa} and I_{Kv} produced by different concentrations of RMF (10^{-10} to 10^{-5} mol/l) on the two types of rats with the holding potential of -40 mV were observed and compared. The cerebral basilar arteries of the SHR and WKY rats were relaxed by RMF in a concentration-dependent manner (P<0.05; n=5). At the same concentration, the diastolic effect of RMF on SHR was weaker than that observed in WKY rats (P<0.05, n=5). When the rats were pre-perfused with 10^{-3} mol/l of the BKCa channel blocker tetraethylammonium (TEA), the diastolic amplitudes of RMF in SHR and WKY rats were decreased, and the fitting curves shifted down (P<0.05; n=7 and 6, respectively). However, no statistically significant difference was observed with 10^{-3} mol/l of the Kv channel blocker 4-aminopyridine (4-AP; n=6 and 9, respectively; P>0.05). Outward currents were increased by RMF in both BASMCs of SHR and WKY rats in a voltage- and dose-dependent manner (P<0.05; n=6). At the same concentration, the effect of RMF on the outward currents in BASMCs of WKY rats was stronger than that on SHR (P<0.05; n=6). The enhancing effect of RMF can be partially blocked by either 10^{-3} mol/l TEA (P<0.05; n=6) or 10^{-3} mol/l 4-AP (P<0.05 or 0.01; n=6 and 9, respectively) however can be totally blocked by the mixture of TEA and 4-AP (P<0.05, n=7). RMF served a diastolic role in the cerebral basilar arteries of rats in a dose-dependent manner, likely by activating the BKCa and Kv channels. However, SHR demonstrated a less pronounced diastolic reaction to RMF than that observed in WKY rats.

Correspondence to: Dr Jun-Qiang Si or Dr Ke-Tao Ma, Department of Physiology, Shihezi University Medical College, 192 Beier Road, Shihezi, Xinjiang 832002, P.R. China
E-mail: sijunqiang@shzu.edu.cn
E-mail: maketao@hotmail.com

*Contributed equally

Key words: remifentanil, cerebrovascular, potassium channel, patch clamp, pressure myograph system

Introduction

In China, the incidence of hypertension in surgical patients has reached 20% (1). In addition, Prys-Roberts (2) identified that hypertension in surgical patients is 24% in Britain. During anesthesia, hypertensive patients, in particular the elderly, are extremely prone to fluctuations in blood pressure. If blood pressure rises or falls by 30% away from the baseline value, this will lead to serious complications (stroke, heart or kidney failure etc.) and even death. Therefore, in the anesthetic management of these patients, particular attention should be given to maintain a stable blood pressure, and analgesics for minimizing fluctuations in blood pressure are very important. Remifentanil (RMF), a new ultra short effect of the μ-opioid receptor agonist, is used for the induction and maintenance of general anesthesia. Loading or maintenance doses of RMF can decrease peripheral vascular resistance, thereby causing hypotension (3-6), which may be directly associated with the relaxation of vascular smooth muscles (7,8). Unlugenc *et al* (9) stated that RMF exerted a dilation effect on isolated thoracic aortas of rats. Paris *et al* (10) demonstrated that the cerebral blood flow velocity was reduced by large doses of RMF. By contrast, Engelhard *et al* (11)

showed that after continuous infusion of RMF, the mean arterial pressure, intracranial pressure, and cerebral blood flow velocity of patients did not change. Thus, the specific mechanisms and effects of RMF on the cerebrovascular system remain unclear. Large-conductance calcium-activated potassium channels (BKCa) and voltage-gated potassium channels (Kv) are two important potassium ion channels in the vascular smooth-muscle cell (VSMC) membrane. When the VSMC membrane potential depolarization occurred, the opening probability of BKCa and Kv channels increased, and the intracellular potassium efflux also increased; the cell then hyperpolarized, the opening of L-type-calcium channel became limited, thereby reducing the calcium influx and decreasing the intracellular calcium concentration, leading to vasodilation (12,13). In the current study, the pressure myograph system and whole-cell patch-clamp technique were used to observe the effects of RMF on the diameter and smooth-muscle cells (SMCs) of isolated cerebral basilar arteries (BAs) of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. The effects on the BAs and the mechanisms involved were also investigated to provide a better basis for clinical use of these two groups of patients undergoing cranial operation.

Materials and methods

Animals. A total of 60 SHR and 60 WKY rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing) [Animal certificate of conformity: SCXK (Beijing) 2012-0001, weighing ~200-250 g, aged 16-20 weeks old, male or female]. Rats were housed in separate cages in a specific pathogen-free environment at 24±3˚C, relative humidity of 40-70%, in a 12 h light-dark cycle, and were provided with free access to food and water. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the Medical College of Shihezi University and consistent with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (14).

Reagents. RMF was purchased from Hubei Yichang People Fook Pharmaceutical Co., Ltd. (batch number, 6141211; Yichang, China). Phenylephrine (PE), acetylcholine (ACh), ethylenediaminetetraacetic acid (EDTA), tetraethylammonium (TEA), 4-aminopyridine (4-AP), collagenase, papain, bovine serum albumin (BSA), DTT, and DMEM culture medium were purchased from Sigma-Aldrich (Merck Millipore, Darmstadt, Germany). KCl and other reagents were acquired locally. All reagents used in the pressure myograph system and whole-cell patch-clamp technique were prepared using sugar-free physiological saline solution (PSS). Extracellular solution was a stock sample prepared before being further diluted with external solution to achieve the final concentration. The formulas of PSS/saline solution with high kalium and the external solution were in accordance with the literature (15,16).

