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RESEARCH PAPER

# Chronic exercise normalizes changes in $Ca_v1.2$ and $K_{Ca}1.1$ channels in mesenteric arteries from spontaneously hypertensive rats

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## BACKGROUND AND PURPOSE

Regular physical activity is an effective non-pharmacological therapy for prevention and control of hypertension. However, the underlying mechanisms are not fully understood. Accumulating evidence shows that the elevated vascular tone in hypertension is a consequence of the 'ion channel remodelling' that occurs during sustained high BP. The present study investigated the effects of aerobic exercise on the electrical remodelling of L-type $Ca^{2+}$ ($Ca_v1.2$) and large-conductance $Ca^{2+}$-activated $K^+$ ($K_{Ca}1.1$) channels in mesenteric arteries (MAs) from spontaneously hypertensive rats (SHRs).

## EXPERIMENTAL APPROACH

SHRs and normotensive (Wistar-Kyoto) rats were subjected to aerobic training or kept sedentary, and vascular mechanical and functional properties were evaluated.

## KEY RESULTS

Exercise did not affect the heart weight, but reduced the heart rate and body weight in SHR. In mesenteric arterial myocytes, exercise normalized the increased $Ca_v1.2$ and $K_{Ca}1.1$ current density in SHRs. Exercise also ameliorated the increased open probability and mean open time of the single $K_{Ca}1.1$ channel in hypertension. The isometric contraction study revealed that both nifedipine ($Ca_v1.2$ channel blocker) and NS11021 ($K_{Ca}1.1$ channel activator) induced concentration-dependent vasorelaxation in MAs precontracted with noradrenaline. Exercise normalized the increased sensitivity of tissues to nifedipine and NS11021 in SHR. Furthermore, protein expression of the $Ca_v1.2$ $\alpha_{1C}$-subunit together with the $K_{Ca}1.1$ $\alpha$- and $\beta1$-subunit was significantly increased in SHRs; and exercise ameliorated these molecular alterations in hypertension.

## CONCLUSIONS AND IMPLICATIONS

Chronic exercise reduces BP and restores vascular function in MAs from SHR, which might be related to the correction of the $Ca_v1.2$ and $K_{Ca}1.1$ channel remodelling during hypertension.

## Abbreviations

BW, body weight; $Ca_v1.2$, L-type $Ca^{2+}$ (channel); DBP, diastolic BP; Em, resting membrane potential; HW/BW, heart weight to body weight ratio; IbTX, iberiotoxin; I-V, current-voltage; $K_{Ca}1.1$, large-conductance $Ca^{2+}$-activated $K^+$ (channel); L-NAME, $N^{\omega}$-nitro-L-arginine methyl ester; MA, mesenteric artery; NA, noradrenaline; Po, open probability; SBP, systolic BP; SHR, spontaneously hypertensive rat; VSMC, vascular smooth muscle cell; WKY, Wistar-Kyoto rat

## Tables of Links

| TARGETS | |
|---|---|
| **Ion channels**[a] | **Enzymes**[b] |
| $Ca_V1.2$ | NOS |
| $K_{Ca}1.1$ | Papain |

| LIGANDS | |
|---|---|
| Iberiotoxin (IbTX) | Nitric oxide (NO) |
| L-NAME | Noradrenaline (NA) |
| Nifedipine | |

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b]Alexander *et al.*, 2013a,b).

## Introduction

Hypertension is one of the primary risk factors for heart disease and stroke, the leading causes of death worldwide. Numerous studies have demonstrated that chronic hypertension is associated with an impaired vascular relaxation caused by an increased vascular tone; however, the underlying mechanisms are not fully understood. It is well recognized that the vascular smooth muscle cells (VSMCs) are more depolarized as a consequence of the 'ion channel remodelling' that occurs during chronic hypertension (Harder *et al.*, 1983). Since $Ca^{2+}$ and $K^+$ channels have a critical role in regulating intracellular $Ca^{2+}$ levels, resting membrane potential ($E_m$) and cell contractility, both of them have attracted considerable attention in the hypertension research area.

There are several families of $K^+$ and $Ca^{2+}$ channels expressed in VSMCs (Sonkusare *et al.*, 2006; Joseph *et al.*, 2013). Large-conductance $Ca^{2+}$-activated $K^+$ ($K_{Ca}1.1$) channels are an important family of $K^+$ channels which are highly expressed in VSMCs and play an important role in regulating the $E_m$. They are activated by membrane depolarization and increased cytosolic $Ca^{2+}$ concentration, and induce a hyperpolarization that opposes vasoconstriction (Brayden and Nelson, 1992; Nelson *et al.*, 1995). Voltage-gated L-type $Ca^{2+}$ ($Ca_V1.2$) channels also open in response to membrane depolarization. Excitation of VSMCs results in depolarization, which leads to the voltage-dependent opening of $Ca_V1.2$. The opening of $Ca_V1.2$ channels allows $Ca^{2+}$ influx, causing a rise in global $Ca^{2+}$ and activation of the cellular contractile machinery (Joseph *et al.*, 2013). Obviously, the balance of $Ca_V1.2$ and $K_{Ca}1.1$ channels is responsible for cellular depolarization and hyperpolarization which is critical for vascular tone.

Regular physical activity is a well-recommended non-pharmacological therapy for the prevention and control of hypertension (Hagberg *et al.*, 2000; Vina *et al.*, 2012). Over the past few years, there has been increasing evidence suggesting the link between exercise and vascular ion channels (Bowles *et al.*, 1998; Albarwani *et al.*, 2010; Zhao and Wang, 2010). During persistent high BP, ion channels on the plasma membrane of VSMCs undergo 'electrical remodelling' such that the arteries maintain a heightened vascular tone (Joseph *et al.*, 2013). However, the effects of chronic exercise on the hypertension-associated pathophysiological remodelling of ion channels are largely unknown. We hypothesized that regular aerobic exercise may normalize the hypertension-related alterations in $Ca_V1.2$ and $K_{Ca}1.1$ channels to restore the vasorelaxation of peripheral resistant arteries.

Therefore, the present study was designed to examine the effects of chronic exercise on $Ca_V1.2$ and $K_{Ca}1.1$ channel structure and function in mesenteric artery (MA) myocytes from spontaneously hypertensive rats (SHRs). The information obtained will help to elucidate the underlying cellular and molecular mechanisms of the beneficial effects of exercise on the hypertension-associated decrease in vasorelaxation of small arteries.

## Methods

### *Animals and exercise training*

All experimental protocols were approved by the ethical committee of Beijing Sport University and were performed in accordance with the Chinese animal protection laws and institutional guidelines. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). Every effort was made to minimize the number of animals used and their suffering. In addition, the target nomenclature (e.g. ion channels) conformed to BJP's Concise Guide to Pharmacology (Alexander *et al.*, 2013a).

Three-month-old male normotensive Wistar-Kyoto rats (WKY, $n$ = 24) and SHRs ($n$ = 24) were separated into a sedentary group (SHR-SED and WKY-SED) and an exercise group (SHR-EX and WKY-EX) at random ($n$ = 12 in each group). After a 1 week acclimatization period, rats in the SHR-EX group were subjected to aerobic exercise (about 55–65% of maximal aerobic velocity, 18–20 $m \cdot min^{-1}$, 0% grade, 60 min, 5 days/week for 8 weeks) on a motor-driven treadmill (Agarwal *et al.*, 2011; Roque *et al.*, 2013). To determine the maximal exercise capacity, rats were subjected to a progressive exercise test using an incremental speed protocol of 5 $m \cdot min^{-1}$ every 3 min and no grade until exhaustion. Rats were considered to be exhausted when they could no longer run at the treadmill speed (Roque *et al.*, 2013).

Body weight (BW) was measured weekly. BP and heart rate (HR) were determined by an indirect tail-cuff method (BL-300A; Chengdu Technology & Market Co, Ltd, Chengdu, China).

### *Electrophysiological measurements*

*Smooth muscle cell dispersion.* Animals were anaesthetized with sodium pentobarbitone (50 $mg \cdot kg^{-1}$, i.p.) and decapi-

tated using a guillotine. The MAs were removed and placed into a $Ca^{2+}$-free solution containing (mM): 137 NaCl, 5.6 KCl, 1 $MgCl_2$, 10 glucose, 10 HEPES, 0.42 $Na_2HPO_4$, 0.44 $NaH_2PO_4$ and 4.2 $NaHCO_3$. The second to third order of arterial branches (A2–A3) were cut into pieces and incubated for 30 min at 37°C with papain (4 mg·mL$^{-1}$), dithiothreitol (1 mg·mL$^{-1}$) and BSA (2 mg·mL$^{-1}$), and then the VSMCs were dispersed.

