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European Journal of Pharmacology 514 (2005) 111 – 119
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Alteration of arterial smooth muscle potassium channel composition and BK_{Ca} current modulation in hypertension

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Received 28 February 2005; accepted 21 March 2005
Available online 28 April 2005

Abstract

We investigated K^+ currents and their regulation by the sarcoplasmic reticulum in mesenteric arterial smooth muscle cells of the spontaneously hypertensive rat (SHR). Using perforated patch-clamp technique, we found the overall K^+ current density was significantly lower in adult SHR compared to adult Wistar–Kyoto rats (WKY). The K^+ currents were almost exclusively of large-conductance Ca^{2+}-dependent (BK_{Ca}) variety in SHR, but largely of voltage-gated (Kv) variety in WKY. Western blot assay showed parallel findings. These differences were not observed in pre-hypertensive rats. Depleting the intracellular Ca^{2+} store inhibited the K^+ currents in adult SHR. Ryanodine augmented the K^+ current at 1 μM, but suppressed it at 10 μM; 2-aminoethoxydiphenyl borate demonstrated concentration-dependent inhibition. We conclude that an alteration of membrane K^+ channel composition has resulted in lower overall K^+ current density. The changes in K^+ current type may indicate an underlying defect in Ca^{2+}-handling that predisposes smooth muscle cells to the hypertensive phenotype.
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Keywords: K^+ current density; Hypertension; Sarcoplasmic reticulum; Ryanodine receptor

1. Introduction

Contraction of vascular smooth muscle is largely driven by voltage-dependent Ca^{2+}-influx. As such, one might expect the smooth muscle cells from hypertensive animals to be more depolarized than their normotensive counterparts, a prediction which has been substantiated in some studies (Cheung, 1984) but not others (England et al., 1993). Since K^+ currents play a major role in setting the resting membrane potential and suppressing membrane excitability, a further prediction is that the membranes of hypertensive animals should exhibit less total K^+-current than normotensive animals. Several studies have tested this prediction using the patch clamp technique, and obtained varying results depending on the experimental conditions and tissue type used. For example, when the holding potential approximated the resting membrane potential, total K^+ currents were approximately equivalent between SHR and WKY mesenteric arteries when endogenous Ca^{2+}-handling mechanisms were left intact (Cox et al., 2001a,b) (by using the perforated patch configuration), but greater in SHR aorta than WKY when $[Ca^{2+}]_i$ is clamped high (Liu et al., 1997). When $[Ca^{2+}]$ was strongly buffered (e.g., using the whole-cell configuration) to suppress Ca^{2+}-dependent K^+-channels, the total K^+-currents evoked by step depolarizations were once again greater in SHR mesenteric (Cox et al., 2001a,b) and cerebral (Liu et al., 1998) arteries compared to WKY, while the opposite was found in aortic cells (Cox, 1996; Rusch et al., 1992). When the holding potential was elevated to inactivate voltage-dependent K^+-channels, the current remaining (primarily Ca^{2+}-dependent K^+ current) was substantially greater in SHR mesenteric arteries (Cox et al., 2001a,b) but not the aorta (Cox, 1996). At the unitary

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doi:10.1016/j.ejphar.2005.03.032

level, the probability of K_{Ca} channel opening is increased in SHR aorta (England et al., 1993), but not different between WKY and SHR cerebral arteries (Amberg and Santana, 2003). Thus, most studies showed total K^+ currents in general, and Ca^{2+}-dependent K^+ current in particular, to be increased in the SHR animals, a finding which contradicts the expected outcome stated above.

This greater amount of K^+ current is not due to changes in the fundamental properties of the channels, since there was no difference between SHR and WKY with respect to K_{Ca} unitary conductance (Amberg and Santana, 2003; England et al., 1993; Liu et al., 1998), nor voltage-dependence of K^+ current activation or inactivation (Cox, 1996; Cox et al., 2001a,b). On the other hand, increased K_{Ca} current may be secondary to increased Ca^{2+}-sensitivity and/or to increased density of the channels in the membrane. Ca^{2+}-sensitivity is indeed increased in SHR aorta (England et al., 1993; Rusch et al., 1992) but equivalent in SHR and WKY cerebral arteries (Liu et al., 1998). With respect to K_{Ca} channel density, aortic cells of SHR animals show greater number of functional channels per patch (England et al., 1993) and greater expression of channel proteins (Liu et al., 1997); however, conflicting data were obtained for the cerebral vasculature, in which there is either greater (Liu et al., 1998) or equivalent (Amberg and Santana, 2003) expression of channel proteins between SHR and WKY membranes, and no difference in number of channels per patch (Amberg and Santana, 2003). K^+ channel expression has not yet been examined in mesenteric arteries.

K_{Ca} channels are activated by voltage-dependent Ca^{2+}-influx as well as by release of internal Ca^{2+} from the sarcoplasmic reticulum. The latter can occur spontaneously in the form of Ca^{2+}-sparks, producing a sudden brief K^+ current response referred to as a spontaneous transient outward current, or STOC. A recent study found no significant difference between SHR and WKY cerebral arteries with respect to spark/STOC frequency nor amplitude (Amberg and Santana, 2003), while STOC activity was somewhat more prevalent in depolarization-evoked currents in SHR aorta (England et al., 1993). STOC and spark activity have not yet been examined in other resistance arteries of the SHR.

