Reduced Calcium Sensitivity of Dihydropyridine Binding to Calcium Channels in Spontaneously Hypertensive Rats

Hitoshi Ebata, Takashi Natsume, Takeshi Mitsuhashi, and Toshio Yaginuma

To explore the role of calcium channels in hypertension, dihydropyridine ([3H]PN200-110) binding to heart, brain, and skeletal muscle microsomes of 4-, 8- and 15-week-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats was measured. At a constant Ca\(^{2+}\) ion concentration (pCa 3.0), maximal binding (B\(_{\text{max}}\)) of dihydropyridine binding to heart and brain microsomes was significantly enhanced in 8- and 15-week-old SHR compared with WKY rats (p<0.01), whereas this phenomenon was not observed in 4-week-old SHR and WKY rats. B\(_{\text{max}}\) and dissociation constant (K\(_d\)) values for skeletal muscle microsomes from SHR showed no difference compared with WKY rats irrespective of age. Dihydropyridine binding to heart microsomes, brain microsomes, and solubilized skeletal muscle microsomes exhibited strong calcium dependence. The Ca\(^{2+}\)-dependent dihydropyridine binding curves for heart showed a Hill slope, and pK\(_{0.5}\) values for 15-week-old SHR and WKY rats were 0.70±0.12 and 4.66±0.12 versus 0.72±0.12 and 5.66±0.08 (n=4, mean±SD), respectively, indicating that 15-week-old SHR require 10-fold higher calcium concentration than WKY rats to promote dihydropyridine binding. The pK\(_{0.5}\) values of calcium for brain and solubilized skeletal muscle calcium channels in 15-week-old SHR were also significantly lower than in WKY rats. This difference first became apparent in SHR and WKY rats as early as 4 and 8 weeks after birth. These results suggest that enhancement of calcium channel density might occur in the heart and brain of SHR in response to elevated blood pressure and that reduced calcium sensitivity of dihydropyridine binding to calcium channels might be a primary characteristic of this rat strain.

(Hypertension 1991;17:234-241)

The intracellular Ca\(^{2+}\) concentration in spontaneously hypertensive rats (SHR) and patients with essential hypertension is reported to be increased in comparison with Wistar-Kyoto (WKY) rats and normotensive individuals, and such an increase in the cytosolic calcium ion concentration, which causes contraction of smooth muscle in the blood vessels, may play an important role in the development of hypertension.

To explain this phenomenon, abnormal calcium handling in the cell membrane of SHR has been predicted. For example, such abnormalities are thought to include degrangements of the calcium pump (Ca,Mg-ATPase), calcium binding to membranes, sodium-calcium exchanges, and the calcium channels themselves.

The effect of a calcium antagonist such as nifedipine on blood pressure in SHR and patients with essential hypertension is greater than in WKY rats or in normotensive individuals, suggesting that an abnormality of the calcium channel may exist in hypertension. Because calcium influx is modulated by the voltage-dependent calcium channel, the role of the calcium channel in the maintenance of intracellular calcium concentration is very important. Among the calcium antagonists, dihydropyridine (DHP) blocks the slow inward current most potently, and its receptor is considered to be a putative calcium channel.

By using [3H]DHP as a biochemical probe, calcium channels in several tissues from SHR and WKY rats have been characterized. After establishment of hypertension, Ishii et al reported that the density of calcium channels was increased in the brain of SHR, whereas in the heart no differences were observed between SHR and WKY rats. On the other hand, Rampe et al reported that there were no changes in
calcium channel density in either the brain or heart between SHR and WKY rats. Any difference in the density of calcium channels between SHR and WKY rats is still controversial. Because DHP binding to brain, heart, and smooth muscle, but not skeletal muscle, is modulated by calcium ions, the density of calcium channels should be measured under a constant free calcium ion concentration. So far, it appears that no previous studies have considered the free calcium ion concentration, an important regulator of DHP binding, when comparing the density of DHP receptors in SHR with that in WKY rats.