Instruments. Pressure myograph system (110P; Danish Myo Technology A/S, Aarhus, Denmark), MyoVIEW software (Danish Myo Technology A/S), Axon MultiClamp 700B patch-clamp amplifier (Axon; Molecular Devices LLC, Sunnyvale, CA, USA), micromanipulator (PCS5001; Siskiyou Design, Oregon, USA), P-97 microelectrode pullers (Sutter Instrument, Novato, CA, USA), heated water bath (HSS-1B; Chengdu Science Instrument Factory, Chengdu, China), and multiple perfusion administration system (supplied in-house by Huazhong University of Science and Technology, China).

Pressure myograph measurement. Cerebral BA (CBA) segments were placed in a 4˚C oxygen-saturated physiological solution containing 118.9 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 5.5 mM glucose. CBA segments were tied to a glass tube using 12-0 nylon monofilament sutures, and placed in a microvascular chamber (Pressure Myograph System; Danish Myo Technology A/S). The chamber was perfused with a physiological solution (pH 7.4, bubbled with 95% O_2 and 5% CO_2) and heated to 37˚C. CBA segments were pressurized to a constant transmural pressure of 60 mmHg. The diameter was continuously determined and recorded via video dimension analyzer and the DMT Vessel Acquisition Suite. CBA segments were treated with progressively increasing doses of RMF (10^{-4}-10 μM), followed by PE (0.1 mM). The results were evaluated via the changes in vascular diameter recorded on the DMT (17).

Whole-cell patch-clamp recording. To isolate single SMCs from CBAs, CBAs were incubated in a low-Ca^{2+} solution for 20 min containing 142 mM NaCl, 5 mM KCl, 0.05 mM $CaCl_2$, 1.0 mM $MgCl_2$, 4.0 mM Na-HEPES, 5.0 mM HEPES and 7.5 mM glucose and cut into 1 mm segments and digested with low-Ca^{2+} solution containing papain (1 mg/ml), collagenase A (0.5 mg/ml), BSA (1 mg/ml) and DL-dithiothreitol (1 mg/ml) for 10 min at 37˚C. Specific operations were performed according to the literature (18).

Conventional whole-cell patch-clamp recording was performed using an Axon 700B amplifier (Axon; Molecular Devices LLC) (19). The pipette had a resistance of approximately 5 MΩ after being filled with internal solution containing 130 mM K-gluconate, 10 mM NaCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM HEPES, 5 mM ethylene glycol-bis (β-aminoethyl ether) N,N',N'-tetraacetic acid and 7.5 mM glucose. The seal resistance commonly reached 1-20 GΩ prior to rupture of the membrane. The membrane current or voltage signals were filtered at 10 kHz and recorded on a computer equipped with a Digidata 1440A AD-interface and pClamp software, version, 10.2 (Axon; Molecular Devices LLC) at a sampling interval of 200 msec.

Statistical analysis. SHR and WKY rats were age-matched to minimize individual differences. The results are expressed as the mean ± standard error. Statistical analysis was performed using the SPSS statistical software package, version 17.0. A two-factor multilevel analysis of variance was used for repeated measurement data, followed by the Neuman-Keuls post hoc test. A two-sample t-test was used between groups, and the paired t-test was applied in the same group. $P<0.05$ was considered to indicate a statistically significant difference.

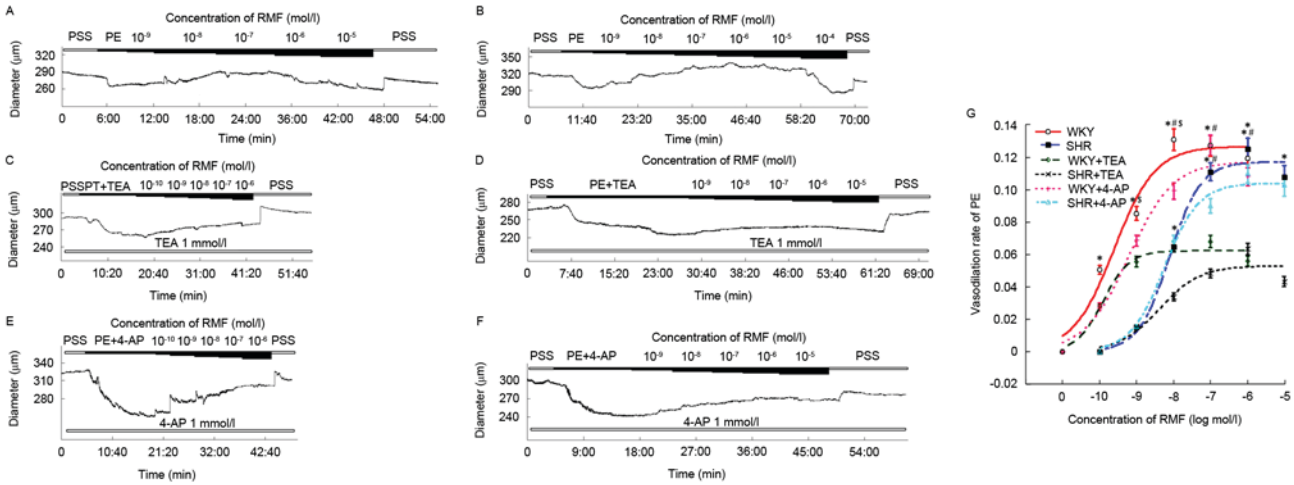

Figure 1. Diastolic effects of RMF on CBA of SHR and WKY rats were in a dose-dependent manner, which were associated with the BKCa channel. The initial image about different concentrations of RMF on cerebral CBA of WKY (A, C and E) and SHR (B, D and F) rats. (G) The fitting curves of RMF on SHR and WKY rats at different concentrations. All data were compared with each other after being standardized. Results are presented as the mean ± standard error, n=5 for WKY and SHR, n=7 for SHR+TEA, n=6 for WKY+TEA, n=6 for SHR+4-AP and n=9 for WKY+4-AP. *P<0.05, vs. pre-administration; $^{\#}$P<0.05, WKY vs. WKY + TEA, SHR vs. SHR + TEA; $^{\$}$P<0.05, WKY vs. SHR. RMF, remifentanil; CBA, cerebral basilar artery; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; TEA, tetraethylammonium; 4-AP, 4-aminopyridine.

