Hypertension is a major risk factor for stroke, myocardial infarction, vascular disease, and chronic kidney diseases. Regular exercise, a readily available therapy that is low in cost and relatively free of adverse effects, is receiving growing attention as a means of preventing and controlling cardiovascular diseases. There is strong epidemiological evidence indicating that exercise is a real polypill with multisystemic effects and numerous benefits, including decreased risk of chronic diseases such as hypertension. However, the mechanisms underlying these effects have not been completely elucidated.

During persistent high blood pressure, small arteries and arterioles undergo extensive biological and structural changes in response to elevated intraluminal perfusion pressure. Mounting evidence suggests that ion channels in the plasma membrane of vascular smooth muscle cells (VSMCs) also undergo electric remodeling such that the arteries maintain a heightened vascular tone. The large-conductance Ca$^{2+}$-activated K$^+$ (BK$_{Ca}$) channels are densely expressed in VSMCs and activated by membrane depolarization and by increases in subsarcolemmal Ca$^{2+}$. They promote homeostasis by countering myogenic constriction because of elevated intraluminal pressure by attenuating depolarization and thereby ensure perfusion to critical organs, such as the brain. The majority of studies show that the functional expression of BK$_{Ca}$ channels increases during hypertension. This important local protective mechanism keeps cerebral blood flow at normal or near-normal levels during chronic hypertension.

**Objective**—Regular exercise is an effective nonpharmacological means of preventing and controlling hypertension. However, the molecular mechanisms underlying its effects remain undetermined. The hypothesis that hypertension increases the functional coupling of large-conductance Ca$^{2+}$-activated K$^+$ (BK$_{Ca}$) channels with ryanodine receptors in spontaneously hypertensive rats (SHR) as a compensatory response to an increase in intracellular Ca$^{2+}$ concentration in cerebral artery smooth muscle cells was assessed here. It was further hypothesized that exercise training would prevent this increase in functional coupling.

**Approach and Results**—SHR and Wistar–Kyoto (WKY) rats were randomly assigned to sedentary groups (SHR-SED and WKY-SED) and exercise training groups (SHR-EX and WKY-EX). Cerebral artery smooth muscle cells displayed spontaneous transient outward currents at membrane potentials more positive than −40 mV. The amplitude of spontaneous transient outward currents together with the spontaneous Ca$^{2+}$ sparks in isolated cerebral artery smooth muscle cells was significantly higher in SHR-SED than in WKY-SED. Moreover, hypertension displayed increased whole-cell BK$_{Ca}$, voltage-gated Ca$^{2+}$ channel, but decreased K$_v$ currents in cerebral artery smooth muscle cells. In SHRs, the activity of the single BK$_{Ca}$ channel increased markedly, and the protein expression of BK$_{Ca}$ (β1, but not α-subunit) also increased, but K$_v$1.2 decreased significantly. Exercise training ameliorated all of these functional and molecular alterations in hypertensive rats.

**Conclusions**—These data indicate that hypertension leads to enhanced functional coupling of ryanodine receptors–BK$_{Ca}$ to buffer pressure–induced constriction of cerebral arteries, which attributes not only to an upregulation of BK$_{Ca}$ β1-subunit function but also to an increase of Ca$^{2+}$ release from ryanodine receptors. However, regular aerobic exercise efficiently prevents augmented coupling and so alleviates the pathological compensation and restores cerebral arterial function. (Arterioscler Thromb Vasc Biol. 2016;36:1607-1617. DOI: 10.1161/ATVBAHA.116.307745.)

**Key Words:** constriction ■ exercise ■ hypertension ■ potassium channel ■ rats

**Exercise Prevents Upregulation of RyRs–BK$_{Ca}$ Coupling in Cerebral Arterial Smooth Muscle Cells From Spontaneously Hypertensive Rats**

Lijun Shi, Yanyan Zhang, Yujia Liu, Boya Gu, Run Cao, Yu Chen, Tengteng Zhao

**Objective**—Regular exercise is an effective nonpharmacological means of preventing and controlling hypertension. However, the molecular mechanisms underlying its effects remain undetermined. The hypothesis that hypertension increases the functional coupling of large-conductance Ca$^{2+}$-activated K$^+$ (BK$_{Ca}$) channels with ryanodine receptors in spontaneously hypertensive rats (SHR) as a compensatory response to an increase in intracellular Ca$^{2+}$ concentration in cerebral artery smooth muscle cells was assessed here. It was further hypothesized that exercise training would prevent this increase in functional coupling.

**Approach and Results**—SHR and Wistar–Kyoto (WKY) rats were randomly assigned to sedentary groups (SHR-SED and WKY-SED) and exercise training groups (SHR-EX and WKY-EX). Cerebral artery smooth muscle cells displayed spontaneous transient outward currents at membrane potentials more positive than −40 mV. The amplitude of spontaneous transient outward currents together with the spontaneous Ca$^{2+}$ sparks in isolated cerebral artery smooth muscle cells was significantly higher in SHR-SED than in WKY-SED. Moreover, hypertension displayed increased whole-cell BK$_{Ca}$, voltage-gated Ca$^{2+}$ channel, but decreased K$_v$ currents in cerebral artery smooth muscle cells. In SHRs, the activity of the single BK$_{Ca}$ channel increased markedly, and the protein expression of BK$_{Ca}$ (β1, but not α-subunit) also increased, but K$_v$1.2 decreased significantly. Exercise training ameliorated all of these functional and molecular alterations in hypertensive rats.

