Voltage-Dependent Ca\(^{2+}\) Channels in Resistance Arteries From Spontaneously Hypertensive Rats

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Alterations in voltage-dependent Ca\(^{2+}\) channels in the arterial smooth muscle cells of spontaneously hypertensive rats (SHR) were investigated using the whole-cell voltage clamp and compared with Wistar-Kyoto (WKY) rats. Single cells were freshly isolated from resistance mesenteric arteries from 4- to 5-week-old (young) and 16- to 18-week-old (adult) SHR. Elevated blood pressure was only evident in adult SHR, not in young SHR. In young rats, the Ca\(^{2+}\) channel current density (current amplitude normalized by cell capacitance) was significantly higher \((P<.01)\) in SHR than in WKY rats at the command potential of \(-10 \text{ mV}\) or higher \((50 \text{ mmol/L Ba}\(^{2+}\))\): The current density at 20 mV was \(-16.8\pm 1.1 \text{ pA/pF in SHR (n=38 cells)}\) and \(-11.0\pm 0.8 \text{ pA/pF in WKY rats (n=30 cells)}\). In adult rats, the difference in current densities disappeared: \(-15.9\pm 1.3 \text{ pA/pF in SHR (n=25 cells)}\) and \(-15.6\pm 1.5 \text{ pA/pF in WKY rats (n=29 cells)}\). The ratio of maximal amplitude of T-type current to that of L-type current was low in young SHR \((0.10\pm 0.01)\) compared with the other three groups \((0.16 \text{ to } 0.20)\). Neither the activation curve nor the steady-state inactivation curve of SHR was different from that of age-matched WKY rats. However, the activation curves in adult rats were shifted to a hyperpolarized direction compared with those of young rats in both strains. These results suggest that the increased activity of voltage-dependent L-type Ca\(^{2+}\) channels of resistance arteries in young SHR may be related to the development of hypertension. The changes observed in adult rats may be due to a secondary modification of the channel during maturation and the presence of hypertension. \((\text{Circ Res.} \ 1993;73:1090-1099)\)

**KEY WORDS** • Ca\(^{2+}\) channels • vascular smooth muscle • spontaneously hypertensive rats • electrophysiology • voltage clamp

An abnormal Ca\(^{2+}\) handling by the plasma membrane of arterial smooth muscle cells has been considered to play a role in the development and maintenance of hypertension. It has been predicted that the voltage-dependent Ca\(^{2+}\) channels may be altered in hypertensive human and animal models compared with their normotensive controls.\(^1\)\(^2\)

The patch-clamp method has enabled the investigation of voltage-dependent Ca\(^{2+}\) channels in vascular smooth muscles. The advantage of using the patch-clamp method is to observe the current moving through channels to evaluate the channel function and properties directly in living cells.\(^3\)\(^4\) However, only a few studies using this method have examined the properties of the Ca\(^{2+}\) currents of vascular smooth muscle cells from hypertensive and normotensive animals.\(^5\)\(^6\) It was reported that the ratio of long-lasting (L-type) current to transient (T-type) current was higher in cultured venous cells from spontaneously hypertensive rats (SHR) than from Wistar-Kyoto (WKY) rats.\(^4\) Since the arterial muscle has much more relevance for blood pressure control than does the venous muscle\(^6\) and since the distribution of T-type current in a cell was reported to change during primary culture,\(^7\) we conducted experiments using freshly isolated single smooth muscle cells from the mesenteric resistance arteries of SHR and WKY rats.

The main purpose of the present study was to evaluate whether Ca\(^{2+}\) channels in the resistance artery are altered in SHR compared with WKY rats. Subsidiary information gained was concerned with changes in the Ca\(^{2+}\) channel current during maturation and the development of hypertension in SHR. For this purpose, we used SHR and WKY rats at two ages, both before and after hypertension had developed.

**Materials and Methods**

**Animals**

Four- to 5-week-old (young) and 16- to 18-week-old (adult) male SHR and WKY rats \((n=7 \text{ to } 9 \text{ rats in each group)}\), which had been maintained at the Institute of Experimental Animals in Kyushu University, were used. The rats were fed standard rat chow and had free access to tap water. The ages of SHR and WKY rats were matched with a difference of less than 3 days. Systolic blood pressure was measured by the tail-cuff method.