Therefore, to explore the role of the calcium channel in hypertension, we measured the density and DHP-binding affinity of calcium channels from heart, brain, and skeletal muscle of 4-, 8- and 15-week-old SHR and WKY rats under the same calcium ion concentration.

Because DHP-sensitive calcium channels are classified into three subtypes (i.e., heart, brain, and skeletal muscle) according to their sensitivity to EDTA, pH, and heparin, we compared the characteristics of calcium channels from these organs. In addition, the calcium sensitivities of DHP binding to these calcium channels were examined in both SHR and WKY rats at each age.

**Experimental Procedures**

Male Okamoto SHR and corresponding WKY normotensive control rats were purchased from Charles River Japan Co., and rats aged 4, 8 and 15 weeks were used. Systolic arterial blood pressure was measured by tail plethysmography (Natsume rat-tail manometer).

(+)-PN200-110 (80 μCi/mmol) was purchased from Amersham Corp., Arlington Heights, Ill. 3-(3-Cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), nifedipine, and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma Chemical Co., St. Louis, Mo. Protein concentration was measured with a liquid scintillation counter (Aloka LSC-700). Nonspecific binding was determined in the presence of 1 μM cold nifedipine.

**Preparation of heart, brain, and skeletal muscle microsomes.** The rats were killed by cervical dislocation, and the heart, brain, and skeletal muscle were removed immediately and placed in 4–5 volumes of cold buffer A (0.25 M mannitol, 70 mM Tris-HCl [pH 7.4], 0.01 mg/ml leupeptin, and 0.1 mM PMSF). All procedures were carried out at 4°C. Leupeptin (0.01 mg/ml) and PMSF (0.1 mM) were added to all buffers used to avoid proteolytic degradation.

Heart and brain microsomes were prepared by the modified procedure of Glossman and Ferry, as described by Moczylowski and Latorre with slight modification. Briefly, minced heart ventricles, whole brain (except the cerebellum), and skeletal muscle dissected from the back were homogenized twice in buffer A using a Polytron homogenizer (Kinematica, Switzerland) for 20 seconds each time at medium speed, with a 20-second interval between. The homogenate was centrifuged at 1,900g for 30 minutes, and then the precipitate was resuspended with buffer A, homogenized, and centrifuged as described above. The procedure was repeated twice, and the resulting supernatants were combined and filtered through two layers of nylon gauze (50 mesh). Solid KCl was added to a final concentration of 0.6 M to the skeletal muscle supernatant to solubilize the contractile proteins. The filtered supernatant was then centrifuged at 70,100g for 40 minutes, and the resulting pellet was suspended in buffer A for use in the DHP binding assay.

To examine the purity of the heart the microsome preparation, ouabain-sensitive Na,K-ATPase in microsomes at each stage, was measured by the method of Fiske and Subbarow. The ouabain-sensitive Na,K-ATPase activity of SHR showed no significant difference in comparison with matched WKY rats (data not shown), indicating similar microsome purity between SHR and WKY rats.

**Radioligand Binding Studies**

**Binding assay with heart, brain, and skeletal muscle microsomes.** The modified binding assay of Glossman and Ferry was carried out. Microsomes (30–100 μg protein) were incubated in 0.5 ml of a solution containing 200 mM 3-(N-morpholino)propanesulfonic acid (MOPS pH 7.0), 90 mM KCl, and 2 mM EGTA at different free Ca²⁺ concentrations with the indicated concentration of [³H]PN200-110 for 40 minutes at 30°C. After incubation, samples were withdrawn and filtered through a Whatman GF/C glass filter under reduced pressure. The filter was washed once for skeletal muscle and three times for heart and brain with 4 ml ice-cold 50 mM MOPS, pH 7.0, and the radioactivity bound to the filter was measured with a liquid scintillation counter (Aloka LSC-700). Nonspecific binding was determined in the presence of 1 μM cold nifedipine.