Altogether, then, there remains considerable conflict and disparity with respect to the changes and regulation of K^+ currents in the spontaneously hypertensive rat model. In this paper, we compare K^+ currents in smooth muscle cells from mesenteric resistance arteries of young SHR (pre-hypertensive) and adult SHR (hypertensive), as well as their normotensive WKY counterparts, under native recording conditions (negative holding potential and intact Ca^{2+}-homeostatic mechanisms). We found total K^+ current to be decreased in SHR membranes (consistent with their more depolarized and hypertensive state), although the net contribution of K_{Ca} currents was dramatically greater in SHR compared to WKY. Furthermore, we found that release of internal Ca^{2+} via ryanodine receptors is a major trigger for the enhanced K_{Ca} activity.

2. Materials and methods

2.1. Isolation of smooth muscle cells from rat mesenteric arteries

Adult (6–8 months old) and pre-hypertensive (3–4 weeks old) SHR and age-matched WKY were obtained from the rat colonies originated from the Charles River strain and maintained at the McMaster University Central Animal Facility. After the rats were euthanized by intraperitoneal injection with sodium pentobarbital (50 mg/kg), the secondary branches (150–200 μm in diameter) of mesenteric artery were carefully dissected under a microscope. The arteries were cleaned of connective tissue and stored at 4 °C in oxygenated Krebs solution. The arteries were minced and transferred to a digestion tube containing collagenase and elastase (see details in Chemicals and Solutions). The tissues were incubated with the enzymes in a 37 °C bath for 45 min and carefully triturated to liberate free smooth muscle cells. The resulting smooth muscle cell suspension was stored at 4 °C and used within 8 h. Individual cells were allowed to adhere to the glass bottom of a 1-ml recording chamber, and then were superfused with Ringers' solution at a rate of 2 ml/min.

2.2. Patch-clamping experiments

Membrane potassium currents were recorded at room temperature using the nystatin perforated patch configuration (0.3 mg/ml). Electrophysiological responses were tested in cells that were phase dense and appeared relaxed. Micropipettes (tip resistance 3 to 5 MΩ) were made from borosilicate glass capillary tubing (Sutter Instrument Co, Novato, CA) using a programmable puller (model P-87, Sutter Instrument Co, Novato, CA) and then fire polished (MF-830, Narishige, Japan). Access resistances ranged from 11 to 40 MΩ (compensated 70%), and whole cell capacitance ranged from 8 to 33 pF. Membrane currents were filtered at 1 kHz and sampled at 2 kHz. Current signals were converted from analog to digital format (DigiData 1200, Axon Instruments, Foster City, CA), and stored on the computer. Acquisition and analysis of data were accomplished using Axopatch 200B and pCLAMP8 software (Axon Instruments, Foster City, CA). Drug application was achieved by a micropuffer (Picospritzer™ II, General Valve Corp, Fairfield, NJ).

Pipettes were sealed to the cell using a negative pressure at 0 mV. Holding potential was set to −70 mV. For perforated patch, cell break-in was achieved by the pore-forming antibiotic nystatin. Access resistance was compensated by 70% prediction and 70% correction. After a suitable access resistance (<40 MΩ) was gained, membrane potential was stepped from −60 to +50 mV in 10 mV increments at 3 s intervals. In most experiments, current–voltage (I/V) relationships were recorded before and after drug application. The K^+ currents were also measured during step depolarization to either +30 or +50 mV, delivered at 10–15 s intervals. The data were later plotted and analyzed using Clampfit 8.0

and SigmaPlot 2000 software. Currents were standardized according to membrane area (using cell capacitance).

2.3. Western blots assay

Mesenteric arteries from different group of rats (4 adult WKY, 4 adult SHR, 3 young WKY, and 3 young SHR) were collected and stored at −80 °C. The tissues were homogenized in buffer containing 20 mM Tris (pH 7.5), 75 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.1% β-mercaptoethanol, 25 μg/ml aprotinin, 25 μg/ml leupeptin, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride. Samples were then centrifuged at 10,000 × *g*. The supernatant was collected for immunoblot analysis. Ten micrograms of denatured protein was fractionated by 10% sodium dodecyl sulfate-polyacrylamide gel (Bio Rad Labs, Hercules, CA) electrophoresis. Proteins were then electrophoretically transferred onto nitrocellulose membranes (Hybond-ECL, Amersham). The membranes were probed with polyclonal rabbit anti-BK_{Ca}, anti-Kv1.2, and anti-Kv1.5 (1:300 dilution; Alomone Labs, Jerusalem, Israel), followed by secondary horseradish peroxidase-conjugated goat anti-rabbit IgG(1:15000 dilution; Sigma). Blots were detected with enhanced chemiluminescence (ECL, Amersham). β-actin was used for loading control.

2.4. Chemicals and solutions

Digestion solution containing collagenase (type F, from Clostridium histolyticum, 1 mg/ml), elastase (type IV, from porcine pancreas, 0.25 mg/ml), papain (from papaya latex, 0.03 mg/ml), 1,4-dithio-L-threitol (0.75 mg/ml), and bovine serum albumin (1 mg/ml), in Ca^{2+}- and Mg^{2+}-free Hanks' solution was used. Electrode solution had the following components (in mM): 140 KCl, 1 $MgCl_2$, 0.4 $CaCl_2$, 20 HEPES, and 1 EGTA (pH = 7.2). For perforated patch recording, the pipette tip was back-filled with nystatin-free electrode solution, and the remainder of the pipette was filled with nystatin solution. The nystatin solution was prepared as 30 mg/ml stock in DMSO, then freshly diluted to a final concentration of 0.3 mg/ml in electrode solution. The cells were constantly perfused using standard Ringer solution which contained the following (in mM): 130 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 20 HEPES, and 10 D-glucose (pH = 7.4).

