Exercise training increases K⁺-channel contribution to regulation of coronary arterial tone

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Bowles, D. K., M. H. Laughlin, and M. Sturek. Exercise training increases K⁺-channel contribution to regulation of coronary arterial tone. J. Appl. Physiol. 84(4): 1225–1233, 1998.—The present study examined whether regulation of coronary tone in conduit arteries (≥1.0 mm ID) is altered by exercise training. Yucatan miniature swine were treadmill trained for 16–20 wk (Ex) and compared with sedentary counterparts (Sed). Endothelium-denuded arterial rings were stretched to optimal length and allowed to equilibrate for 60 min. Inhibition of either Ca²⁺-activated channels (1 mM triethylammonium (TEA) or 10 nM iberiotoxin (IBTX)) or voltage-dependent K⁺ channels (1 mM 4-aminopyridine (4-AP)) significantly increased resting tension in both groups; however, the effect of all K⁺-channel blockers was greater in Ex. Addition of 1 mM sodium nitroprusside reduced resting tension in both groups, confirming the presence of active basal tone; however, sodium nitroprusside-sensitive tone was increased approximately twofold in Ex compared with Sed group. Perforated patch-clamp experiments on isolated smooth muscle cells demonstrated no effect of exercise training on whole cell TEA-sensitive, 4-AP-sensitive, or basal K⁺ current. Similarly, whereas TEA, 4-AP, and IBTX all decreased resting membrane potential, there was no difference in depolarization between groups. The greater effect of TEA on resting tension in Ex could be mimicked in Sed by addition of the Ca²⁺-channel agonist BAY K 8644. In conclusion, the greater response to K⁺-channel blockers after exercise training is consistent with an increased contribution of K⁺ channels to regulation of basal tone in conduit coronary arteries. The lack of an effect of training on K⁺ current characteristics or membrane potential responses in isolated cells suggests that a requisite factor for enhanced K⁺-channel activation in arteries from Ex, possibly stretch, is absent in isolated cells.

voltage-activated; amphotericin; voltage-dependent potassium ion channels; calcium-activated potassium ion channels; tetraethylammonium; 4-aminopyridine; iberiotoxin; porcine

Physical inactivity is an independent risk factor for the development of coronary artery disease (10). Regular exercise is associated with a reduction in the occurrence and severity of cardiovascular disease, independent of changes in other risk factors (10), suggesting that this protective effect may result from intrinsic adaptations within the coronary vascular bed. Recent studies have shown that exercise training produces numerous adaptations within the coronary circulation (20, 21, 23, 30). These adaptations have been generalized as an overall enhanced vasodilation and attenuated vasoconstriction to vasoactive agonists (20, 30). Interestingly, these training-induced adaptations are not uniform but heterogeneous (20, 30). For example, the enhanced responsiveness to adenosine after exercise training seen in conduit and small arteries is absent in smaller resistance arteries (24, 29, 30). Conversely, enhanced endothelium-dependent vasodilations after exercise training have been reported in resistance, but not conduit, coronary arteries (24, 29, 30). This heterogeneous adaptation to exercise training alters the typical pattern of functional heterogeneity in the coronary circulation, whereby coronary vessels respond to physiological and pharmacological stimuli in a heterogeneous, size-dependent manner (16, 19).

The superimposition of a heterogeneous, training-induced adaptation on an underlying functional heterogeneity makes extrapolation of training-induced adaptations from one arterial size to another tenuous. Previously, Muller et al. (23) reported an increase in the myogenic response of resistance coronary arteries (75–150 µm in diameter) after exercise training. Myogenic tone is typically associated with small-diameter (<500 µm) vessels under physiological intravascular pressures (22). However, the presence of active resting tone in the absence of neural or humoral influences, i.e., myogenic tone, has been reported in larger vessels with the use of isometric tension techniques (1, 2, 15, 28). The mechanisms producing myogenic tone are proposed to be initiated by the mechanical stimulus of stretch, by increasing either intravascular pressure or length (22). Mechanical stretch depolarizes smooth muscle in an endothelium-independent manner, likely through activation of a nonselective stretch-activated cation (SAC) channel (9, 22). As currently modeled (22, 27), this depolarization activates dihydropyridine-sensitive, voltage-gated Ca²⁺ channels (VGCC), allowing Ca²⁺ influx, in addition to Ca²⁺ entry, directly through SAC channels, thus producing smooth muscle contraction. Membrane depolarization and Ca²⁺ influx are also proposed to activate K⁺ channels, which act as a negative-feedback mechanism to limit depolarization and VGCC activation and contraction (6, 42). Accordingly, inhibition of Ca²⁺-activated (KCa) and voltage-dependent K⁺ (Kh) channels by using pharmacological blockers has been shown to produce vasoconstriction of pressurized arteries (6, 17, 25) and increases in resting tension in isometric arterial preparations (1, 2, 15), providing strong evidence for a role for these K⁺ channels in regulating basal tone.