PN200-110 binding assay with solubilized skeletal muscle microsomes. This assay was carried out by a modification of the procedure of Glossman and Ferry. Skeletal muscle microsomes were solubilized with 0.3% CHAPS. The solubilized samples (40 μg) were incubated with 0.5 ml of solution (0.1% CHAPS, 1 mg/ml bovine serum albumin [BSA] as a carrier protein, 200 mM MOPS, pH 7.0, 90 mM KCl, 2 mM EGTA, and the indicated concentration of [³H]PN200-110) at 30°C for 40 minutes under different pCa²⁺ conditions. Then 4 ml ice-cold 10% polyethylene glycol, 50 mM MOPS, pH 7.0, was used to precipitate the solubilized microsomes onto Whatman GF/C filters. Nonspecific binding was determined in the presence of 1 μM cold nifedipine.

**Determination of Free Ca²⁺ Concentration**

Free Ca²⁺ concentration was calculated as described by Johnson and Potter. Ca²⁺ titrations were conducted in a buffer composed of 200 mM MOPS, pH 7.0, 90 mM KCl, and 2 mM EGTA. To determine
TABLE 1. Body Weight, Systolic Blood Pressure, and Heart and Brain Weights

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SHR (n)</th>
<th>WKY (n)</th>
<th>SHR (n)</th>
<th>WKY (n)</th>
<th>SHR (n)</th>
<th>WKY (n)</th>
<th>SHR (n)</th>
<th>WKY (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>58.8±5.4</td>
<td>79±2.74</td>
<td>119±8.0</td>
<td>117±12.0</td>
<td>138±14.5</td>
<td>127±8.7</td>
<td>132±7.3</td>
<td>141±4.5</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>128±13.4</td>
<td>112±2.0</td>
<td>142±8.9</td>
<td>138.8±3.2</td>
<td>158±8.29</td>
<td>158±12.0</td>
<td>138±6.7</td>
<td>140±10.0</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>340±32*</td>
<td>377±22</td>
<td>548±33.7*</td>
<td>482±19.8</td>
<td>1,034±130*</td>
<td>784±119</td>
<td>502±36.4*</td>
<td>437±34.1</td>
</tr>
<tr>
<td>Brain weight (g)</td>
<td>1.06±0.099</td>
<td>1.08±0.063</td>
<td>1.70±0.21</td>
<td>1.66±0.25</td>
<td>1.76±0.279</td>
<td>2.24±0.207</td>
<td>1.46±8.8</td>
<td>1.46±8.8</td>
</tr>
</tbody>
</table>

Values given are mean±SD. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

Data Analysis

Data are given as mean±SD. Hill plots were analyzed by an interactive nonlinear regression Hill program, and $B_{\text{max}}$ was determined by best fit of the data to the Hill equation. The statistical significance of differences in body weight and blood pressure between two age-matched groups was analyzed by unpaired Student's $t$ test.

Results

The number of rats in each group, their body weight, systolic blood pressure, and some other parameters are shown in Table 1. Blood pressure of SHR was significantly higher than that of WKY rats at 6 weeks of age, and hypertension developed at about 7–8 weeks. Therefore, we selected 4-week-old SHR as representing the prehypertension stage, 8-week-old SHR as the developing hypertension stage, and 15-week-old SHR as the established hypertension stage.

The ratio of heart weight-to-body weight (mg/g×100) for SHR was significantly higher than for WKY rats at each stage. There was no difference in whole brain weight between age-matched SHR and WKY rats.

PN200-110 Binding to Heart, Brain, and Skeletal Muscle Microsomes

The density and affinity of PN200-110 binding to each tissue were measured at a constant free calcium ion concentration (pCa=3.0) because Ca$^{2+}$ is an important regulator of DHP binding. The results were compared between age-matched SHR and WKY rats. Table 2 shows the results of $B_{\text{max}}$ and $K_d$ for [3H]PN200-110 binding to heart, brain, and skeletal muscle microsomes from Scatchard analysis.

