Calcium Current in Smooth Muscle Cells From Normotensive and Genetically Hypertensive Rats

Dixon W. Wilde, Philip B. Furspan, James F. Szocik

Abstract Genetic hypertension results from numerous phenotypic expressions. We hypothesized that increased calcium current in vascular smooth muscle of genetically hypertensive animals is partly responsible for observed increases in agonist sensitivity, contractility, and calcium influx. Using adult, spontaneously hypertensive stroke-prone rats (SHRSP) and normotensive Wistar-Kyoto (WKY) controls from an inbred colony, we characterized calcium current in smooth muscle cells isolated from cerebral arteries. Calcium current in WKY cells reached a maximum of $-27.7\pm2.7$ pA (n=32) at $+20$ mV. Peak inward current at $+20$ mV in SHRSP cells had a mean amplitude of $-44.4\pm3.0$ pA (n=72, P<.05). SHRSP cells exhibited a higher calcium current density. Maximal inward current normalized to cell capacitance yielded mean values of $2.07\pm0.11$ pA/pF for WKY (n=32) and $2.80\pm0.12$ pA/pF (n=79) for SHRSP (P<.05) cells. Transient-type Ca$^{2+}$ channel current had the same magnitude and current-voltage relation in both cell types, giving an L-type/T-type ratio of 3.85 for WKY and 6.25 for SHRSP cells. The voltage-dependent inactivation curve for SHRSP calcium current was shifted to the right only over the range of $-50$ to $-30$ mV, but the half-maximal inactivation voltages and Boltzmann coefficients were not significantly different between cell types. Increased calcium inward current in this model of genetic hypertension could account in part for altered calcium homeostasis and increased vascular reactivity, contributing to hypertension and vasospasm. (Hypertension. 1994;24:739-746.)

Key Words • hypertension, genetic • cerebral artery • calcium • rats, inbred SHR

Development of essential hypertension is thought to have a genetic basis involving increased contractile activity and agonist sensitivity of vascular smooth muscle that may arise from altered function of vascular muscle membrane Ca$^{2+}$ channels. Relatively few studies have examined electrophysiological differences between normotensive and hypertensive vascular smooth muscle. Increased Ca$^{2+}$ influx in vascular smooth muscle may contribute to increased contractility and promote the rise of blood pressure. In general, Ca$^{2+}$ influx is greater in vascular smooth muscle cells (VSMCs) from the spontaneously hypertensive rat (SHR) compared with the normotensive Wistar-Kyoto (WKY) control rat at rest and during norepinephrine and K$^+$ stimulation of the cell membrane.1-3 VSMCs from genetically hypertensive or induced hypertensive animals also exhibit greater sensitivity to the Ca$^{2+}$ channel agonist Bay K 8644.4-6

The best direct evidence for alterations of Ca$^{2+}$ channel activity in vascular smooth muscle from hypertensive animals comes from a series of studies of the electrophysiological characteristics of VSMCs from SHR and normotensive rats. Whole-cell voltage-clamp analyses have been used to examine the relative contributions of L-type and T-type Ca$^{2+}$ channels to total cell inward Ca$^{2+}$ current (I$_{Ca}$) in SHR and WKY VSMCs.7-10 In cultured VSMCs from neonatal azygous vein, cells from SHR exhibited a greater proportion of total inward Ca$^{2+}$ current arising from L-channel activity, even though total inward current magnitude was similar in VSMCs from both SHR and WKY rats. In addition, the effects of intracellular [Ca$^{2+}$] on the amplitude of calcium current (I$_{Ca}$) were more pronounced in SHR cells than in WKY cells. A recent study of normotensive and hypertensive rat mesenteric artery VSMCs (not from the stroke-prone strain) by Ohye et al11 clearly indicated that Ca$^{2+}$ channel activity is increased in SHR. However, this difference was observed only in young animals that had not developed elevated blood pressure. Adult SHR showed no differences in I$_{Ca}$ compared with adult WKY rats, and there were no differences in the voltage dependence for channel inactivation.