*Whole-cell* $Ca_v1.2$ *current recordings.* Whole-cell $Ca_v1.2$ currents were measured with conventional voltage-clamp configuration (Wilde *et al.*, 1994). The cell was held at –80 mV and then stepped in 10 mV increments from –70 to +70 mV. Voltage steps were 200 ms in duration. Membrane currents were low-pass filtered at 2 kHz, digitized at a sampling frequency of 10 kHz. To increase unitary currents, $Ba^{2+}$ replaced $Ca^{2+}$ as charge carrier and the divalent cation concentration was elevated in the bath solution (Rubart *et al.*, 1996). The external solution comprised (mM): 20 $BaCl_2$, 10 HEPES, 5 glucose, 1 $MgCl_2$ and 124 choline chloride (pH 7.4 with CsOH). To minimize the outward $K^+$ current, $Cs^+$ instead of $K^+$ was used in the pipette solution. It contained (mM): 130 CsCl, 10 HEPES, 3 $Na_2ATP$, 0.1 $Na_2GTP$, 1.5 $MgCl_2$, 10 glucose, 10 EGTA and 0.5 MgATP, pH 7.3 titrated with CsOH.

*Whole-cell* $K^+$ *current recordings and Em.* Resting Em was measured with the current-clamp configuration of the patch-clamp technique while the cell was held at zero membrane current. Whole-cell $K^+$ currents were also measured using the voltage-clamp configuration. The external solution comprised (mM): 134 NaCl, 6 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose and 10 HEPES (pH 7.4). The internal solution comprised (mM): 110 potassium aspartate (K-Asp), 30 KCl, 1 EGTA, 3 $Na_2ATP$, 0.85 $CaCl_2$, 10 glucose and 10 HEPES (pH 7.2 with KOH). Current-voltage (I-V) relationships were generated in voltage-clamp cells held at an Em of –80 mV and then stepped in 10 mV increments to +70 mV. Voltage steps were 350 ms in duration. $K_{Ca}1.1$ currents were defined as the 100 nM iberiotoxin (IbTX)-sensitive component.

*Single-channel* $K_{Ca}1.1$ *current recordings.* Single-channel $K_{Ca}1.1$ currents were recorded in excised inside-out membrane patches under symmetrical $K^+$ (145 mM) (Shi *et al.*, 2013). As an index of channel steady-state activity, we used the product of the number of channels in the patch ($N$) and the channel open probability (Po). The $Ca^{2+}$-dependent activation was fitted with the Hill equation. All electrophysiological studies were performed using Axon700B amplifier, pCLAMP 10.2 and Clampfit 10.2 software (Axon Instruments Inc., Foster City, CA, USA).

## Isometric contraction studies

The MA and its branches were removed at the age of 21 weeks ($n$ = 6 each group), and placed in cold Krebs solution with the following composition (mM): 131.5 NaCl, 5 KCl, 1.2 $NaH_2PO_4$, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 11.2 glucose, 13.5 $NaHCO_3$ and 0.025 EDTA; gassed with 95% $O_2$ and 5% $CO_2$ (pH 7.4). Short segments of A2 were used for contractile studies by using Multi Myograph System (620M; DMT, Aarhus, Denmark).

The contractile response for tension was evaluated by measuring the maximum peak height and expressed as a percentage of contraction to 120 mM $K^+$ ($K_{max}$). In some experiments, the non-selective NOS inhibitor $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME, 100 μM) was added after $K_{max}$ measurement. To investigate the contribution of $K_{Ca}1.1$ and $Ca_v1.2$ channels in vascular tone regulation, the vascular responses to IbTX ($K_{Ca}1.1$ blocker, $10^{-7}$ M), NS 11021 ($K_{Ca}1.1$ opener, $10^{-9}$–$10^{-5}$ M) and nifedipine ($Ca_v1.2$ inhibitor, $10^{-9}$–$10^{-5}$ M) were examined.

## Western immunoblotting

Membrane proteins were isolated and pooled for use in Western blots as previously described (Shi *et al.*, 2013). The primary antibodies involved were as follows: polyclonal anti-$\alpha_{1C}$ (1:200), polyclonal anti-$K_{Ca}1.1$ (1:300) and polyclonal anti-*slo*$\beta$1 (1:300), which are all products from Alomone Laboratories (Jerusalem, Israel). After incubation with a secondary antibody (anti-rabbit IgG-HRP, 1:4000; Sigma-Aldrich, Beijing, China (Mainland)), immunoreactive brands were visualized with enhanced chemiluminescence and signals were recorded with Bio-Rad ChemiDOC XRS+ (Bio-Rad Laboratories, Hercules, CA, USA). β-actin was used to correct equal loading of all samples.

## Statistical analysis

Data are expressed as mean ± SEM. The term $n$ represents the number of cells or animals used in each experiment. Statistical analysis was conducted via one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons. Student's $t$-test was used for comparisons between two groups. $P < 0.05$ was considered significant and $P < 0.01$ was considered highly significant.

# Results

## Physical characteristics of experimental animals

There were no significant differences between the body weights (BWs) of the rats in the WKY and SHR groups at baseline (12 weeks). However, they were significantly lower in both the WKY and SHR exercise groups at study end (21 weeks) when compared with their sedentary counterparts (Table 1). Heart weight (HW) and HW to BW ratio (HW/BW) in both the WKY-EX and SHR-SED groups were significantly higher than those in the WKY-SED group, whereas there were no significant differences in either HW or HW/BW between the SHR-SED and SHR-EX groups.

There were no significant differences in systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP) and HR in both the WKY and SHR exercise groups at baseline when compared with their sedentary counterparts. However, SBP and MAP, but not DBP and HR in SHR-SED were significantly higher than those in WKY-SED. After 8 weeks of exercise, the SBPs of rats in both the WKY and SHR exercise groups were significantly lower than those of their sedentary counterparts. So were DBP and MAP in SHR-EX when compared with SHR-SED. However, DBP and MAP in WKY-EX were not significantly different from that in WKY-SED. As shown in Table 1, HR was significantly higher in SHR-SED when compared with WKY-SED at the end of the study. It then significantly dropped in SHR-EX compared with SHR-SED.

**Table 1**
Physical characteristics of rats

| | | WKY-SED | WKY-EX | SHR-SED | SHR-EX |
|---|---|---|---|---|---|
| BW (g) | Initial | 238.3 ± 3.3 | 242.4 ± 4.8 | 245.8 ± 4.5 | 244.8 ± 6.0 |
| | Final | 350.2 ± 5.1$^{\$\$}$ | 340.3 ± 4.7*$^{\$\$}$ | 346.4 ± 6.2$^{\$\$}$ | 333.4 ± 5.8*$^{\$\$}$ |
| HW(g) | Initial | – | – | – | – |
| | Final | 1.13 ± 0.01 | 1.32 ± 0.01* | 1.40 ± 0.04** | 1.38 ± 0.06 |
| HW (mg)/BW (g) | Initial | – | – | – | – |
| | Final | 3.23 ± 0.02 | 3.88 ± 0.04* | 4.05 ± 0.07** | 4.14 ± 0.14 |
| SBP (mmHg) | Initial | 123.3 ± 4.3 | 124.8 ± 5.3 | 172.1 ± 6.3** | 166.5 ± 6.8 |
| | Final | 128.5 ± 4.7 | 119.6 ± 4.1* | 192.3 ± 4.5**$^{\$}$ | 145.6 ± 5.2$^{\#\#\$}$ |
| DBP (mmHg) | Initial | 98.5 ± 4.6 | 97.8 ± 4.7 | 112.5 ± 4.2 | 110.4 ± 5.6 |
| | Final | 104.0 ± 5.8 | 101.5 ± 5.5 | 124.2 ± 5.3**$^{\$}$ | 115.4 ± 3.1$^{\#}$ |
| MAP (mmHg) | Initial | 106.7 ± 6.0 | 106.7 ± 5.1 | 132.2 ± 5.7** | 128.9 ± 5.5 |
| | Final | 112.1 ± 5.3 | 107.5 ± 4.9 | 146.7 ± 5.4**$^{\$}$ | 125.4 ± 4.8$^{\#}$ |
| HR (beats min-1) | Initial | 386 ± 19 | 382 ± 21 | 398 ± 17 | 395 ± 18 |
| | Final | 393 ± 29 | 368 ± 18 | 432 ± 22* | 389 ± 20$^{\#}$ |

*$P$ < 0.05 and **$P$ < 0.01 versus WKY-SED; $^{\#}P$ < 0.05 and $^{\#\#}P$ < 0.01 versus SHR-SED; one-way ANOVA. $^{\$}P$ < 0.05 and $^{\$\$}P$ < 0.01 versus initial (Student's $t$-test). $n$ = 12 in each group. Initial: 12 weeks. Final: 21 weeks.