In the heart binding study, there were no significant differences in $B_{\text{max}}$ and $K_d$ between 4-week-old SHR and WKY rats. $B_{\text{max}}$ for the heart of 8-week-old SHR was significantly enhanced ($p<0.05$) as com-
pared with 8-week-old WKY rats. B\text{max} of 15-week-old SHR was higher (p<0.01) than that of WKY. There were no significant differences in K\text{d} between SHR and WKY rats from 8 to 15 weeks of age. This result suggests that before the development of hypertension, there were no differences in the density of heart calcium channels or their affinity, whereas at the stages of development and establishment of hypertension in SHR, the density of heart calcium channels was greater than in age-matched WKY rats.

Table 2 also shows the B\text{max} and K\text{d} for [3H]PN200-110 binding to brain microsomes. There was no difference in B\text{max} and K\text{d} between 4-week-old SHR and WKY rats, but the B\text{max} of [3H]PN200-110 binding to SHR at 8 and 15 weeks of age was significantly greater than that of age-matched WKY rats (p<0.01). This result obtained with brain is similar to the result for heart, suggesting that these differences in DHP receptor density might be due to the secondary effects of hypertension.

B\text{max} and K\text{d} for the skeletal muscle microsomes of 4-, 8-, and 15-week-old SHR and WKY rats are shown in Table 2. There were no significant differences in B\text{max} and K\text{d} between age-matched SHR and WKY rats at any stage, indicating that the density and affinity of skeletal muscle calcium channels might not be affected by elevation of blood pressure.

Statistical analysis (ANOVA followed by the Bonferroni method) showed that the density and affinity of calcium channels from the heart and brain in both SHR and WKY rats decreased with age, whereas those of skeletal muscle increased with age.

**Calcium Dependence of PN200-110 Binding to Heart, Brain, and Solubilized Skeletal Muscle Microsomes**

**Difference in calcium dependence of dihydropyridine binding to heart calcium channels.** As reported by Luchowski et al.,\textsuperscript{33} DHP binding to heart calcium channels showed strong calcium dependence, suggesting that the calcium channel is a calcium-binding protein. Figure 1 shows Scatchard analysis of DHP binding at pCa 3.0 and pCa 9.0 to microsomes from the heart of 15-week-old SHR. The B\text{max} at pCa 3.0 and pCa 9.0 was 220 and 57 fmol/mg protein, respectively, whereas the value of K\text{d} for pCa 3.0 and pCa 9.0 was 70 and 69 pM, respectively. This suggests that the increase of DHP binding to the heart was due to the B\text{max} effect, with little affect on K\text{d}.

Figure 2 shows the calcium dependence of DHP binding to heart calcium channels from age-matched SHR and WKY rats under almost identical [3H]PN200-110 concentrations. Figure 2A demonstrates the calcium dependence of PN200-110 binding to heart microsomes from 4-week-old SHR and WKY rats. Calcium increased the PN200-110 binding to SHR heart microsomes from 175±10 fmol/mg protein (pCa 9.0) to 423±7.0 fmol/mg protein. Calcium produced an increase in PN200-110 binding to WKY rat heart microsomes with a pK 0.5 of 4.7±0.06 with a Hill coefficient of 0.68±0.18 (r=0.986). When free calcium was increased from pCa 9.0 to 3.0, PN200-110 binding to the WKY rat heart microsomes increased from 188±3.49 fmol/mg protein to 426±6.41 fmol/mg protein. Calcium produced an increase in PN200-110 binding to WKY rat heart microsomes with a pK 0.5 of 6.1±0.19 and a Hill coefficient of 0.697±0.16 (r=0.988), indicating that SHR requires a calcium concentration 25 times higher than WKY.
Ca\(^{2+}\)– DH\(_{\text{H}}\) BINDING Curves

(Heart)

![Graph showing Ca\(^{2+}\)– DH\(_{\text{H}}\) BINDING Curves](image)