Results

CBAs of SHR and WKY rats were relaxed by RMF in a concentration-dependent manner. Following measuring the activity to be efficient, restoring stability, and pre-shrinking by 10^{-4} mol/l PE to steady state, the blood vessel diameters of SHR and WKY rats were 270.11±9.79 and 242±10.27 μm. Subsequent to adding RMF prepared by 10^{-4} mol/l PE from low to high concentrations to the chamber, the diastolic range of SHR with 10^{-9} to 10^{-5} mol/l RMF was identified to be 4.29±1.56, 17.65±2.84, 30.06±6.42, 33.86±7.48 and 29.25±7.28 μm (Fig. 1B); compared with the pre-administration phase. Excluding the concentration of 10^{-9} mol/l, all concentrations were statistically different (n=5; F=12.98; P<0.05; Fig. 1G). However, the diastolic amplitude of WKY rats with 10^{-10}-10^{-6} mol/l RMF was 12.39±7.44, 21.05±9.01, 32.13±14.71, 31.28±13.90 and 29.51±15.52 μm, respectively (Fig. 1A); and compared with those not given RMF, a statistical significance was observed in all concentrations (n=5; F=11.81; P<0.05; Fig. 1G). Subsequent to drawing the dose-response curve, it was identified that the EC_{50} values of CBA of SHR and WKY rats relaxed by RMF were (4.32±1.22)x10^{-9} mol/l and (3.09±0.58) x10^{-10} mol/l, and the difference was statistically significant (n=5; P<0.05) (Fig. 1G). Compared with WKY rats, the vasodilation rate curve of SHR by RMF shifted right and downwards, which indicated that the relaxation response of BA in SHR to RMF was weaker than that of WKY rats, and the difference was statistically significant (n=5; F=20.34; P<0.01; Fig. 1G).

The diastolic effects of RMF on CBA of SHR and WKY rats were mediated by the BKCa channel. Following pre-incubation of the mixture of 1 mmol/l TEA and 10^{-4} mol/l PE for 20 min to a steady state, the vessel diameters of SHR and WKY rats were 231.62±5.26 and 239.75±7.04 μm, respectively. Successively adding RMF formulated by 1 mmol/l TEA and 10^{-4} mol/l PE from a low to high concentration to the chamber, it was identified that the diastolic range of SHR by 10^{-9}-10^{-5} mol/l RMF was 3.74±0.59, 8.58±2.67, 12.14±3.05, 15.89±4.07 and 11.37±5.39 μm, respectively (Fig. 1D); while the diastolic amplitude of WKY rats with 10^{-10}-10^{-6} mol/l RMF was 6.78±1.26, 13.34±1.94, 15.59±2.09, 16.34±2.37 and 13.49±2.53 μm, respectively (Fig. 1C). Although different concentrations of RMF produced diastolic effects on BAs in a dose-dependent manner, compared with no administration of TEA, the relaxation rate curve of SHR shifted down during 10^{-8}-10^{-5} mol/l, and a statistically significant difference was identified at the concentrations of 10^{-7} and 10^{-6} mol/l (n=7; F=15.47; P<0.05; Fig. 1G). However, the vasodilation rate curve of WKY rats significantly shifted down during 10^{-9}-10^{-6} mol/l, and the statistical difference was observed with the concentrations of 10^{-8} and 10^{-7} mol/l (n=6; F=22.36; P<0.05; Fig. 1G). These indicated that CBA of SHR and WKY rats were relaxed by RMF, which was associated with the opening of BKCa channels. Prior and subsequent to incubation with TEA, the EC_{50} value of CBA of SHR relaxed by RMF were (4.32±1.22)x10^{-9} mol/l and (7.54±3.17)x10^{-9} mol/l, and the difference between them was not statistically significant (n=7; P>0.05; Fig. 1G); while the EC_{50} value of WKY rats by RMF were (3.09±0.58)x10^{-10} mol/l and (2.26±0.55)x10^{-10} mol/l, and neither was statistically different (n=6; P>0.05; Fig. 1G).

RMF relaxed CBA, which was not related to Kv channel. Following pre-incubation of BA with the mixture of 1 mmol/l 4-AP and 10^{-4} mol/l PE for 20 min to a steady state, the diameters of SHR and WKY rats were 257.67±5.36 and 273.93±12.79 μm, respectively. Subsequently, RMF that had been prepared with 1 mmol/l 4-AP and 10^{-4} mol/l PE was added to the chamber from a low to high concentration, and it was identified that the diastolic amplitudes of BA of SHR with 10^{-9}-10^{-5} mol/l RMF were 6.73±0.77, 17.12±4.48, 22.64±4.99, 27.6±5.36 and 26.20±6.07 μm, respectively (Fig. 1F); while the diastolic range of WKY rats with 10^{-10}-10^{-6} mol/l RMF were 7.43±1.72, 17.71±3.51, 25.63±4.33, 34.93±5.25 and

