To elucidate functional changes in the vascular smooth muscle of spontaneously hypertensive rats (SHR) after chronic inhibition of angiotensin converting enzyme, we examined the contractile responses to different pharmacological interventions in the isolated aortas from SHR treated with a novel angiotensin converting enzyme inhibitor, CS-622 (10 mg/kg/day) for 20 weeks. In normal K+ medium, a marked contraction was elicited by increasing Ca2+ concentration from 0 to 3 mM in aortas from a control group of SHR, but not in aortas from SHR treated with CS-622. In 60 mM K+ medium, however, the sensitivity of aorta to Ca2+ was almost the same in the two groups. A calcium channel activator, CGP-28392 (10^-7 to 10^-4 M), induced a marked contraction in the aortas from control SHR, but not in the aortas from CS-622–treated SHR. When slightly depolarized in 10 or 12 mM K+ solution, the aortas from CS-622–treated SHR contracted in response to CGP-28392. The aortic sensitivity to KCl contraction was much lower in CS-622–treated SHR than in untreated SHR, whereas the sensitivity to phenylephrine contraction was little different in the two groups. These contractile profiles of aortas from CS-622–treated SHR were very similar to those from normotensive Wistar-Kyoto rats but not to those from hydralazine-treated SHR. These data suggest that contractions due to Ca2+ through voltage-dependent calcium channels are exaggerated in SHR aorta and that long-term treatment with angiotensin converting enzyme inhibitor suppresses the abnormal contractility of SHR vascular smooth muscle, probably through alterations of voltage-related functions of calcium channels. (Hypertension, 1989;14:652–659)
effects in contrast to classical calcium channel blockers. Such agents (e.g., Bay K-8644, CGP-28392, and YC-170) have been shown to activate voltage-dependent calcium channels, and are called "calcium channel activators" or "calcium agonists". The calcium channel activators are useful pharmacological tools for investigation of the voltage-dependent calcium channels. Aoki and Asano have shown that Bay K-8644 elicits a marked contraction in femoral arteries of SHR, but only a small contraction in those of WKY rats. Their study indicates that calcium channels in SHR femoral arteries are modulated by dihydropyridines to a greater extent than occur in WKY rats.

The purpose of the present study was to delineate the pharmacological characteristics of calcium channels in the vascular smooth muscle from SHR chronically treated with an ACE inhibitor. Vascular contractile profiles of the aorta to agonists such as calcium channel activator (CGP-28392), CaCl2, KCl, and the α1-agonist phenylephrine were examined in SHR and WKY rats and SHR treated chronically with the ACE inhibitor CS-622 or with hydralazine. The results suggest that the exaggerated contractile responses, probably due to alterations of voltage-sensitive calcium channels in vascular smooth muscle of SHR, are suppressed by the chronic inhibition of ACE, whereas vascular contractions related to receptor-operated calcium channels are not.

Materials and Methods

Drug Treatment Regimen and Measurement of Blood Pressure

Male SHR and WKY rats were obtained from Hoshino Laboratory Animals (Yashio, Saitama, Japan). In one series of experiments, 23-week-old SHR were divided into four groups: group 1 (15 rats) received vehicle for CS-622 (NaHCO3 1 g/l and KHCO3 1 g/l in their drinking water); group 2 (15 rats), CS-622 in their drinking water at a concentration of 100 mg/l; group 3 (13 rats), distilled water (vehicle for hydralazine); group 4 (14 rats), hydralazine. The water intake was estimated at a factor of three (10^-5 to 3 x 10^-3 M) at 20-minute intervals. The contractile response to Ca^2+ in both normal K+ KHS or in Ca^2+-free high K+ KHS (60 mM K^+ substituted for Na^+) was 15 minutes. Thereafter, contraction-concentration curves for each agonist were determined as described below.

Dose-response curves for Ca^2+ were determined in normal and high K^+ KHS in the aortas from drug-treated or vehicle-treated SHR (groups 1-4) and untreated SHR and WKY rats. In this experiment, the tissue was washed several times over 30 minutes in Ca^2+--free KHS, exposed to 0.4 mM EGTA for 5 minutes, and washed again in Ca^2+-free normal K^+ KHS or in Ca^2+-free high K^+ KHS (60 mM K^+ substituted for Na^+) for 15 minutes. Thereafter, cumulative dose-response curves for Ca^2+ were obtained by increasing Ca^2+ concentration by a factor of three (10^-5 to 3 x 10^-3 M) at 20-minute intervals. The contractile response to Ca^2+ in both normal K^+ and high K^+ was expressed as the percentage of the control contraction induced by 60 mM K^+.