Although HR in WKY-EX was somewhat lower than in WKY-SED, this was not significant.

## *Exercise training attenuated SHR-associated increase in $Ca_v1.2$ and $K_{Ca}1.1$ currents*

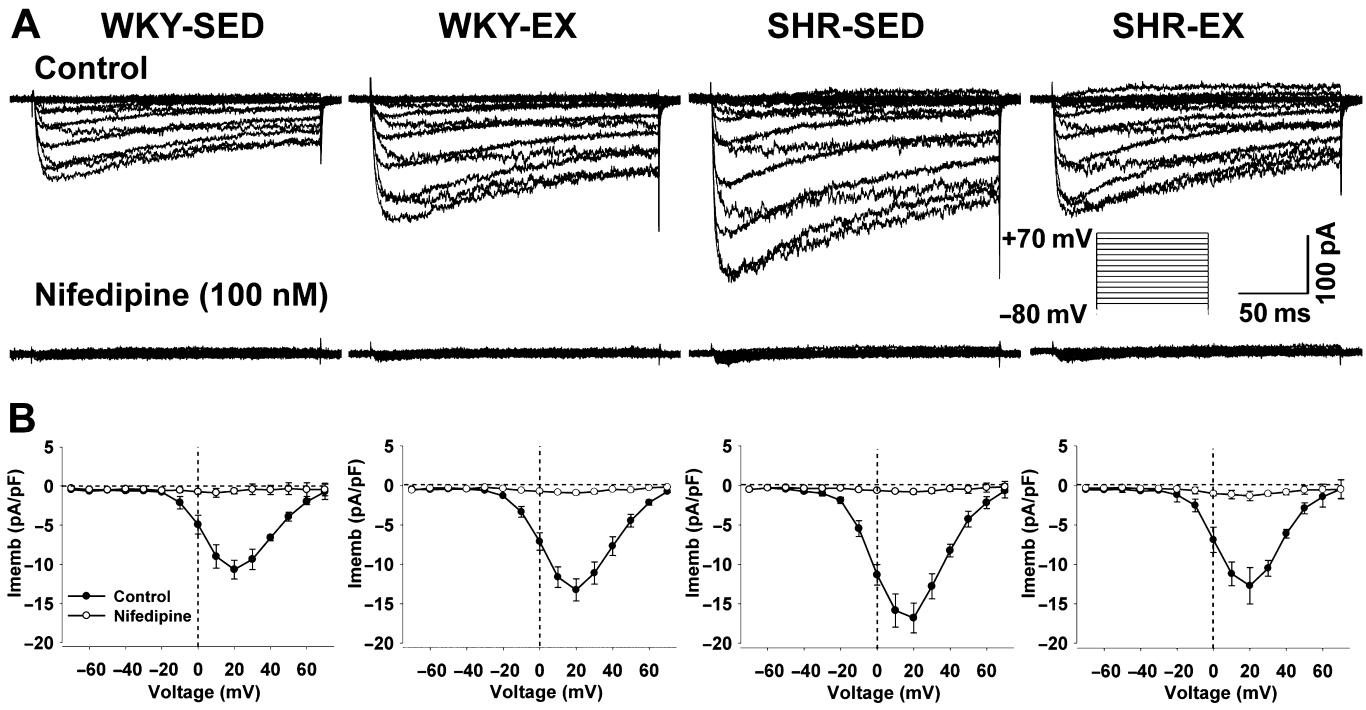
The electrophysiological properties of $Ca_v1.2$ and $K_{Ca}1.1$ channels in myocytes from small MAs were determined. Figure 1A illustrates typical records of whole-cell $Ca^{2+}$ currents. Peak inward current was recorded at +20 mV in all three groups. Nifedipine (100 nM) suppressed the inward currents almost completely, which indicates that the inward currents recorded were $Ba^{2+}$ currents through $Ca_v1.2$ channels. To compensate for differences in cell size, membrane $Ca^{2+}$ currents are expressed relative to cell capacitance (pA/pF). Cell capacitance in myocytes from four groups was 17.1 ± 1.1 pF (WKY-SED, $n$ = 24 cells), 17.5 ± 1.3 pF (WKY-EX, $n$ = 22 cells), 17.4 ± 1.6 pF (SHR-SED, $n$ = 30 cells) and 16.9 ± 1.4 pF (SHR-EX, $n$ = 28 cells) respectively. In this electrophysiological study, each $n$ represents cells from six animals unless stated otherwise. Maximal calcium current density was –10.7 ± 1.2 pA/pF for WKY-SED ($n$ = 18 cells), –12.8 ± 1.0 pA/pF for WKY-EX ($n$ = 20 cells), –16.9 ± 2.1 pA/pF for SHR-SED ($n$ = 20 cells) and –12.7 ± 2.3 pA/pF for SHR-EX ($n$ = 21 cells, all from six animals, Figure 1B). Obviously, the maximal calcium current density in SHR-SED was much higher than that of WKY-SED ($P$ < 0.01). Exercise training markedly decreased the $Ca_v1.2$ currents in hypertensive rats but increased them in normotensive rats (both $P$ < 0.05).

Compared with WKY-SED (–50.0 ± 4.2 mV, $n$ = 35 cells), the resting Em measured in SHR-SED was depolarized (–37.2 ± 3.5 mV, $n$ = 33 cells). After exercise training, the resting Em in SHR-EX was –45.3 ± 3.7 mV ($n$ = 40 cells), which was higher than SHR-SED, but was not significantly different from that of WKY-SED. Exercise training had no effect on resting Em in WKY (WKY-EX, –51.3 ± 4.9 mV, $n$ = 26 cells/6 rats). As shown in Figure 2, after IbTX (100 nM) treatment for 10 min, the whole-cell $K^+$ currents were markedly inhibited. The IbTX-sensitive ($K_{Ca}1.1$) currents were higher in SHR-SED and WKY-EX when compared with that of WKY-SED (both $P$ < 0.01). For example, at +70 mV, the current density was 42.7 ± 3.6 pA/pF in SHR-SED ($n$ = 12 cells/6 rats), 26.5 ± 3.2 pA/pF in WKY-EX ($n$ = 14 cells/6 rats) and 14.9 ± 0.8 pA/pF in WKY-SED ($n$ = 15 cells/6 rats). In hypertensive rats, exercise training significantly decreased the current density (SHR-EX, 23.4 ± 1.4 pA/pF, $n$ = 16 cells/6 rats) when compared with their sedentary counterparts ($P$ < 0.01).

All of these data indicate that hypertension is associated with an enhancement in the functional expression of $Ca_v1.2$ and $K_{Ca}1.1$ channels, whereas exercise training attenuates these changes. However, in normotensive rats, exercise training did not decrease but increased the whole-cell $Ca_v1.2$ and $K_{Ca}1.1$ channel currents.