These findings suggest not only quantitative differences in Ca$^{2+}$ channel expression in SHR and WKY systemic vessels but differences in the regulation of the channels by intracellular free Ca$^{2+}$ or perhaps other factors. Evidence for quantitative differences in Ca$^{2+}$ channel expression has also been obtained from radio-labeled dihydropyridine binding studies in SHR and WKY heart, brain, and skeletal muscle microsomes.12 At constant [Ca$^{2+}$], [H]dihydropyridine binding is significantly higher in SHR than in WKY preparations, supporting the hypothesis that hypertensive animals express greater numbers of Ca$^{2+}$ channels (L-type in this case). Increased dihydropyridine binding in SHR preparations correlates well with the increase in systolic blood pressure.13

The cerebral arterial circulation is an important regional vascular bed in the end-stage pathology of hypertension. In the cerebral arterial circulation, changes in
transmural pressure have been shown to initiate contraction that may involve alteration of the ionic permeability of the VSMC membrane. In SHR, in which elevation of intravascular and transmural pressure is chronic, the cerebral vessels undergo both structural and functional changes, including increased sensitivity to serotonin, an activator of Ca$^{2+}$ influx, and greater maximal response to the agonist.

We hypothesized that in arterial muscle from hypertensive animals, increased vascular contractility results in part from altered expression and regulation of sarcoplasmic Ca$^{2+}$ channels. The objective of these experiments was the characterization of some of the basic phenotypes of Ca$^{2+}$ channels in cerebral arterial VSMCs from the stroke-prone SHR (SHRSP) and its normotensive WKY control.

**Methods**

**Isolated Cell Preparation**

All animal use protocols were given prior approval by the University of Michigan Committee on the Use and Care of Animals. WKY rats and SHRSP were obtained through the inbred colony (approximately 70 brother-sister mating generations) maintained at the Department of Anatomy and Cell Biology at the University of Michigan Medical School. A total of 28 WKY rats and 34 SHRSP were used for all experiments. Blood pressures of nonanesthetized animals were measured by tail cuff before euthanasia. Mean systolic pressure in the WKY animals averaged 125±2 mm Hg (n=17 animals). Mean systolic pressure for the SHRSP was 190±5 mm Hg (n=17 animals). Cerebral artery VSMCs were isolated from adult (>120 days old) age- and sex-matched pairs (whenever possible) of SHRSP and WKY rats. Under pentobarbital anesthesia (50 mg/kg, IP), the animal was killed by pneumothorax and decapitation. The brain was removed to a beaker of chilled 0.1 mmol/L Ca$^{2+}$ buffered salt solution containing (mmol/L) NaCl 140, KCl 5.4, KH$_2$PO$_4$ 0.44, Na$_2$HPO$_4$ 0.42, NaHCO$_3$ 4.17, CaCl$_2$ 0.1, HEPEXS 5, and glucose 5.55 at pH 7.35. Basilar and middle cerebral arteries and the Circle of Willis were removed and pooled to provide sufficient tissue for dispersion.

Vessels were cleaned of dura and glial tissue and subjected to papain-collagenase dispersion according to the method of Fursspan and Webb. Briefly, vessels were minced and placed into 5 mL of 0.1 mmol/L Ca$^{2+}$ Hanks' buffered salt solution containing 0.05 g bovine serum albumin (type 1), 5 mg soybean trypsin inhibitor (type 1), 5 mmol/L taurocholic acid, 2 mg dithiothreitol, 3 mg type I collagenase, and 3 mg papain (all from Sigma Chemical Co). Vessel fragments were incubated at 37°C with gentle shaking for 25 minutes. After incubation, vessel fragments were removed by pipette and placed in 15mL plastic capped tubes and resuspended in 0.1 mmol/L Ca$^{2+}$ Hanks' solution containing the albumin, trypsin inhibitor, and taurocholate as detailed above. Fragments were washed three times in this solution to remove enzyme and were then stored capped at 2°C. Single, relaxed cells were released from vessel fragments by gentle pipette agitation. Isolated VSMCs in suspension were transferred to a controlled-environment (atmosphere and temperature) recording chamber on the stage of an inverted microscope and maintained at 37°C. The 0.5-mL chamber was constantly superfused at 3 mL/min by bicarbonate-buffered Tyrode's solution equilibrated with 95% O$_2$/5% CO$_2$. The Tyrode's solution contained (mmol/L) NaCl 120, KCl 5.4, MgCl$_2$ 1.2, glucose 5, and NaHCO$_3$ 14 adjusted to pH 7.3. Initially, cells were superfused with Tyrode's containing 1.8 mmol/L Ca$^{2+}$. However, for whole-cell voltage-clamp recording, 10 mmol/L Ba$^{2+}$ replaced Ca$^{2+}$ as charge carrier. Bath pH was adequately maintained at 7.3 to 7.4 (as determined in chamber calibration runs with a micro-pH electrode, Microelectrodes, Inc) because of the equilibration of both superfusate and chamber atmosphere with 95% O$_2$/5% CO$_2$.