**Figure 2.** Line graphs showing Ca\(^{2+}\) dependence of dihydropyridine (DH\(_{\text{P}}\)) binding to heart calcium channels. Panel a: Difference in calcium dependence of PN200-110 binding to heart microsomes between 4-week-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Computer analysis of calcium data from SHR and WKY rats demonstrated good fit to a one-site model. In 4-week-old SHR, PN200-110 binding was increased from 175±10 fmol/mg protein (pCa 9.0) to 423±7.0 fmol/mg protein (pCa 3.0), and the pK\(_{0.5}\) for Ca\(^{2+}\) and Hill coefficient were 4.7±0.06 and 0.64±0.18 (r=0.986, n=4, mean±SD), respectively. In 4-week-old WKY rats, PN200-110 binding was increased from 188±3.49 fmol/mg protein (pCa 9.0) to 426±6.41 fmol/mg protein (pCa 3.0) and the pK\(_{0.5}\) for Ca\(^{2+}\) and Hill coefficient were 0.72±0.18 (n=5, mean±SD), respectively. Panel b: PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. In SHR, PN200-110 binding was increased from 59.3±11.9 fmol/mg protein (pCa 9.0) to 244±14 fmol/mg protein (pCa 3.0), and the pK\(_{0.5}\) and Hill slope were 4.5±0.10 and 0.72±0.18 (n=5, mean±SD), respectively. In WKY rats, PN200-110 binding was increased from 55.8±13.6 fmol/mg protein (pCa 9.0) to 219±7.28 fmol/mg protein (pCa 3.0), and the pK\(_{0.5}\) and Hill slope were 4.6±0.12 and 0.70±0.12 (n=4, mean±SD), respectively. Panel c: PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. In SHR, PN200-110 binding was increased from 78±6.3 fmol/mg protein (pCa 9.0) to 348±6.41 fmol/mg protein (pCa 3.0), and the pK\(_{0.5}\) and Hill slope were 5.66±0.08 and 0.72±0.12 (n=4, mean±SD), respectively. Concentration of [H]PN200-110 used was 0.909±0.05 nM (n=5, mean±SD).

**Figure 3.** Illustrates the calcium dependence of DH\(_{\text{P}}\) binding to brain calcium channels. Figure 3 illustrates the calcium dependence of DH\(_{\text{P}}\) binding to brain microsomes from 8-week-old SHR and WKY rats. Calcium increased PN200-110 binding to brain microsomes from SHR and WKY rats from 34.1±1.97 fmol/mg protein (pCa 9.0) to 100±4.32 fmol/mg protein (pCa 3.0), and 20±3.2 fmol/mg protein (pCa 9.0) to 78±6.3 fmol/mg protein (pCa 3.0), respectively. The pK\(_{0.5}\) and Hill coefficient for SHR and WKY rats were 4.6±0.18, 0.70±0.12 and 6.0±0.12, 0.72±0.06, respectively. As was the case for heart calcium channels, this calcium-dependent increase of PN200-110 binding to brain calcium channels was modulated by the B\(_{\text{max}}\) effect with little change in K\(_{d}\) (data not shown). Significantly reduced sensitivity of DH\(_{\text{P}}\) binding to calcium was observed in 4- and 15-week-old SHR as compared with age-matched WKY rats (Table 3).

**Figure 4.** Shows the DH\(_{\text{P}}\) binding-calcium ion curves for solubilized skeletal muscle calcium channels. DH\(_{\text{P}}\) binding to skeletal muscle microsomes is known to be calcium independent, whereas the solubilized microsomes are known to exhibit strong calcium dependence for DH\(_{\text{P}}\) binding. Presumably this difference is caused by contamination due to calcium ions entrapped within everted T tubule vesicles, which promote DH\(_{\text{P}}\) binding to microsomes. To determine whether the calcium sensitivity of DH\(_{\text{P}}\) binding to skeletal muscle calcium channels from SHR differs from that of WKY rats, DH\(_{\text{P}}\) binding to the solubilized skeletal muscle microsomes was examined. CHAPS at 0.3% solubilized about 20% of the total protein and preserved about 10% of the specific activity of DH\(_{\text{P}}\) binding as compared with the original microsome activity.

