Effects of a Chronic Treatment by Nisoldipine, a Calcium Antagonistic Dihydropyridine, on Arteries of Spontaneously Hypertensive Rats

Théophile Godfraind, Stanislav Kazda, and Maurice Wibo

Earlier studies have shown that relaxation in response to several agents is impaired in arteries from spontaneously hypertensive rats (SHR). We had previously reported that SHR aortas present a delayed relaxation when first exposed for 35 minutes to a 100 mM KCl solution and then transferred into physiological solution. The first phase of relaxation appeared similar in SHR and Wistar-Kyoto rat arteries, but the second phase was markedly slowed down in SHR arteries, giving rise to a postcontraction tone. In this study, we found that this postcontraction tone could be demonstrated not only in the aorta but also in the mesenteric artery, was independent of the presence of endothelium, increased with the age of SHR, and disappeared progressively when arterial segments were submitted to successive cycles of KCl depolarization followed by reimmersion in physiological solution. Chronic treatment of SHR with nisoldipine at doses that blocked the development of hypertension and attenuated the concomitant hypertrophy of heart and aorta, or in vitro pretreatment of SHR arteries with nisoldipine, decreased the contractile force developed by arteries in response to KCl depolarization and normalized the subsequent relaxation. \([^3]H\)(+)-PN200-110 binding studies on heart and brain homogenates indicated an increase in apparent \(K_d\) in nisoldipine-fed rats without significant change in \(B_{max}\). Binding data were compatible with the view that occupation of dihydropyridine receptors by nisoldipine after chronic oral administration was responsible for the modifications observed ex vivo in the mechanical activity of arteries. We conclude that the postcontraction tone of SHR arteries was mainly due to an abnormally prolonged activation of calcium channels after transfer of depolarized arteries into the physiological solution and that a labile or slowly releasable factor was probably involved in this phenomenon. We suggest that the antihypertensive action of nisoldipine might be related to the mechanisms involved in the suppression of the postcontraction tone as observed in vitro and that this mode of action could be more important than the vasodilating effect of this drug. (Circulation Research 1991;68:674–682)

Dihydropyridine calcium antagonists are now established for the management of essential hypertension, but the mechanism of their therapeutic action still needs to be characterized.\(^1\)\(^–\)\(^3\) Because dihydropyridines are potent blockers of potential-operated calcium channels, it has been proposed that their antihypertensive effect could be related to vasodilation. However, unlike other vasodilators, calcium antagonists are more effective as blood pressure lowering agents in hypertensive than in normotensive rats and humans.\(^4\)\(^–\)\(^6\) It has been hypothesized that this could be due to abnormalities of calcium channels in the cell membrane of arterial muscle in hypertension.\(^7\) Several authors have proposed that vascular smooth muscles from hypertensive animals present abnormalities with respect to the relation between the cellular metabolism of \(Ca^{2+}\) and excitation–contraction coupling. Cauvin and van Bree\(^8\) and Kazda et al\(^9\) have reported that the sensitivity of receptor-operated calcium channels to dihydropyridines is altered in hypertensive rats. Winquist and Bohr\(^10\) have observed that arteries from spontaneously hypertensive rats (SHR) display an endogenous tone that is suppressed in calcium-free EGTA-containing solution.
In 1972, Field et al. reported that aortic segments from hypertensive rats, which had been contracted in a solution containing 80 mM KCl, relaxed more slowly than segments from control rats when the depolarizing solution was washed from the bath with normal Krebs’ solution. In their study of aortic segments from SHR, Godfraind et al. confirmed these observations and showed that this anomalous relaxation was corrected in the presence of a calcium antagonist. This suggested that this behavior was primarily related to alterations in calcium channel functioning rather than in the pumping mechanisms that restore a low concentration of cytoplasmic Ca²⁺.