**Whole-Cell Recording**

Single, relaxed cells were voltage-clamped in whole-cell mode using 6-M1 glass suction electrodes (R-6 glass, Garner Glass). Pipettes were filled with an intracellular dialysis containing (mmol/L) Cs-glutamate 110, CsCl 20, tetraethylammonium-Cl 20, HEPES 5, EGTA 10, 2 Mg-ATP 5, and phosphocreatine 5 (all from Sigma) at pH 7.2. Pipettes were coated with a nitrocellulose/butyl acetate/toluene sulfonamide/formaldehyde resin to minimize pipette capacitance, and bath depth was maintained at 1 to 2 mm. Standard protocols included generation of current-voltage plots at holding potentials (V_h) of −80 and −40 mV using pCLAMP software (version 5.6, Axon Instruments). This allowed for digital subtraction of current records to permit visualization of T-channel and L-channel currents that were then quantified for comparison between SHRSP and WKY cells. Additional protocols examined voltage-dependent inactivation of the Ca$^{2+}$ channels using 2-second conditioning prepulses to vary cell holding potential. Current evoked at a test potential to +20 mV was normalized to peak inward current. Graphed normalized current was fit by a Boltzmann function of the form

$$y=A_0+A_1e^{-V/k}$$

where $V$ is the command potential, $V_{1/2}$ is the potential for half-maximal inactivation, and $k$ is the Boltzmann constant for the fitted curve. Time constants ($\tau$) for the activation and inactivation phases of inward current was best described by single exponential functions and were determined using the CLAMPFIT subroutine in the pCLAMP program according to the equation

$$v=A_0+A_1e^{-V/k}$$

Voltage-clamp protocols, data acquisition, and off-line data analyses were performed on an 80386 IBM clone computer driving an Axopatch 200A amplifier (Axon Instruments). After establishment of a gigaohm seal and whole-cell recording mode, series resistance and membrane capacitance were compensated electronically using the on-board circuitry of the clamp amplifier. Membrane capacitance compensation was maintained routinely at 70% to 85%. Estimated voltage drop across uncompensated residual series resistance was less than 4 mV. Experimental control and data analyses were accomplished using pCLAMP software (Axon Instruments, version 5.6). Analog current recordings were digitized at 20 microseconds per point, averaged, and stored on the hard drive of the computer. Compensation for nonspecific membrane leakage current was accomplished using the P/6 protocol of pCLAMP. For current-voltage runs, membranes were stepped for 200 ms from a holding potential of −80 mV to −1/6 of the command step, averaged, scaled, and subtracted from the current resulting from the command step. The P/6 protocol from a holding potential of −80 mV ensured that no active currents were evoked that would have confounded the recorded current. For the inactivation protocols, the same leak protocol was used, but step lengths were 2.2 seconds. Comparison of the P/6 protocol with an off-line leak subtraction protocol consisting of ten −10-mV steps from a holding potential of −80 mV showed no difference in the leak-corrected currents because both methods yielded leak estimated from the zero-current range of the current-voltage relation. No differences in the size of leak currents were noted between WKY and SHRSP cells. Isolation 5, at the fast inactivating T-channel component of inward current was performed by digital subtraction of current records recorded from V_h of −80 and −40 mV. The T-current component was very small and was not observed in all cells (see "Results"). Voltage-dependent inactivation of inward current was determined by varying holding potential for 2 seconds in

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10-mV increments with a test potential to +20 mV. Inactivation was plotted as maximal relative inward current against the conditioning potential. The 2-second conditioning pulse has been successfully used by Bean et al.\(^{19}\) in studies of rat mesenteric artery Ca\(^{2+}\) channel current. Preliminary recordings at the beginning of the study revealed that a 2-second period was approximately four to five times longer than the minimum time sufficient for complete inward current decay. Effects of drugs were monitored with a constant pulse protocol (200-ms steps from \(V_h\) of -80 to +20 mV) during administration and washout. Since inward current generally decrements because of "run down" phenomena, we discarded any cells not showing recovery from drug application to at least 85% of the control amplitude. Generally, recording periods ranged from 5 to 20 minutes. The use of ADP and phosphocreatine in the pipette solution significantly reduced the rate of run down.