**Conclusions**—These data indicate that hypertension leads to enhanced functional coupling of ryanodine receptors–BK$_{Ca}$ to buffer pressure–induced constriction of cerebral arteries, which attributes not only to an upregulation of BK$_{Ca}$ β1-subunit function but also to an increase of Ca$^{2+}$ release from ryanodine receptors. However, regular aerobic exercise efficiently prevents augmented coupling and so alleviates the pathological compensation and restores cerebral arterial function. (Arterioscler Thromb Vasc Biol. 2016;36:1607-1617. DOI: 10.1161/ATVBAHA.116.307745.)

**Key Words:** constriction ■ exercise ■ hypertension ■ potassium channel ■ rats
that the functional coupling of RyRs with BKCa channels in hypertension is enhanced in cerebral arteries as a compensatory mechanism to attenuate hypertension.

Exercise training, especially regular aerobic exercise, has beneficial effects on cerebral vascular health. One possible mechanism underlying this protection is altered Ca2+ regulation because of its critical role in the contraction–relaxation cycle of CASMCs, and, most importantly, Ca2+ homeostasis is affected by chronic endurance exercise. The close functional relationship between Ca2+/current and the activity of K+ channels makes alterations in K+ channel activity another candidate for the observed effect of exercise-induced cerebral vascular protection.

For this reason, this study was designed to determine whether genetic hypertension (spontaneously hypertensive rats [SHR]) results in alterations in the coupling of Ca2+ regulation and K+ channel activity and whether the changes in SHR can be prevented by exercise training. It is here hypothesized that hypertension causes in greater coupling of the release of Ca2+ from the SR to BKCa activation as a compensatory mechanism in an attempt to prevent increased vasoconstriction in cerebral arteries, and this pathological remodeling is prevented by aerobic exercise training.

Materials and Methods
Materials and Methods are available in the online-only Data Supplement.

Results
Body Weight and Blood Pressure
No significant differences were observed in body weights of Wistar–Kyoto rats (WKYS) and SHRs before experiments. The basic systolic blood pressure in SHRs (192.6±2.2 mmHg, n=72) were significantly higher than those in WKYS (184.2±2.5 mmHg, n=72, P<0.01) before the study. After 8 weeks of exercise training, the body weights were significantly lower in both the exercise groups (WKY-EX: 338.2±2.2 g and SHR-EX: 301.7±2.2 g) than in their sedentary counterparts (WKY-SED: 349.1±2.5 g, P=0.02) and SHR-SED: 318.1±2.7 g, P=0.004). Similarly, systolic blood pressure in SHR-EX (181.3±2.2 mmHg) was significantly lower than that of the sedentary counterparts (SHR-SED: 197.8±2.2 mmHg, P<0.01, n=56 in each group).

Exercise Prevents Increases in STOCs in CASMCs From SHR
Cerebral myocytes displayed STOCs at membrane potentials (Em) more positive than −40 mV; depolarization increased STOC amplitude and frequency (Figure 1 in the online-only Data Supplement). STOC activity was found to be abolished by 100-nmol/L iberiotoxin. The RyRs agonist caffeine activated STOCs effectively. At the holding potential (HP) of −30 mV, the amplitude and the frequency of STOCs were increased by 32.9±2.3% and 438.1±51.8%, respectively, after caffeine (1 mmol/L) treatment. These characteristics indicate that STOCs in cerebral myocytes are BKCa channel mediated and RyRs dependent.

At the same HP (−40 to 0 mV), the amplitude STOCs in SHR-SED was significantly higher than that in WKY-SED (Figure 1) (134.2±2.5 mmHg, n=72) (about 1.24−, 1.95−, 1.67−, 1.90−, and 1.86-fold of WKY-SED at −40, −30, −20, −10, and 0 mV, respectively). After exercise training, it was significantly higher in WKY-EX than in WKY-SED (at −30 to 0 mV, ie, 1.49−, 1.20−, 1.18−, and 1.12-fold increase over WKY-SED, respectively), but it was significantly lower in the SHR-EX group than in the SHR-SED group (−40−0 mV, 0.94−, 0.86−, 0.79−, 0.69−, and 0.68-fold of SHR-SED, respectively). There was no significant difference of the frequency of STOCs among 4 groups from −20 to 0 mV. At −40 and −30 mV, however, the frequency of STOCs was significantly higher in SHR-SED than in WKY-SED (ie, 1.28 and 2.46 fold of WKY-SED, respectively). However, after exercise training, no significant changes in frequency were observed between WKY-EX and WKY-SED groups or between SHR-EX and SHR-SED groups.

Exercise Inhibits the Alteration of the Components of Whole-Cell Ik in CASMCs From SHR
Compared with in the WKY-SED group (−51.5±4.8 mmHg, n=40 cells/6 rats), the resting Em measured in SHR-SED was depolarized (−38.2±3.4 mmHg, n=48 cells/6 rats). After exercise training, the resting Em in SHR-EX was −45.3±3.3 mmHg (n=42 cells/6 rats), which was higher than in SHR-SED. Exercise training did not change the resting Em in WKY (WKY-EX, −48.6±4.3 mmHg, n=45 cells/6 rats).