The present experiments were designed to investigate if the anomalous tone after KCl-evoked contraction (postcontraction tone) could be related to the age-dependent evolution of hypertension and if it could be observed in arteries other than the aorta. In addition, we examined if treatment of rats by a dose of nisoldipine already reported to attenuate the development of hypertension could modify both the ex vivo responsiveness of aortas and mesenteric arteries to KCl depolarization and their postcontraction tone. We also performed ligand studies to estimate the occupation of dihydropyridine receptors in various tissues when nisoldipine was given in the food during prolonged periods.

The results show that the enhanced postcontraction tone was also present in SHR mesenteric arteries and was age related. It disappeared when isolated arteries were repeatedly stimulated in organ baths for prolonged periods and was suppressed after chronic administration of nisoldipine at doses that prevented the development of hypertension or by pretreatment of isolated arteries with nisoldipine. These observations suggest that the enhanced postcontraction tone is attributable primarily to a persistent activation of calcium channels after removal of the depolarizing stimulus, rather than to defective relaxation processes, and that this abnormal state of calcium channels might be caused by a labile or slowly exchangeable factor. Thus, calcium antagonists might prevent the development of hypertension by interfering with mechanisms related to this putative dysfunctioning of calcium channels.

**Materials and Methods**

**Animals**

At the age of 8 weeks, male SHR and Wistar-Kyoto (WKY) rats (Moelgaard, Mejby, Denmark) were divided at random into two groups; one group was used as a control and received ordinary food (Sniff GmbH, Soest, FRG), and the other received the same food, but it also contained 1,000 ppm nisoldipine. They were kept in the same environment and received food and water ad libitum. The average food utilization of each group was similar. Systolic blood pressure was measured every week by the tail-cuff method in conscious animals prewarmed to 35°C in thermostatic cages.

Several randomized groups of age-matched SHR and WKY rats in equal number were used in these experiments. To validate our initial observations on the influence of age on the parameters so far examined, we also designed two randomized experiments with 36 SHR and 36 WKY rats; each of these groups was subdivided into three subgroups maintained alive during 9–12, 14–16, and 34–36 weeks, respectively.

**Drugs**

The dihydropyridine nisoldipine (isobutyl methyl 1,4 dihydro-2,6-dimethyl-4-[2-nitrophenyl]-3,5-pyridinedicarboxylate) was mixed into the commercial diet (1,000 ppm), which was formulated in the usual pellet form (“Sniff R”). For in vitro studies, nisoldipine was dissolved in ethanol as a stock solution of 10⁻³ M. This solution was thereafter diluted in the experimental medium.

**Ex Vivo Study of Arterial Contraction and Relaxation**

Immediately after the rats were killed by decapitation, the thoracic aortas and superior mesenteric arteries were removed and cleaned of connective tissue. From each animal, four rings (2 mm wide) of thoracic aorta and superior mesenteric artery were cut and suspended in organ chambers (50 ml) filled with physiological solution under a resting tension of 20 and 10 mN, respectively. In some experiments, the intimal surface of the rings had been gently rubbed with the ends of a small forceps to remove endothelial cells (effective endothelium removal was confirmed by the absence of relaxation to acetylcholine). Rings were connected to an isometric level with two strain gauges as part of a balanced bridge. The output was fed into an analog/digital converter, which was connected to a Kontron microcomputer (model Psi 80 D, Kontron GmbH, Eching, FRG). The program allowed input from up to eight force transducers simultaneously. After suitable calibration, tensions were displayed on the screen of the microcomputer, recorded on a printer, and stored on disk at predetermined time intervals.

After being mounted in organ chambers, rings of arteries were allowed to equilibrate for 90 minutes in physiological solution (37°C), which was aerated with a gas mixture of 95% O₂-5% CO₂ and contained (mM) NaCl 112, KCl 5, NaHCO₃ 25, KH₂PO₄ 1, MgSO₄ 1.2, CaCl₂ 1.25, and glucose 11.5. The physiological solution was then replaced by a depolarizing solution, which contained (mM) NaCl 17, KCl 100, CaCl₂ 1.25, NaHCO₃ 25, KH₂PO₄ 1, and glucose 11.5. The subsequent contraction was recorded during 35 minutes. Thereafter, the 100 mM KCl solution was replaced by the physiological solution (a procedure achieved in 8 seconds), and the tone was recorded as long as necessary (20–80 minutes). The contraction/relaxation cycle was repeated three times for each preparation, unless otherwise stated, at 90, 180, and 360 minutes after suspension of the vessels in the organ chambers. Four rings from a control artery and
four rings from an artery prepared from a nisoldipine-treated rat were recorded simultaneously.