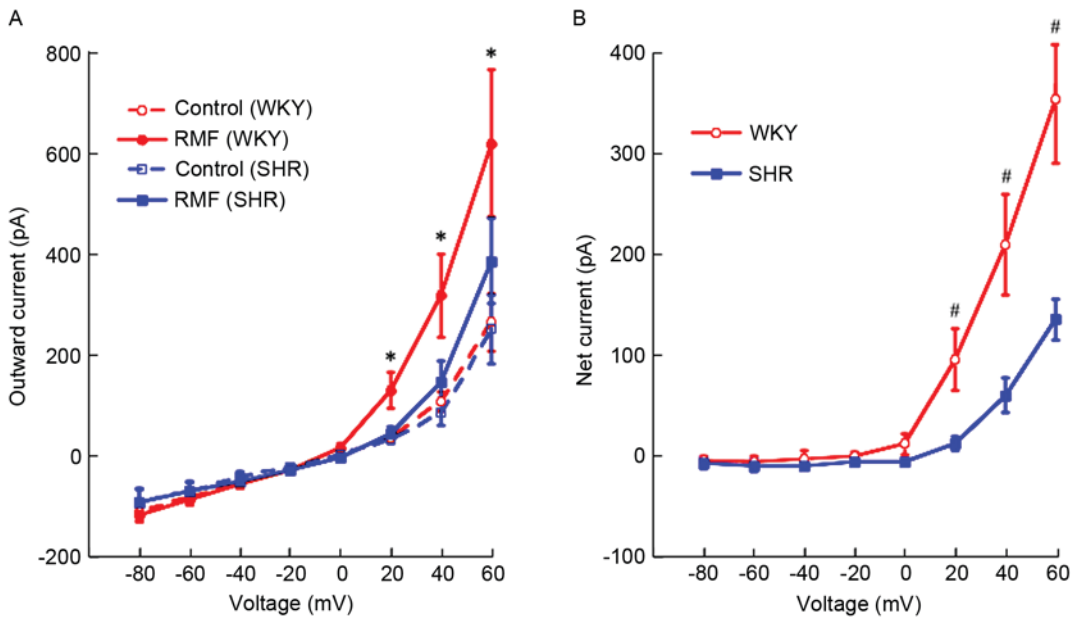

Figure 2. Outward currents were increased by RMF in vascular smooth-muscle cells of the cerebral basilar artery of SHR and WKY rats in a voltage-dependent manner. (A) The outward currents and (B) the increasing effect of RMF on outward currents in BASMCs of SHR and WKY rats at voltage from -80 to +60 mV. Results are presented as the mean ± standard error, net current=I_{RMF}-$I_{control}$, n=6. *P<0.05, vs. pre-administration and WKY vs. SHR groups. RMF, remifentanil; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

29.66±4.53 μm, respectively (Fig. 1E). Different concentrations of RMF still caused diastolic reactions on the vessels of SHR and WKY rats in a dose-dependent manner. Compared with pre-incubation without 4-AP, the diastolic rate curves of SHR and WKY rats marginally moved down, and no significant difference was observed (n=6 and 9; F=2.13 and 2.59; P>0.05; Fig. 1G). Prior and subsequent to incubation with 4-AP, the EC_{50} values of RMF on BA of SHR were (4.32±1.22)x10^{-9} mol/l and (4.12±1.16) x10^{-9} mol/l, and the difference between them was not statistically significant (n=6; P>0.05; Fig. 1G). The EC_{50} values of WKY rats produced by RMF were (3.09±0.58)x10^{-10} mol/l and (10.75±3.41)x10^{-10} mol/l, and neither were statistically different (n=9; P>0.05; Fig. 1G).

Outward currents were increased by RMF in BASMCs of rats in a voltage-dependent manner. At 0 to 60 mV, RMF (3x10^{-7} mol/l) enhanced the outward currents in BASMCs of SHR and WKY rats in a voltage-dependent manner, however no significant difference at 0 mV was observed (Fig. 2A). In addition, the increasing effect of RMF on outward currents in BASMCs of SHR was weaker than for that of WKY rats with the same voltage (Fig. 2B).

Outward currents were increased by RMF in BASMCs of rats in a dose-dependent manner. Approximately 10^{-10}-10^{-5} mol/l RMF made outward currents (+60 mV) in BASMCs of SHR increase from 317±33 to 326±30, 363±28, 430±38, 504±32, 572±72 and 619±89 pA, respectively; the outward currents in BASMCs of WKY rats were from 381±68 to 542±49, 632±45, 721±58, 859±69, 1217±19 and 1610±50 pA, respectively. Subsequent to fitting the dose-response curve, the EC_{50} values of the enhancing effect of RMF on outward currents in BASMCs of SHR and WKY rats were (9.58±5.1)x10^{-8} mol/l and (2.93±1.4)x10^{-9} mol/l, and the two exhibited a statistically significant difference (n=6; P<0.01). Compared with pre-administration, the outward currents of SHR were enhanced in a dose-dependent manner, with exception of the concentration of 10^{-10} mol/l (n=6; F=13.21; P<0.05). These effects were additionally observed in the WKY rats at all concentrations (n=6; F=21.57; P<0.05). Compared with WKY rats, the increasing effect of RMF on outward currents of SHR was weaker at the same concentration. In addition to 10^{-10}, 10^{-6} and 10^{-5} mol/l, the remaining concentrations had a statistically significant difference (n=6; F=8.65; P<0.05; Fig. 3A). Fig. 3B presents the comparison of the effect of RMF (3x10^{-7} mol/l) on outward currents in BASMCs of SHR and WKY rats, which suggested that the relaxant effect on CBA of SHR by RMF was weaker in WKY rats (n=6; P<0.05).