On the basis of the findings of Muller et al. (23) in resistance arteries, we hypothesized that, if this training adaptation were homogeneous within the coronary circulation, conduit arteries (>1.0 mm in diameter) would possess an enhanced K⁺-channel activation and thus be more responsive to K⁺-channel blockade after exercise training. Although conduit arteries contribute little to total coronary vascular resistance in the absence of disease, the presence of disease increases the
contribution of conduit arteries to coronary flow regulation (13). In addition, although coronary artery disease can produce functional abnormalities throughout the coronary arterial tree, lesion formation is primarily a macrovascular phenomenon (7). Therefore, understanding exercise-induced adaptations in conduit coronary arteries is vital to understanding the interaction of physical activity and coronary artery disease.

MATERIALS AND METHODS

Animals. Adult female miniature swine weighing 25–40 kg were obtained from the breeder (Charles River) and housed in pens at the College of Veterinary Medicine until use. Animal protocols were approved by the University of Missouri Animal Care and Use Committee in accordance with the “Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training.”

Training procedures. Animals were randomly divided into exercise-trained (Ex) or sedentary (Sed) groups. During the first week, the Ex pigs ran on the treadmill at 3 miles/h (mph), 0% grade, for 20–30 min (endurance) and at 5 mph for 15 min (sprint). The speed and duration of running were increased progressively at a rate dependent on the tolerance of each pig. During the 12th wk of training, a typical training session consisted of the following 85-min workout: 1) 5-min warm-up run at 2.5 mph, 2) 15-min sprint at speeds of 5–8 mph, 3) 60-min endurance run at 4–5 mph, and 4) 5-min warm-down run at 2 mph. Ranges of running speed are presented because the exercise training program is customized to the exercise ability of each pig. The pigs were given positive reinforcement to exercise by being fed after each training bout.

Preparation of coronary arteries. At least 24 h after completion of exercise training (or sedentary confinement), the pigs were anesthetized with ketamine (30 mg/kg) and pentobarbital sodium (35 mg/kg), heparinized, and the hearts were removed and placed in iced (4°C) Krebs bicarbonate solution for vessel dissection. Segments of conduit right coronary artery (>1.0 mm ID) were trimmed of fat and connective tissue in sterile modified Eagle’s minimal essential storage medium containing 20 mM HEPES plus 2% horse serum on ice.

Isometric tension determination. Standard isometric tension-recording techniques were used as previously described (29). Briefly, arterial rings (3-mm axial length) were cut under a dissection scope by using a micrometer eyepiece scaled to 25 µm per division. The endothelium was removed by gently rubbing the luminal surface with a tapered wooden dowel. Each vessel ring was mounted on two intraluminal wires (0.45 mm in diameter), one of which was fixed to a force transducer and the other attached to a linear-displacement transducer. The rings were submerged in a 25-ml organ bath containing physiological buffer plus 294 U/ml collagenase (CLS II, Worthington), 5 U/ml elastase (Worthington), 2 mg/ml bovine serum albumin (fraction V, Sigma Chemical), 1 mg/ml soybean trypsin inhibitor (type I-S, Sigma Chemical), and 0.4 mg/ml DNase I (type IV, Sigma Chemical). Smooth muscle cells were enzymatically dispersed by incubation for 45–60 min in a shaking water bath at 37°C with subsequent gentle trituration by micropipette. After dispersion, 2.5 µmol/l fura 2-AM (Molecular Probes) was added, and incubation continued at 37°C for an additional 20 min. Cell suspensions were then washed and stored in low-Ca2+ (0.5 mM) buffer at 4°C until use (0–6 h).