HEPES, $MgCl_2$ and KCl were purchased from BioShop Canada Inc. (Burlington, ON). D-glucose was purchased from Merck Inc. (Darmstadt, Germany). DMSO was obtained from Caledon Laboratories Ltd. (Georgetown, ON). All other chemicals were purchased from Sigma (St. Louis, MO). Anti-BK_{Ca}, anti-Kv1.2, and anti-Kv1.5 antibodies were purchased from Alomone Labs Ltd. (Jerusalem, Israel).

2.5. Data analysis

All values are expressed as means ±S.E.M. unless otherwise stated. Statistical comparison of membrane K^+ current density of four groups of rats was performed by two-way analysis of variance (ANOVA) using SigmaStat™ (Version 1.01), followed by a Tukey's post-hoc test using Statsoft™. $P < 0.05$ was considered statistically different.

3. Results

3.1. Membrane K^+ current components in adult SHR and WKY

Systolic blood pressure of adult SHR (197 ± 10 mm Hg, $n = 8$) was significantly higher than WKY (131 ± 7 mm Hg, $n = 8$, $P < 0.05$). Incrementing step depolarizing commands (+10 mV increments from holding potential of −70 mV) were used to compare the K^+ currents present in smooth muscle cells from adult SHR and WKY. These commands evoked families of K^+ currents that differed between the two strains of rat in at least two fundamental ways.

First, the overall current densities were markedly smaller in SHR compared to WKY. At +50 mV, these were 10.6 ± 2.0 pA/pF ($n = 6$) and 18.6 ± 3.4 pA/pF ($n = 6$), respectively (as determined 170–340 ms after onset of the voltage pulses, at which time a stable current activation was achieved; Fig. 1A). The mean *I*/*V* relationships for these two groups are given in Fig. 1B. We estimated the resting membrane potential (V_R) in each cell by interpolating each individual current–voltage relationship and deriving the voltage at which total membrane current was zero (which is, by definition, V_R). In this way, we found V_R to be −33.3 ± 5.7 mV ($n = 6$) and −55.8 ± 5.5 mV ($n = 6$, $P < 0.05$) in SHR and WKY, respectively.

Second, the currents differed markedly with respect to time-course. In the WKY, the K^+ currents activated monotonically and reached a relatively stable plateau within 100 ms, whereas cells from SHR were comprised largely of spike-like transient currents reminiscent of STOCs. These transient currents occurred randomly over the duration of the depolarizing pulse; integration over the same period of the voltage pulse gave mean current densities which were significantly smaller in the SHR than WKY ($P < 0.05$) (Fig. 1B).

3.2. Membrane K^+ current components in pre-hypertensive SHR and WKY

In our inbred colonies, the blood pressure in young SHR (3∼4 weeks) is not elevated as compared with age-matched WKY (Dickhout and Lee, 1998). K^+ currents in the cells from young SHR and WKY rats were similar in appearance to those evoked in adult WKY rats, with a largely monotonically activating outward current superimposed by fewer and smaller STOC-like currents, and a mean current density at +50 mV of 40.3 ± 13.3 pA/pF and 29.9 ± 5.5 pA/pF, respectively (Fig. 1A and B). Two-way ANOVA showed that the currents from these three groups of rats (young SHR and WKY, and adult WKY) were not statistically different from each other, but were significantly greater ($P < 0.05$) than the currents evoked in the cells from adult SHR (Fig. 1B). We also examined VR as described above and found no significant difference between young WKY and SHR (−41.7 ± 7.0 mV versus −40 ± 12.4 mV).

3.3. Effects of different channel blockers on SHR membrane K^+ currents

To confirm that the K^+ currents recorded in adult SHR smooth muscle cells were in fact primarily Ca^{2+}-dependent