To determine whether there were any differences in K+ current (Ik) in cerebral arterial myocytes among the 4 groups, Ik measurements were taken from an HP of −60 mV using conventional whole-cell methods. To compensate for differences in cell size, membrane Ik are expressed relative to cell capacitance (pA/pF). Cell capacitance, an index of cell membrane area, did not show significant differences among WKY-SED (20.2±1.3 pF, n=44 cells), WKY-EX (19.5±1.0 pF, n=46 cells), SHR-SED (18.9±1.6 pF, n=48 cells), and SHR-EX (18.4±1.2 pF, n=48 cells). Each n represents cells from 6 animals unless stated otherwise. As shown in Figure 2, at an HP of +80 mV, whole-cell Ik density in SHR-SED (51.3±4.8 pA/pF, n=24 cells) was significantly higher than that of WKY-SED (30.3±2.9 pA/pF, n=24 cells). Exercise training resulted in significantly lower Ik density in SHR-EX (28.9±2.6 pA/pF, n=18 cells) than in SHR-SED, but it did not differ significantly in WKY (WKY-EX: 31.6±2.7 pA/pF, n=18 cells; Figure 2).

The effects of selective BKCa channel inhibitor iberiotoxin (100 nmol/L) or selective K+ channel inhibitor 4-aminopyridine (4-AP) (3 mmol/L) on Ik were determined to directly assess the
Exercise and Arterial RyRs–BKCa Coupling in SHR

Shi et al

1609

relative contribution of BKCa and Kv channels to Iκ in Figure 2A through 2D. As shown in Figure 2E and 2F, the iberiotoxin-sensitive (BKCa) currents were larger in SHR-SED and WKY-EX than in WKY-SED (both P<0.01). For example, at +80 mV, the current density was 26.8±2.8 pA/pF in SHR-SED (n=24 cells/6 rats), 9.8±1.3 pA/pF in WKY-EX (n=18 cells/6 rats), and 6.1±0.8 pA/pF in WKY-SED (n=24 cells/6 rats). In hypertensive rats, exercise training rendered the current density significantly lower (SHR-EX, 6.9±0.6 pA/pF, n=18 cells/6 rats) than in their sedentary counterparts (P<0.01). However, 4-AP–sensitive currents (Kv) were significantly lower in hypertensive rats than in age-matched normotensive animals (P<0.01). At +80 mV, the current density was 15.4±1.5 pA/pF in SHR-SED (n=16 cells/6 rats) and 22.4±2.4 pA/pF in WKY-SED (n=20 cells/6 rats, P<0.01). Exercise training did not cause Kv currents in WKY (WKY-EX: 23.3±2.5 pA/pF, n=20 cells/6 rats) to differ significantly from those of sedentary counterparts (P=0.252). In hypertensive rats, exercise training rendered the Kv current density significantly lower (SHR-EX, 6.9±0.6 pA/pF, n=18 cells/6 rats) than in their sedentary counterparts (P<0.01).

However, 4-AP–sensitive currents (Kv) were significantly lower in hypertensive rats than in age-matched normotensive animals (P<0.01). At +80 mV, the current density was 15.4±1.5 pA/pF in SHR-SED (n=16 cells/6 rats) and 22.4±2.4 pA/pF in WKY-SED (n=20 cells/6 rats, P<0.01). Exercise training did not cause Kv currents in WKY (WKY-EX: 23.3±2.5 pA/pF, n=20 cells/6 rats) to differ significantly from those of sedentary counterparts (P=0.252). In hypertensive rats, exercise training rendered the Kv current density significantly lower (SHR-EX, 6.9±0.6 pA/pF, n=18 cells/6 rats) than in their sedentary counterparts (P<0.01).

All these data indicate that hypertension is associated with enhancement of functional expression of BKCa channels but decreased functional expression of Kv channels, whereas exercise training attenuated these changes. However, in normotensive rats, exercise training did not decrease but rather increased the whole-cell BKCa currents without causing any significant change in Kv currents.

Exercise Suppresses the Altered Single-Channel Properties of BKCa Channels in CASMCs From SHR

Subsequent experiments examined the possibility that alterations in the properties of single channels could account for the increased BKCa current in CASMCs from SHRs. As shown in Figure 3A, the single-channel recording of BKCa channels at the Em of 0.3 and 1 µmol/L [Ca2+]free levels (HP=+40 mV). The Po of BKCa channels from SHR-SED was larger than that of channels from WKY-SED (P<0.01, n=6 patches each). After exercise training, the Po in hypertensive rats (SHR-EX) became lower than in SHR-SED (P<0.01). However, in normotensive rats, the Po after exercise training (WKY-EX) was significantly higher than in their sedentary counterparts (P<0.01). As shown in Figure 3B, at the testing potential of +40 mV, 1 µmol/L [Ca2+]free, the mean open time (TO, dwell time of open state) of single BKCa channel was profoundly higher in SHR-SED (44.2±5.5 ms) than in WKY-SED (9.2±2.2 ms, P<0.01). However, the mean close time (TC, dwell time of close state) was significantly lower in SHR-SED (16.6±4.1 ms) than in WKY-SED (87.5±7.6 ms, P<0.01). However, exercise training attenuated both of these changes (SHR-EX: To=16.0±2.0 ms, P<0.01; Tc=24.3±2.5 ms, n=6, both P<0.01) without significantly affecting the conductance of BKCa channels. In normotensive rats, exercise training increased TO but decreased TC significantly (WKY-EX: TO=14.7±3.6 ms; TC=29.1±5.1 ms, n=6) when compared with WKY-SED (both P<0.01). These results indicate that single-channel activity was involved in the changes of whole-cell BKCa currents in all 4 groups.