**Cell Dispersion**

Single smooth muscle cells were obtained by collagenase treatment from the resistance mesenteric arterial branch \((\text{diameter, } <300 \mu m)\), similar to the method previously reported.\(^8\)\(^−10\) A rat was stunned by a blow to the head and then decapitated. The whole superior mesenteric bed was dissected and placed on an organ chamber. The tissue was continuously superfused, and the arterial lumen was perfused via an inserted polyethylene...
ethylene tube (heat-reformed to have a small tip diameter) by modified Krebs’ solution (mmol/L: Na+, 137.4; K+, 5.9; Ca2+, 2.0; Mg2+, 1.8; HCO3-, 15.5; H2PO4-, 1.2; Cl-, 134; and glucose, 11.2; pH 7.2 to 7.3 bubbled with 95% O2-5% CO2). Connective tissues were carefully removed to isolate the arteries with surgical microscissors under a dissecting microscope. The superfusion and perfusion solutions were then changed to a Ca2+-free solution (mmol/L: NaCl, 145; KCl, 6; glucose, 10; and HEPES, 10; pH 7.3 titrated with NaOH). The tissue with the perfusion tube was then transferred into an incubation chamber containing the Ca2+-free solution. After about a 15-minute incubation at 36°C, the solutions were changed to the Ca2+-free solution, which contained 0.3% collagenase (Wako Chemical Co, Tokyo, Japan). After another 40- to 50-minute incubation at 36°C, the peripheral segments of the mesenteric artery (resistance artery) were removed by microscissors and were transferred to another tubing that contained the Ca2+-free solution without collagenase. The digested tissues were gently agitated with a glass pipette to disperse single cells. Cells were then placed in a Ca2+-free solution containing 1 mmol/L MgCl2 and 0.2% bovine serum albumin (essentially fatty acid free, Sigma Chemical Co, St Louis, Mo) in a refrigerator (6°C to 8°C) until use. Patch-clamp experiments were performed within 4 hours after cell dispersion to avoid any possible deterioration of the channel activity. Six to 10 cells were used for current recordings from one cell dispersion.

Electrical Recording

Whole-cell voltage clamp was performed with a patch pipette through a voltage-clamp amplifier (Axopatch 1-D, Axon Instruments Inc, Foster City, Calif) by the method of Hamill et al. In our preliminary experiments, the cell capacitances obtained in this method agreed closely with that obtained from the integral of the capacitive transient. The differences between the two methods were within 5% of the values. The leak and residual capacitances were subtracted using the P/4 protocol, since the cells did not have active background currents with high Cs+ solution in the pipette and high Ba2+ solution in the bath. In our preliminary experiments, Cd2+-sensitive inward current and the current obtained by the P/4 protocol were quite similar, as reported previously. The liquid junction potential was not corrected, because it was small (less than 3 mV). All experiments were performed at room temperature (22°C to 24°C). The cell was held at −80 mV, and command potentials were applied every 10 seconds, unless otherwise stated. Data were acquired after the current amplitude was stabilized (usually 2 to 3 minutes after obtaining the whole-cell configuration).

The cell suspension was placed into a small chamber (0.2 mL) on the stage of an inverted microscope (TMD-Diaphot, Nikon, Tokyo, Japan). While making the seal between the recording pipette and cell surface (when the tip of the pipette approached a cell), the cell was continuously superfused by the Ca2+-free solution containing 4 mmol/L MgCl2 to avoid contraction. This was because the pipette solution contained ATP, an agonist, which contracts the cell when applied to the cell surface. After obtaining the seal of over 5 GΩ (usually 8 to 20 GΩ initially), the patch membrane was disrupted by further negative pressure to obtain the whole-cell configuration. The bath solution was then exchanged for a proper solution to record the current.

Solutions and Chemicals

To isolate the inward Ca2+ channel currents, the pipette was filled with a high-Cs+ solution of the following composition (mmol/L): CsCl, 150; Na2-ATP, 3; MgCl2, 3; EGTA, 10; and HEPES, 10; pH 7.3 titrated with CsOH. Ba2+ ion was used as a charge carrier of the Ca2+ channel. The bath solution contained (mmol/L) BaCl2, 50; Tris-Cl, 50; Na+; 10; and HEPES, 10; pH 7.3 titrated with CsOH.