Simultaneous whole cell voltage clamp and fura 2 microfluorometry. Whole cell currents were determined by using the amphoterocin-perforated patch-clamp technique (32) simultaneously with fura 2 microfluorometry, as used routinely in our laboratory (35, 36, 38). Cells were superfused with physiological saline solution containing (in mM) 138 NaCl, 5 KCl, 2 CaCl2, 10 glucose, 24 HEPES, pH 7.4. Pipette solutions contained (in mM) 45 KCl, 75 K2SO4, 10 NaCl, 8 MgCl2, 10 HEPES, pH 7.1 with KOH, and 50 µg/ml amphoterocin B. Ionic currents were amplified by a List EPC-7 patch-clamp amplifier containing a headstage with switchable feedback resistors of 0.5 and 50 GΩ. Whole cell currents were filtered through an eight-pole, low-pass filter with a cutoff frequency of 400 Hz, digitized at 600-µs intervals, and stored and analyzed on a computer with customized AxoBASIC 1.0 software (Axon Instruments). Fire-polished patch pipettes (>2 MΩ) were sealed (seal resistance >1 GΩ) against the cell membrane with gentle suction. After seal formation, series resistance was monitored for determination of sufficient whole cell access, defined as a reduction in series resistance below 25 MΩ. Whole cell capacitance at 50 GΩ were obtained for each cell by normalization of whole cell current to cell capacitance. Ramp depolarizations (2,000 ms) from −90 to +70 mV were recorded for determination of voltage relationship during each experimental condition. Membrane potential (Vm) was determined by using current clamp mode.

Myoplasmic Ca2+ determination. Fura 2 was used for determination of myoplasmic Ca2+ concentration ([Ca2+]i), as done routinely in our laboratory (35, 36) and described in detail (37, 38). During patch clamp, cells were exposed to excitation light from a 150-W Xe arc lamp passed via liquid light guide through a rotating interference 340- and 380-nm filter wheel (50-ms rotation period) and reflected by a dichroic mirror (DM 400, Nikon) through a ×40 phase contrast oil-immersion objective. Fluorescence emission (480-nm barrier filter) was spatially defined to a single cell with an adjustable rectangular aperture. Fluorescence emission was amplified with a photomultiplier tube with high sensitivity at 510 nm (peak fura 2 emission). Sample-and-hold circuitry was used to separate 340- and 380-nm excitation-associated emission. Data acquisition and analysis were accomplished by using a Labmaster analog-to-digital converter and a microcomputer equipped with AxoBASIC 1.0 data-acquisition software (Axon Instruments). All experiments were conducted at room temperature (22–25°C). Cells were superfused with physiological saline solution under gravity flow.
Statistics. Data are means ± SE, with each animal counted as one observation. Repeated-measures ANOVA was used to compare current-voltage relationships within groups with paired t-test used for post hoc analysis. ANOVA and unpaired t-tests were used for comparison between groups. A P value <0.05 was set as the criterion for significance in all comparisons.

RESULTS

Efficacy of exercise training. Consistent with previous reports that used the treadmill-trained miniature swine model (20, 21, 23, 29), the Ex animals in the present study demonstrated marked training adaptations including a 43% increase in citrate synthase activity in the long head of the triceps brachii (Sed, 12.12 ± 0.86 vs. Ex, 17.29 ± 0.95 µmol·min⁻¹·g⁻¹; P < 0.05), an increased heart weight-to-body weight ratio (Sed, 4.28 ± 0.22 vs. Ex, 5.35 ± 0.17 g/kg; P < 0.05), and a 42 ± 6% increase in submaximal endurance time (P < 0.05 compared with Sed, –3.7 ± 4.1%).

Effect of K⁺-channel blockers on resting tension. Figure 1 shows a typical experimental tracing depicting the effect of K⁺-channel blockers on resting tension in conduit coronary arterial rings from both Sed and Ex animals. Inhibition of K⁺ channels by addition of either tetraethylammonium (TEA), iberiotoxin (IBTX), or 4-AP resulted in an increase in resting tension. The increase in resting tension to all K⁺-channel blockers was inhibited by the L-type Ca²⁺-channel antagonist diltiazem, administered either before or during K⁺-channel block (data not shown). These data indicate that K⁺ channels that are sensitive to TEA, IBTX, and 4-AP contribute to regulation of resting tension in isolated coronary arteries. Inhibition of these contractions by diltiazem is consistent with the hypothesis that the hyperpolarizing influence of K⁺-channel blockers depolarizes arteries and releases Ca²⁺-channel activation and contraction.