## *Exercise training inhibited SHR-associated alterations in $K_{Ca}1.1$ channel gating properties*

To examine whether the changes in $K_{Ca}1.1$ currents are related to alterations in single-channel activity in MA myocytes, the single-channel activity was recorded in inside-out recording mode. At the testing potential of +40 mV, 1 μM $[Ca^{2+}]_{free}$, the $NPo$ was significantly increased in hypertension (SHR-SED: 0.58 ± 0.07 vs. WKY-SED: 0.21 ± 0.04). After exercise training, the $NPo$ in hypertensive rats (SHR-EX, 0.30 ± 0.04) was decreased compared with SHR-SED ($P$ < 0.05). However, in normotensive rats, the $NPo$ after exercise training (WKY-EX, 0.45 ± 0.06) was significantly increased com-




## Figure 1

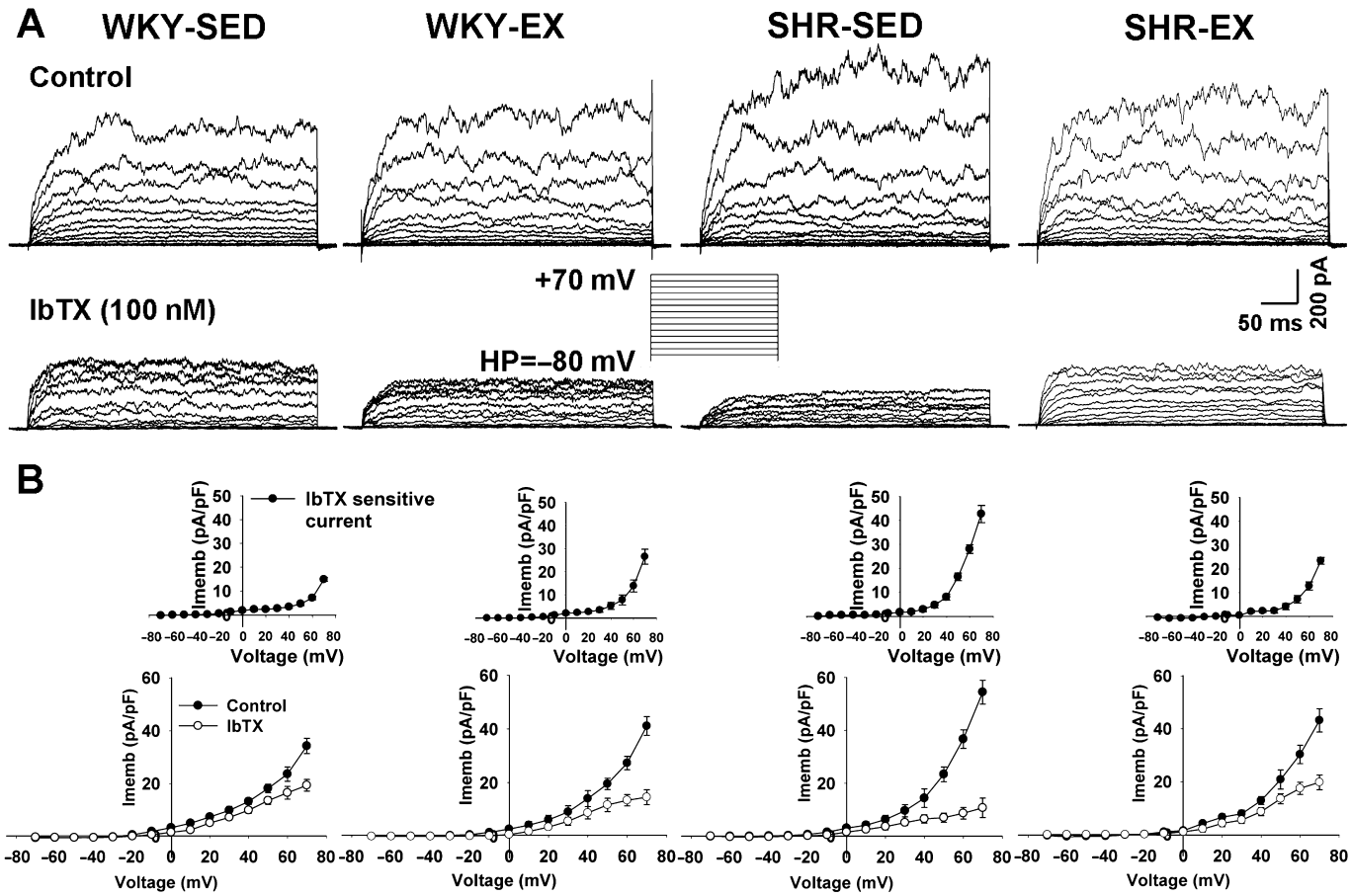
Whole-cell L-type $Ca^{2+}$ channel currents recorded in myocytes of MAs. (A) Traces of $Ca^{2+}$ channel currents evoked by command potentials in the absence (upper panel) or presence of nifedipine (100 nM) (lower panel). (B) Current-voltage relationships of $Ca_v1.2$ currents in VSMCs. Current amplitudes at various command potentials were normalized to cell capacitance and plotted.

pared with its sedentary counterparts ($P < 0.01$, Figure 3A and B). As shown in Figure 3C, the mean open time (To, dwell time of open state) of single $K_{Ca}1.1$ channel was profoundly increased in SHR-SED (26.2 ± 2.1 ms) compared with WKY-SED (7.8 ± 2.5 ms) ($n = 6$, $P < 0.01$). In contrast, the mean close time (Tc, dwell time of close state) was significantly decreased in SHR-SED (SHR-SED: 24.9 ± 3.5 ms; WKY-SED: 70.5 ± 6.8 ms, $P < 0.01$). However, exercise training attenuated both of these changes (SHR-EX: To = 17.5 ± 3.2 ms; Tc = 37.4 ± 4.6 ms, $n = 6$, both $P < 0.01$) without affecting the conductance of $K_{Ca}1.1$ channels (Figure 3D). In normotensive rats, exercise training increased To and decreased Tc significantly (WKY-EX: To = 36.2 ± 4.5 ms; Tc = 48.6 ± 5.6 ms, $n = 6$) when compared with WKY-SED (both $P < 0.01$). These results indicate that single-channel activity was involved in the change in whole-cell $K_{Ca}1.1$ currents in all four groups.

The $Ca^{2+}$ dependence of the $K_{Ca}1.1$ channels was also examined. Figure 3E plots the relationship between *N*Po normalized to their maximum value against $[Ca^{2+}]_{free}$. The Po–$Ca^{2+}$ curves were fitted (using least squares) by use of the Hill equation with a $K_D$ value of 1.09 ± 0.15 (SHR-SED, $n = 6$) and 3.12 ± 0.30 μM (WKY-SED, $n = 6$, $P < 0.05$). A leftward shift in $Ca^{2+}$-dependent activation was observed with hypertension. However, in hypertensive rats, after exercise training, the Po–$Ca^{2+}$ curve was rightward shifted ($K_D$ in SHR-EX: 2.08 ± 0.21 μM, $n = 6$) compared with SHR-SED ($P < 0.05$). In WKY-EX, exercise training induced a leftward shift in the Po–$Ca^{2+}$ curve with a $K_D$ value of 2.32 ± 0.29 μM (WKY-EX, $n = 6$, $P < 0.05$) when compared with its sedentary counterparts (WKY-SED). No significant differences in the Hill coefficient ($n_H$) were observed between the four groups [1.11 ± 0.09 (WKY-SED), 1.05 ± 0.11 (WKY-EX), 1.34 ± 0.21 (SHR-SED) and 1.14 ± 0.11 (SHR-EX)].

### *Exercise training attenuated SHR-associated changes in $Ca_v1.2$ and $K_{Ca}1.1$ channel contribution to vascular tone regulation in MAs*

To determine the physiological relevance of these findings, the effect of a $Ca_v1.2$ channel and $K_{Ca}1.1$ activator or inhibitor on the contraction/relaxation of MA rings was measured. In each MA ring, KCl (120 mM) was first applied to induce the maximal contraction. As shown in Table 2, the response induced by KCl (120 mM) was similar in the four groups. Then two sets of experiments with or without L-NAME (100 μM) treatment were conducted in separate artery rings. Noradrenaline (NA, $10^{-5}$ M) also induced a significant increase in vascular tone. In the presence of L-NAME, the maximal NA-induced contraction in SHR-SED was 128.5 ± 8.0%$K_{max}$, which was higher than that of WKY-SED (105.8 ± 7.2%$K_{max}$, $P < 0.01$) and SHR-EX (112.6 ± 7.8%$K_{max}$, $P < 0.01$). No significant differences were observed in NA-induced contractions between WKY-EX (104.6 ± 6.3%$K_{max}$) and WKY-SED. In the absence of L-NAME, NA-induced tension increase was lower than that in the presence of L-NAME (Table 2) in each group, (all $P < 0.05$). In order to examine the contribution of the $K_{Ca}1.1$ channel to the vascular tone, in one experiment,