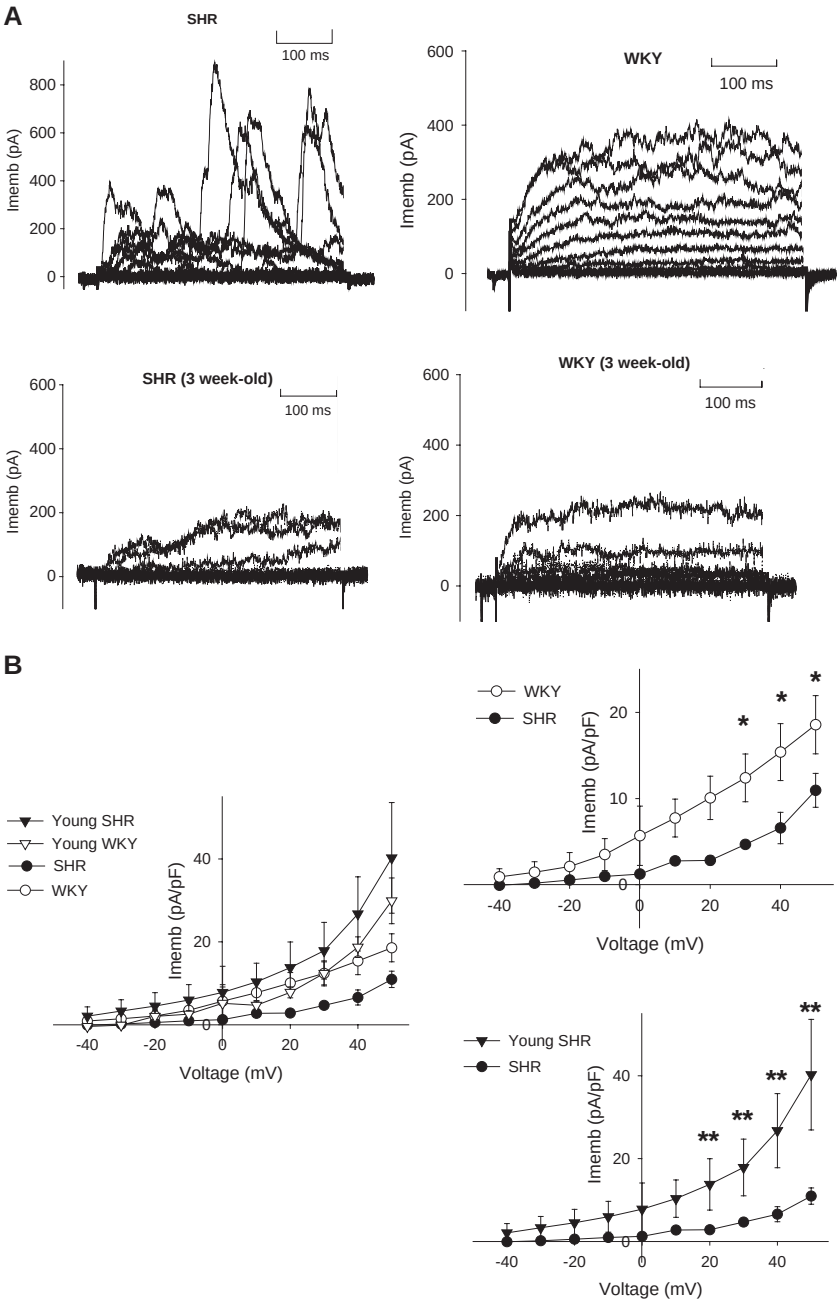


Fig. 1. A: Current–voltage relationships were investigated using incrementing depolarizing steps (from −60 to +50 mV; 10 mV increments at 1.2 s interval; $V_h = -70$ mV). Representative original traces of each group are shown. Note the presence of STOC-like currents only in the adult SHR, and their absence in the other 3 groups. B: Average K^+ current densities (normalized by cell capacitance; range of integration: 170 ms to 340 ms) were compared among adult and young (3-week-old) SHR and WKY. Each value represents the mean±S.E.M. ($n = 6$). *$P < 0.05$ and **$P < 0.01$.

currents (accounting for their STOC-like appearance), we examined the effects of a number of K^+ channel blockers on these currents in SHR. The BK_{Ca} channel inhibitor iberiotoxin (100 nM) suppressed the membrane K^+ currents in SHR by 56±14% ($n = 4$), while the non-selective K_{Ca} channel blocker tetraethylammonium (1 mM) suppressed the membrane K^+ currents by 68±12% ($n = 5$) (Fig. 2); this inhibition was easily reversed by washout. The Kv channel blocker 4-aminopyridine (1 mM), on the other hand, had little or no effect on these currents (12±8% inhibition; $n = 4$). The portion of K^+ currents affected by different channel blockers is shown by the I/V plots (Fig. 2).

3.4. Expression of K^+ channel proteins in SHR versus WKY

To test whether the differences in K^+ current activity are associated with changes in the relative populations of K^+ channels, we compared the relative levels of K^+ channel proteins in the four groups of rats (adult SHR and WKY, young SHR and WKY) using antibodies against BK_{Ca}, Kv1.2 and Kv1.5 proteins, since others