One of the difficulties in comparing Ca2+ channels in vascular smooth muscle cells of SHR and WKY rats by the voltage-clamp method is the unstable recording of the Ca2+ channel current. We previously reported that the use of ATP in the pipette solution enabled us to record Ca2+ channel currents constantly and stably in the whole-cell configuration. Thus, we used 3 mmol/L ATP in the pipette solution, in which the concentration maximized the Ca2+ channel activity. As a consequence, ATP minimized the cell-to-cell variation of the current densities.

Nicardipine was a gift from the Yamanouchi Pharmaceutical Co, Tokyo, Japan. Nicardipine was dissolved in distilled water to make the 2 mmol/L stock solution. The drug solution was made every day before experiments. The bath solution was exchanged by flushing the test solution, and the excess fluid was siphoned off. It took 15 to 30 seconds to exchange the bath solution.

Cell Characteristics

The obtained cells were spindle shaped. These cells contracted in response to bath applications of high-K+ solution and agonists such as norepinephrine in a physiological salt solution. Lengths of single smooth muscle cell from young SHR (n=10 cells) and young WKY rats (n=10 cells) were 94±7 and 93±8 μm, respectively. Areas measured under the microscope (plate area) of the cell from young SHR and young WKY rats were 3810±280 and 3740±310 μm2, respectively. Calculated surface areas of SHR and WKY rats cells were 11 960±820 versus 11 740±910 μm2, respectively, assuming the cell as a spindle shape (3.14×plate area). Cell capacitances were 15±1 pF in young SHR and 16±2 pF in young WKY rats. The calculated specific membrane capacitances in young SHR and young WKY rats showed no substantial difference (young SHR, 1.39±0.10 μF/cm2; young WKY rats, 1.42±0.11 μF/cm2). Length, plate area, and surface area of the cells from adult SHR (n=10 cells) and adult WKY rats (n=10 cells) were as follows: adult SHR, 120±6 μm, 4850±360 μm2, and 15 230±1210 μm2, respectively.
and adult WKY rats, 117±7 μm, 5020±440 μm², and 15760±1310 μm², respectively. The absence of differences in the length and plate area of single cells between SHR and WKY rats at this age was also reported in another study (10- to 14-week-old SHR and WKY rats). The cell capacitances and the membrane-specific capacitance from adult SHR and adult WKY rats were nearly the same (adult SHR, 20±2 pF and 1.4±0.11 μF/cm², respectively; and adult WKY rats, 18±2 pF and 1.40±0.09 μF/cm², respectively). These results suggest that the relation between the cell capacitance and the cell-membrane area did not differ between SHR and WKY rats as well as between the two ages. The values of the specific capacitance in the present study were similar to those shown in other reports. The presence of infolding, which could not be determined by the microscope, might be one explanation for these values (over 1.0 μF/cm²).

Data Analysis

For obtaining the current density, the peak amplitude was normalized by the cell capacitance, since the specific capacitances of single cells from SHR and WKY rats as well as young and adult rats were nearly the same. For the current-voltage (I-V) curve, the current densities were plotted against the corresponding command potentials.

Conductance (G) was obtained as follows: G = I_peak/(V_reversal − V), where I_peak is the peak amplitude of the current, V is the command potential (−80 to 60 mV), and V_reversal is the reversal potential. In the present study, the potential at the zero current level was tentatively used as the reversal potential, which was estimated in the I-V curve by extrapolating the I-V relation linearly to a further positive direction using points of 30, 40, 50, and 60 mV.

For the activation curve, the conductances were plotted against the corresponding command potentials. The curve was drawn by fitting the data to the Boltzmann equation: P = 1/[1 + exp((V − Vh)/k)], where P is the relative conductance normalized by the maximal conductance, V is the command potential, Vh is the potential required for half activation of the current, and k is the Boltzmann coefficient.

The steady-state inactivation curve was obtained by a double-pulse protocol. After applying various levels of conditioning holding potential (HP) for 5 seconds, the test pulse of 20 mV evoked the current. The conditioning and test pulses were separated with a short return to −80 mV for 10 milliseconds to assign channels either into the closed (resting) or inactivated state. This short interval did not virtually affect the ratio of the inactivated to total channels, which defines the availability of channels at a given membrane potential. Amplitudes of the current were normalized by those evoked without the conditioning potential. The normalized (relative) amplitudes were plotted against the conditioning potentials. The steady-state inactivation curve was drawn by fitting the data to the Boltzmann distribution: P = 1/[1 + exp((V − Vh)/k)], where P is the relative amplitude, V is the conditioning potential, Vh is the potential required for half inactivation of the current, and k is the Boltzmann coefficient.