Fig. 1. Effect of K⁺-channel blockers on resting tension in conduit coronary arteries. Typical experimental tracings showing effect of K⁺-channel blockers tetraethylammonium (TEA; 1 mM), iberiotoxin (IBTX; 10 nM), and 4-aminopyridine (4-AP; 1 mM) on resting tension in conduit coronary arterial rings from sedentary (Sed; A) and exercise-trained (Ex; B) animals. Arterial rings were stretched to optimal length and allowed to equilibrate for 60 min before addition of K⁺-channel blockers (●). All K⁺-channel blockers produced a sustained increase in resting tension in arterial rings from both Sed and Ex animals.

Fig. 2. Effect of K⁺-channel blockers on resting tension after exercise training. Group data show increase in resting tension induced by K⁺-channel blockers (TEA, 1 mM; IBTX, 10 nM; and 4-AP, 1 mM) in coronary arteries from Sed and Ex animals. Change in resting tension is expressed both relative to developed tension response to 60 mM K⁺ (A) and in grams of developed tension (B). All K⁺-channel blockers produced a significant, similar increase in resting tension in arteries from Sed animals. After exercise training, all K⁺-channel blockers examined produced an increase in resting tension that was significantly greater than in Sed. In addition, differential effect of K⁺-channel blockers was found in Ex group, i.e., 4-AP > TEA > IBTX. *P < 0.05, Sed vs. Ex; #P < 0.05, vs. TEA; n = 13, 7, and 8 pigs (Sed), and 14, 7, and 7 pigs (Ex) for TEA, IBTX, and 4-AP, respectively.
both normalized and absolute resting tension was greater in arteries from Ex animals compared with those from Sed animals. Although the contractile response to 60 mM K\(^+\) was ~20% greater in Ex compared with Sed (13.76 ± 0.54 vs. 11.19 ± 0.53 g, P < 0.05), the contractile response to K\(^+\)-channel block was ~200–300% greater in Ex, even when normalized to the 60 mM K\(^+\) contraction. Thus the greater contraction induced by K\(^+\)-channel block after training is not simply a nonspecific response to depolarization, but, rather, the contribution of K\(^+\) channels sensitive to TEA, IBTX, and 4-AP to resting tension appears to be increased after exercise training.

Effect of exercise training on basal tone. Coronary rings from both groups were stretched to a similar extent at L\(_\text{o}\) (189 ± 7 and 188 ± 6%, Sed and Ex, respectively; P > 0.05). Effective inner diameter at L\(_\text{o}\) was 1.57 ± 0.04 and 1.54 ± 0.02 mm for Sed and Ex groups, respectively (P > 0.05). Although resting tension at L\(_\text{o}\) tended to be higher in Ex compared with Sed, this difference was not significant (Fig. 3). Supramaximal SNP (1 mM) significantly reduced resting tension in both groups; however, this reduction was approximately twofold greater in Ex compared with Sed, indicating an enhanced SNP-sensitive basal tone after exercise training.

Effect of K\(^+\)-channel blockers on K\(^+\) current in coronary smooth muscle cells. To determine whether the increased contribution of K\(^+\) channels to resting tension after exercise training was due to an increase in whole cell K\(^+\) current, we examined the effect of TEA and 4-AP on K\(^+\) current in perforated patch-clamped coronary smooth muscle cells. This technique allows control of intracellular monovalent ion concentrations and V\(_\text{m}\) while leaving the metabolic and second-messenger systems intact (32). Figure 4B shows representative experimental tracings showing current-voltage relationship obtained during ramp depolarization from -90 to +70 mV (A) in absence (a) and presence of either 1 mM TEA (b) or 4-AP (c) (B). Isolated smooth muscle cells were patch clamped by using perforated patch-clamp technique under physiological ion conditions (K\(^+\)-equilibrium potential approximately -90 mV). C: TEA-sensitive (a-b) and 4-AP-sensitive (a-c) currents were obtained by subtraction of currents in B. V\(_\text{m}\), membrane potential.

![Figure 4](http://jap.physiology.org/content/journal/jappl/2017/10.1152/jappl.00434.2017)

Fig. 4. Inhibition of whole cell K\(^+\) current by TEA and 4-AP. Representative experimental tracings showing current-voltage relationship obtained during ramp depolarization from -90 to +70 mV (A) in absence (a) and presence of either 1 mM TEA (b) or 4-AP (c) (B). Isolated smooth muscle cells were patch clamped by using perforated patch-clamp technique under physiological ion conditions (K\(^+\)-equilibrium potential approximately -90 mV). C: TEA-sensitive (a-b) and 4-AP-sensitive (a-c) currents were obtained by subtraction of currents in B. V\(_\text{m}\), membrane potential.