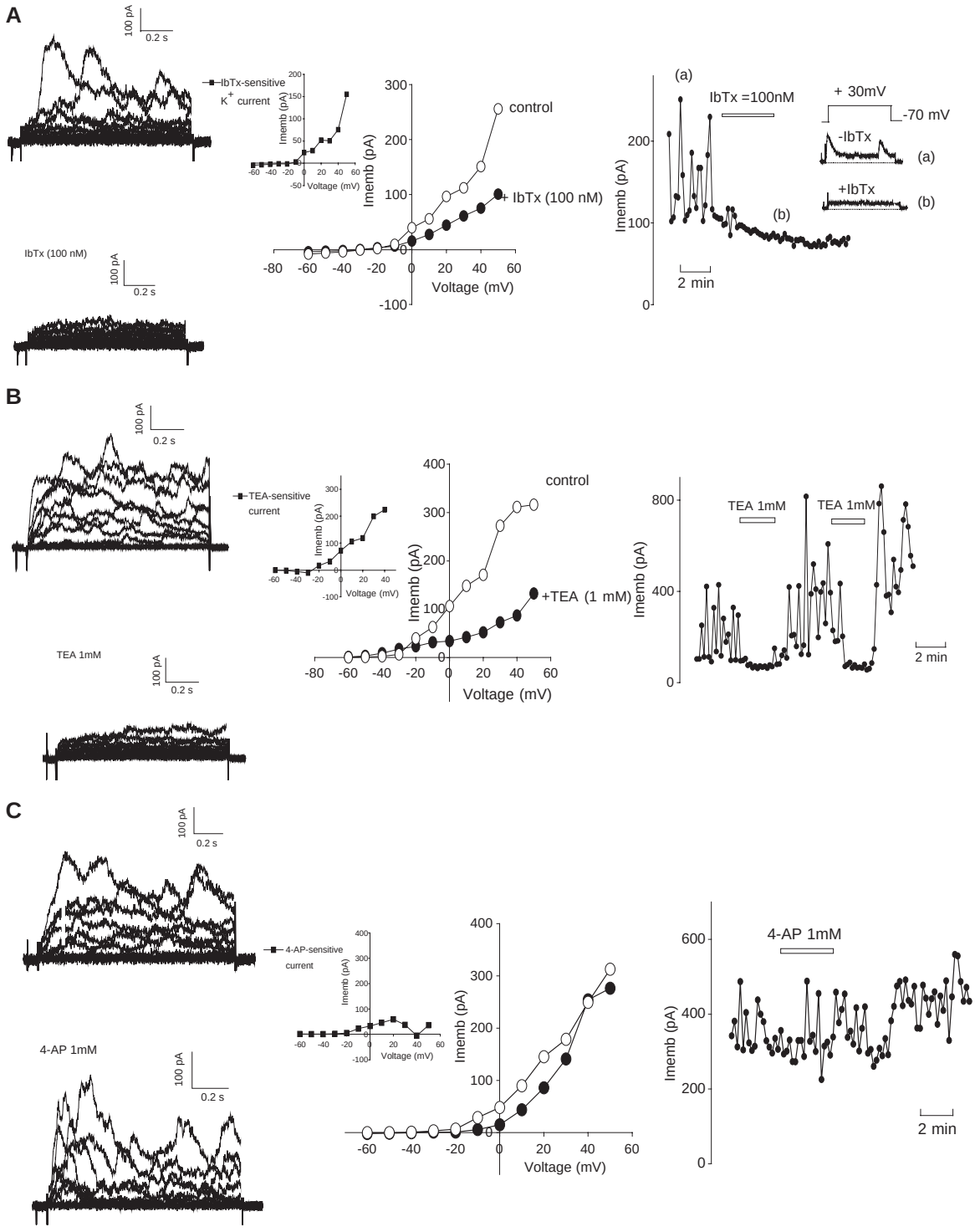


Fig. 2. Depolarizing step commands (as described in Fig. 1) were used to elicit K^+ currents from mesenteric SMCs of SHR in the presence or absence of iberiotoxin (100 nM; A), tetraethylammonium (1 mM; B) or 4-aminopyridine (1 mM: C). The raw currents (control versus inhibitors) are shown in the left panel, and the mean *I*/*V* relationships are plotted in the middle panel; inset of middle panel shows the difference current obtained upon subtraction of currents obtained in the presence of blocker from control currents. Right panels: Mean magnitude of K^+ currents evoked using a single depolarizing step to +30 mV, from a holding potential of −70 mV, delivered at 15 s intervals: these mean currents were highly variable in magnitude due to the STOC-like currents, and were reversibly suppressed by iberiotoxin and TEA, but not by 4-AP.

have shown that these channels predominate in this tissue (Cox et al., 2001a,b; Grissmer et al., 1994). Western blot analysis of the membrane protein extractions demonstrated that BK_{Ca} channel proteins were significantly higher in cells from adult SHR than adult WKY. The expression of Kv1.2 and Kv1.5 were significantly lower in cells from SHR than WKY (Fig. 3A-1). Such differences were absent between young SHR and WKY (Fig. 3A-2). Protein/β-actin ratios in the four groups of rats are shown in Fig. 3B.

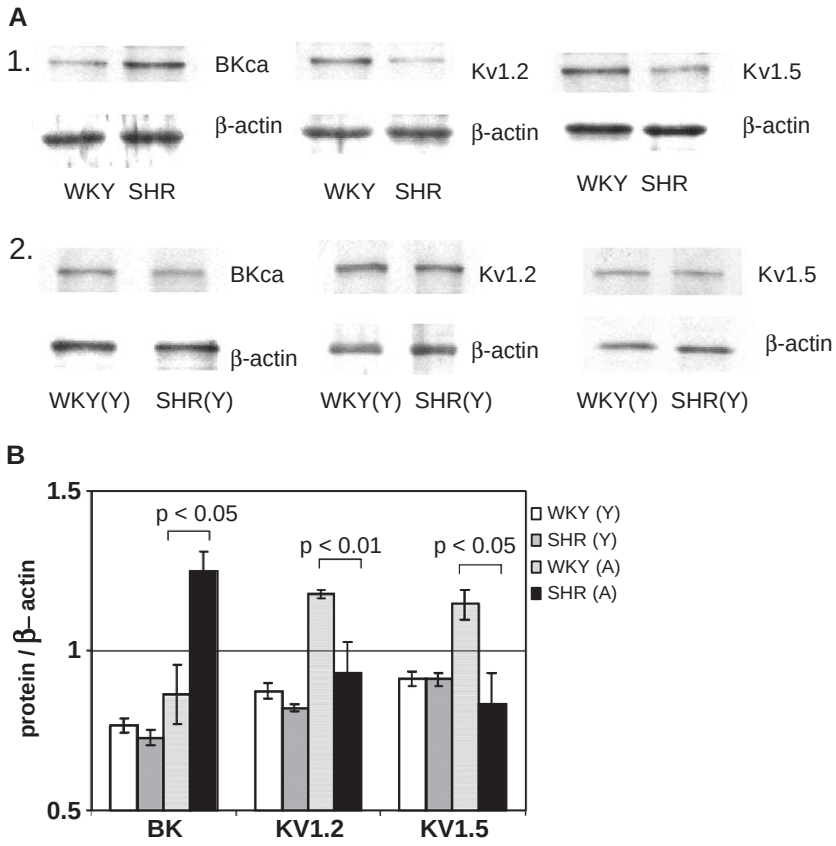


Fig. 3. A-1. Western blot analysis of membrane proteins extracted from adult SHR and adult WKY rats. A-2. Analysis of proteins extracted from young SHR and young WKY. Extracts were separated by SDS gel electrophoresis, then probed using polyclonal rabbit anti-BK_{Ca}, anti-Kv1.2, or anti-Kv1.5 antibodies (see Section 2). Equal loading of proteins was later confirmed by stripping the gels and re-probing with polyclonal anti-β-actin antibody. Each gel was representative of four independent experiments. B. The 4 groups of protein levels were normalized against β-actin control using densitometry, then plotted to show the statistical difference.