Bay K 8644 (Sigma) was prepared as a stock solution in 100% ethanol and diluted before use. Final ethanol concentration in working solutions was 0.01% (vol/vol) and was shown in preliminary control runs to have no effect on the inward current. All data are presented as mean±SEM. Values of \(n\) indicate number of cells unless otherwise stated. Data were compared by unpaired Student's \(t\) test with Bonferroni correction for multiple comparisons. A value of \(P<.05\) was considered significant.

**Results**

**Characteristics of Isolated Cells**

The isolated cells were approximately 100 to 150 \(\mu m\) in length and 6 to 9 \(\mu m\) in diameter and contracted in response to superfusion by high extracellular \([K^+]_o\). Total membrane capacitance, a rough measure of cell surface area, averaged 13.4±0.7 pF (\(n=40\)) for WKY cells and 15.9±0.67 pF (\(n=89\)) for SHRSP cells (\(P<.05\) by unpaired Student's \(t\) test), consistent with the descriptions of cellular hypertrophy in hypertensive vascular smooth muscle.\(^{20}\)

**Comparison of Ca\(^{2+}\) Channel Current in WKY and SHRSP Cells**

Inward Ca\(^{2+}\) channel current in SHRSP cerebral artery VSMCs was approximately twice as large as that from WKY cells (Fig 1A and 1B). Inward current for both cell types activated at \(-40\) mV using holding potentials of \(-80\) or \(-40\) mV and peaked at \(+20\) mV. For WKY cells, peak inward current reached \(-27.7\pm2.7\) pA (\(n=32\) cells) at \(+20\) mV (\(-80\)-mV holding potential), whereas peak inward current at \(+20\) mV in SHRSP cells had a mean amplitude of \(-44.4\pm3.0\) pA (\(n=72\) cells, \(P<.05\) compared with WKY cells). Whole-cell currents recorded from SHRSP and WKY cerebral artery VSMCs were very small compared with inward Ca\(^{2+}\) channel currents reported in other VSMCs.\(^{21}\) Separate control experiments using fura 2-loaded cells indicated that intracellular \([Ca^{2+}]_i\) was increased in both SHRSP and WKY cells exposed to serotonin and to membrane depolarization by elevation of extracellular \([K^+]_o\), indicating that membranes were intact and functional after enzyme treatment. It was therefore unlikely that the enzyme dispersion artificially reduced the current magnitudes.

Differences in cell surface area could account for the observed differences in maximal whole-cell Ca\(^{2+}\) channel current. Normalization of peak inward current at \(+20\) mV against cell capacitance yielded mean values of 2.1±0.1 pA/pF for WKY cells (\(n=32\)) and 2.8±0.1 pA/pF (\(n=79\)) for SHRSP cells (\(P<.05\)) (Fig 1C). It therefore appears that the increased amplitude of Ca\(^{2+}\) channel current observed in the SHRSP cells is in part due to increased channel density.

The contribution of T-channels to total membrane current (as established by digital subtraction of current records obtained from holding potentials of \(-80\) mV and \(-40\) mV) was \(-7.3\pm1.2\) pA (\(-0.6\pm0.1\) pA/pF) in WKY cells (\(n=23\)) and \(-7.2\pm0.8\) pA (\(-0.5\pm0.1\) pA/pF) in SHRSP cells (\(n=37\)) (Fig 2). This component of inward current had a threshold at \(-50\) mV and peaked at \(-20\) mV for both cell types. Evidence for T-channel contribution to total current was observed in only 23 of 32 WKY cells (72%) and 37 of 72 SHRSP cells (51%). The finding that T-current amplitude was equal in those...
cells exhibiting this component supports the hypothesis that SHRSP cells exhibit elevated numbers of L-type channels to produce total membrane currents larger than in WKY cells. Based on the mean values for T-type and L-type components, WKY cells exhibited an L/T ratio of 3.85; SHRSP cells had an L/T ratio of 6.25.

Investigations into differential regulation of the L-type channels indicated that small but important differences may exist in the regulation of these channels in the two vessel types. Comparison (by unpaired Student's t test) of relative current magnitudes during inactivation runs revealed significant differences in relative current magnitude over the range of -50 to -30 mV (Fig 3A).