Fig. 3. Increased basal tone after exercise training. Comparison of resting tension and ramp tension in presence of 1 mM sodium nitroprusside (SNP) and SNP-sensitive tone in coronary arteries from Sed (n = 13) and Ex (n = 14) animals is shown. Resting tension was not affected by training (Sed, 5.21 ± 0.33 vs. Ex, 5.91 ± 0.54 g). Addition of SNP significantly reduced resting tension in both groups; however, SNP-sensitive component of resting tension was significantly greater in Ex compared with Sed. *P < 0.05, Sed vs. Ex.
In isolated single cells, the TEA- and 4-AP-sensitive components of whole cell K⁺ current were also unaffected by exercise training (Fig. 6). Similar results were obtained by using current-voltage relationships derived from short-ramp (200-ms) or 330-ms step depolarizations. Long-ramp depolarization data were provided, as this protocol had no effect on myoplasmic [Ca²⁺], in contrast to step depolarizations, which significantly increased myoplasmic [Ca²⁺] at positive test potentials. Thus in isolated single smooth muscle cells, basal and TEA- and 4-AP-sensitive macroscopic K⁺ currents appear unchanged by exercise training.

Effect of exercise training and K⁺-channel blockers on Vₘ. In addition to examining whole cell K⁺ current by using voltage clamp, we also examined the effect of TEA, IBTX, and 4-AP on resting Vₘ by using current-clamp techniques (Fig. 7). Basal Vₘ was unchanged by exercise training (−48.2 ± 4.6 vs. −47.2 ± 3.8 mV, Sed vs. Ex, respectively; P > 0.05). Consistent with the effect of K⁺-channel blockers on resting tension and whole cell K⁺ current, all K⁺-channel blockers examined produced a significant depolarization in both Sed and Ex groups. However, there was no effect of exercise training on the depolarization produced by K⁺-channel blockade.

Ca²⁺-channel activation potentiates TEA-induced rise in resting tension. As mechanical stretch is postulated to increase K⁺-channel activation secondary to increased depolarization and Ca²⁺ influx (6, 17, 22), we predicted that the increase in resting tension produced by K⁺-channel blockers in the present study would be potentiated by increasing Ca²⁺ influx. Figure 8 summarizes an experiment in which a coronary arterial ring from a Sed animal is exposed repeatedly to 1 mM of TEA in the presence of increasing concentrations of the L-type Ca²⁺-channel agonist BAY K 8644. As hypothesized, the TEA-induced increase in resting tension was increased by BAY K 8644 in a concentration-dependent manner (see Fig. 8, inset). Inhibition of L-type Ca²⁺ channels by diltiazem reversed the rise in tension in both the absence and presence of BAY K 8644, demonstrating that the effect of exercise training can be mimicked by Ca²⁺-channel activation.

DISCUSSION

Endurance exercise training produces numerous adaptations within the coronary circulation that alter regulation of coronary tone (5, 20, 23, 30). Although these adaptations are complex and heterogeneous, in general, exercise training is associated with an enhanced vasodilation and reduced vasoconstriction to vasoactive agonists (20, 30). Previously, Muller et al. (23) demonstrated an enhanced myogenic tone in porcine coronary resistance arteries (75–150 µm ID) after exercise training. With the use of an identical endurance training model, the present study demonstrated a similar increase in basal tone in conduit coronary arteries (>1.0 mm ID). Furthermore, exercise training was associated with an increased K⁺-channel contribu-
tion to regulation of basal tone, consistent with increased myogenic tone (6, 22, 25). Thus it appears that enhanced coronary tone is an adaptation of both resistance and conduit arteries to endurance exercise training.

Recently, Jain et al. (15) proposed a model in which resting tension is composed of two components: basal (active) tone and passive tone. In the present study, a supramaximal concentration of SNP was used to abolish the active component of resting tension. This SNP-sensitive tone was approximately twofold greater after exercise training, indicative of an increased basal tone after exercise training. Jain et al. further divided basal tone into an SNP-sensitive component (myogenic tone) and an SNP-insensitive component (intrinsic tone).