**Calculation of Results and Statistical Analysis**

Means (±SEM) of contractile forces developed during observation of arteries were calculated at 10, 20, and 30 seconds, and 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, and 35 minutes after each change of solution. For each group of preparations the number of segments and the number of rats are reported. The significance of differences between means was checked by Student’s t test; values of p<0.05 were considered to be significant.

**Binding Studies**

Binding studies were performed on homogenates from heart and forebrain. These tissues were obtained from the same animals from which arteries were taken for contractility studies. The use of whole homogenates avoided loss of nisoldipine that would have occurred in washing steps associated with purification of membranes from nisoldipine-fed rats. Tissues (about 1 g) were minced in 10 ml ice-cold Tris-buffered physiological solution ([mM] NaCl 145, KCl 5, MgCl$_2$ 1.25, CaCl$_2$ 1.25, and Tris 20; pH 7.5) and then homogenized in an all glass Potter-Elvehjem grinder. Homogenates were filtered through a nylon sieve (200-µm mesh). Homogenates from nisoldipine-treated rats were protected from light.

$[^3]$H$^+(+)$-PN200-110 binding was measured at 37°C as described previously, using about 100 µg protein (as estimated by the method of Lowry et al$^{17}$) in a final volume of 0.5 ml. Total and nonspecific binding (1 µM nifedipine added) were determined in triplicate at five $[^3]$H$^+(+)$-PN200-110 concentrations (from 30 to 600 pM). $K_d$ and $B_{max}$ values were obtained from Scatchard plots of specific binding. $[^3]$H$^+(+)$-PN200-110 (86 Ci/mmol) was from New England Nuclear Research Products, Boston.

**Results**

**Biometric Parameters of WKY and SHR**

The evolution of blood pressure in control rats and in rats fed with nisoldipine was compatible with already published observations.$^{13}$ As illustrated in Table 1, for 20–22 weeks old rats, nisoldipine treatment attenuated the rise in blood pressure of SHR ($p<0.001$) and did not alter the blood pressure of WKY rats. This treatment did not affect the weight of rats (data not shown), but it significantly reduced heart and aorta hypertrophy observed in SHR (Table 1).

**KCl-Evoked Contraction and Postcontraction Tone of the Aorta and Mesenteric Artery**

Figure 1 shows that tension development in response to KCl depolarization was very similar in aortas isolated from untreated WKY and SHR and that it was not greatly influenced by the age of rats. The contractile response was depressed after chronic treatment with nisoldipine, but the inhibition (measured at 35 minutes) was more pronounced in WKY (43±3.7%, $n=36$) than in SHR (30.5±3.5%, $n=36$) ($p<0.05$). For mesenteric arteries, the force of contraction was lower in younger than in older rats, but there was no major difference between SHR and WKY rats (see Figure 2). In mesenteric arteries from nisoldipine-treated rats (not shown), the reduction of the developed tension was significantly more important in WKY than in SHR only at the age of 9–12 weeks ($p<0.01$).

Figure 2 also illustrates the time course of the decrease in tension after washing out the 100 mM KCl solution. With WKY rat arteries, return to precontraction tone occurred as a monophasic function of time, whereas with SHR arteries, a fast and a slow phase could be distinguished. As Figure 2 illustrates, the fast phase corresponded to an initial decrease in tension of about 20% in aorta and 40% in mesenteric artery, and it was not age related. The half-life of the slow phase was related to the age of the SHR, the postcontraction tone at 10 minutes being much higher in older than in younger rats ($p<0.001$). In arteries from the oldest groups, this postcontraction tone was still elevated after 60 minutes. A convenient parameter related to the magnitude of this tone is the time required to reach 50% of the KCl-induced force. As shown in Table 2, for aortas, this time was higher in SHR than in WKY rats ($p<0.001$) and it increased with aging of SHR but not of WKY rats. Interestingly, as shown in Figure 3, when SHR arteries were submitted to a second and a third prolonged exposure to the KCl solution, the postcontraction tone was lower than after the first contraction.