According to the modulated receptor hypothesis, assuming a 1:1 binding of the drug to the channel, a binding constant (K_i) to channels in the resting state (K_r) was estimated from the current inhibition at negative HPs from the following equation: I_drug/I_control = 1/(1+[drug]/K_i), where I_control and I_drug are the amplitude of the current before and after application of the drug, respectively, and [drug] is the drug concentration. In the modulated receptor hypothesis, a shift in the steady-state inactivation curve induced by the drug comes from different sensitivities of the drug for different states of the channel. Accordingly, K_i of nicardipine for channels in the inactivated state (K_i) and in the resting state (K_r), was obtained from the following equation: V_h(control) − V_h(drug) = k ln((1+[drug]/K_i)/(1+[drug]/K_r)), where V_h(control) and V_h(drug) are the V_h values before and after nicardipine, respectively, and k is the Boltzmann coefficient.

Fitting the data to each equation was performed using the nonlinear least-squares method. The data are expressed as mean±SEM. Statistical significance was determined by an analysis of variance, followed by a multiple comparison test (Scheffé’s test). An unpaired t-test, if applicable, and Mann-Whitney test (nonparametric method) were also used to compare the groups. A value of P<0.05 was considered to be statistically significant.

Results

Blood Pressure and Body Weight

The systolic blood pressures of young (4- to 5-week-old) WKY rats (104±5 mm Hg, n=10 rats) and SHR (108±6 mm Hg, n=9 rats) were not different. Body weight did not differ between the two strains (WKY rats, 54±5 g; SHR, 53±4 g). At the adult age of 16 to 18 weeks, the systolic blood pressure of SHR (237±4 mm Hg, n=9 rats) was significantly (P<0.01) higher than that of WKY rats (167±3 mm Hg, n=11 rats). The body weights of SHR (273±8 g) and WKY rats (288±9 g) were not significantly different.

Current Density

The voltage-dependent Ca²⁺ channel current was recorded with the whole-cell voltage-clamp method using 50 mmol/L Ba²⁺ in the bath solution. Mean current densities of the Ca²⁺ channels in young SHR (n=35 to 38 cells; cell capacitance, 16±1 pF) and young WKY rats (n=28 to 30 cells; cell capacitance, 16±1 pF) at various command potentials are plotted in Fig 1A. The mean current densities of SHR were significantly larger than those of WKY rats (P<0.01). Differences were most evident at command potentials of 0 mV or higher.

In adult rats, the mean current densities of SHR (n=22 to 25 cells; cell capacitance, 19±1 pF) and WKY rats (n=26 to 29 cells; cell capacitance, 18±2 pF) at all command potential levels did not differ (Fig 1B).

The distributions of the current densities at a command potential of 20 mV are depicted in Fig 2. It is evident that the distribution of the current density of young SHR was skewed to the right compared with young WKY rats (Fig 2A versus 2B). The mean current densities at 20 mV in SHR and WKY rats were −16.8±1.1 pA/pF (n=38 cells) and −11.0±0.8 pA/pF (n=30 cells), respectively. Median values of the current density of young SHR and young WKY rats were −14.2
pA/pF and −10.4 pA/pF, respectively. When the difference in young rats was assessed by unpaired t test and the Mann-Whitney test, the differences were significant (P<.01 and P<.01, respectively).

The distributions for adult SHR and WKY rats were not different (Fig 2C versus 2D). The mean current densities at 20 mV in SHR and WKY rats were −15.9±1.3 pA/pF (n=25 cells) and −15.6±1.5 pA/pF (n=29 cells), respectively. Median values of the current densities of SHR and WKY rats were −14.3 pA/pF and −15.9 pA/pF, respectively.

When within-group ages were compared, the current density for WKY rats significantly increased with maturation (P<.01) (Fig 2B versus 2D). On the contrary, the current densities for young SHR and adult SHR were not significantly different (Fig 2A versus 2C).