The apparent Ca2+ sensitivity of BKCa channels in patches excised from CASMCs was evaluated. The relations of normalized Po (Po/PoMax) against [Ca2+]free are shown in Figure 3C. The maximal open probability (PoMax) was determined at a voltage of +40 mV with 100 µmol/L free Ca2+ in the bath solution at the end of channel recording. The Po–Ca2+ curve fitted (using least squares) by Hill equation with a Kd value of 0.75±0.11 (SHR-SED, n=6) and 3.24±0.41 µmol/L (WKY-SED, n=6, P<0.01). A leftward shift in Ca2+-dependent
activation was observed with hypertension. However, in hypertensive rats, the Po–Ca\x20+ curve was further to the right ($K_d$ in SHR-EX: 1.26±0.11 μmol/L, n=6) after exercise training than in SHR-SED ($P<0.01$). In WKY-EX, exercise training induced a leftward shift in the Po–Ca\x20+ curve with a $K_d$ value of 2.20±0.24 μmol/L (WKY-EX, n=6, $P<0.01$) relative to the sedentary counterparts (WKY-SED). No significant differences in the Hill coefficient ($H$) were observed in any of the 4 groups (0.98±0.09 [WKY-SED], 1.06±0.09 [WKY-EX], 1.17±0.17 [SHR-SED], and 1.14±0.09 [SHR-EX]).

The effects of tamoxifen on BK\textsubscript{Ca} channels were examined (Figure 3D). Tamoxifen, a β1 subunit–specific BK\textsubscript{Ca} channel activator, a (xeno)estrogen, is known to activate BK\textsubscript{Ca} channels only when they are associated with β1-subunit.\textsuperscript{23} In the presence of 100 nmol/L [Ca\textsuperscript{2+}]\textsubscript{free}, application of tamoxifen (1 μmol/L) evoked a 3.4-fold increase in the Po of BK\textsubscript{Ca} channels in WKY-SED patches, whereas, in SHR-SED patches, it evoked 8.9-fold increase (Figure 3E).

After exercise training, tamoxifen showed a 4.3-fold increase in WKY-EX patches, which was significantly higher than in WKY-SED ones ($P<0.01$, n=6). However, in hypertensive rats, exercise training inhibited tamoxifen-evoked effects (4.6-fold increase of NPo, $P<0.01$). These results indicate that an SHR-induced decrease in
Ca²⁺ sensitivity of single BKCa channels may be caused by decrease in the functional expression of the BKCa β₁ accessory subunit. However, this change can be prevented by regular exercise training.

**Exercise Inhibits the Increases in CaV₁.2 Currents in CASMCs From SHR**

Voltage-gated Ca²⁺ channels (Caᵥ₁.₂), which mediate Ca²⁺ influx into the VSMCs, are particularly important to vascular excitation and contraction. To determine whether Caᵥ₁.₂ channel activity differed in the hypertension or exercise training groups, measurements of whole-cell Ca²⁺ currents were conducted from an HP of −30 mV, and Ba²⁺ served as the charge carrier (Figure 4). Nifedipine (100 nmol/L) suppressed the inward currents almost completely, which indicates that the inward currents recorded were Ba²⁺ currents through Caᵥ₁.₂ channels. The current–voltage (I–V) relationship curves were bell-shaped; the inward currents began to appear at ≈−20 mV and peaked at +20 mV. Maximal calcium current density was −6.2±1.2 pA/pF for WKY-SED (n=16 cells/6 rats), −7.2±1.1 pA/pF for WKY-EX (n=18 cells/6 rats, P=0.016 versus WKY-SED), −13.2±1.7 pA/pF for SHR-SED (n=18 cells/6 rats, P<0.01 versus WKY-SED), and −9.6±1.5 pA/pF for SHR-EX (n=20 cells/6 rats, P<0.01 versus SHR-SED). The maximal calcium current density in SHR-SED was much higher than that of WKY-SED. Taken together, these results suggest that exercise training markedly decreased the Caᵥ₁.₂ currents in hypertensive rats but increased them significantly in normotensive rats.

**Exercise Restrains the Changes in BKCa and KV Channel Protein Expression and Their Contribution to Vascular Tone Regulation in Cerebral Arteries From SHR**

It has been reported that exercise training improves the bioavailability of NO in the endothelium in small mesenteric arteries from exercised SHR relative to sedentary SHR and WKY.²⁴ To exclude the effects of endothelium nitric oxide synthase, nonselective nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (100 μmol/L) was added after measurement of Kᵥout. To examine the functional contribution of BKCa and KV channels to the regulation of vascular tone, selective channel inhibitors were used on the cerebral...
arteries. As shown in Figure 5A, the iberiotoxin-induced increase in tension in the SHR-SED group was higher than in the WKY-SED group. Exercise training markedly inhibited this increase in hypertensive rats. However, in normotensive rats, exercise training did not inhibit but rather enhanced the increase in iberiotoxin-induced tension. However, the increase in 4-AP–induced tension was less pronounced in SHR-SED than in WKY-SED. Exercise training markedly inhibited this decrease in hypertensive rats. However, in normotensive rats, exercise training did not change the 4-AP–induced increase in tension. These data support the hypothesis that hypertension increases BKCa channel activity but decreases Kv channel activity, and that exercise training reverses these alterations.