The postcontraction tone of SHR aortas (24–25 weeks) was not appreciably affected by removal of endothelium. In the presence of endothelium, the residual tone 35 and 70 minutes after washout of the 100 mM KCl solution amounted to 9.21±0.81 and

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**Table 1. Biometric Parameters of Wistar-Kyoto and Spontaneously Hypertensive Rats**

<table>
<thead>
<tr>
<th></th>
<th>Blood pressure (mm Hg)</th>
<th>Heart weight (g/100 g)</th>
<th>Aorta weight (mg/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>139±4.4</td>
<td>0.29±0.01</td>
<td>0.70±0.03</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>132±3.2</td>
<td>0.29±0.01</td>
<td>0.69±0.01</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>230±2.7*</td>
<td>0.37±0.01*</td>
<td>1.00±0.02*</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>151±5.1†</td>
<td>0.33±0.01†</td>
<td>0.84±0.02†</td>
</tr>
</tbody>
</table>

Peak systolic blood pressure, heart weight per 100 g body weight and aorta weight per unit length were measured in 20–22-week-old rats. Results are mean±SEM from seven rats, except for aorta weight for which Wistar-Kyoto (WKY) rat data refer to three rats. SHR, spontaneously hypertensive rats.

*Values from control SHR are significantly higher than those from control WKY rats ($p<0.001$ for blood pressure and heart weight; $p<0.01$ for aorta weight).

†Values from nisoldipine-treated SHR are significantly lower than those in control SHR ($p<0.001$ for blood pressure and heart weight; $p<0.05$ for aorta weight).
5.65 ± 0.14 mN, respectively (n = 4). In the absence of endothelium, the corresponding values were 7.48 ± 0.30 and 5.10 ± 0.57 mN (n = 5).

Figure 4 compares postcontraction tone in mesenteric arteries from untreated and nisoldipine-treated rats. It shows that the age-related difference between WKY and SHR vessels found in untreated rats was suppressed in nisoldipine-treated rats. A similar conclusion can be drawn from data obtained on aortas for which the times to 50% of the KCl-induced force are compared (Table 2). Furthermore, we examined the action of an in vitro treatment of SHR aortas with various concentrations of nisoldipine on the contraction evoked by potassium depolarization and on the subsequent endogenous tone. As illustrated in Table 3, when isolated SHR aortas were preincubated for 90 minutes in physiological solution with nisoldipine (30 pM), the KCl-evoked contraction was inhibited by about 30% and the postcontraction tone measured after 10 minutes was inhibited by about 70%.

**Properties of Dihydropyridine Receptors in Heart and Brain**

To examine the influence of the chronic administration of nisoldipine on dihydropyridine receptors, the specific binding of the calcium channel ligand \[^{3}H\](+)-PN200-110 was measured in homogenates prepared from hearts and brains of WKY and SHR. Results of saturation experiments are reported in Table 4. The number of receptors, as estimated by the maximum binding capacity \(B_{\text{max}}\), when expressed in femtomoles per milligram protein, was significantly lower in hearts but not in brains of SHR. The difference in \(B_{\text{max}}\) between SHR and WKY rat hearts is probably related to the cardiac hypertrophy of SHR, since it is no longer significant when results are expressed per gram tissue wet weight (Table 5). \(K_d\) values of WKY and SHR were not significantly different.