However, based on Boltzmann equation fits to the data, half-maximal inactivation voltages were no different between WKY and SHRSP cells (-13.0±1.5 and -12.2±1.0 mV, respectively, P>.05). Small but insignificant differences were noted in the Boltzmann coefficients of the fitted curves. For WKY cells, $k$ was $-16.2±2.0$ mV; for SHRSP cells, $-13.8±1.5$ mV (P>.05) (Table 1).

No significant differences were detected in the voltage dependence for current activation in SHRSP and WKY cells (Fig 3B). For WKY cells, $I_{\alpha}$ reached half-maximal activation at $-3.4±5.1$ mV, with a Boltzmann coefficient of $9.4±1.3$ mV. Similarly, values for the voltage-dependent activation of $I_{\alpha}$ in SHRSP were $-1.3±4.1$ mV and $8.6±2.1$ mV, respectively (P>.05 compared with WKY cells).

Comparison of Sensitivities of SHRSP and WKY Currents to Bay K 8644

The effects of the Ca$^{2+}$ channel agonist Bay K 8644 at 0.1 and 1.0 μmol/L were investigated on depolarization-evoked Ca$^{2+}$ channel current (Fig 4). We chose this Ca$^{2+}$
Fig 4. Line graphs and recordings show effect of 1.0 μmol/L Bay K 8644 on development of inward L-type Ca\(^{2+}\) channel current recorded using a holding potential of -40 mV. Top, Current-voltage relation for Wistar-Kyoto (WKY, n=4) cells with representative recordings. Bottom, Current-voltage relation for stroke-prone spontaneously hypertensive rat (SHRSP) cells (n=9) with representative recordings. *Significant difference from control (no Bay K 8644) at P<.05. Bay K 8644 increased peak inward L-channel current, shifted peak activation voltage by 10 mV toward more negative, and increased current decay rate (see Table 2).

channel agonist to avoid diminution of the already small whole-cell current. At a holding potential of -40 mV, 1.0 μmol/L Bay K 8644 increased inward current in WKY VSMCs from -35.8±4.0 to -58.8±12.0 pA (n=4, P<.05 compared with control) and shifted the voltage for maximal activation from +30 mV in control to +20 mV during Bay K 8644. The increase in inward current during Bay K 8644 constituted a difference of 23.0 pA or 64%. In contrast, 1.0 μmol/L Bay K 8644 applied to SHRSP cells increased \(I_{\text{Ca}}\), from -44.4±6.3 to -84.1±13.2 pA (n=9, P<.05 compared with control), a difference of 39.3 pA or 89% (P<.05 compared with the effect on peak inward Ca\(^{2+}\) current in WKY cells). Maximal activation voltage in SHRSP cells was also shifted during 1.0 μmol/L Bay K 8644 from +30 to +20 mV.

Examinations of voltage-dependent inactivation and activation during Bay K 8644 exposure revealed similar effects on \(I_{\text{Ca}}\) in both WKY and SHRSP cells (Figs 5 and 6, Table 1). Bay K 8644 at 1.0 μmol/L shifted the half-maximal current inactivation voltage to the left in both WKY and SHRSP cells (both P<.05) (Table 1). In control cells, \(V_{1/2}\) values were -13.0±1.5 and -12.2±1.0 for WKY and SHRSP cells, respectively. Bay K 8644 at 1.0 μmol/L shifted these values to -25.0±3.2 and -25.5±3.4, respectively (P<.05 for both compared with control, n=4 for WKY and n=9 for SHRSP cells). Interestingly, 1.0 μmol/L Bay K 8644 slightly but significantly increased the half-maximal activation voltage for both WKY and SHRSP cells (Fig 6, Table 1). This shift to the right was observed in SHRSP cells at 0.1 μmol/L Bay K 8644 (P<.05, n=16) but not in WKY cells (P>.05, n=16). Boltzmann constants for the inactivation plots revealed similar significant depressions of k for both SHRSP and WKY cells during Bay K 8644 (Table 1).