The enhanced effect of RMF on BASMCs outward current was mediated by the BKCa channel. Approximately 10^{-3} mol/l TEA was pre-perfused for 3 min. The mixture of 10^{-3} mol/l TEA and 3x10^{-7} mol/l RMF was administered. The increasing effect of RMF on BASMCs outward current was partially blocked and outward current (at +60 mV) of WKY rats reduced to 115.15±19.88 pA, which was only 0.44±0.03 times higher than that of the control group (n=6; P<0.05; Fig. 4A); while outward current of SHR decreased to 83.21±20.69 pA, which was only 0.33±0.02 times higher than that of the control group (n=6; P<0.05; Fig. 4B); these indicated that the BKCa channel-mediated outward current contributed to the outward current of the BASMCs of SHR and WKY rats induced by RMF (Fig. 4C). In addition, the inhibition rate of TEA on I_{BKCa} in BASMCs of SHR and WKY rats was 0.67±0.02 and 0.56±0.03, and the difference between them was statistically significant (n=6, P<0.05), which suggested that the influence of BKCa channels in the BASMCs of SHR was more than in WKY rats.

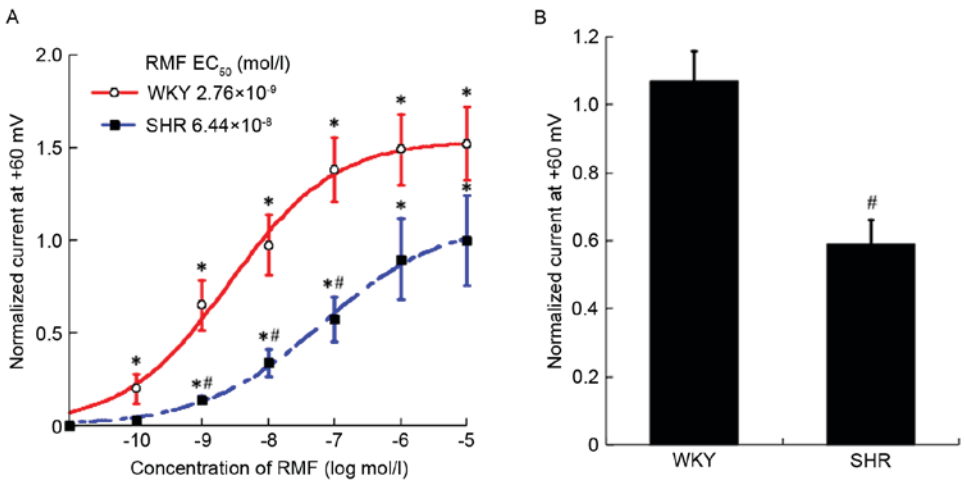

Figure 3. Outward currents were increased by RMF in both vascular smooth-muscle cells of the cerebral basilar artery of SHR and WKY rats in a dose-dependent manner. (A) The fitting curves of RMF on outward currents in BASMCs of SHR and WKY rats at different concentrations. (B) The comparison of the effect of RMF ($3x10^{-7}$ mol/l) on outward currents in BASMCs of SHR and WKY rats. All data were compared after the current was normalized=$(I_{RMF}-I_{control})/I_{control}$. Results are presented as the mean ± standard error, n=6. *P<0.05, vs. pre-administration; #P<0.05, vs. WKY and SHR groups. RMF, remifentanil; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

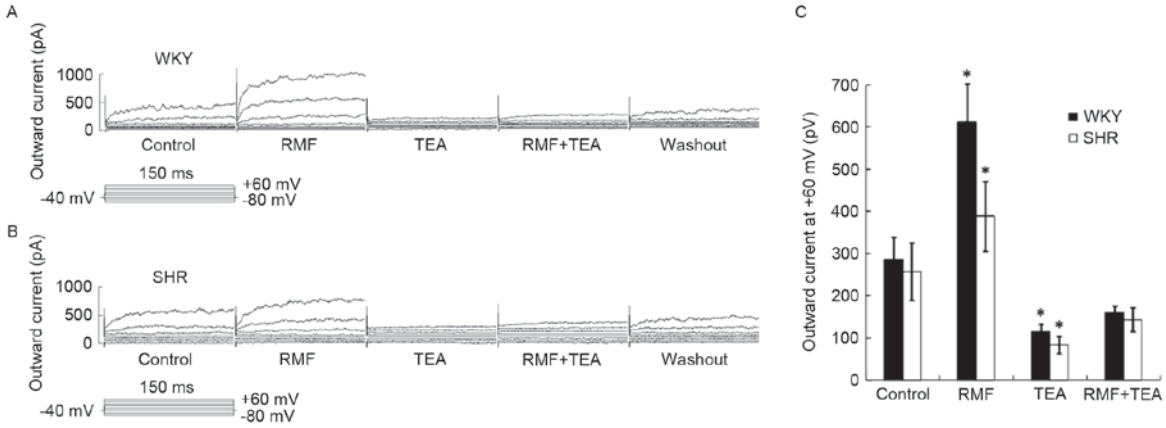

Figure 4. Enhanced effect of RMF can be partially blocked by 10^{-3} mol/l TEA. Raw current images with respect to the effect of RMF and TEA (BKCa channel blocker) on (A) WKY and (B) SHR rats. (C) Bar chart showing the effects of RMF and TEA on SHR and WKY rats. Results are presented as the mean ± standard error, n=6. *P<0.05, vs. control group. RMF, remifentanil; TEA, tetraethylammonium; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

Enhanced effect of RMF on BASMCs outward current was partially blocked by 4-AP (Kv channel blocker). Approximately 10^{-3} mol/l of 4-AP was pre-perfused for 3 min, and the mixture of 10^{-3} mol/l 4-AP and $3x10^{-7}$ mol/l RMF was administered. The increasing effect of RMF on the BASMCs outward current was still partially blocked, and the outward current (at +60 mV) of WKY rats was reduced to 105.12±12.18 pA, which was 0.52±0.04 times greater than that of the control group (n=9; P<0.01) (Fig. 5A); while outward current of SHR decreased to 274.96±81.92 pA, which was only 0.36±0.05 times higher than that of the control group (n=6; P<0.05; Fig. 5B); these suggested that the outward current of the BASMCs of SHR and WKY rats were enhanced by RMF, and involved the outward current mediated by Kv channel (Fig. 5C).