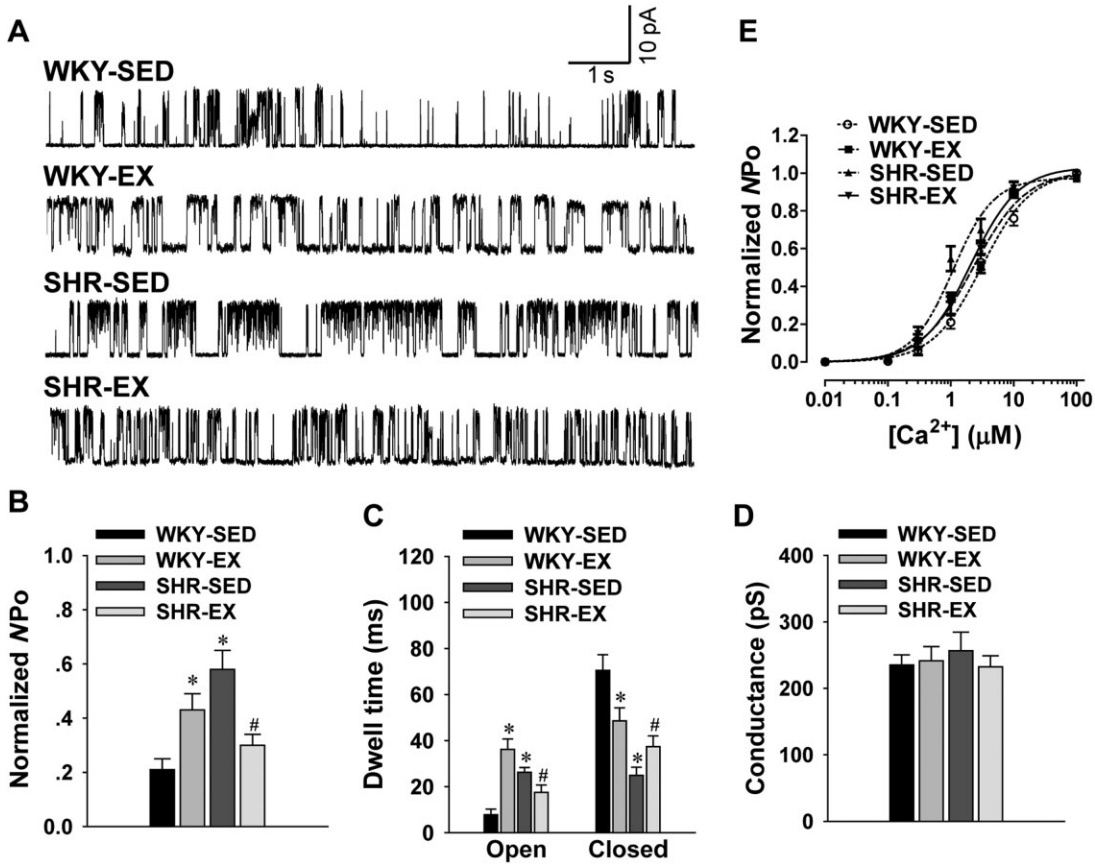



## Figure 2

Whole-cell $K^+$ currents in myocytes of MAs. (A) Typical recordings of whole-cell $K^+$ currents measured during depolarizing voltage steps in the absence (upper panel) or presence of IbTX (100 nM) (lower panel). (B) The mean current density versus voltage plot in the absence or presence of IbTX in myocytes.

IbTX (a specific $K_{Ca}1.1$ channel blocker, $10^{-7}$ M) was added to the bath. It was observed that in both L-NAME treated and untreated groups, IbTX produced a slight increase in resting tension in all four groups (Table 2, Figure 4A(panel a) and B). The IbTX-induced tension increase in SHR-SED was higher than that in the WKY-SED. Interestingly, exercise training markedly inhibited this increase in hypertensive rats. However, in normotensive rats, exercise training did not inhibit but enhanced IbTX-induced tension increase. It can be seen that IbTX-induced force was lower in arteries without L-NAME than in those treated with L-NAME in WKY-SED, WKY-EX and SHR-EX groups, but in SHR-SED, no significant differences were observed (Table 2). In another experiment, the effects of NS 11021 ($K_{Ca}1.1$ channel activator) on MAs precontracted with $10^{-5}$ M NA were examined. As shown in Table 2, Figure 4A(b) and C, NS11021 ($10^{-5}$ M) reduced the contractions of the vessels to NA significantly in all four groups, and the reduction was SHR-SED > WKY-SED, SHR-SED > SHR-EX, and WKY-EX > WKY-SED ($n = 6$ in each group, all $P < 0.05$). Furthermore, no significant differences were observed in L-NAME treated groups compared with L-NAME untreated groups. These data reveal that compared with WKY-SED, there is a profound effect of $K_{Ca}1.1$ channel activation in decreasing vascular tone in SHR-SED. Exercise training diminishes this change in hypertensive rats. However, in normotensive rats, exercise training increases but does not inhibit the contribution of the $K_{Ca}1.1$ channel in vascular tone regulation.

In another experiment, at the plateau of the NA-induced contraction, nifedipine ($Ca_v1.2$ channel inhibitor, $10^{-9}$ to $10^{-5}$ M) was given in half-log increments. A parallel leftward shift of the concentration–relaxation curve was detected in hypertensive rats. Here, $10^{-5}$ M NA-induced maximal tension increase was treated as 100% in each group. In L-NAME-treated groups, the sensitivity of MA to nifedipine was SHR-SED > SHR-EX ($P < 0.05$) and WKY-EX > WKY-SED ($P < 0.05$). The $pIC_{50}$, a negative logarithm of the 50% effective concentration, was 7.27 ± 0.07 (WKY-SED), 7.38 ± 0.05 (WKY-EX), 7.52 ± 0.09 (SHR-SED) and 7.30 ± 0.12 (SHR-EX) respectively ($n = 6$ in each group, Figure 4A(panel c) and D). These results indicate that the contribution of $Ca_v1.2$ channels to vascular tone regulation was significantly increased in hypertension, whereas exercise training significantly ameliorates this effect. However, in normotensive animals, exercise training increases rather than decreases the contribution of $Ca_v1.2$ channels to vascular tone regulation.




## Figure 3

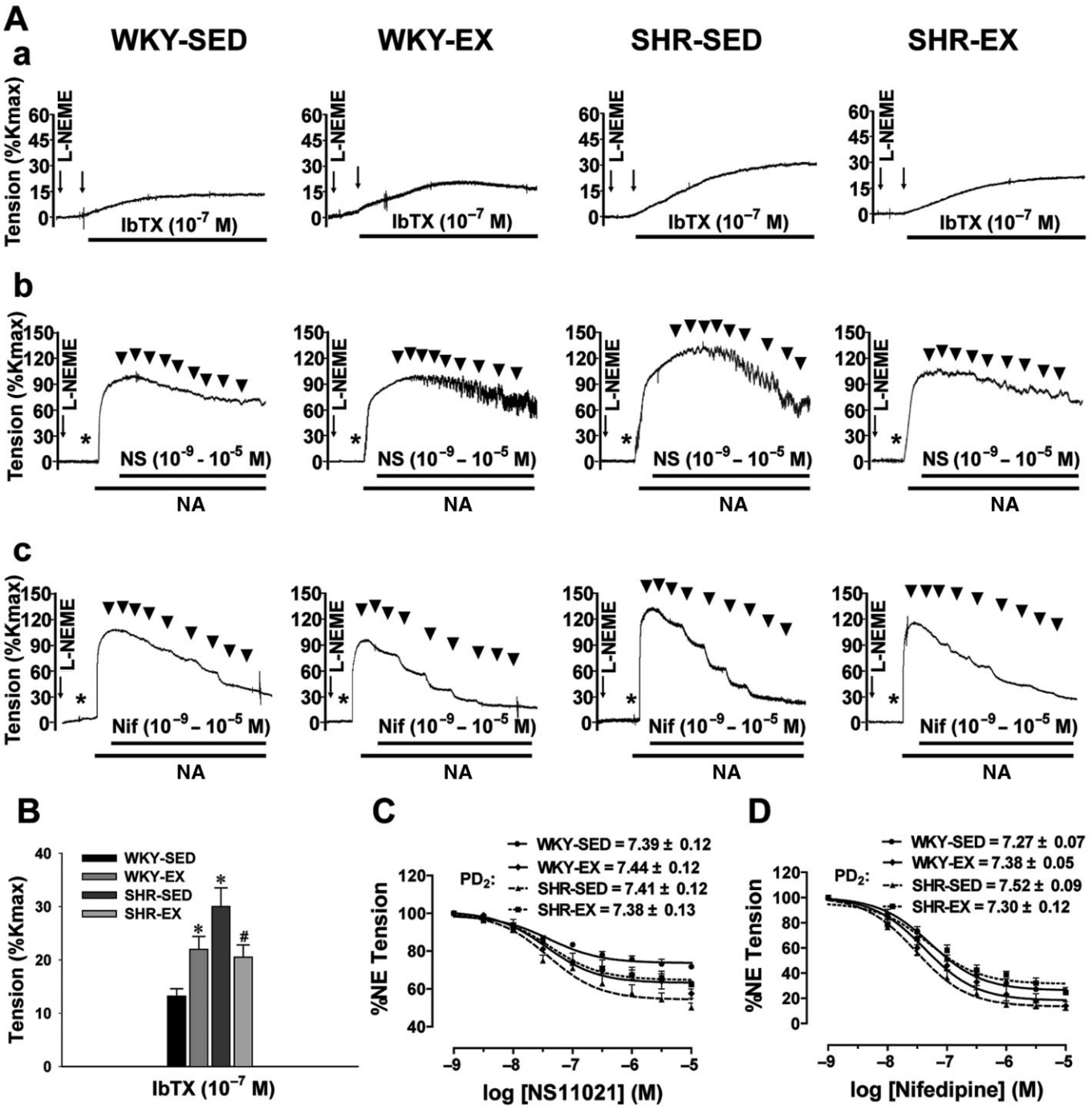
Effects of exercise training on SHR-associated alteration in $K_{Ca}1.1$ channel activity and gating properties in MA myocytes. (A) Representative recordings of single-channel currents at a membrane voltage of +40 mV in WKY, SHR, and SHR-EX groups (1 μM $[Ca^{2+}]_{free}$ in bath solution) respectively. (B, C, D) Summary of the $K_{Ca}1.1$ channel normalized *N*Po (C), dwell time of open and closed state (D), and conductance (E). (E) Effects of exercise on the $Ca^{2+}$ dependence of $K_{Ca}1.1$ channels. HP = +40 mV. The data points were fitted with the Hill equation to obtain the calcium concentration necessary to open half of the channels ($K_D$) and the Hill coefficient. **P* < 0.05 compared with WKY-SED; #*P* < 0.05 compared with SHR-SED; one-way ANOVA. *n* = 6 in each group.