Comparison of Current-Time Constants
Analyses of current activation and decay time constants (τ) were performed on cells in which capacitance neutralization was at 80% or better. For WKY cells, inward current activation and decay time constants were 2.50±0.21 and 35.10±4.80 ms, respectively (Table 2). In SHRSP cells, these values were 2.67±0.16 and 32.16±3.18 ms, respectively. There was no significant difference between these values when compared between cell types (P>.05 in all cases, n=32 for WKY and n=71 for SHRSP cells). Bay K 8644 at 0.1 μmol/L lowered the time constant for the decay phase of the current similarly in both WKY and SHRSP cells. For WKY cells, the decay constant fell to 19.04±3.72 ms and...
This study demonstrates that Ca\(^{2+}\) channel current in VSMCs from adult SHRSP is significantly larger than that in VSMCs from adult WKY rat. This difference persists after normalization of current amplitude to membrane capacitance even though the SHRSP cells appear to have significantly larger membrane surface area. The voltage dependence for current activation and inactivation was different between the cell types although over a narrow range of voltage, individual values for normalized current inactivation exhibited some significant differences, with the SHRSP cells showing less inactivation over the range of \(-50\) to \(-30\) mV. SHRSP cells were more than twice as sensitive to the L-type Ca\(^{2+}\) channel agonist Bay K 8644 as WKY cells.

These data indicate an increased L-channel expression in the SHRSP model of genetic hypertension compared with the normotensive WKY control. Recently, Ohya et al\(^{11}\) demonstrated increased inward L-type Ca\(^{2+}\) channel current in young SHR mesenteric artery smooth muscle cells. This difference was lost as the animals grew to adulthood and the SHR developed hypertension. Not surprisingly, these results confirm the early findings of Hermansmeyer et al\(^{7-10}\) in studies of Ica in cultured neonatal azygous vein cells from SHR and WKY rats. However, our results differ somewhat from those of Ohya et al and Hermansmeyer et al in that the enhanced inward calcium channel current apparently persists in the adult animal. Two considerations that might explain this difference from previously published results are (1) a selection for a different genetic constitution during breeding of the SHRSP (compared with the SHR model) and (2) the specialized regional circulatory elements used in the experiments described above. The latter hypothesis is supported by results of studies examining the effect of Bay K 8644 on conduit and small muscular arteries in hypertensive rats.\(^{4,6}\) The absence of electrophysiological information on L-channel overexpression in adult hypertensive animals is surprising, considering the abundant evidence for altered dihydropyridine sensitivity and binding and increased Ca\(^{2+}\) permeability in adult animal models of hypertension.\(^{25-26}\)

The mechanism or mechanisms underlying the increased L-channel expression in the SHRSP cells is yet to be determined, as are putative differences in the intracellular regulation of these channels by factors such as intracellular pH, cyclic nucleotides,\(^{27}\) and GTP-binding proteins.\(^{28}\) Preliminary data from our laboratory have also implicated

### Table 1. Boltzmann Fit Parameters for WKY and SHRSP Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(V_{1/2})</th>
<th>(k)</th>
<th>(V_{1/2})</th>
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<tbody>
<tr>
<td>WKY, control (n=21)</td>
<td>(-13.0\pm1.5)</td>
<td>(-16.2\pm2.0)</td>
<td>(-3.4\pm0.7)</td>
<td>(9.4\pm1.3)</td>
</tr>
<tr>
<td>WKY, Bay K 0.1 (\mu)mol/L (n=16)</td>
<td>(-13.6\pm2.0)</td>
<td>(-11.6\pm1.8)</td>
<td>(-3.8\pm1.6)</td>
<td>(6.6\pm1.2) *</td>
</tr>
<tr>
<td>WKY, Bay K 1.0 (\mu)mol/L (n=4)</td>
<td>(-25.0\pm3.2)</td>
<td>(-11.4\pm2.1)</td>
<td>(4.7\pm1.1) *</td>
<td>(5.6\pm1.1) *</td>
</tr>
<tr>
<td>SHRSP, control (n=71)</td>
<td>(-12.2\pm1.0)</td>
<td>(-13.8\pm1.5)</td>
<td>(-1.3\pm1.8)</td>
<td>(8.6\pm2.1)</td>
</tr>
<tr>
<td>SHRSP, Bay K 0.1 (\mu)mol/L (n=16)</td>
<td>(-15.3\pm2.4)</td>
<td>(-9.1\pm2.0)</td>
<td>(3.6\pm2.0) *</td>
<td>(6.6\pm1.7)</td>
</tr>
<tr>
<td>SHRSP, Bay K 1.0 (\mu)mol/L (n=9)</td>
<td>(-25.5\pm3.4)</td>
<td>(-8.7\pm1.2)</td>
<td>(3.4\pm1.3) *</td>
<td>(6.6\pm1.4)</td>
</tr>
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\(V_{1/2}\) indicates potential for half-maximal inactivation; \(k\), Boltzmann constant for fitted curve; WKY, Wistar-Kyoto; Bay K, Bay K 8644; and SHRSP, stroke-prone spontaneously hypertensive rat.