**Activation and Inactivation Curves**

The activation curve for the Ca²⁺ channel current was obtained from each cell (n=27 to 39 cells in each group), as described in “Materials and Methods” (Fig 3A). The curves for SHR and WKY rats nearly overlapped for both young and adult ages. The curves for adult rats shifted in the hyperpolarizing direction compared with those for young rats. The mean values of the parameters for the Boltzmann distribution were as follows: young SHR (n=39 cells), V₅₀=6.3±1.1 mV and k=−7.7±0.3 mV; young WKY rats (n=31 cells), V₅₀=5.6±0.8 mV and k=−8.2±0.5 mV; adult SHR (n=27 cells), V₅₀=−0.2±1.0 mV and k=−7.6±0.6 mV; and adult WKY rats (n=30 cells), V₅₀=−1.4±1.0 mV and k=−7.8±0.4 mV. The V₅₀ values for adult rats were significantly smaller than those for young rats in both WKY and SHR strains (P<.01 and P<.01, respectively), whereas the k values were not significantly different.

The steady-state inactivation curves, obtained by the double-pulse protocol (n=12 to 15 cells in each group), were nearly overlapped in the four groups (Fig 3B). Parameters for the Boltzmann distribution were not different: young SHR (n=12), V₅₀=0.0±2.4 mV and k=11.5±0.9 mV; young WKY rats (n=12), V₅₀=0.9±2.3 mV and k=11.1±0.4 mV; adult SHR (n=15), V₅₀=−2.8±1.9 mV and k=11.2±0.5 mV; and adult WKY rats (n=13), V₅₀=−4.5±1.9 mV and k=10.4±1.0 mV.

**Types of Ca²⁺ Channels**

In arterial smooth muscle cells, the characteristics of T-type current are reported as follows: the current (1) was evoked at the lower command potentials, (2) disappeared within 100 milliseconds after depolarization, (3) was inactivated by lower HPs, and (4) was relatively resistant to dihydropyridines. The presence of the T-type current was evaluated by changing HPs using a double-pulse protocol. A test potential of −10 mV was used, as this potential was shown to evoke a relatively large T-type current.
Current tracings at various levels of HPs are superimposed in Fig 4A. Peak amplitudes were inhibited by HPs more positive than -40 mV. The transient (fast) component was nearly abolished and the sustained component was mostly preserved at an HP of -50 mV (Fig 4A). This figure suggests that the transient (fast) component is a T-type current and that the sustained component is an L-type current.

Actions of nickel (Ni\(^{2+}\)) on the transient (fast) component of the inward current were examined (Fig 4B). It was reported that 40 \(\mu\)mol/L Ni\(^{2+}\) completely inhibited the T-type current of sinoatrial nodal cells recorded with 2.5 mmol/L Ca\(^{2+}\) in the bath solution,\(^{20}\) whereas a higher concentration was required to abolish the T-type current of neuroblastoma cells (IC\(_{50}\) was 47 \(\mu\)mol/L) using 50 mmol/L Ba\(^{2+}\) in the bath.\(^{21}\) In the present study, the current was recorded with 50 mmol/L Ba\(^{2+}\), and the transient component was inhibited by 80 \(\mu\)mol/L Ni\(^{2+}\) (Fig 4B). The sustained component was relatively preserved. This result further suggests that the transient (fast) component observed at lower command potentials is a T-type current.

Fig 5A shows actual tracings of the Ca\(^{2+}\) channel current evoked by the command potential of -10 mV from five different cells of both young SHR and young WKY rats. It was reported in cultured azygous venous cells that the ratio of maximal amplitude of L-type current to that of T-type current (L/T ratio) was larger in SHR than in WKY rats.\(^{4}\) To clarify these results in the resistance artery, the ratio of the maximal amplitude of T-type current to that of L-type current (T/L ratio) was obtained for each cell. Several cells did not have any T-type current (T/L ratio=0) (4 cells of 36 cells in young SHR, 3 cells of 30 cells in young WKY rats, 4 of 31 cells in adult SHR, and 4 of 26 cells in adult WKY rats). In our previous study\(^{6}\) and our present experiments in high Ba\(^{2+}\), maximal amplitude of the T-type current was observed at negative command potentials (from -20 to 0 mV), and a maximal amplitude of the L-type current was observed at a command potentials of 20 to 30 mV. Furthermore, the T-type current nearly disappeared at positive command potentials above 20 mV. Thus, the transient component of the inward current at -10 mV (difference between peak amplitude and amplitude at 100 milliseconds) may correspond to the maximal amplitude of the T-type current. Similarly, the peak amplitude at 20 mV may correspond to the maximal amplitude of the L-type current. Since we used an HP of -80 mV, the current at 20 mV may contain a small T-type current; however, it was negligible as compared with the L-type current.