Intrinsic tone was identified by the increase in tension produced by warming a vessel from 6°C to 37°C. Therefore, the observed increase in basal tone after exercise training could be due to either an increased intrinsic tone or an increased myogenic tone. Several lines of evidence suggest that the increased basal tone after exercise training was myogenic, not intrinsic. First, the increased basal tone in the present study was SNP sensitive, unlike the intrinsic component described by Jain et al. Second, there was no difference in the reduction of resting tension produced by cooling vessels to 6°C compared with SNP (data not shown), indicating an absence of SNP-insensitive, temperature-sensitive tone. In addition, the increased basal tone produced by exercise training was measured in the absence of endothelium or neurohumoral influences and thus was truly “myogenic.” Finally, as discussed below, myogenic tone is associated with an increased sensitivity to K^+^-channel block. Thus the evidence suggests that, similar to resistance coronary arteries (23), conduit

Fig. 7. Effect of exercise training and K^+^-channel blockers on V_m in isolated coronary smooth muscle cells. V_m was determined during perforated patch-clamp experiments by using current clamp. Addition of K^+^-channel blockers produced significant reduction of V_m in both Sed (n = 6 pigs) and Ex (n = 5 pigs). Exercise training had no effect on basal V_m or K^+^-channel blocker-induced depolarization. *P < 0.05 vs. basal.

Fig. 8. Potentiation of TEA-induced contraction by BAY K 8644. Experimental tracings show effect of increasing concentrations of L-type Ca^2+^-channel agonist BAY K 8644 on TEA-induced increase in resting tension (conduit artery, Sed animal). Repetitive additions of TEA (1 mM) were added after 20-min preincubation with BAY K 8644 (10^{-10} to 3 \times 10^{-9} M). TEA-induced increase in resting tone was potentiated by BAY K 8644 in a concentration-dependent manner (inset). Diltiazem addition (arrows) inhibited TEA effect in absence and presence of BAY K 8644. BAY K 8644 alone had minimal effect on resting tension at concentrations used. Thus effect of exercise training can be mimicked by Ca^2+^-channel activation. Horizontal lines, TEA exposure. Tension is expressed as developed tension (DT) normalized to DT response to 60 mM K^+ (%60K).
coronary arteries demonstrate increased myogenic tone after endurance exercise training.

Although the mechanisms for generation of myogenic tone are incompletely understood, recent models propose that mechanical perturbation, either pressure or stretch, activates SAC channels in the smooth muscle membrane, resulting in membrane depolarization (22, 27). Depolarization activates dihydropyridine-sensitive VGCC, allowing Ca$^{2+}$ influx, in addition to Ca$^{2+}$ entry directly through SAC channels, thus producing smooth muscle contraction. Depolarization and Ca$^{2+}$ influx are also proposed to activate K$^{+}$ channels, which act as a negative-feedback mechanism to limit depolarization and VGCC activation and contraction (6, 42), and thus act as a brake on vasoconstriction. This negative-feedback contribution of K$^{+}$ channels to vascular tone regulation has been determined by use of selective K$^{+}$-channel blockers (1, 2, 6, 17, 25, 28). In vascular smooth muscle, K$_c$ channels are strongly inhibited by external TEA (K$_{50} = 150–300$ μM), whereas ATP-sensitive K$^{+}$ channels and K$_v$ channels are largely insensitive to TEA [K$_{50} > 7$ and >50 mM, respectively (26)]. IBTX is a highly selective blocker of K$_c$ channels [IC$_{50} = 250$ pmol/l (11)]. In contrast, K$_v$ channels, sometimes described as voltage-dependent delayed rectifier channels, are inhibited at relatively low 4-AP concentrations (26, 27, 33). Furthermore, in agreement with others (33), preliminary studies in our laboratory have determined that 1 mM 4-AP has no effect on single K$_c$-channel kinetics (data not shown). Thus, at the concentrations used in the present study, TEA and IBTX should preferentially block K$_c$ channels, whereas 4-AP should preferentially block K$_v$ channels.

In coronary arteries from both Sed and Ex animals, TEA, IBTX, and 4-AP all produced significant increases in resting tension, indicating a significant contribution of both K$_c$ and K$_v$ channels to regulation of resting tone. Interestingly, the increase in resting tension produced by all K$^{+}$-channel blockers was significantly greater in the Ex group, providing evidence for an enhanced role for both K$_c$ and K$_v$ channels in regulation of coronary tone after exercise training. In physiological ion conditions, V$_m$ of smooth muscle cells in pressurized arteries and arterioles has been measured between –40 and –60 mV, significantly more positive than the equilibrium potential for K$^{+}$ [approximately –85 mV (27)]. Thus, when activated, K$^{+}$ channels shift V$_m$ toward equilibrium potential for K$^{+}$, causing membrane hyperpolarization, thus limiting depolarization and vasoconstriction. Therefore, the increased K$^{+}$-channel activation in arteries from trained animals is consistent with an increased negative feedback, which limits membrane depolarization and vasoconstriction.