Cumulative dose-response curves for CGP-28392 (10^-4 to 10^-6 M) were determined in the aortas from chronically dosed SHR (groups 1-4), untreated SHR, and WKY rats. CGP-28392 was added to the bath in a cumulative manner at 20-minute intervals. After the aorta was washed, 60 mM K^+ was applied to obtain the maximum contraction. In the aortas from CS-622--treated SHR and untreated WKY rats, the dose-response curves for CGP-28392 were also obtained in 10 or 12 mM K^+ medium that would slightly depolarize the membrane of vascular smooth
muscle cells. K+ concentration of the bathing solution was increased to 10 or 12 mM K+ 15 minutes before addition of CGP-28392. The contractile response to CGP-28392 was expressed as the percentage of the maximum response to 60 mM K+ contraction.

Dose-response curves for K+ and phenylephrine were determined in the aortas from CS-622-treated and vehicle-treated SHR (groups 1 and 2) and untreated SHR and WKY rats. K+ was added in a cumulative manner ranging from 5.9 to 60 mM at 15-minute intervals. Phenylephrine (3×10⁻⁹ to 10⁻³ M) was added at 10-minute intervals. The contractile responses to both agonists were expressed as the percentage of the maximum response induced by these agonists. From these dose-response curves, ED₅₀ values (concentration that caused a 50% response) were obtained for KC1 and phenylephrine.

The effects of nicardipine and CS-622 diacid (active form of CS-622) on Ca²⁺ contraction and CGP-28392 contraction were examined in the aortas from untreated SHR. Nicardipine (10⁻⁸ or 10⁻⁷ M) and its vehicle (3×10⁻⁶ M HCl) and CS-622 diacid (10⁻⁶ M) and its vehicle (0.001% NaHCO₃) were added to the bath 30 minutes before addition of these agonists.

**Drugs**

Drugs used were CS-622 [α-((2S,6R)-6-[(1S)-1-ethoxy carbonyl-3-phenylpropyl]amino-5-oxo-2-(2-thienyl)perhydro-1,4-thiazepin-4-yl] acetic acid hydrochloride] (Research Laboratories, Sankyo Co., Ltd., Tokyo, Japan), hydralazine hydrochloride (Sigma Chemical Co., St. Louis, Missouri), CGP-28392 (Ciba-Geigy Ltd., Basel, Switzerland), phenylephrine hydrochloride (Sigma Chemical Co.), nicardipine (synthesized at Sankyo Co. Research Laboratories), CS-622 diacid (Sankyo Co.), acetylcholine chloride (Ovisot, Daichi Seiyaku Co., Ltd., Tokyo, Japan), angiotensin I (Peptide Institute Inc., Osaka, Japan), EGTA (Tokyokasei-kogyo Co., Ltd., Tokyo, Japan), and N,N-dimethylacetamide (Wako Pure Chemical Industries Ltd., Osaka, Japan).

### TABLE 1. Systolic Blood Pressure of Spontaneously Hypertensive Rats Before and After Drug Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>n</th>
<th>Before (mm Hg)</th>
<th>20 Weeks After (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>15</td>
<td>198±4</td>
<td>199±5</td>
</tr>
<tr>
<td>2</td>
<td>CS-622 (100 mg/l)</td>
<td>15</td>
<td>198±4</td>
<td>123±3*</td>
</tr>
<tr>
<td>3</td>
<td>Vehicle</td>
<td>13</td>
<td>192±4</td>
<td>206±7</td>
</tr>
<tr>
<td>4</td>
<td>Hydralazine (50 mg/l)</td>
<td>14</td>
<td>194±4</td>
<td>133±5*</td>
</tr>
</tbody>
</table>

Vehicle for CS-622 was distilled water containing NaHCO₃ and KHCO₃ (1 g/l each). Vehicle for hydralazine was distilled water. Values are mean±SEM.

*Significantly different (p<0.001) from values in control groups by Duncan’s test.

CGP-28392, CS-622 diacid, and nicardipine were dissolved in N,N-dimethylacetamide, 0.25% NaHCO₃, and 0.01 N HCl, respectively, to make the stock solution. EGTA was dissolved in distilled water, and the pH was adjusted to 7.0 with NaOH. Other drugs were dissolved in distilled water.