The mean T/L ratios for the four groups are shown in Fig 5B (young SHR [n=36], 0.10±0.01; young WKY rats [n=30], 0.16±0.02; adult SHR [n=31], 0.20±0.03; and adult WKY rats [n=26], 0.20±0.04). The T/L ratio for young SHR was significantly smaller than that for young WKY rats (P<.05) (Fig 5B).

Effects of Dihydropyridine on L-Type Channels

The binding of Ca\(^{2+}\) channel blocker to the L-type Ca\(^{2+}\) channels was evaluated according to a simple 1:1 binding version of the modulated receptor hypothesis.\(^{17,18}\) We examined an action of nicardipine on the current amplitude and the steady-state inactivation.

![Fig 2. Bar graphs showing distributions of the current densities at the command potential of 20 mV for young spontaneously hypertensive rats (SHR) (A), young Wistar-Kyoto (WKY) rats (B), adult SHR (C), and adult WKY rats (D).](http://circres.ahajournals.org/Downloadedfrom)
curve for the estimation of the binding constant $K_d$. A test command potential of 20 mV was used, because the command potential of 20 mV (in 50 mmol/L Ba$^{2+}$) or 30 mV (in 100 mmol/L Ba$^{2+}$) evoked mostly the L-type current and very little T-type current. Fig 6 shows the results from adult SHR and adult WKY rats. The steady-state inactivation curves were obtained by the double-pulse protocol before and after application of nickel $80 \mu$M
0.2 μmol/L nicardipine. Nicardipine inhibited the amplitude of inward current and shifted the steady-state inactivation curve to a hyperpolarized direction.

The $K_d$ values for the channels in the resting state ($K_r$) and inactivated state ($K_i$) were calculated for each cell, as described in "Materials and Methods," and are illustrated in Fig 7. The $K_d$ values were nearly the same in all groups (young SHR [n=12], 0.80±0.36 μmol/L; young WKY rats [n=12], 0.88±0.26 μmol/L; adult SHR [n=15], 0.60±0.28 μmol/L; and adult WKY rats [n=13], 0.62±0.19 μmol/L). The $K_i$ in adult SHR was lower than that in adult WKY rats (SHR [n=15], 2.0±0.5 nmol/L; WKY rats [n=13], 5.1±1.3 nmol/L; $P<.05$), whereas such a difference between SHR and WKY rats was not significant in the young rats (SHR [n=12], 4.0±1.0 nmol/L; WKY [n=12], 4.3±0.8 nmol/L).

**Discussion**

In the present study using the whole-cell voltage clamp, we tested the hypothesis that the voltage-dependent $Ca^{2+}$ channels in resistance mesenteric arteries of SHR are altered compared with those of normotensive WKY rats. The main findings were as follows: (1) In young SHR before developing hypertension, the current density of the voltage-dependent $Ca^{2+}$ channels was higher than that in young WKY rats; this difference disappeared after the development of hypertension. The current density increased with maturation in WKY, but not SHR, rats. (2) The voltage-dependent kinetics of activation and inactivation of the channels did not differ between SHR and WKY rats. (3) The estimated $K_d$ of the dihydropyridine derivative for the channel in the inactivated state was lower in adult SHR than in adult WKY rats; no differences were found in the young animals.

In the present study, the increased current density in young SHR was significant at command potentials of −10 mV or higher. Since command potentials above 0 mV mainly evoke the L-type current in vascular tissues in high Ba$^{2+}$ solution, the increased current component obtained in the present study was considered to be primarily of the L-type channels. Rusch and Hermsmeyer also reported that the L-type current was relatively predominant in SHR compared with WKY rats in cultured azygous venous cells from neonatal rats. In our results, the T/L ratio was smaller in young SHR than in young WKY rats because of the greater L-type current. On the basis of these two studies, we can postulate that enhanced L-type $Ca^{2+}$ channel activity already exists in neonatal SHR but disappears in the adult when the hypertension is present. The high current density of the $Ca^{2+}$ channels before the development of hypertension could play a role in the development of hypertension in SHR.