In another experiment, at the plateau of the phenylephrine (PHE, 10⁻⁴ M)-induced contraction, tamoxifen (10⁻⁴ 3×10⁻⁶ M) reduced the contractions of the vessels to PHE significantly in all the 4 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).

To identify the molecular mechanisms underlying the functional alteration of BKCa and Kv channels in vascular tone regulation, channel protein expression was examined in CA VSMCs. Representative immunoblots, depicted using bar graphs (Figure 5B), showed hypotension to be associated with a marked increase in protein expression of β1-subunits without significant changes in α-subunits. The β1/α ratio showed a significant increase in hypertension (SHR-SED: 2.17±0.47 versus WKY-SED: 1.00±0.07, P<0.01), suggesting that the composition stoichiometry of BKCa channels was modified by hypertension. At the cellular level, hypertension induced a larger increase in the number of β1-subunits with respect to α-subunits. However, after exercise training, the α- and β1-subunit expression and β1/α ratio were markedly lower (SHR-EX, β1/α: 1.39±0.50). In normotensive rats, exercise training induced significant increases in β1-subunit expression and β1/α ratio without affecting α-subunits (WKY-EX, α-subunit: 1.12±0.21, P>0.05; β1-subunit: 1.54±0.25, P<0.01; β1/α: 1.41±0.30, P<0.01).

Regarding the K1.1 channel, Western blotting showed that, in hypertensive rats, K1.2 protein expression was significantly lower than in normotensive rats (SHR-SED: 0.37±0.11 versus WKY-SED: 1.00±0.09, P<0.01) without any visible difference in the K1.5 channel. Exercise training markedly inhibited this decrease (SHR-EX: 1.33±0.22, P<0.01). However, in normotensive rats, exercise training did not induce any significant change in K1.2 or K1.5 protein expression (Figure 5B).

Exercise Alleviates the Increased Spontaneous Ca²⁺ Sparks From SHR

Cytosolic Ca²⁺ is a ubiquitous signaling molecule, and VSMCs express numerous Ca²⁺ signaling mechanisms that play a crucial role in determining and regulating their functional roles. The spontaneous Ca²⁺ sparks and caffeine-induced Ca²⁺ oscillations were examined to assess the effects of exercise training on the ryanodine-sensitive release of Ca²⁺ in cerebral arteries. Spontaneous Ca²⁺ sparks were observed in CASMCs during an 8.5-s period of line scan in 4 groups. The spark amplitude in WKY-EX did not change (1.39±0.06 F/F₀, n=90 events from 14 cells), but it was higher in SHR-SED cells (1.61±0.07 F/F₀, n=129 events from 18 cells) than in WKY-SED (1.42±0.07 F/F₀, n=105 events from 16 cells, P<0.01). After exercise training, the amplitude in SHR-EX was 1.53±0.07 F/F₀ (n=110 events from 18 cells, P<0.01), which was smaller than in SHR-SED. In addition, as shown in Figure 6B, the rise time in SHR-SED was smaller but the decay half-time was greater than in WKY-SED, which indicated that Ca²⁺ sparks in SHR-SED cells rose faster but decayed slower than in normotensive cells. However, exercise training reduced these gaps. In normotensive rats, exercise training had no effect on rise time but decreased the average decay half-time of Ca²⁺ sparks.

Rapid application of 10 mmol/L caffeine evoked similar Ca²⁺ transients in 4 groups (Figure 6C through 6E), excluding a change in the SR Ca²⁺ store as the major cause of the different sparks amplitude. Neither hypotension nor exercise training changed the function of ryanodine-sensitive release of Ca²⁺ in cerebral arteries. These data show that the change in Ca²⁺ spark activity may partially account for the change in BKCa channel activity in cerebral arterial myocytes observed in the hypertension and exercise training group (SHR-EX).