## Table 2

Peak responses of vascular tension

| | | Δ Maximal tension | | | |
|---|---|---|---|---|---|
| | | WKY-SED | WKY-EX | SHR-SED | SHR-EX |
| $K_{max}$ (120 mM) (mN) | (–) L-NAME | 14.4 ± 1.5 | 14.3 ± 1.3 | 15.1 ± 1.8 | 14.6 ± 1.6 |
| NA ($10^{-5}$ M) as %$K_{max}$ | (–) L-NAME | 90.0 ± 6.4 | 88.6 ± 5.4 | 120.1 ± 5.1** | 96.4 ± 6.6# |
| | (+) L-NAME | 105.8 ± 7.2$ | 104.6 ± 6.3$ | 128.5 ± 8.0** | 112.6 ± 7.8#$ |
| IbTX ($10^{-7}$ M) as %$K_{max}$ | (–) L-NAME | 8.7 ± 1.3 | 9.4 ± 1.2* | 25.8 ± 3.0** | 11.2 ± 2.1## |
| | (+) L-NAME | 13.2 ± 1.4$ | 16.1 ± 1.4*$ | 30.0 ± 3.5** | 18.5 ± 1.8##$ |
| NA–NS11021 as %NA | (–) L-NAME | 65.5 ± 4.3 | 57.4 ± 4.0* | 45.1 ± 5.2* | 55.3 ± 4.6# |
| | (+) L-NAME | 71.6 ± 3.8 | 57.5 ± 5.5* | 48.6 ± 4.3* | 62.2 ± 3.0# |

**P* < 0.05 and ***P* < 0.01 compared with WKY; #*P* < 0.05 and ##*P* < 0.01 compared with SHR-SED. $*P* < 0.05 compared with (–) L-NAME (Student's *t*-test). *n* = 12 in each group. NA: $10^{-5}$ M; NS11021: $10^{-5}$ M.



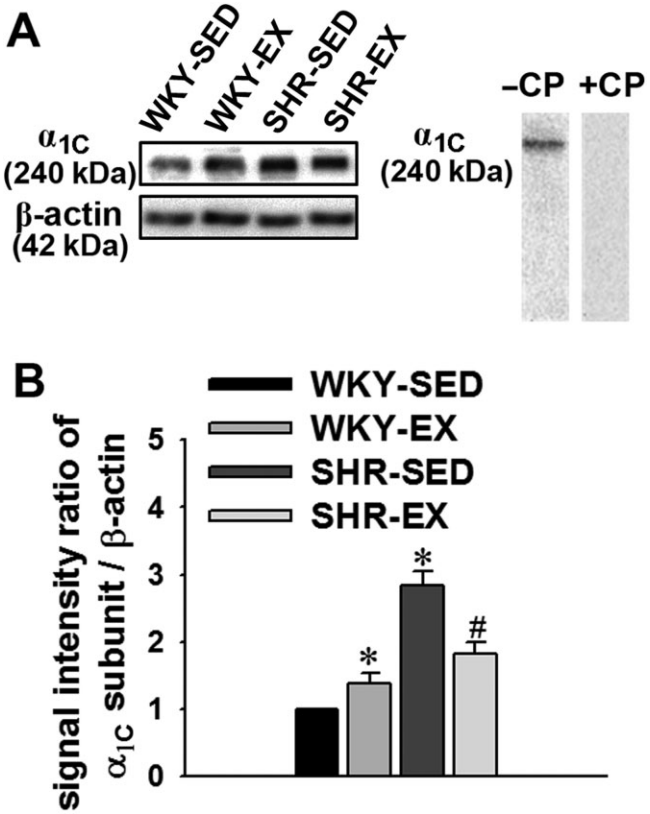

## Figure 4

Effects of a $Ca_v1.2$ and $K_{Ca}1.1$ channel inhibitor and activator on the vascular tension in MAs from WKY-SED, WKY-EX, SHR-SED, and SHR-EX. (A) Typical experimental tracings showing: the effect of $K_{Ca}1.1$ blocker IbTX (100 nM) on resting tension (a); the effect of $K_{Ca}1.1$ channel activator NS11021 ($10^{-9}$ to $10^{-5}$ M) on NA-induced vessel contraction (b); and the effect of $Ca_v1.2$ channel inhibitor nifedipine ($10^{-9}$ to $10^{-5}$ M) on NA-induced vessel contraction (c). In each experiment, the arteries were pre-incubated with non-selective NOS inhibitor L-NAME (100 μM) for 20 min (arrows). Asterisks mark the time of addition of NA ($10^{-5}$ M). Black triangles in (A, b and c) mark the time of addition of NS11021 (b) or nifedipine (c) ($10^{-9}$, $3 \times 10^{-9}$, $10^{-8}$, $3 \times 10^{-8}$, $10^{-7}$, $3 \times 10^{-7}$, $10^{-6}$, $3 \times 10^{-6}$, $10^{-5}$ M). (B) Summary of effects of IbTX on NA-induced vessel contraction. $^{*}P < 0.05$ compared with WKY-SED; $^{\#}P < 0.05$ compared with SHR-SED; one-way ANOVA. (C) Concentration–response curves for effects of NS11021 on NA-induced contraction. (D) Concentration–response curves for effects of nifedipine on NA ($10^{-5}$ M)-induced contraction; $n = 6$ in each group. NS, NS 11021; Nif, nifedipine.

### *Exercise training attenuated SHR-associated alterations in the expression of $Ca_v1.2$ and $K_{Ca}1.1$ channels*

Finally, we determined the expression of $Ca_v1.2$ and $K_{Ca}1.1$ channels in the VSMCs of MA. As shown in Figure 5A, the presence of a 240 kDa band in MA myocytes from Wistar rats corresponds to the $Ca_v1.2$ channel $\alpha_{1C}$-subunit. The band was abolished by pre-absorption of the antibody with its antigenic competing peptide (1:200), confirming antibody specificity for its recognition sequence. A striking up-regulation of this $\alpha_{1C}$-subunit was observed in SHR-SED (2.83 ± 0.21) compared with WKY-SED (1.00 ± 0.00), which was ameliorated by exercise training (SHR-EX, 1.82 ± 0.17, $n$ = 6 in each group, Figure 5B). However, in normotensive rats, exercise training significantly increased but did not inhibit the $\alpha_{1C}$ protein expression (WKY-EX, 1.38 ± 0.15, $n$ = 6) when compared with its sedentary counterparts ($P$ < 0.05).