3.5. Effects of sarcoplasmic reticulum Ca^{2+} release on membrane K^+ current in adult SHR and WKY

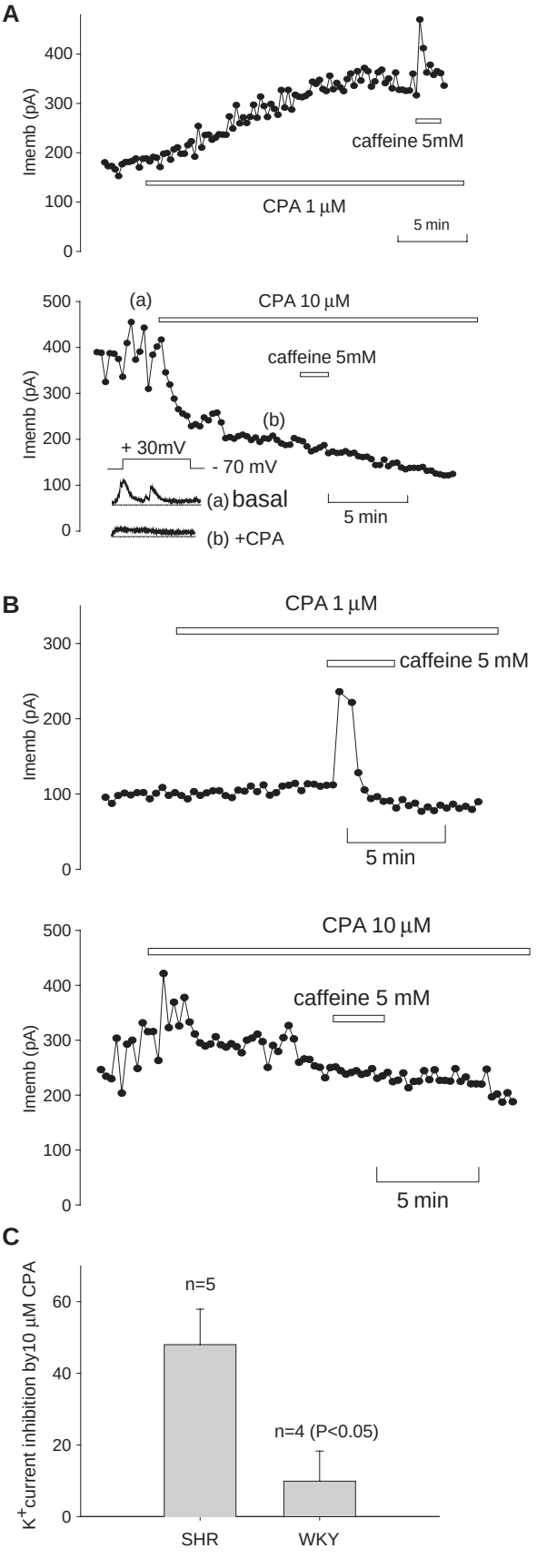
The time-course and pharmacological sensitivity of the currents recorded from adult SHR smooth muscle cells suggested that they might be regulated in large part by the release of internal Ca^{2+} (in the form of "sparks"). We therefore examined the contribution of the sarcoplasmic reticulum Ca^{2+} to these currents.

The suppressive effect of 10 μM cyclopiazonic acid (CPA) on adult SHR membrane K^+ currents became evident within 2 min after its application to the bathing medium, and maximal inhibition (48.0±9.9%, $n=5$) was reached within 10 min. At this point, the internal pool of Ca^{2+} was depleted, since caffeine (5 mM) did not evoke any change in K^+ current (Fig. 4A) or contraction (data not shown). At a lower concentration (1 μM), CPA gradually augmented membrane K^+ currents, after which caffeine (5 mM) was able to trigger some Ca^{2+} release, as indicated by a small outward K^+ current. In the WKY control animals, 10 μM CPA induced an inhibition of 9.8±8.4% ($n=4$, $P<0.05$); the depletion of internal Ca^{2+} was confirmed by the fact that 5 mM caffeine did not evoke a current pulse. On the other hand, 1 μM CPA had little effect on the K^+ current, and subsequent application of caffeine triggered a response (Fig. 4B). The comparison of the effects of CPA are plotted in Fig. 4C.

The effects of ryanodine receptor-mediated Ca^{2+} release was studied using ryanodine applied from a micropuffer placed close to the cells. At 10 μM, ryanodine suppressed the K^+ currents (38±13%, $n=4$) within 6 to 8 min after the application. At a lower concentration (1 μM), ryanodine exerted an excitatory effect on membrane K^+ currents, with maximal augmentation (15±14%, $n=5$) at 6 min after application (Fig. 5). Moreover, the STOCs were largely abrogated in response to 10 μM ryanodine, but increased in both magnitude and frequency in response to 1 μM ryanodine. These observations further suggest that ryanodine only influenced the Ca^{2+}-dependent BK component, with Kv being less affected. The inositol 1, 4, 5-trisphosphate ($InsP_3$) receptor antagonist 2-aminoethoxydiphenyl borate inhibited membrane K^+ currents by 22±7% at 100 μM, but had little effect at 10 μM concentration (Fig. 5).