In arteries from Sed animals, there was no significant difference in the contractile response to any K$^{+}$-channel blocker. However, after exercise training, there appeared a differential response to the type of K$^{+}$-channel blocker, i.e., 4-AP > TEA > IBTX. Thus, in addition to a general increase in the contribution of K$^{+}$ channels to regulation of basal tone, exercise training appears to preferentially enhance the contribution of K$_v$ channels. In both groups, there was a tendency for TEA to produce a greater response than the selective K$_c$-channel blocker IBTX. This could be an indication of the partial nonselectivity of K$_c$-channel block by TEA or an inability of the large IBTX peptide to completely penetrate the medial layer of this large-artery preparation, thus leaving the innermost portion unblocked. This limited diffusion of IBTX compared with TEA could also explain the slower rate of the contractile response seen with the former (see Fig. 1). However, as both TEA and IBTX showed similar directional effects in arteries from Sed and Ex animals, we conclude that K$_c$ channels contribute to regulation of basal tone and that this contribution is increased after exercise training. Together, these data provide strong evidence for an enhanced role for both K$_c$- and K$_v$-channel activation in regulating coronary tone after exercise training.

On the basis of the enhanced effect of K$^{+}$-channel blockers on resting tension after exercise training in the intact artery, we hypothesized that there would be an associated increase in TEA- and 4-AP-sensitive K$^{+}$ current in coronary smooth muscle from Ex animals. To test this, we studied isolated smooth muscle cells using perforated patch-clamp techniques, in the presence and absence of the K$^{+}$-channel blockers TEA and 4-AP. Similar to previous reports that used whole cell dialyzing voltage clamp (35), exercise training had no effect on whole cell K$^{+}$ currents. We found, consistent with the effect of K$^{+}$-channel blockers on resting tension, a significant reduction in whole cell K$^{+}$ current and V$_m$ by all K$^{+}$-channel blockers in both Sed and Ex groups. However, contrary to our original hypothesis, exercise training had no effect on either the TEA- or 4-AP-sensitive components of whole cell K$^{+}$ current or the depolarization response to any K$^{+}$-channel blocker. Thus the enhanced K$^{+}$-channel contribution seen after exercise training in the intact vessel was not apparent in the isolated single-cell preparation. Whereas the reason for this discrepancy is presently unknown, we conclude that a requisite factor necessary for detection of the training-induced adaptation is absent in the isolated cell preparation.

One obvious difference between the intact artery and single-cell experiments is the lack of a mechanical stimulus, or stretch, in single cells. Previous studies have shown that K$_c$- and K$_v$-channel blockers depolarize and constrict small arteries only when pressurized, i.e., stretched (6, 17). Thus one plausible explanation is that the enhanced K$^{+}$-channel contribution after training is a consequence of stretch. Exercise training may increase the expression or activation of SAC channels to produce a greater depolarization to a given stretch or pressure stimulus, thus producing greater activation of both K$_c$ and K$_v$ channels.

Exercise training also increases the expression or activation of VGCC (4); thus a given depolarization may result in more Ca$^{2+}$ influx to produce a greater myogenic response and an enhanced K$_c$ channel activation. Although further studies are needed to fully
describe this training adaptation, evidence supporting a role for enhanced VGCC in \(K_{\text{Ca}}\) channel activation is suggested by the action of the selective VGCC-agonist BAY K 8644. As shown in Fig. 8, an increase in concentrations of BAY K 8644 potentiated the effect of TEA on resting tension. At 1 nM, BAY K 8644 produced only a minor increase in resting tension; however, the contractile response to TEA was increased more than fourfold. Thus the effect of exercise training on \(K_{\text{Ca}}\) channel activation can be mimicked by activation of VGCC. In addition, we have previously reported an enhanced \(Ca^{2+}\) influx in response to agonist stimulation in coronary arteries from trained animals (5). These data support an enhanced \(Ca^{2+}\) influx via VGCC as a mechanism for the enhanced \(K^+\)-channel activation after exercise training.