Nisoldipine treatment did not affect \(B_{\text{max}}\) but increased the apparent \(K_d\) values of \[^{3}H\](+)-PN200-110 (\(K_{d(\text{PN})}\)). However, this increase was statistically significant \((p < 0.05)\) only for WKY rats. In binding studies carried out on thoroughly washed membranes, the apparent \(K_d\) of dihydropyridine radioligands was not changed after chronic administration of nifedipine or nitrendipine. Therefore, we may assume that, in our experiments with whole, unwashed homogenates, the increase in apparent \(K_d\) values was due to the persistence in the membranes of the nisoldipine that had been chronically administered. Thus, we may write

\[
K_{d(\text{app,PN})} = K_{d(\text{PN})}(1 + [\text{Niso}]/K_{d(\text{Niso})})
\]

from which

\[
[N\text{iso}] = (K_{d(\text{app,PN})} - K_{d(\text{PN})})K_{d(\text{Niso})}/K_{d(\text{PN})}
\]

[Niso] represents the theoretical free concentration of nisoldipine required to occupy a proportion of dihydropyridine receptors similar to the estimated occupancy in tissue homogenates of nisoldipine-fed rats. Assuming that \(K_{d(\text{Niso})}/K_{d(\text{PN})}\) is close to 1,\(^{16,19}\) we can estimate from Table 4 that this theoretical nisoldipine concentration is between 15 and 75 pM.

**Discussion**

The present experiments confirm that continuous treatment of SHR from the age of 8 weeks with food
containing 1,000 ppm nisoldipine inhibits the development of hypertension. They also show that the blood pressure of WKY rats treated in the same way was unaffected. The growth of the animals was not modified by the treatment, but the hypertrophy of SHR heart and aorta was considerably reduced. A similar observation has recently been reported in mesenteric arteries with isradipine. Reduction of hypertrophy was not caused by loss of tissue water and was also quantified in aorta by measuring the thickness of the vessel wall (data not shown). It thus appears that chronic treatment with dihydropyridines suppresses structural changes in hypertensive rats.

Results reported in Figure 1 show that tension development on KCl depolarization was inhibited by 25–45% in aortas from nisoldipine-treated rats. The experiment reported in Table 3 suggests that a 30% inhibition can be achieved by exposing SHR aortas to 30 pM nisoldipine. This concentration is within the range of concentrations estimated from apparent \( K_d \) values of [H](+)PN200-110 in heart and brain homogenates. Thus, the weaker tension development in KCl-stimulated arteries from nisoldipine-fed animals might be attributed to inhibition of calcium channels by nisoldipine in these arteries. This view is reinforced by the observation that both inhibition of tension of arterial rings (Figure 1) and increase of apparent \( K_d \) values in heart (but not brain) homogenates (Table 4) were more pronounced in nisoldipine-treated WKY than in nisoldipine-treated SHR. The apparently greater tissular load of nisoldipine after chronic treatment in WKY rats does not
TABLE 2.  Time (in Seconds) to 50% Fading of KCl-Induced Tone in Rat Aorta

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>9–12 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>141±23</td>
<td>233±8*</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>40±2†</td>
<td>88±7†</td>
</tr>
<tr>
<td>14–16 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>144±10</td>
<td>324±22*</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>52±3†</td>
<td>52±2†</td>
</tr>
<tr>
<td>34–36 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>131±11</td>
<td>1,804±222*</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>68±7†</td>
<td>438±79†</td>
</tr>
</tbody>
</table>

Aorta rings from control and nisoldipine-treated rats were depolarized in a solution containing 100 mM KCl for 35 minutes. Thereafter, they were reimmersed in physiological solution and the time (seconds) required to reach 50% of the KCl-induced tone was measured. Results are mean±SEM from 12 rings (three rats). WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

*Significantly different from WKY (p<0.01).
†Significantly different from control (p<0.01).

seem to be related to a difference in food uptake, since the weights of the animals were not significantly different. Clearly, a pharmacokinetic analysis is needed to shed light on this observation. We cannot exclude the possibility that nisoldipine could be more rapidly washed out from SHR tissues.