Western blot analysis using α-subunit ($K_{Ca}1.1$) and β1-subunit (KCNMB1) specific antibodies detected bands of 125 and 28 kDa respectively. As shown in Figure 6A, hypertension is associated with a significant increase in the protein expression of both α- and β1-subunits. Specificity of the antibody was also tested (Figure 6B). Compared with the normotensive control group (WKY-SED, 1.00 ± 0.00), the protein expression level of the α- and β1-subunits observed in SHR-SED was significantly increased (1.48 ± 0.18 and 3.78 ± 0.40, respectively, Figure 6C and D). The β1/α ratio showed a significant increase in hypertension (SHR-SED: 2.55 ± 0.28 vs. WKY-SED: 1.00 ± 0.00) (Figure 6E). These data show that at the cellular level, hypertension induced a larger increase in β1-subunits with respect to α-subunits. However, after exercise training, the α- and β1-subunit expression and β1/α ratio were markedly decreased (SHR-EX, α-subunit: 1.03 ± 0.15; β1-subunit: 1.54 ± 0.21; β1/α: 1.49 ± 0.14). Interestingly, in normotensive rats, exercise training induced significant increases of β1-subunit expression and β1/α ratio without affecting the α-subunit (WKY-EX, α-subunit: 1.05 ± 0.13, $P$ > 0.05; β1-subunit: 2.17 ± 0.23, $P$ < 0.01; β1/α: 2.07 ± 0.18, $P$ < 0.01).



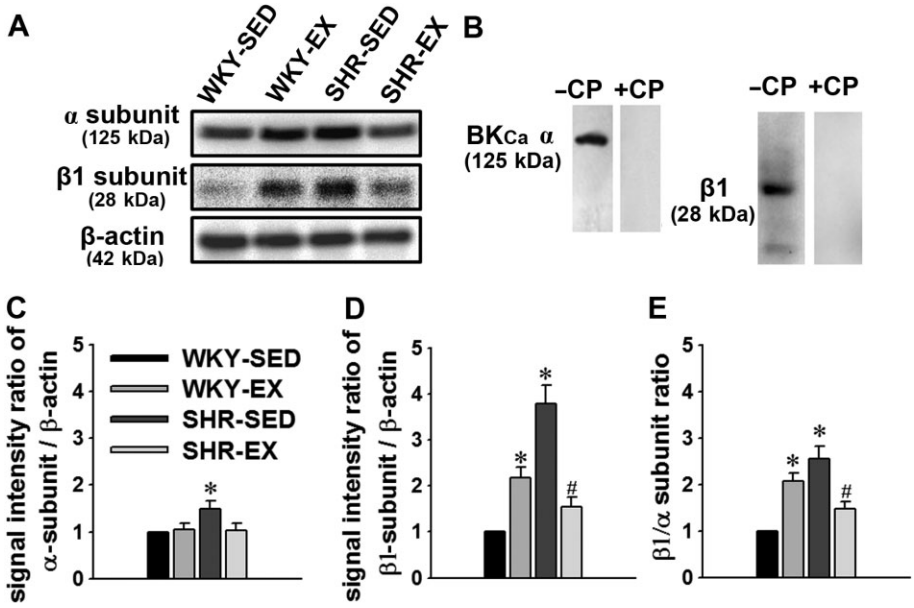

## Figure 5

Protein expression of $Ca_v1.2$ channel ($\alpha_{1C}$) subunit in mesenteric myocytes. (A) Immunoreactive bands corresponding to $\alpha_{1C}$-subunit and β-actin detected by Western blot (left panel). In the right panel, the $\alpha_{1C}$-subunit was identified in the left lane as a 240 kDa band in Wistar rats' MAs (–CP), but was absent in the right lane after competing peptide inhibition (+CP). (B) Summarized data of $\alpha_{1C}$-subunit protein levels expressed as a ratio to β-actin ($n$ = 6 in each group). *$P$ < 0.01 compared with WKY-SED; #$P$ < 0.01 compared with SHR-SED; one-way ANOVA.

## Discussion

The results of this study demonstrate that in addition to lowering BP, aerobic exercise training is associated with alterations in $Ca_v1.2$ and $K_{Ca}1.1$ channel function in SHR. Differences in biophysical properties and molecular expression of $Ca_v1.2$ and $K_{Ca}1.1$ channels between WKY and SHR were eliminated by regular exercise training. To our knowledge, this is the first study to provide functional and molecular evidence that chronic exercise effectively reverses the remodelling of $Ca_v1.2$ and $K_{Ca}1.1$ channels in hypertension to restore the vascular function in MAs. The other interesting but unexpected result was that in normotensive rats, exercise training enhanced rather than inhibited the molecular and functional expression of $Ca_v1.2$ and $K_{Ca}1.1$ channels. The completely different effects of exercise training on normotensive and hypertensive rats suggest the different mechanisms and significance of exercise-induced $Ca_v1.2$ and $K_{Ca}1.1$ channel alterations in physiological and pathological situations.

After 8 weeks of exercise training, rats in the hypertensive group showed decreased BP and HR. This decrease in BP may involve various mechanisms. For example, it is attributed to the attenuation of sympathetic activity to the heart leading to bradycardia and, consequently, to a reduction in cardiac output (Negrão *et al.*, 1993; Véras-Silva *et al.*, 1997). This lower sympathetic activity induced by exercise training could also be attributed to an improvement in the arterial baroreflex and chemosensitive cardiopulmonary baroreflex sensitivity in SHR (Silva *et al.*, 1997; Krieger *et al.*, 1999; Zamo *et al.*, 2011), which modulate the peripheral autonomic nervous system. In addition, other peripheral mechanisms are also involved, including an attenuation of total peripheral vascular resistance (Azevedo *et al.*, 2003) and an improvement in endothelial function (Sherman, 2000; Sun *et al.*, 2008; Roque *et al.*, 2013). The reverse of the pathological ion channel remodelling in arterial myocytes associated with hypertension may also be related to the BP decrease, which was induced by exercise training.




## Figure 6

Protein expression of $K_{Ca}1.1$ (α and β1) subunits in mesenteric smooth muscle cells. (A) Immunoreactive bands corresponding to $K_{Ca}1.1$ α-subunit, β1-subunit and β-actin. (B) Specificity of the antibody tested on mesenteric arterial smooth muscle lysate in Wistar rats pre-incubated with the corresponding control antigen abolished the immunoreactive band in the arterial tissue, indicating specificity of the antibody for its intended epitope. (C and D) Summarized data of α-subunit (C) and β1-subunit (D) protein levels expressed as a ratio to β-actin ($n = 6$ in each group). (E) Summarized β1/α ratio. $^*P < 0.01$ compared with WKY-SED, $^{\#}P < 0.01$ compared with SHR-SED; one-way ANOVA.

By using electrophysiological methods, we observed that both $Ca_v1.2$ and $K_{Ca}1.1$ channel currents were increased in SHR, a result consistent with those from many previous studies (Asano *et al.*, 1993; Rusch and Runnells, 1994; Lozinskaya and Cox, 1997; Pesic *et al.*, 2004; Sonkusare *et al.*, 2006). There are considerable data suggesting that an up-regulation of $Ca_v1.2$ channel function in VSMCs is a hallmark of hypertension. Augmented $Ca^{2+}$ influx through $Ca_v1.2$ channels contributes to the increased peripheral resistance and contractile responses of VSMCs in various hypertensive animal models (Lozinskaya and Cox, 1997; Pesic *et al.*, 2004; Sonkusare *et al.*, 2006). However, data from the literature also show that there is a positive correlation between BP and the $Ca^{2+}$ current density in the VSMCs from SHR (Lozinskaya and Cox, 1997; Pesic *et al.*, 2004). The enhanced voltage-dependent $Ca^{2+}$ channel currents during hypertension have been demonstrated to be mediated by an up-regulation of the channel itself (Wilde *et al.*, 1994) and membrane potential depolarization (Harder *et al.*, 1985; Cox and Rusch, 2002). In fact, a high BP up-regulates functional $Ca_v1.2$ channels in arteries by promoting the expression of this $\alpha_{1C}$-subunit. Moreover, the depolarization of VSMCs, a fundamental response of the vasculature to high BP, is a powerful stimulus for α1C up-regulation. In the present study, the resting Em in MA myocytes from SHR-SED was more depolarized than that in their normotensive counterparts. Therefore, VSMCs exhibited a higher $Ca^{2+}$ current density. After a period of exercise training, with a reduction in BP and Em hyperpolarization (vs. SHR-SED), there was a concomitant decrease in $Ca_v1.2$ current densities, α1C expression and an elevation in $Ca^{2+}$-dependent tone in SHR. However, how is this process initiated? What comes first – BP lowering or a reduction in $Ca_v1.2$ channel number? The answer to this question is still not clear. The results of the present experiments alone cannot answer this question and more studies need to be done.