Enhanced effect of RMF on BASMCs outward current was totally inhibited by the mixture of TEA and 4-AP. The mixture of 10^{-3} mol/l TEA and 4-AP was pre-perfused for 3 min, and the mixture of 10^{-3} mol/l TEA, 4-AP and $3x10^{-7}$ mol/l RMF were administered. The increasing effect of RMF on BASMCs outward current was totally inhibited and outward current (at +60 mV) of WKY rats reduced to 139.69±18.04 pA, which was 0.32±0.034 times higher than that of the control group (n=7; P<0.05; Fig. 6A); while outward current of SHR decreased to 99.23±27.23 pA, which was only 0.32±0.04 times higher than that of the control group (n=7; P<0.05; Fig. 6B); these suggested that the enhanced effects of RMF on BASMCs outward current of SHR and WKY rats were completely and collectively mediated by BKCa and Kv channels (Fig. 6C).

Discussion

In the current study, RMF was observed to relax BA in a concentration-dependent manner, however this can be suppressed by TEA. The results suggested that the relaxant effect of RMF on BA was ascribed to the BKCa channel activity. The whole-cell patch-clamp technique was used to show that RMF enhanced I_{BKCa} and I_{Kv} in BASMCs in a

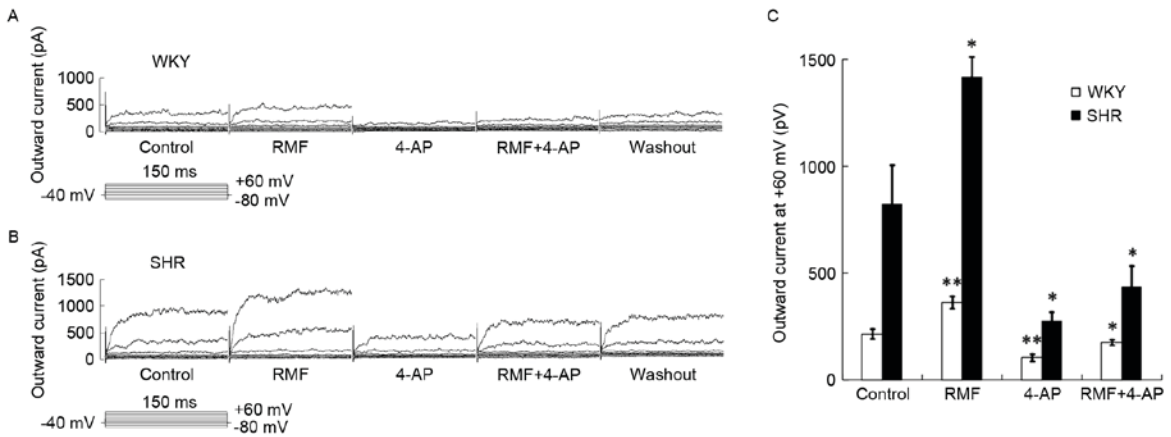

Figure 5. Enhanced effect of RMF can be partially blocked by 10^{-3} mol/l 4-AP. Raw current images with respect to the effect of RMF and 4-AP on (A) WKY and (B) SHR rats. (C) Bar chart showing the effect of RMF and 4-AP on SHR and WKY rats. Results are presented as the mean ± standard error, n=6 for SHR and n=9 for WKY. *P<0.05, **P<0.01, compared with control group. RMF, remifentanil; 4-AP, 4-aminopyridine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

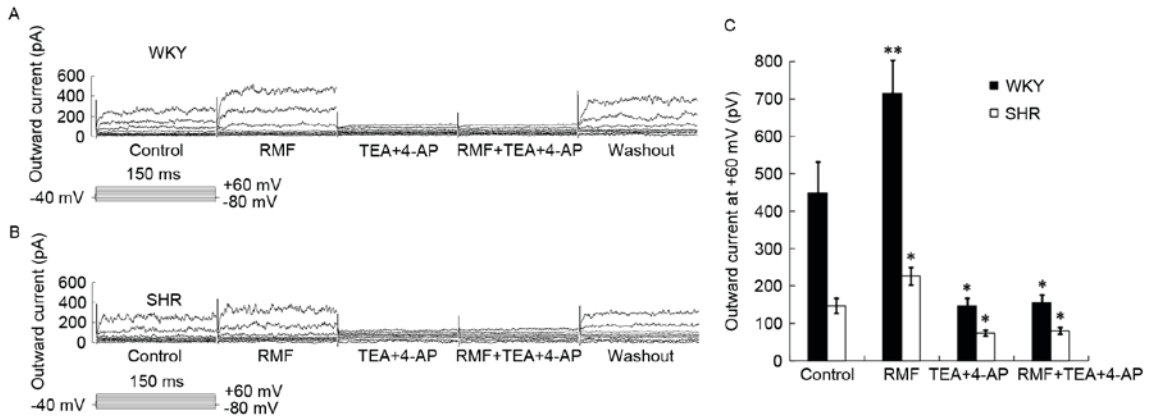

Figure 6. Enhanced effect of RMF can be totally blocked by 10^{-3} mol/l TEA and 4-AP. Raw current images with respect to the effect of RMF, TEA and 4-AP on (A) WKY and (B) SHR rats. (C) Bar chart showing the effect of RMF, TEA and 4-AP on SHR and WKY rats. Results are presented as the mean ± standard error, n=7. *P<0.05, **P<0.01, vs. control group. RMF, remifentanil; TEA, tetraethylammonium; 4-AP, 4-aminopyridine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