Distribution of $Ca^{2+}$ channels of arterial muscle tissues from SHR and WKY rats has been examined by
The maximal binding ($B_{\text{max}}$) of PN200-110 to aorta of SHR and WKY (4- and 13-week-old) rats was not significantly different. The $B_{\text{max}}$ values for nifedipine in the tail artery of SHR and WKY (6-week-old) rats were also nearly the same. If the channel density of the membrane in the resistance mesenteric arteries did not differ between SHR and WKY rats, as in the aorta and tail artery, then the increased current density of the Ca$^{2+}$ channel current in young SHR could be explained mainly by an increased availability of the channel for opening, namely an increased open probability.

The activation curves were shifted in a negative direction in adult rats compared with young rats to the same degree in both SHR and WKY rats. It is thus likely that maturation, but not hypertension, caused this change. Because the steady-state inactivation curve was not different between young and adult rats, the window current through Ca$^{2+}$ channels was larger in adult rats than in young rats, which would cause a continuous Ca$^{2+}$ influx. Thus, Ca$^{2+}$ influx via voltage-dependent Ca$^{2+}$ channels may be greater in adult rats than in young rats.

The current densities were the same levels in adult SHR and WKY rats after the hypertension had become established. Earlier development of L-type Ca$^{2+}$ channel occurred in SHR. It is not clear whether a factor for
Fig 7. Bar graphs showing estimated binding constants for channels in the resting state (Kᵦ, A) and in the inactivated state (Kᵢ, B), assessed from the modulated receptor hypothesis. SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto. The vertical axis is of log scale. Data are shown as mean±SEM. *P<.05 compared with WKY rats.

an upregulation disappears or a factor for a downregulation appears during the development of hypertension.

The estimated Kₐ of nicardipine for the inactivated channel was smaller in adult SHR than in adult WKY rats. Some factors, perhaps associated with hypertension, may secondarily produce this change, because no difference was seen between young SHR and young WKY rats. These observations may relate with the effectiveness of Ca²⁺ channel blockers in treating hypertension clinically.

In the binding studies of dihydropyridine derivatives (PN200-110 and nitrendipine) on membrane preparations from the aorta and tail artery of SHR and WKY rats, no difference in Kᵦ value has been reported between the two strains. The discrepancy between our results and those in the binding studies may be due to the use of different methods to obtain Kᵦ: biochemical versus electrophysiological, and membrane preparation versus single smooth muscle cell. Another possible explanation for the discrepancy might be a regional difference in the arteries: the aorta versus resistance mesenteric arteries.

Enhanced electrical activities have been reported in adult SHR and adult stroke-prone SHR compared with WKY rats in various vascular tissues. Spontaneous oscillation of membrane potentials was observed in small cerebral arteries of adult SHR. Norepinephrine produced regenerative electrical activities in tail arteries of adult stroke-prone SHR. The membrane potentials in adult SHR arteries were depolarized or unchanged compared with age-matched WKY rats. No enhanced membrane activities nor alterations of membrane potential were reported either in the tail artery or mesenteric artery of young SHR before established hypertension compared with the age-matched WKY rats.

\[[Ca^{2+}] \] in vascular smooth muscle cells from SHR and WKY rats has been studied using Ca²⁺ indicators. Most studies showed that the cells from adult SHR after the development of hypertension had higher [Ca²⁺] than those from adult WKY rats. Although the cells from young SHR before the development of hypertension had a resting [Ca²⁺], similar to that from WKY cells, agonists such as angiotensin II and vasopressin caused greater increases in SHR cells. Erne and Hermansmeyer reported that [Ca²⁺] in the submembrane area of cultured azygous venous cells (from neonates) was higher in SHR cells than in WKY cells, whereas the mean [Ca²⁺] in whole cytosol was not different. Although the increased activity of Ca²⁺ channels observed in the present study may not be directly linked with the resting [Ca²⁺], it may be related to the increased level of submembrane Ca²⁺ or to the enhanced entry of Ca²⁺ produced by agonists.

In summary, we have compared the Ca²⁺ channels of resistance arteries in SHR and WKY rats using the patch-clamp technique. The most obvious difference was that the current density was higher in young SHR than in young WKY rats. The present study thus provides additional support for the hypothesis that functional alterations of Ca²⁺ channels in the resistance artery may exist and play a role in the development of hypertension in SHR. The Ca²⁺ channels could be secondarily modified by maturation and hypertension.

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