Receptor numbers in WKY and SHR heart and brain homogenates were not significantly modified by the chronic nisoldipine treatment. This finding is compatible with the absence of clear evidence of tolerance or withdrawal symptoms on chronic treatment with dihydropyridines in humans.21 In agreement with our results, Nishiyama et al.22 who administered nifedipine to rats by stomach tube during 2 weeks, found no modification of receptor number in heart. However, other groups have reported either downregulation of dihydropyridine binding sites in heart (but not brain) after intravenous administration of nifedipine (36–360 mg/kg/hr) for 20 days21 or upregulation in heart after oral administration of nitrendipine (300 ppm in the diet) for 21 days.23 These discrepancies might be related to either the plasma levels attained or the particular dihydropyridine used.

Alteration in vascular reactivity is a well-documented property of arteries from SHR, but functional studies of voltage-operated calcium channels have provided variable results, probably related to methodology.24 Several authors have reported that calcium concentrations higher than the physiological one elevate tone in aortic strips from SHR but not in strips from WKY rats.25,26 Furthermore, Winquist and Bohr20 have shown the existence of a small but consistent tone of SHR basilar artery, which is suppressed by EGTA treatment. We report here that the contraction evoked by KCl depolarization was similar in arteries isolated from age-matched SHR and WKY rats, but that after readmission of physiological solution to the bath, relaxation of SHR arteries was delayed and showed a complex time course when compared with WKY rat arteries. The behavior of SHR arteries was described as an enhanced postcontraction tone that could be observed for prolonged periods after the contraction. Several investigators have shown that relaxation in response to several agents is impaired in SHR vessels27–29 and that this reduced sensitivity is age related.30 Others31–33 have reported that ATP-dependent microsomal calcium pumps are altered in SHR tissues. The enhanced postcontraction tone that we observed after washout of the depolarizing solution was sensitive to pretreatment with nisoldipine, which suggests that a prolonged activation of voltage-dependent calcium channels was primarily involved. However, a deficiency in the mechanisms responsible for calcium extrusion, as demonstrated by Daniel's group31,33,34 in vascular and nonvascular smooth muscle from SHR, could contribute to the delayed relaxation. Indeed, such a deficiency could remain latent in the presence of nisoldipine, since this drug limits the rise in intracellular calcium. The enhanced postcontraction tone

![Figure 3. Fading of postcontraction tone of spontaneously hypertensive rat (SHR) mesenteric artery after repeated exposure to KCl solution. Rings of mesenteric artery from 20–22-week-old (panel a) and 34–36-week-old (panel b) SHR and Wistar-Kyoto (WKY) rats were submitted to three successive 35-minute periods of KCl depolarization (see "Materials and Methods"). Data show postcontraction tone 10 minutes after reimmersion in physiological solution. Results are mean±SEM (vertical bars) from 12 rings (three rats).](http://circres.ahajournals.org/lookup/doi/10.1161/01.RES.85.12.679)
was not appreciably modified after endothelium removal and, therefore, appears unrelated with the reported production of an endothelium-derived contracting factor by SHR aortas.\textsuperscript{35} Alterations in the properties of voltage-dependent calcium channels in cultured muscle cells from SHR have been recently described.\textsuperscript{36}

The postcontraction tone of SHR arteries was greatly attenuated when the in vitro incubation was prolonged and when the tissue was submitted to successive long-lasting stimulations by KCl-rich solution. These observations indicate that a labile factor, or at least a factor that can be slowly washed out of the tissue, could be responsible for this anomalous tone. In addition, because the postcontraction tone was sensitive to acute treatment with nisoldipine, it is unlikely that this anomaly could be directly related to the hypertrophy of SHR arteries, which was obviously unaffected by successive depolarization/repolarization cycles or by acute administration of nisoldipine. We observed the postcontraction tone in both aorta and mesenteric artery, and, as this phenomenon appears unrelated to the size of the artery, it could also occur in resistance vessels, which are more directly involved in the development of hypertension.