Discussion

In this study, data supported the novel hypothesis that, in SHRs, a genetic model of hypertension, enhancement of the functional...
Figure 5. Large-conductance Ca^{2+}-activated K^+ channel (BK_{ca}) and K_v channel protein expression and contribution to the regulation of vascular tone in cerebral arteries from spontaneously hypertensive rats (SHR). A. Effects of BK_{ca} and K_v channel modulators on the vascular tension in cerebral arteries. a to c, Typical experimental tracings showing the effect of (a) BK_{ca} blockers iberiotoxin (IbTX, 100 nmol/L) or (b) K_v channel inhibitor 4-aminopyridine (4-AP; 3 mmol/L) on resting tension; (c) the effect of tamoxifen (10^{-9} to 3×10^{-6} M) on PHE-induced vessel contraction. In each experiment, the arteries were preincubated with nonselective nitric oxide synthase inhibitor N^ω-nitro-l-arginine methyl ester (L-NAME, 100 μmol/L) for 20 min (arrows). Asterisks indicate the time of addition of phenylephrine (PHE, 10^{-4} M). The black triangles in (c) mark the time of addition of tamoxifen (10^{-9}, 10^{-8}, 3×10^{-8}, 10^{-7}, 3×10^{-7}, 10^{-6}, and 3×10^{-6} M). d and e, Statistical diagram of (b) IbTX and (c) 4-AP on vascular tone. f, Concentration–response curves for effects of tamoxifen on PHE-induced contraction. B. Protein expression of BK_{ca} (α and β1) subunits, K_v1.2 and K_v1.5 channels in cerebral artery smooth muscle cells. a and b, Immunoreactive bands corresponding to the BK_{ca} (α) α-subunit and β1-subunit, (b) K_v1.2 and K_v1.5 channels, and β-actin. c, d, and e, Summarized data of the (c) α-subunit and β1-subunit, (d) β1/α ratio, and (e) K_v1.2 and K_v1.5 channel protein levels expressed as a ratio to β-actin (n=6 for each group). *P<0.01, relative to Wistar–Kyoto rats (WKY)-SED; #P<0.01, relative to SHR-SED; 1-way ANOVA. n=6 in each group.
coupling of BKCa channels, and the release of Ca2+ releases is an adaptive mechanism that opposes pressure-induced constriction and so prevents against ischemic events in the brain and that long-term regular aerobic exercise reverses such pathological compensation to restore the cerebral arterial function. The current work also provides evidence that the increased functional coupling in cerebral arterial myocytes from SHRs attributes not only to the increase of RyRs function but also to the augmented β1-subunit function of sarcoplasma BKCa channels.

Perforated whole-cell recording demonstrated a significant increase in STOC amplitude in cerebral arterial myocytes from SHRs at the same HP. Subsequently, a series of experiments were conducted to explore the mechanisms underlying this change. An increase of the whole-cell I\(_{\text{K}}\) (conventional whole-cell recording) was observed in CASMCs from hypertensive rats. Selective BKCa and Kv channel blockers showed that the components of whole-cell I\(_{\text{K}}\) were different in SHRs. Specifically, there were significantly more BKCa channel components and fewer Kv channel components in hypertensive subjects. The contractile studies in isolated cerebral arteries were consistent with the findings of electrophysiological studies. In previous studies, similar results have been reported in other vascular beds. Results demonstrated larger K\(_{\text{Ca}}\) and smaller Kv components in mesenteric arterial smooth muscle cells from SHR. In addition, this study demonstrated that exercise training can reverse these changes in K+ components in CASMCs from hypertensive rats. However, in normotensive rats, after exercise training, the only increase was in the contribution of BKCa channels to the regulation of vascular tone. Neither the density of Kv channel currents nor their contribution to the regulation of vascular tone was observed between WKY-EX and WKY-SED. These results indicate that the mechanisms by which exercise affects normotensive animals may differ from those in hypertensive animals, thus reinforcing the heterogeneity of the BKCa control mechanism.

As mentioned above, the amplitude of STOCs was higher in CASMCs from SHRs than in those from WKYS, which indicates that the coupling of BKCa channels on the cell membrane with the release of Ca2+ from SR increased significantly during hypertension. This increase could have been caused by...
any of several mechanisms, including (1) increased release of Ca\(^{2+}\) release from the SR, (2) increased coupling efficiency between the release of Ca\(^{2+}\) and BK\(_{ca}\) activation, or (3) an increase in BK\(_{ca}\) pore-forming α-subunit protein. Western blot analysis showed there to be no significant differences in BK\(_{ca}\) α-subunit protein expression among 4 groups, whereas the expression of β1-subunit was significantly enhanced in SHRs. In this way, the increased expression of β1-subunit protein and subsequent altered open-time distribution and voltage sensitivity of BK\(_{ca}\) single channels may explain the increase in the activity of STOCs in CASMCs from SHRs. Single-channel recording showed a significant increase in open probability of BK\(_{ca}\) channels in SHRs and greater effectiveness (sensitivity) of Ca\(^{2+}\) in activating these channels. Moreover, the open dwell times of hypertension BK\(_{ca}\) channels were higher than in those from normotensive arteries. This was consistent with increased expression of the BK\(_{ca}\) β1-subunit in hypertensive arteries. Tamoxifen has been reported to directly activate BK\(_{ca}\) channels through the β1-subunit, so it is often used as a pharmacological probe to assess β1-subunit function. The results of BK\(_{ca}\) single-channel recording and the measurement of vascular tone showed tamoxifen-induced BK\(_{ca}\) channel activation and vasodilation to be greater in SHR-SED than in WKY-SED, and exercise inhibited these increases in hypertensive rats. These data were consistent with the findings of Western blot analysis that β1 function was increased in hypertensive cerebral artery myocytes and that this increase can be markedly attenuated by exercise training. Taken together, these results suggest that the increase in STOC amplitude and BK\(_{ca}\) components in I\(_{ca}\) in cerebral myocytes from SHR can be attributed primarily to the enhancement of single BK\(_{ca}\) channel activity, which itself stemmed from upregulation of β1-subunit expression.