In addition to increased \(Ca^{2+}\) influx as a source of \(Ca^{2+}\) for activation of \(K_{\text{Ca}}\) channels, \(Ca^{2+}\) release from the sarcoplasmic reticulum (SR) is known to activate \(K_{\text{Ca}}\) channels (3, 25, 35). Spontaneous outward \(K^+\) currents (STOC) are the result of \(Ca^{2+}\) release via ryanodine receptors, which activates \(K_{\text{Ca}}\) channels (3, 25, 35). Recently, Nelson et al. (25) visualized discrete subcellular SR \(Ca^{2+}\) release events in cerebral arterial smooth muscle, i.e., \(Ca^{2+}\) sparks, which were correlated with STOC activity. As modeled by Nelson et al., STOCs produced by \(Ca^{2+}\) sparks summate to hyperpolarize and relax (or inhibit contraction of) arterial smooth muscle. Accordingly, the increased \(K_{\text{Ca}}\) activation seen in the present study after exercise training could be due to an increased \(Ca^{2+}\) spark and thus STOC activity. However, using a training model identical to that of the present study, Stehno-Bittel et al. (35) demonstrated a reduction in STOC activity in coronary smooth muscle after exercise training, despite an increased \(Ca^{2+}\) release. As modeled by Stehno-Bittel et al., exercise training increases the slow release of \(SR Ca^{2+}\) toward the sarcolemma for extrusion from the cell. As a consequence of the restricted subsarcolemmal space, this \(SR Ca^{2+}\) unloading establishes a high subsarcolemmal \(Ca^{2+}\) gradient, which can produce a steady-state increase in \(K_{\text{Ca}}\) current (36), as opposed to a transient \(K^+\)-current activation. Thus well-documented changes in \(SR Ca^{2+}\) regulation by exercise training may also contribute to enhanced \(K_{\text{Ca}}\) channel activation.

The enhanced role of \(K_v\) channels in regulation of coronary tone after training is especially intriguing. These channels are activated by depolarization (17, 40), mechanical stretch (41), and phosphorylation (8, 18), and are inhibited by intracellular \(Ca^{2+}\) (12, 31). Thus a number of possible mechanisms could be altered by exercise training to increase \(K_v\)-channel activation. However, given the central role of stretch-induced depolarization in activating these channels (17), one could speculate that a greater depolarization response to stretch induced by training may underlie the enhanced role of \(K_v\) channels found in the present study.

Whereas an increased tone in resistance (23) and conduit (present study) arteries after exercise training would appear counterintuitive in relation to the overall pattern of enhanced vasodilation and reduced vasoconstriction associated with exercise training (20, 30), the training-induced increase in tone may actually provide the basis for enhanced vasodilation. A recent study in humans (14) compared proximal (i.e., conduit) coronary artery diameter in ultradistance runners and sedentary counterparts by using coronary arteriography. Under basal conditions, the proximal right coronary artery cross-sectional area was not affected by training; however, intracoronary infusion of nitroglycerin produced a 2.2-fold greater increase in arterial cross-sectional area in runners than in sedentary controls. On the basis of the finding that basal coronary artery diameter was similar in runners and sedentary controls, despite an increased maximal diameter in the former, the authors (14) concluded that the coronary arteries of runners may exhibit greater vascular tone. This enhanced tone, especially in resistance size arteries, could contribute to the enhanced coronary vasodilatory capacity seen after exercise training (20, 21).

In conclusion, endurance exercise training was found to increase basal tone and the contribution of both \(K_{\text{Ca}}\) and \(K_v\) channels to regulation of tone in conduit coronary arteries, similar to previous reports of increased myogenic tone in coronary resistance arteries (23). The inability to correlate the enhanced sensitivity of intact arteries to \(K^+\)-channel blockade with an enhanced \(K^+\) current or \(V_m\) change in isolated single cells may indicate that a requisite factor for expression of this training-induced adaptation, possibly stretch, is absent in isolated cells.

We thank Charles Williams, Qicheng Hu, Pam Thorne, and Tammy Strawn for invaluable assistance in this study. We also thank Dr. R. Shebuski of Pharmacia-Upjohn for the gift of iberiotoxin.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-52490 (to M. H. Laughlin, M. Sturek, D. K. Bowles), HL-41033 and HL-02872 (to M. Sturek), and HL-36531 (to M. H. Laughlin).

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Received 18 june 1997; accepted in final form 13 November 1997.

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Exercise training and coronary tone.