4. Discussion

As outlined in the Introduction, there is considerable disparity and contradiction with respect to whether/how K^+ currents are altered in the SHR model. Much of the discrepancy probably arises from the differences in experimental recording conditions (particularly holding potential and buffering of $[Ca^{2+}]_i$) and types of vascular preparations used. None of the studies of K^+ currents to date have compared the changes in the SHR animals before and during



development of hypertension. In this study, we examined K^+ currents in the smooth muscle cells from resistance arteries of young and adult rats of the SHR and WKY using experimental conditions that approximate the native state.

Hypertension is expected to be associated with membrane depolarization, resulting in increased probability of voltage-dependent Ca^{2+} channel opening. Consistent with this, we found that the voltage at which net membrane current was zero (a condition which defines resting membrane potential) to be significantly more positive in SHR compared to WKY (approximately −33 mV and −56 mV, respectively). This more depolarized membrane potential would result in higher resting tone of the arterioles, and contribute to the vascular resistance seen in hypertension. Others have found no difference between the mean membrane potentials in SHR and WKY rats (England et al., 1993): however, those measurements were made under conditions in which Ca^{2+}-homeostatic mechanisms were disrupted (by dialyzing the cells internally with a zero-Ca^{2+} solution containing EGTA).

Consistent with our observation that SHR membranes are considerably depolarized relative to their WKY counterparts, we also found overall K^+ current density to be decreased in the former group. While others have shown total K^+-currents to be increased in SHR, this is normally associated with an inhibitory effect on vasomotor response: it is therefore difficult to put those other observations in the context of the hypertensive status.

Interestingly, although total K^+ currents were decreased in the adult SHR (hypertensive) animals, we also found that the net contribution of Ca^{2+}-dependent K^+ currents was greatly increased in these animals compared to pre-hypertensive SHR and age-matched young WKY. In particular, STOC activity was consistently present in cells obtained from adult SHR animals, but was essentially absent in the pre-hypertensive young SHR as well as in the young and adult WKY. Cox et al. have reported their findings of K^+ channel subtypes in WKY with selective channel blockers (Cox, 1996; Cox et al., 2001a,b), we have also observed that Kv current predominated in WKY (Gao et al., 2003). These electrophysiological findings were further supported by Western blot assay in this study, which revealed a parallel change in the membrane K^+ channel protein. In particular, we found considerably lower staining of Kv1.2 and a greater staining of

Fig. 4. A. K^+ currents were evoked from SHR myocytes using single depolarizing steps as described in Fig. 2. Left panel: 1 μM CPA caused a gradual enhancement of mean amplitude of currents evoked by pulses to +30 mV, delivered at 15 s intervals: mean amplitudes reached a peak 15 min after introduction of CPA, at which point the store was not completely depleted (as indicated by a small response to caffeine). Right panel: 10 μM CPA rapidly and markedly suppressed the mean magnitudes of the evoked currents; loss of responsiveness to caffeine confirmed depletion of the Ca^{2+} store. Insets show raw tracings evoked before and during application of 10 μM CPA. B. CPA effect on K^+ currents in WKY control. Left panel: 1 μM CPA had little effect on K^+ currents, and caffeine evoked a transient increase on mean amplitudes of K^+ currents. Right panel: 10 μM CPA showed little suppression of evoked current, compared with that in SHR. C. Percent inhibition of K^+ currents by 10 μM CPA in SHR and WKY.

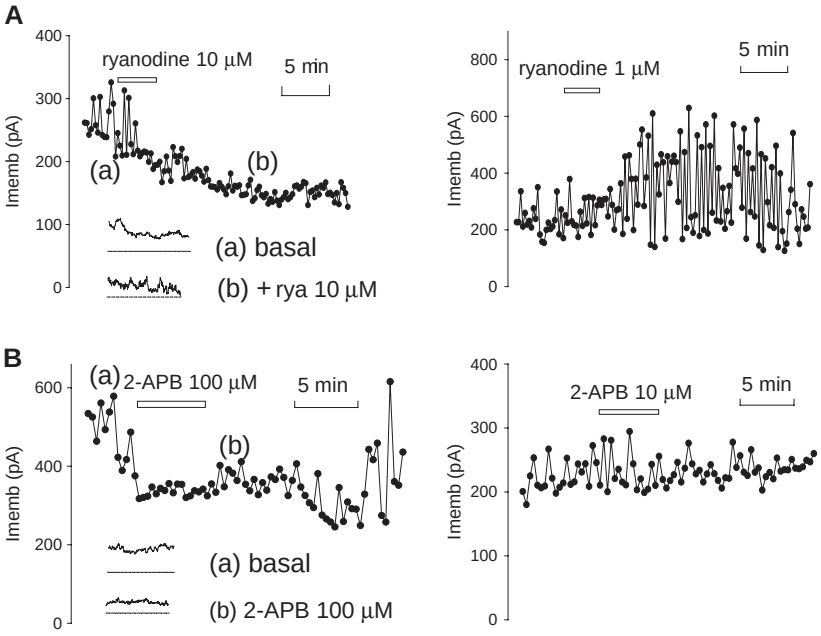


Fig. 5. The effects of ryanodine and of 2-aminoethoxydiphenyl borate on depolarization-evoked K^+ currents were investigated using a similar strategy as described in Fig. 4. (A) Ryanodine exerted similar time- and concentration-dependent changes in K^+ currents as did CPA. That is, the currents were gradually suppressed by 10 μM ryanodine (left panel), but slightly enhanced by 1 μM ryanodine (right panel); the latter was accompanied by a marked increase in STOC-like activity. (B) 2-aminoethoxydiphenyl borate suppressed the magnitude of K^+ currents at 100 μM (left panel) but had little or no effect at 10 μM (right panel).