Despite the intrinsic alteration in biophysical properties of BK\(_{ca}\) channels, the increase in STOC observed in SHRs could have been caused by the increase in the release of Ca\(^{2+}\) from the ryanodine-sensitive stores. Ca\(^{2+}\) sparks in CASMCs represent the spontaneous and coordinated opening of an undefined number of RyRs within a cluster. The Ca\(^{2+}\) spark–BK\(_{ca}\) coupling is changed in some vascular disorders; which has the enhancement of single BK\(_{ca}\) channel activity, which itself may explain the increase in the amplitude or frequency of Ca\(^{2+}\) sparks between WKY-EX and WKY-SED groups. These results indicate that, in normotensive rats, the upregulation of BK\(_{ca}\) β1-subunit is the major cause of the exercise-induced increase of BK\(_{ca}\) currents and their contribution to the regulation of vascular tone regulation, as reported previously. However, in SHRs, the upregulation of the coupling of Ca\(^{2+}\) sparks with BK\(_{ca}\) channels can be attributed not only to the upregulation of the BK\(_{ca}\) β1-subunits but also to the increase in the activity of Ca\(^{2+}\) sparks, and exercise acts as an inhibitor of the pathological remodeling during chronic hypertension. The effects of exercise on cerebral arteries in SHR are similar to those observed in conduit coronary arteries in a porcine model of diabetic dyslipidemia. Mokelke et al demonstrated that diabetic dyslipidemia led to elevated whole-cell I\(_{ca}\) and increased functional coupling of K\(_{ca}\) and Ca\(^{2+}\) release in conduit coronary smooth muscle, whereas endurance exercise prevented increased coupling of the release of Ca\(^{2+}\) to activation of K\(_{ca}\) channels. However, in another study in the same animals, Mokelke et al observed decreases in STOC and Ca\(^{2+}\) sparks in coronary microvessels, and exercise training also prevented this reduction. These results indicate that the contribution of Ca\(^{2+}\) sparks and STOCs to vasoregulation is complicated and dependent on the diameter of the artery even within the same vascular bed, the same animal, and the same laboratory using the same methods. However, exercise seems to be able to prevent or correct the pathological alterations regardless.

Early in the 1970s, Jones observed increased K\(^+\) turnover in the arteries of experimental rats serving as a model of hypertension. Since then, most studies of the matter have documented increased functional expression of BK\(_{ca}\) channels in vasculature during hypertension, although small number of papers have produced different results. For example, Amberg et al reported decreased sensitivity in BK\(_{ca}\) channels caused by a decreased expression of the regulatory β1-subunit of the channel in both angiotensin II–induced hypertensive rats and genetic hypertension models (SHRs). The results of the current work seem to contradict their findings. The reasons for some of these disparate results are not completely clear and must be studied further. Despite the data disparity on the function of BK\(_{ca}\) in chronic hypertension, it is clear that RyR–BK\(_{ca}\) regulates arterial function in hypertension as an entirety.

These results also provide strong evidence that the increase in the coupling of RyRs and BK\(_{ca}\) channels is not a cause but a result of hypertension to compensate the increase of the intraluminal pressure in cerebral arteries. In previous studies, Liu et al demonstrated that cerebral arterioles from SHR and WKY rats had similar resting diameters, indicating that the upregulation of BK\(_{ca}\) channels during hypertension does not produce active dilation but rather nullifies the increased tendency of the cerebral resistance vessels to constrict. Regarding Ca\(_{1.2}\) channels, the observation that the whole-cell Ca\(_{1.2}\) currents in CASMCs from hypertensive rats were higher than those from normotensive ones is completely consistent with previous reports. Several previous studies have demonstrated that the profound upregulation in vascular Ca\(_{1.2}\) channel function can be attributed primarily to increased expression of the pore-forming α1C-subunit of this channel. However, the mechanisms underlying this upregulation have not been clearly elucidated. Currently available data points to the involvement of multiple cellular mechanisms in the upregulated protein expression of vascular Ca\(_{1.2}\) channels during hypertension, including transcriptional activity and post-transcriptional mechanisms. This study did not involve measuring the protein expression of the α1C-subunit. However, the maximum whole-cell currents of Ca\(_{1.2}\) channels were visibly augmented in CASMCs from SHRs, which may partially account for the elevated blood pressure in hypertension.

The K\(_{ca}\) channels are major contributors to resting Em, and they play an important role in the regulation of the diameters of small arteries. Previous studies from other laboratories have demonstrated that the depolarization and
elevated vascular tone seen in small cerebral arteries for SHR and renal hypertensive rats are associated with a loss of functional $K_{\text{Ca}}$ channels.\textsuperscript{42} In this study, complementing the electrophysiological and contractile studies, Western blotting experiments further showed that arteries from hypertensive animals had lower $K_{\text{Ca}}$ than normotensive animals but not lower $K_{\text{Ca}}$ protein expression, and aerobic exercise training ameliorated this difference. Although it is difficult to determine whether $K_{\text{Ca}}$ channels were sufficiently upregulated (corrected) after exercise to restore cerebral artery function because of the effects of exercise depend on exercise intensity, duration, frequency, and other factors, it has been clear that exercise-induced correction of $K_{\text{Ca}}$ channels in SHR does to some extent help restore cerebral artery function.