Circulating fatty acids are increased in the blood of hypertensive animals and humans,\textsuperscript{37} and various agents from this chemical group exert an inhibitory action on transport ATPases.\textsuperscript{38,39} We have not observed significant differences in cellular sodium content between SHR and WKY rats (data not shown). This result, however, does not disprove the possibility that circulating inhibitors of the Na,K-ATPase are increased in hypertension, as proposed by Hamlyn et al.\textsuperscript{40} Indeed, small variations in intracellular sodium activity may not give rise to detectable changes in net ionic content. Inhibitors of the sodium pump may depolarize vascular muscle cells, as reported for ouabain.\textsuperscript{41} This would not only activate calcium chan-

**TABLE 3. Effect of Nisoldipine (In Vitro) on KCl-Evoked Contraction and Postcontraction Tone in Spontaneously Hypertensive Rat Aorta**

<table>
<thead>
<tr>
<th></th>
<th>Contractile force (mN)</th>
<th>Postcontraction tone after 10 min (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.0±0.9</td>
<td>8.79±1.74</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>18.7±1.5*</td>
<td>2.74±0.44\†</td>
</tr>
</tbody>
</table>

\*Significantly different from control (p<0.01).
\†Significantly different from control (p<0.05).

**FIGURE 4. Effect of chronic nisoldipine treatment on postcontraction tone of mesenteric artery.** Rings were taken from Wistar-Kyoto rats (open symbols) and spontaneously hypertensive rats (closed symbols) belonging to the following age groups: 9–12 weeks (top panels), 14–16 weeks (middle panels), and 34–36 weeks (bottom panels). Results are mean±SEM (vertical bars) from 12 rings (three rats).


**Table 4.** Specific Binding of [\(^{3}H\)](+)-PN200-110 in Heart and Brain Homogenates From Wistar-Kyoto and Spontaneously Hypertensive Rats (19–25 Weeks)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Nisoldipine-fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>93.7±7.3 (9)</td>
<td>169.5±26.1* (7)</td>
</tr>
<tr>
<td>SHR</td>
<td>82.9±6.4 (12)</td>
<td>103±8.5 (7)</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>62.9±4.6 (7)</td>
<td>81.4±3.8* (7)</td>
</tr>
<tr>
<td>SHR</td>
<td>73.8±9.0 (10)</td>
<td>91.4±13.0 (10)</td>
</tr>
</tbody>
</table>

Results are mean±SEM from n rats (indicated in parentheses). *Significantly different from WKY (p<0.01).
†Significantly different from WKY (p<0.05).

**Table 5.** Specific Binding (B\(_{\text{max}}\)) Values of [\(^{3}H\)](+)-PN200-110 in Heart Homogenates From Wistar-Kyoto and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>WKY (9)</th>
<th>SHR (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein content (mg/g tissue)</td>
<td>143.5±6.3</td>
<td>152.6±8.8</td>
</tr>
<tr>
<td>B(_{\text{max}}) (fmol/mg protein)</td>
<td>101.9±3.5</td>
<td>85.1±4.8†</td>
</tr>
<tr>
<td>(pmol/g tissue)</td>
<td>14.6±0.8</td>
<td>13.1±0.6</td>
</tr>
<tr>
<td>(pmol/heart)</td>
<td>14.5±1.0</td>
<td>15.6±0.7</td>
</tr>
</tbody>
</table>

Results are mean±SEM from n rats (indicated in parentheses). WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.
*Significantly different from WKY (p<0.01).
†Significantly different from WKY (p<0.05).


cannels indirectly but would also account for the antihypertensive efficacy of nisoldipine since binding of this drug and its blocking action on calcium channels are enhanced by depolarization. Circulating factors such as fatty acid derivatives or peptides may also activate calcium channels directly. Thus, endogenous factors acting either directly or indirectly could be responsible for a persistent activation of voltage-operated calcium channels after a period of prolonged depolarization.

In summary, we observed that the enhanced postcontraction tone, a characteristic, short-lived feature of arteries isolated from hypertensive rats, was absent in arteries from SHR that had been chronically treated with nisoldipine and that it was suppressed in arteries isolated from untreated SHR after incubation of the vessels in the presence of 30 pM nisoldipine, which is close to the active concentration in vivo. If a process akin to the postcontraction tone occurs in vivo, the antihypertensive efficacy of nisoldipine might be attributable to its action on this process and not only to its vasodilating effect.

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**References**


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