voltage- and dose-dependent manner. In conclusion, RMF served a diastolic role in the basilar arteries of rats, likely via activation of BKCa and Kv channels. The two techniques in the present study demonstrated different responses to the inhibitory effects of 4-AP on vasodilation of the RMF, possibly due to different drug sensitivities at tissue and cellular levels.

K^{+} efflux results in smooth-muscle cell membrane hyperpolarization following channel opening, and it additionally limits the opening of the L-type-calcium channel, reducing Ca^{2+} influx, decreasing the intracellular calcium concentration, and thus leading to vasodilation (12,13). A previous study confirmed that BKCa and Kv channels exist in the BASMCs of rats (16). Lin *et al* (20) identified that RMF relaxed human mesenteric arteries by activating BKCa channels in SMCs in a concentration-dependent manner via whole-cell patch-clamp technique, which was in agreement with the results of the current study.

However, in view of the current literature, the effects of RMF on cerebral arteries remain controversial. Paris *et al* (10) identified that RMF reduced the cerebral blood flow velocity with large doses, however Engelhard *et al* (11) suggested that the mean arterial pressure, intracranial pressure, and cerebral blood flow velocity of patients remained unchanged following continuous infusion of RMF. The current study demonstrated that RMF relaxed the BA of rats *in vitro*, and this inconsistency may be attributed to the following: More uncontrollable factors of macro-indicators detected in clinical studies, differences between the species, or possible existence of contraction mechanisms of RMF on BA which remain to be identified. The effect of this contraction mechanism should be greater than the relaxation mechanism, and it should be a new direction for future research.

In addition, two opposite views were presented on the comparison between SHR and WKY rats from local and international studies. Among these views, Liu *et al* (21) showed that the KCa current density sensitive to IBTX in cerebral artery smooth-muscle cell membrane of SHR was 4.7 times higher than that of WKY rats, and Hu *et al* (22) identified that the BKCa current density of SHR was

2.5 times higher than that of WKY rats. Other views were represented by Yang *et al* (23); they suggested the functions of BKCa channels in mesenteric artery smooth-muscle cells of Han hypertensive patients. It was observed that whole-cell current density, spontaneous transient outward currents, and the Ca^{2+} sensitivity of hypertensive patients were significantly reduced (23). In the current study, the BKCa channel activity in BASMCs of SHR was higher than those in WKY rats. Yang *et al* (23) presented a different reason regarding the difference between experimental objects. In the current study, it was concluded that the diastolic effect of RMF on BASMCs of SHR was weaker than that on WKY rats, and this observation may be ascribed to SHR being less sensitive than WKY rats; RMF served a role in SHR via another contraction mechanism, which required further experiments to be confirmed. It was hypothesized that RMF may serve a diastolic role in the BA of normotensive patients and produce an increase in cerebral blood flow, which increases intracranial pressure. When intracranial pressure exceeded 30~40 mmHg, cerebral blood flow declined, and eventually caused cerebral ischemia and hypoxia, which can lead to the formation of brain midline shift or herniation in severe cases (24). However, the diastolic effect of RMF on cerebral arteries of hypertensive patients was weaker than in normotensive ones, which was more likely to cause serious complications in chronic hypertension patients with risk for ischemia and hypoxia. Therefore, RMF should be carefully used in hypertensive patients with increased intracranial pressure (traumatic brain injury) under anesthesia.

RMF is a new μ-opioid receptor agonist with ultra short effect. Previous studies have indicated that the opioid peptides significantly increased the whole-cell I_{BKCa} and the opening probability of BKCa channels and inhibited Ca^{+} channels of neurons and chromaffin cells through the opioid receptors, which indicated a tight coupling between receptors and channels (25,26). In addition, G-protein-coupled receptors can exist in equilibrium with active and inactive states (27). Consequently, it was hypothesized that RMF was likely to serve an activation effect on BKCa and Kv channels via G-protein-coupled approach, however, the specific mechanisms require further elucidation.

In summary, RMF served a diastolic role in the CBA of rats, likely by activating BKCa and Kv channels in avoltage- and dose-dependent manner. SHR demonstrated a weaker diastolic reaction to RMF than WKY rats. These results may provide guidance regarding the two groups, particularly in hypertensive patients undergoing cranial surgery.