**Statistical Analysis**

Data were expressed as mean±SEM. Statistical difference was calculated by Student’s t-test, by Cochran-Cox test for single comparison, and by Duncan’s multiple range test for multiple comparison if these variances were uniform.

**Results**

**Blood Pressure of Rats**

SHR of each group were given the respective drug in their drinking water for 20 weeks. Administered solution had a concentration of 100 mg/l for CS-622 and 50 mg/l for hydralazine. Based on the water intake of these rats (about 100 ml/kg), measured at week 16 during drug treatment, daily doses of CS-622 and hydralazine were estimated to be about 10 and 5 mg/kg, respectively. SBP before and after 20-week treatment is shown in Table 1. The table indicates that vehicle alone did not lower SBP and that CS-622 and hydralazine produced similar lowering of SBP.

![Figure 1](http://hyper.ahajournals.org/)

**FIGURE 1.** Line graphs showing dose-response relations of CaCl₂-induced contraction in normal (5.9 mM) K+ solution in aortas from spontaneously hypertensive rats (SHR) treated with CS-622 (about 10 mg/kg/day) or its vehicle for 20 weeks (Panel A), in aortas from SHR treated with hydralazine (about 5 mg/kg/day) or its vehicle for 20 weeks (Panel B), and in aortas from untreated SHR and age-matched Wistar-Kyoto (WKY) rats (Panel C). CaCl₂ was added in cumulative manner ranging from 10⁻⁵ to 3×10⁻³ M. Contractile response was expressed as percentage of contraction induced by 60 mM K+ (in 2.5 mM Ca²⁺). Values are mean±SEM of five to eight aortas.
Dose-Response Curves for CaCl₂ in the Isolated Aortas

Figure 1 shows the dose-response curves for Ca²⁺ in normal K⁺ (5.9 mM) solution in the aortas from SHR treated with CS-622, hydralazine, and vehicles for 20 weeks and in the aortas from untreated SHR and WKY rats. In the aortas from vehicle-treated SHR, Ca²⁺ at 10⁻⁴ M caused a contraction, and the contractile response reached 60-70% of the maximum contraction (induced by 60 mM K⁺, 2.5 mM Ca²⁺) when Ca²⁺ concentration was raised to 3×10⁻³ M. On the other hand, such a contraction in response to Ca²⁺ in normal K⁺ was not observed in the aortas from SHR treated with CS-622 (about 10 mg/kg/day) for 20 weeks (Figure 1A). However, this contraction was still present in the aortas from SHR treated with hydralazine (about 5 mg/kg/day) (Figure 1B), although the agent lowered SBP of SHR to the same degree as CS-622 did (Table 1). Unlike the aortas of SHR, WKY rat aortas did not contract in response to Ca²⁺ up to 3×10⁻³ M (Figure 1C), indicating that the SHR aorta has an abnormal contractility to Ca²⁺ in normal K⁺ solution. Our previous report demonstrated that such Ca²⁺ contraction in the SHR aortas was suppressed in the presence of the calcium channel blocker nicardipine (10⁻⁸ M), but not in the presence of CS-622 diacid (10⁻⁶ M).

Figure 2 shows dose-response curves for Ca²⁺ in the fully depolarized aortas in 60 mM K⁺ solution. No difference was noted in contraction of the aorta in response to Ca²⁺ between CS-622-treated SHR and vehicle-treated SHR (Figure 2A) and also between hydralazine-treated SHR and vehicle-treated SHR (Figure 2B). In high K⁺ solution, the contractile response to Ca²⁺ was almost the same in aorta of SHR and in WKY rat aorta at each concentration of Ca²⁺ (Figure 2C), whereas in normal K⁺ solution the contraction was greater in SHR than WKY rats (Figure 1C).