\(^{*}P<.05\).
possible differences in the lipid constitution of the SHRSP and WKY membranes, based on differential actions of volatile anesthetics with different lipid solubilities. These latter findings have led to the hypothesis that an alteration in the lipid milieu of the hypertensive membrane might change the basic and regulated functions of the L-channels. This, coupled with increased L-channel expression, could significantly affect vascular smooth muscle Ca\(^{2+}\) metabolism, particularly under agonist activation or membrane depolarization. Dominiczak et al\(^{30}\) reported increased cell membrane viscosity in VSMCs from SHRSP although phospholipid composition did not differ between SHRSP and WKY cells. Similar results were reported by Tsuda et al\(^{31}\) in studies of SHR and WKY animals. These findings suggest an essential defect in the sarcolemma of genetically hypertensive vascular smooth muscle cells that could have a significant effect on membrane protein function, including Ca\(^{2+}\) channel activities. In one recent study, cholesterol enrichment of cell membranes produced increased L-type Ca\(^{2+}\) channel activity in VSMCs.\(^{31}\) Further suggestion of altered channel function may be indicated by the results of experiments with Bay K 8644. Although Bay K 8644 increased inward Ca\(^{2+}\) channel current and shifted the current-voltage and inactivation curves to the left in a manner similar to other VSMCs,\(^{19}\) it failed to also shift the activation plots to the left as in the manner of cardiac myocytes.\(^{22}\) This indicated that the interaction of this drug with the channel may differ in cerebral vascular muscle. However different the cerebral Ca\(^{2+}\) channels may be from channels in other vascular beds, the absence of significantly different channel behavior in the presence of Bay K 8644 supports the conclusion that the SHRSP cells simply express greater numbers of L-channels without any fundamental change in the behavior of the individual channel when compared with WKY cells.

Based on the results of these preliminary studies, we may hypothesize only an increased Ca\(^{2+}\) channel density in SHRSP cerebral artery cells. Although fundamental biophysical differences may exist between WKY and SHRSP cells (ie, increased opening probability or differences in the number of channel states), we do not feel comfortable extrapolating to such speculation. Based on these results, it seems plausible that increased L-channel expression in SHRSP, which persists into adulthood, may contribute to the stroke-prone nature of the animals. Studies are currently underway to examine age-related differences in \(I_{\text{Ca}}\) between these animal strains.

The results from the cerebral arterial studies indicate that examinations of specialized regional circulatory beds may reveal significant differences in Ca\(^{2+}\) channel activity and expression in genetic hypertension models. In the SHRSP, increased Ca\(^{2+}\) channel expression and larger Ca\(^{2+}\) currents may contribute to the stroking behavior of these vessels. Rat cerebral arterial muscle has been shown to respond to increases in transmural pressure by undergoing membrane depolarization, developing spontaneous action potentials and contracting in a manner dependent on extracellular Ca\(^{2+}\).\(^{32}\) Elevation of transmural pressure also increases the sensitivity of feline cerebral arteries to 5-hydroxytryptamine.\(^{33}\) Although the stretched-induced activation of cerebral muscle may revolve around stretch-activated membrane channels, localized autonomic nerve endings, or both, the development of Ca\(^{2+}\)-dependent spiking involves the activation of sarcolemmal Ca\(^{2+}\) channels in the VSMCs.

Although the works of Hermansmeyer et al\(^{10-11}\) and Ohyama et al\(^{34}\) suggest that the Ca\(^{2+}\) channel expression defect is present in SHR neonates, there has been no causal function assigned to this defect with respect to the development of high blood pressure. Elevation of cerebral transmural pressure in vitro is also a different condition from chronic vascular pressure increases in hypertension, and it is as yet unclear whether the resultant increased Ca\(^{2+}\) channel activity in SHRSP cerebral artery VSMCs contributes to or results from the hypertrophy and elevation in blood pressure. Certainly an increased L-type Ca\(^{2+}\) channel density in the SHRSP would add to the potential for vasospastic behavior and might contribute to the stroking phenotype in this rat strain.

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