On the basis of these findings, a model for a mechanistic explanation of increase of RyR–$K_{\text{Ca}}$ channel communication observed in SHRs is here proposed (Figure 7). During hypertension, decreases in $K_{\text{Ca}}$ currents elicit depolarization of the cell membranes of CASMCs. In this way, $K_{\text{Ca}}$ channels are activated with a subsequent elevation of Ca$^{2+}$ influx, which increases the RyR-sensitive release of Ca$^{2+}$ from SR. In addition, because of SHR-induced high expression of the $K_{\text{Ca}}$ sub-units, $K_{\text{Ca}}$ becomes hypersensitive to high local Ca$^{2+}$ produced by Ca$^{2+}$ sparks, which renders Ca$^{2+}$ spark–STOC coupling more efficient by virtue of STOC amplitude and charge transfer. This tends to hyperpolarize the Em and then deactivate Ca$^{2+}$ influx via voltage-operated Ca$^{2+}$ influxes, leading to vasodilatation of cerebral arteries. Although it is accepted that the $K_{\text{Ca}}$ channel provides an endogenous compensatory mechanism to buffer vasoconstriction, the complexity of $K_{\text{Ca}}$ channel regulation in hypertension and the heterogeneity in species, endocirne profile, arterial bed, conduit versus microvessel, hypertension model, sex, age of the experimental animal, and other factors must all be taken into account as well.\textsuperscript{14}

Scientific studies have confirmed the beneficial effects of the physical exercise by reducing the blood pressure and improving the arterial function in hypertension.\textsuperscript{41} Increasing amounts of evidence have shown that exercise has positive effects on the levels of calcium-handling proteins and ion channels\textsuperscript{13,21,29,31,35,44} The current work provides the first evidence that regular aerobic exercise can prevent the upregulation of RyR–$K_{\text{Ca}}$ coupling in cerebral arteries in genetic hypertension, which indicates that exercise has beneficial effects on arterial function homeostasis.

In summary, the current work showed enhanced coupling between Ca$^{2+}$ sparks and $K_{\text{Ca}}$ in CASMCs from SHRs, which may represent an adaptive response to the maintenance of their functional integrity. The increase in $K_{\text{Ca}}$ sensitivity to spark Ca$^{2+}$ stems from an upregulation of the regulatory $K_{\text{Ca}}$, $\beta1$ subunit and an increase in the release of Ca$^{2+}$ from SR. As a result, CASMCs showed far greater STOC amplitude than control cells, contributing to membrane hyperpolarization, which promoted adequate microcirculation to the brain. However, regular exercise effectively prevented this pathological compensation during the process of hypertension and restored cerebral arterial function. These findings provide new insight into the beneficial effects of exercise training on cerebrovascular homeostasis and remodeling during hypertension.

Sources of Funding
This work was supported by the National Natural Science Foundation of China (31371201), the Chinese Universities Scientific Fund (2015ZD008, 2016RC001), Research project of General Administration of Sport of China (2015B035), and the Beijing Natural Science Foundation (to L. Shi).

Disclosures
None.

References


22. Sturek M. Ca2+ regulatory mechanisms of exercise protection against coro-


31. Mokelke EA, Hu Q, Song M, Toro L, Reddy HK, Sturek M. Altered func-

32. Rueda A, Fernández-Velasco M, Benitah JP, Gómez AM. Abnormal Ca2+-


34. Shi L, Liu B, Li N, Xue Z, Liu X. Aerobic exercise increases BKCa channel contribution to regulation of mesenteric arterial tone by upregu-


38. Nieves-Cintrón M, Amberg GC, Nichols CB, Molkentin JD, Santana LF. Activation of NFATC3 down-regulates the beta1 subunit of large conduc-


42. Tobin AA, Joseph BK, Al-Kindi HN, Albarwani S, Madden JA, Nemetz LT, Rusch NJ, Rhee SW. Loss of cerebrovascular Shaker-type K(+) chan-


44. Albarwani S, Al-Siaybi S, Baomar H, Hassan MO. Exercise training attenu-

**Highlights**

- This study provided first functional and molecular evidence that in spontaneously hypertensive rats, there is an enhanced coupling between the ryanodin receptors and the large-conductance Ca2+-activated K+ channels in cerebral artery smooth muscle cells, which may represent an adaptive mechanism to oppose pressure-induced constriction, and thus defend against ischemic events of the brain.

- The increased functional coupling in hypertensive cerebral small mesenteric smooth muscle cells attributes not only to the increase of ryanoadin receptors but also to the augmented β1-subunit function of sarcoplasmic large-conductance Ca2+-activated K+ channel.

- Regular aerobic exercise reverses such pathological compensation to restore the arterial function in hypertension. The present work provides new insight for the beneficial effects of exercise on cerebrovascular homeostasis and remodeling during hypertension.
Exercise Prevents Upregulation of RyRs–BKCa Coupling in Cerebral Arterial Smooth Muscle Cells From Spontaneously Hypertensive Rats
Lijun Shi, Yanyan Zhang, Yujia Liu, Boya Gu, Run Cao, Yu Chen and Tengteng Zhao

Arterioscler Thromb Vasc Biol. 2016;36:1607-1617; originally published online June 23, 2016; doi: 10.1161/ATVBAHA.116.307745
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/36/8/1607

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/