Our results indicate that a high BP increases the expression of $Ca_v1.2$ channels so that a chronic BP decrease might have an opposite effect. This could be an explanation for hypertensive rats, but does not appear to apply to normotensive rats; in normotensive WKY, exercise training induced a small decrease in SBP without changing the DBP and MAP, and the Em of MA myocytes did not change compared with WKY-SED. However, the expression of the $Ca_v1.2$ channel α1C and current density was still markedly increased. Moreover, $K_{Ca}1.1$ channel activity was also up-regulated. This indicates that in normotensive animals, the exercise-induced up-regulation of $Ca_v1.2$ and $K_{Ca}1.1$ channels is independent of BP.

Although it is difficult to give a full explanation for the completely opposite effects induced by exercise on $Ca_v1.2$ and $K_{Ca}1.1$ channels of normotensive and hypertensive rats, we will still try to explain the results from the complicated mechanisms. Firstly, we speculate that exercise *per se* may activate $K_{Ca}1.1$ and $Ca_v1.2$ channels on VSMCs. It was demonstrated that exercise training can increase $K_{Ca}1.1$ channel activity by changing its gating properties in rat thoracic aorta smooth muscle cells (Zhao and Wang, 2010). Our recent study also demonstrated that exercise training significantly increases the contribution of $K_{Ca}1.1$ channel to the regulation of vascular tone in MAs, which is possibly mediated by up-regulating the β1-subunit protein to increase $K_{Ca}1.1$ channel activity (Shi *et al.*, 2013). These data support the idea

that exercise training directly activates $K_{Ca}1.1$ channels in VSMCs. In regard to $Ca_v1.2$ channel, Bowles *et al.* (1998) reported that endurance exercise training induced an increase in $Ca_v1.2$ current density in coronary artery, thus a given depolarization may result in more $Ca^{2+}$ influx to produce a greater myogenic response and an enhanced $K_{Ca}1.1$ channel activation. These data support an enhanced $Ca^{2+}$ influx via $Ca_v1.2$ channels as a mechanism for the enhanced $K_{Ca}1.1$ channel activation after exercise training. In addition to increased $Ca^{2+}$ influx as a source of $Ca^{2+}$ for activation of $K_{Ca}1.1$ channels, $Ca^{2+}$ release from the sarcoplasmic reticulum is known to activate $K_{Ca}1.1$ channels (Nelson *et al.*, 1995).

In the present study, in normotensive rats, in addition to the direct activation of exercise training on $K_{Ca}1.1$ and $Ca_v1.2$ channels, enhanced $Ca_v1.2$ channel activity also subsequently activated the $K_{Ca}1.1$ channels. The net effect of exercise training is an up-regulation of $Ca_v1.2$ and $K_{Ca}1.1$ channel function. However, in hypertensive rats, both $K_{Ca}1.1$ and $Ca_v1.2$ channels on MA myocytes are up-regulated in response to the persistent increase in chronic intraluminal pressure (Joseph *et al.*, 2013). Although exercise training can activate $Ca_v1.2$ directly, as mentioned above, BP has a positive relationship with $Ca_v1.2$ current density. Exercise training effectively blunts the high BP in SHR, which subsequently down-regulates $Ca_v1.2$ channel activity. Thus, the decreased $Ca^{2+}$ influx via $Ca_v1.2$ following exercise training is a prominent mechanism for the decreased $K_{Ca}1.1$ channel activity, which undermines the direct activation by exercise itself on $K_{Ca}1.1$ channels. All of the above would explain the differential effects of exercise on $K_{Ca}1.1$ and $Ca_v1.2$ channels in VSMCs from normotensive and hypertensive rats. However, further studies are needed to obtain solid evidence for this hypothesis.

Isometric contraction experiments on MA rings provided further corroborated the electrophysiological results. The pharmacological $Ca_v1.2$ channel blocker nifedipine induced an increased vasodilatation in myocytes from SHR compared with WKY. The sensitivity of MA to nifedipine was higher in SHR, which was in line with previous reports (Pesic *et al.*, 2004; Sonkusare *et al.*, 2006). However, after exercise training, both the NA-induced vasoconstriction and the sensitivity of MA to nifedipine were decreased compared with untreated SHR, which provided strong support that exercise may correct the SHR-associated up-regulation of $Ca_v1.2$ channel function to maintain the vascular tone. In contrast, in normotensive WKY, exercise training increased the contribution $Ca_v1.2$ channels to the vascular tone regulation.

As mentioned above, endothelial dysfunction is also one of the pathophysiological processes in hypertension. Recently, it was reported that exercise training improves the bioavailability of NO in endothelium in small MAs from exercised SHR in comparison to sedentary SHR and WKY (Roque *et al.*, 2013). To clarify the real effect of exercise on $K_{Ca}1.1$ channels, vascular function was then analysed in MAs treated with or without the eNOS inhibitor L-NAME. Although IbTX-induced force was less in arteries with L-NAME than without L-NAME treatment in each group, the IbTX-induced force was higher in SHR-SED than in WKY-SED in both situations, and exercise training markedly inhibited this increase in SHR-EX. However, in normotensive rats, exercise training did not inhibit but enhanced IbTX-induced contraction. Similarly, the NS11021-induced vessel relaxation (as a % of the NA-induced contraction) was also not significantly different between the two groups with or without L-NAME, although the former group was slightly more than the latter. These data indicate that exercise-induced changes in the contribution of the $K_{Ca}1.1$ channels to MA vascular tone regulation are not subsequent to the effect of exercise-mediated increased NO bioavailability. This is supported by the results of Chen *et al.* (2001), who demonstrated that in MAs, in addition to NO, activation of $K_{Ca}1.1$ channels in the vascular beds, at least in part, also contributes to vasodilatation in animals with exercise training.

Structurally, $K_{Ca}1.1$ channels are assembled by the pore-forming α-subunit and a regulatory â1-subunit (Brenner *et al.*, 2000). The $K_{Ca}1.1$ â1-subunit confers $Ca^{2+}$ sensitivity to $K_{Ca}1.1$ channels, and mediates the coupling of $Ca^{2+}$ sparks to $K_{Ca}1.1$ channel activation (Brenner *et al.*, 2000; Plüger *et al.*, 2000). Western blotting revealed that the protein expression levels of both α- and β1-subunits in SHR were significantly increased. Moreover, the β1/α ratio showed a significant increase in SHR-SED compared with WKY-SED. These data indicate that at the cellular level, hypertension induces a larger increase in β1-subunits with respect to α-subunits. After exercise training, the α- and β1-subunit expression together with the β1/α ratio was markedly decreased compared with sedentary SHR. These results may explain why the $Ca^{2+}$ sensitivity of $K_{Ca}1.1$ channels was highest in SHR-SED and lowest in WKY-SED. Obviously, all of these Western blotting results provide strong support for the results of the functional study.

As a limitation of this study, the use of only one specific type of sex, male rats, must be considered. Of course, the data cannot predict the outcome if female rats are used. However, in a recent meta-analysis of studies using SHR and exercise, we could not find any major differences between male and female rats with respect to the influence of exercise on BP (Schlüter *et al.*, 2010). Another limitation is that we did not adjust training intensity during the 8 week training period. Maximal exercise tests should be repeated for each rat at the fourth week to adjust training intensity and to compare the efficacy of the training protocol.

In conclusion, using a combination of mechanical, electrophysiological and biochemical approaches, the present study provides functional and molecular evidence for the different effects of exercise in normotensive and hypertensive rats in regarding to $Ca_v1.2$ and $K_{Ca}1.1$ channels on MA myocytes. In normotensive rats, exercise training up-regulates $Ca_v1.2$ and $K_{Ca}1.1$ channels' functional and molecular expression, whereas in hypertensive rats, exerciser training normalizes SHR-associated $Ca_v1.2$ and $K_{Ca}1.1$ channel up-regulation to restore mesenteric arterial function. These data further suggest that exercise can also be considered as a coadjutant or an alternative therapy to pharmacological treatments for hypertension.

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## Author contributions

L. S. conceived and designed the experiments. L. S., H. Z., Y. C., Y. L., N. L. and T. Z. collected, analysed and interpreted the data. L. S. drafted and H. Z., Y. C. and L. Z. revised the article. All authors approved the final version of the manuscript.

## Conflict of interest

None.

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