BK_{Ca} in adult SHR compared to adult WKY using the specific antibodies to these channels. The change in BK_{Ca} expression may vary between arterial beds, since another group (Amberg and Santana, 2003) found no statistical difference in expression of BK_{Ca} proteins (neither α- nor β1-subunits) in basilar or cerebral arteries of SHR versus WKY rats.

The marked increase in STOC activity in SHR suggests a fundamental change in Ca^{2+} handling in mesenteric arteries, with more frequent discharge from the sarcoplasmic reticulum (Ca^{2+} sparks). Consistent with this, we found both the STOC activity and the majority of the depolarization-evoked currents were largely abrogated by completely inhibiting sarcoplasmic–endoplasmic reticulum Ca^{2+} ATPase activity using cyclopiazonic acid. If Ca^{2+}-influx contributed to any substantial degree to these currents, we would have expected the K^+ currents to instead be augmented under this condition (since SERCA activity would scavenge incoming Ca^{2+}).

We next examined the effects of manipulating ryanodine and $InsP_3$ receptors on BK_{Ca} current activity in the mesenteric smooth muscle cells from SHR. Ryanodine receptor channels are localised in both the peripheral and central sarcoplasmic reticulum in all muscle types. Three receptor subtypes have been identified, with subtype 2 being the predominant one in smooth muscle cells (Jaggar et al., 2000). Several studies have documented that the effect of ryanodine on the receptor-sensitive Ca^{2+} release is concentration-dependent. These receptors are inhibited by 10–30 mM but activated by <2 mM ryanodine (Jaggar et al., 2000; Mironneau et al., 1996). Our results showed that 1 mM ryanodine enhanced membrane K^+ currents, while 10 mM suppressed them. Furthermore, STOC-like current were largely abolished after applying 10 mM ryanodine, but increased in both magnitude and frequency after applying 1 mM ryanodine, consistent with the proposal that there is more frequent Ca^{2+} discharge (Ca^{2+} sparks) in SHR cells.

To test whether increased Ca^{2+} discharge from the sarcoplasmic reticulum might also involve $InsP_3$-receptors, we examined the effect of 2-aminoethoxydiphenyl borate. This agent is regarded as an inhibitor of $InsP_3$-induced Ca^{2+} release in previous studies (Ascher-Landsberg et al., 1999), although recent evidence suggests that 2-aminoethoxydiphenyl borate may also inhibit Ca^{2+} influx through store-operated channels (Gregory et al., 2001; Ma et al., 2000). The results in our study showed that 2-aminoethoxydiphenyl borate only weakly inhibited membrane K^+ currents at a concentration that was maximally effective in other studies (i.e., 100 mM). These findings suggest $InsP_3$-induced Ca^{2+} release and store-operated Ca^{2+}-influx contribute little to basal regulation of membrane K^+ currents in SHR.

Our data suggest, therefore, that sarcoplasmic reticulum plays a major role in the regulation of membrane BK_{Ca} current activity in SHR, discharging Ca^{2+} in the form of sparks and leading to STOC activity. Elevation of $[Ca^{2+}]_i$ which results from this discharge is expected to have a negative impact on Kv current activity, and possibly even Kv channel expression, in light of findings published elsewhere that there is an inverse relationship between $[Ca^{2+}]_i$ and the magnitude of Kv currents in these tissues (Cox and Rusch, 2002).

The change in K_{Ca} and apparent defect in Ca^{2+}-handling are not congenital in nature (i.e., brought on by the inbreeding which produces the SHR phenotype), since

young SHR and WKY animals show no difference in their Kv1.2 and Kv1.5 channel protein expression nor current densities. However, after the development of hypertension in adult SHR, the expression of Kv1.2 and Kv1.5 channels became lower, that of BK_{Ca} became elevated, and STOCs which were absent in young SHR also became apparent, indicating that these changes are coincident with the development of hypertension. This concept is supported by the results from a study which showed that resting membrane potential in the SMC from the tail artery was similar between young SHR and WKY, but was lower in the adult SHR with established hypertension. More importantly, treatment with captopril prevented the development of hypertension as well as the decrease in membrane potential (Cheung, 1984). Our results complement that functional study by clarifying the nature of the electrophysiological changes that contribute to the hypertensive phenotype. The causal relationships between the electrophysiological changes and development of hypertension can not be determined on the basis of our data alone. Previous studies have interpreted the changes in K_{Ca} current to be an adaptive response to the development of the hypertensive state, an attempt to hyperpolarize the membrane, reduce its excitability and decrease voltage-dependent Ca^{2+}-influx (Cox and Rusch, 2002). We would propose an additional (though not necessarily mutually exclusive) explanation: that the development of the hypertensive status is secondary to defects in Ca^{2+}-handling, manifesting in greater release of internally stored Ca^{2+} (Ca^{2+}-sparking): the resultant change in $[Ca^{2+}]_i$ in the deep cytosol results in enhanced tone (hypertension), while coincident changes in the cytosolic space between the sarcoplasmic reticulum and the plasmalemma produces STOCs and enhances K_{Ca} activity.

In summary, we provide electrophysiological and biochemical evidence for divergent changes in Kv and BK_{Ca} currents in mesenteric arterial smooth muscle cells of SHR. These changes coincide with the development of hypertension, and result in an overall decreased membrane K^+ current which in turn accounts for the decreased membrane potentials seen in these animals. The data further suggest that there is a fundamental change in Ca^{2+}-handling in the SHR animals, one that may produce the electrophysiological changes and hypertension.

Acknowledgement

This work was supported by research grants from the Canadian Institute of Health Research and the Heart and Stroke Foundation of Ontario. We thank Dr. Mark Inman for kindly providing help with statistical analysis.

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