References

1. Guo J, Yu CQ, Lyu J, Guo Y, Bian Z, Zhou H, Tan Y, Pei P, Chen J, Chen Z, *et al*: Status of prevalence, awareness, treatment and controll on hypertension among adults in 10 regions, China. Zhonghua Liu Xing Bing Xue Za Zhi 37: 469-474, 2016 (In Chinese).
2. Prys-Rroberts C: Anaesthesia and hypertension. Br J Anaesth 56: 711-724, 1984.
3. Elliott P, O'Hare R, Bill KM, Phillips AS, Gibson FM and Mirakhur RK: Severe cardiovascular depression with remifentanil. Anesth Analg 91: 58-61, 2000.
4. Sebel PS, Hoke JF, Westmoreland C, Hug CC Jr, Muir KT and Szlam F: Histamine concentrations and hemodynamic responses after remifentanil. Anesth Analg 80: 990-993, 1995.
5. Ouattara A, Boccara G, Köckler U, Lecomte P, Leprince P, Léger P, Riou B, Rama A and Coriat P: Remifentanil induces systemic arterial vasodilation in humans with a total artificial heart. Anesthesiology 100: 602-607, 2004.
6. Kazmaier S, Hanekop GG, Buhre W, Weyland A, Busch T, Radke OC, Zoelffel R and Sonntag H: Myocardial consequences of remifentanil in patients with coronary artery disease. Br J Anaesth 84: 578-583, 2000.
7. Noseir RK, Ficke DJ, Kundu A, Arain SR and Ebert TJ: Sympathetic and vascular consequences from remifentanil in humans. Anesth Analg 96: 1645-1650, 2003.
8. Gursoy S, Bagcivan I, Yildirim MK, Berkan O and Kaya T: Vasorelaxant effect of opioid analgesics on the isolated human radial artery. Eur J Anaesthesiol 23: 496-500, 2006.
9. Unlugenc H, Itegin M, Ocal I, Ozalevli M, Güler T and Isik G: Remifentanil produces vasorelaxation in isolated rat thoracic aorta strips. Acta Anaesthesiol Scand 47: 65-69, 2003.
10. Paris A, Scholz J, von Knobelsdorff G, Tonner PH and Schulte AM Esch J: The effect of remifentanil on cerebral blood flow velocity. Anesth Analg 87: 569-573, 1998.
11. Engelhard K, Reeker W, Kochs E and Werner C: Effect of remifentanil on intracranial pressure and cerebral blood flow velocity in patients with head trauma. Acta Anaesthesiol Scand 48: 396-399, 2004.
12. Hill MA, Yang Y, Ella SR, Davis MJ and Braun AP: Large conductance, Ca2+-activated K+ channels (BKCa) and arteriolar myogenic signaling. FEBS Lett 584: 2033-2042, 2010.
13. Nelson MT and Quayle JM: Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 268: C799-C822, 1995.
14. Laboratory animal welfare: Public Health Service policy on humane care and use of laboratory animals by awardee institutions; notice. Federal register 50: 19584-19585, 1985.
15. Zhang W, Ma KT, Wang Y, Si JQ and Li L: Inhibitory effect of 18beta-glycyrrhetinic acid on KCl- and PE-induced constriction of rat renal interlobar artery in vitro. Sheng Li Xue Bao 66: 195-202, 2014.
16. Tian WW, Zhao L, Ma KT, Li L and Si JQ: Isoliquiritigenin relaxes the cerebral basilar artery by enhancing BKCa current in spontaneously hypertensive rat: Role of sGC/cGMP. Sheng Li Xue Bao 67: 329-334, 2015.
17. Li L, Wang R, Ma KT, Li XZ, Zhang CL, Liu WD, Zhao L and Si JQ: Differential effect of calcium-activated potassium and chloride channels on rat basilar artery vasomotion. J Huazhong Univ Sci Technolog Med Sci 34: 482-490, 2014.
18. Li L, Zhang W, Shi WY, Ma KT, Zhao L, Wang Y, Zhang L, Li XZ, Zhu H, Zhang ZS, *et al*: The enhancement of Cx45 expression and function in renal interlobar artery of spontaneously hypertensive rats at different age. Kidney Blood Press Res 40: 52-65, 2015.
19. Li XZ, Ma KT, Guan BC, Li L, Zhao L, Zhang ZS, Si JQ and Jiang ZG: Fenamates block gap junction coupling and potentiate BKCa channels in guinea pig arteriolar cells. Eur J Pharmacol 703: 74-82, 2013.
20. Lin PT, Liao DQ and Luo NF: Effect of remifentanil on calcium-activated potassium currents in human mesenteric arterial smooth muscle cells. Chin J Anesthesiol 30: 1307-1309, 2006.
21. Liu Y, Hudetz AG, Knaus HG and Rusch NJ: Increased expression of Ca2+-sensitive K+ channels in the cerebral microcirculation of genetically hypertensive rats: Evidence for their protection against cerebral vasospasm. Circ Res 82: 729-737, 1998.
22. Hu Z, Ma A, Tian HY, Xi YT, Fan LH and Wang TZ: Activity and changed expression of calcium-activated potassium channel in vascular smooth muscle cells isolated from mesenteric arteries of spontaneous hypertensive rats. J Xi'an Jiao tong University (Medical Sciences) 31: 424-428, 2010.
23. Yang Y, Li PY, Cheng J, Mao L, Wen J, Tan XQ, Liu ZF and Zeng XR: Function of BKCa channels is reduced in human vascular smooth muscle cells from Han Chinese patients with hypertension. Hypertension 61: 519-525, 2013.
24. Xu QM: Clinical Anesthesiology. People's Medical Publishing House, Beijing pp242-243, 2008 (In Chinese).
25. Su X, Wachtel RE and Gebhart GF: Inhibition of calcium currents in rat colon sensory neurons by K- but not mu- or delta-opioids. J Neurophysiol 80: 3112-3119, 1998.
26. Twitchell WA and Rane SG: Opioid peptide modulation of Ca(2+)-dependent K+ and voltage-activated Ca2+ currents in bovine adrenal chromaffin cells. Neuron 10: 701-709, 1993.
27. Leff P: The two-state model of receptor activation. Trends Pharmacol Sci 16: 89-97, 1995.