**Panel a:** Difference in calcium dependence of dihydropyridine (DH\(_{\text{P}}\)) binding to heart calcium channels. **Panel b:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel c:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel d:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel e:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel f:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel g:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel h:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel i:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel j:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel k:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel l:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel m:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel n:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel o:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel p:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel q:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel r:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel s:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel t:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel u:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel v:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel w:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats. **Panel x:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 8-week-old SHR and WKY rats. **Panel y:** PN200-110 binding Ca\(^{2+}\) curves for heart microsomes from 15-week-old SHR and WKY rats.

**Higher than that of WKY rats to promote DH\(_{\text{P}}\) binding. Figures 2B and 2C also demonstrate the calcium dependence of DH\(_{\text{P}}\) binding to heart microsomes for 8- and 15-week-old SHR and WKY rats, respectively. The values of pK\(_{0.5}\) and Hill coefficient for 8-week-old SHR and WKY rats were 4.5±0.10, 0.72±0.18 and 5.6±0.13, 0.67±0.13, respectively. In addition, the pK\(_{0.5}\) and Hill coefficient for 15-week-old SHR and WKY rats were 4.66±0.12, 0.70±0.12 and 5.66±0.08, 0.72±0.12, respectively. The pK\(_{0.5}\) value for SHR tested at any stage was significantly greater than that of age-matched WKY rats (p<0.01, Table 3). There were no significant changes in Hill slopes, but a reduced calcium sensitivity for DH\(_{\text{P}}\) binding in the heart calcium channel of SHR as compared with that of WKY rats was observed at all ages tested.
Table 3. pK 0.5 and Hill Slope for Dihydropyridine Binding-Calcium Ion Curve

<table>
<thead>
<tr>
<th>Tissue</th>
<th>4 weeks old</th>
<th>8 weeks old</th>
<th>15 weeks old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pK 0.5</td>
<td>Hill</td>
<td>pK 0.5</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>*4.7±0.06</td>
<td>0.68±0.18</td>
<td>*4.5±0.10</td>
</tr>
<tr>
<td>WKY</td>
<td>6.1±0.05</td>
<td>0.70±0.16</td>
<td>5.6±0.13</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>*4.7±0.08</td>
<td>0.68±0.08</td>
<td>*4.6±0.18</td>
</tr>
<tr>
<td>WKY</td>
<td>5.8±0.07</td>
<td>0.70±0.14</td>
<td>6.0±0.12</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>*5.6±0.13</td>
<td>0.71±0.13</td>
<td>*5.5±0.06</td>
</tr>
<tr>
<td>WKY</td>
<td>6.2±0.20</td>
<td>0.68±0.12</td>
<td>6.1±0.08</td>
</tr>
</tbody>
</table>

Values given are mean±SD (n=4). SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

Discussion

Ishii et al reported that the density of calcium channels (DHP receptors) was increased in the brain of SHR as compared with WKY rats, whereas Rampe et al reported that there was no such strain difference in calcium channel density in the brain. Lee et al reported that the calcium channel density in the brain stem of rats with deoxycorticosterone-NaCl hypertension was decreased. Therefore, it appears necessary to explain this disparity among the results obtained by different investigators. To our knowledge, no previous reports have taken into account the free calcium ion concentration by which DHP binding to brain and heart is modulated. Ishii et al used Tris-HCl buffer without addition of calcium ions, and Rampe et al used the Tris-HCl buffer with added 1.2 mM magnesium ion, which like calcium ion also modulates DHP binding to calcium channels.

Because the free Ca²⁺ ion concentration was not controlled in previous studies, it is not surprising that affinity observed in skeletal muscle (fourfold change versus 25-fold in 4-week-old heart) may be due to partial inactivation of the calcium channels by detergent.

The values of pK 0.5 and Hill coefficient for the heart, brain, and solubilized skeletal muscle calcium channels are summarized in Table 3.
the results disagree. For example, if calcium channel density were compared at pCa 6.0 (without chelating agents such as EDTA or EGTA, it is very difficult to remove contamination due to calcium ion, which is usually about 1 μM), there would be no significant difference between SHR and WKY rats (in fact there was no difference in heart calcium channel density, according to Ishii et al22; also see Figure 2). If the density were compared at pCa 3.0, the density of brain calcium channels for 8-week-old SHR would be greater than that for WKY rats (see Figure 3).

In the present study, the calcium ion concentration was determined accurately using a calcium-sensitive dye, and under a constant free calcium ion concentration, the density (Bmax) and affinity (Kd) of SHR before and after hypertension were compared with the age-matched WKY rats.

Before hypertension, there was no difference in Bmax and Kd for heart calcium channels between SHR and WKY rats, but after the elevation of blood pressure, the density of heart calcium channels from 8- and 15-week-old SHR was higher than in age-matched WKY rats. As in the case of heart, brain calcium channel density in SHR was higher than that in WKY rats after development of hypertension but not before. This result suggests that the increased calcium channel density in these organs of SHR may be secondary to the onset of hypertension. Unlike the heart and brain, there were no differences in Bmax and Kd for skeletal muscle microsomes between SHR and WKY rats. It is still unknown why the result for skeletal muscle differs from those for heart and brain.

Using an accurately determined free calcium ion concentration condition, the calcium dependence of DHP binding to heart, brain, and solubilized skeletal muscle calcium channels was examined. DHP binding to the heart, brain, and solubilized skeletal muscle microsomes revealed calcium-dependent DHP binding, indicating that Ca channels in all three preparations have calcium domains that regulate DHP binding, and that the calcium channel is similar in this respect to Ca2+ binding proteins such as calmodulin and troponin C.30,51

The calcium sensitivity of calcium domains of calcium channels from the heart, brain, and skeletal muscle of SHR was reduced as compared with age-matched WKY rats. This reduced sensitivity of SHR was observed at all stages tested, and therefore this might be a primary characteristic change of SHR. The calcium domains of skeletal muscle calcium channels that regulate DHP binding are reported to be located at an extracellular site, although Schilling52 has reported that the calcium domains of heart calcium channels might exist intracellularly.

It has been reported that cytosolic calcium ions in several tissues of SHR and patients with essential hypertension are increased,1-8 and that abnormal calcium handling exists in the membrane system in SHR.9-26 Therefore, it might be possible for these calcium domains to modulate calcium entry through the calcium channels by changing their calcium sensitivity, and the reduced calcium sensitivity of the calcium channel in SHR might lock the protective mechanism against any cytosolic increase in calcium ions.

Although we have thus described the calcium dependence of DHP binding to heart, brain, and skeletal muscle calcium channels and reduced calcium sensitivity of DHP binding in SHR, further comparison of calcium channels between SHR and WKY rats should be done. In the future, to avoid many factors that might affect DHP binding in microsomes (membrane sidedness, proteases, phospholipase), purified calcium channels from each organ should be used for comparative studies between SHR and WKY rats.

Acknowledgments

We are grateful for the excellent secretarial assistance of Sachiko Tanaka, Hiromi Shimada, and Etsuko Chiku. We are indebted to Professor J. David Johnson and Dr. John S. Mills (Department of Physiological Chemistry, Ohio State University) for fruitful discussion and advice.

References


17. Pospelov YV, Orlov SN, Pokudin NI: Decrease of calcium binding by the red blood cell membrane in spontaneously hypertensive rats and in essential hypertension. Pflügers Arch 1979;379:191-195


34. Schilling WP: Effect of divalent cation chelation on dihydropriydine binding in isolated cardiac sarcolemma vesicles. Biochim Biophys Acta 1984;792:220-230


KEY WORDS • ion channels • dihydropriydines • calcium • calcium antagonists
Reduced calcium sensitivity of dihydropyridine binding to calcium channels in spontaneously hypertensive rats.

H Ebata, T Natsume, T Mitsuhashi and T Yaginuma

Hypertension. 1991;17:234-241
doi: 10.1161/01.HYP.17.2.234

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/17/2/234

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

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

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/