Contractile Responses to CGP-28392 in the Isolated Aortas

Figure 3 shows dose-response curves for the calcium channel activator CGP-28392 in the aortas from SHR treated with CS-622, hydralazine, and vehicles and in the aortas from untreated SHR and WKY rats. In the aortas from vehicle-treated SHR, CGP-28392 at a concentration of 10⁻⁷ M evoked a marked contraction with an oscillatory activity. The contractile response to CGP-28392 reached about 80% of the maximum contraction induced by 60 mM K⁺ at a concentration of 10⁻⁶ M. A higher concentration (10⁻⁵ M) of CGP-28392 produced a slight relaxation, probably due to a calcium antagonistic action inherent in this agent. Long-term treatment with CS-622, but not with hydralazine, abolished the contraction due to CGP-28392 (Figure 3A and B). WKY rat aortas produced only a small contraction in response to CGP-28392, whereas aortas from age-matched SHR produced a marked contraction to the agent (Figure 3C). The contractile response to CGP-28392 in SHR aortas was suppressed in the...
presence of nicardipine (10^{-8} to 10^{-7} M) (Figure 4). These data indicate that abnormal contractility to calcium channel activator in SHR aorta is reversed by long-term treatment with CS-622.

We examined whether an acute inhibition of aortic ACE could suppress the contractile response to CGP-28392 in SHR aortas. CS-622 diacid was used in vitro because CS-622 is converted to the active diacid in vivo. As described elsewhere,22 CS-622 diacid at a concentration of 3×10^{-8} M completely abolished the contraction induced by angiotensin I (3×10^{-8} M) in rat aorta. Therefore, 10^{-6} M of CS-622 diacid was considered sufficient to inhibit ACE of the aortic smooth muscle. At this concentration, CS-622 diacid did not affect the dose-response curves of CGP-28392 contraction in SHR aortas (n=5; not significantly different, p>0.05, in each concentration of CGP-28392 by Student's t test, data not shown as figure). Thus, acute inhibition of ACE in vitro could not reproduce the effects of chronic ACE inhibition on the contractile response to CGP-28392 in SHR aorta.

Although CGP-28392 did not evoke any contraction in the aortas from SHR treated chronically with CS-622, this agent produced a clear contraction in these aortas when K+ concentration in the bathing solution was slightly increased (from 5.9 to 10 or to 12 mM) (Figure 5A). In other words, the aortas from CS-622–treated SHR, when slightly depolarized, had a sensitivity for CGP-28392 similar to those from untreated SHR. Such a contractile profile to CGP-28392 was also seen in WKY rat aorta; WKY rat aorta that did not respond to CGP-28392 in normal (5.9 mM) K+ solution contracted markedly in response to this agonist in 10 mM K+ solution (Figure 5B).

Dose-Response Curves for KCl and Phenylephrine in the Isolated Aortas

Figure 6 shows dose-response curves for KCl and for the α1-agonist phenylephrine in the aortas from SHR treated chronically with CS-622 or its vehicle. The aortas from CS-622–treated SHR were much less sensitive to KCl than those from vehicle-treated SHR (Figure 6A), but little difference was observed in the sensitivity to phenylephrine between the two aortas (Figure 6B). The ED50 values for KCl contraction were 18.6±1.0 and 11.6±0.3 mM for CS-622–treated and vehicle-treated SHR, respectively.
alteration of Ca\(^{2+}\) concentration does not evoke any
tensional change in aortas from normotensive WKY rats.\(^{22,24}\) Recently, we found that such a high resting tension in SHR aorta was suppressed by chronic administration of CS-622, a selective ACE inhibitor.\(^{13}\) However, we did not clearly answer the following question in the previous paper: Does the chronic inhibition of ACE alter the activity of calcium channels, and if so, what type of calcium channels? To answer these questions, we analyzed in the present study the pharmacological behavior of calcium channels that were affected by long-term treatment with CS-622.

CS-622 and hydralazine, when administered for
20 weeks lowered SBP of SHR to a similar degree
(Table 1). In the aortas isolated from these rats, contractile response to Ca\(^{2+}\) in normal K\(^{+}\) solution was suppressed by long-term treatment with CS-622, but not with hydralazine (Figure 1). These data suggest that the suppression by CS-622 of high resting tension in SHR aorta did not result from prolonged reduction of blood pressure.

In the present study, CGP-28392, a calcium channel activator, contracted SHR aorta but not WKY rat aorta (Figure 3C). The contractile response to this agonist in SHR aorta was antagonized by a calcium antagonist, nicardipine (Figure 4). These data confirm a similar observation of Aoki and Asano,\(^{19}\) who used femoral arteries, and suggest that calcium channels sensitive to dihydropyridines are abnormally activated in vascular smooth muscles of SHR. The vasoconstriction induced by CGP-28392, which was seen only in SHR, was virtually abolished by chronic administration of CS-622 (Figure 3A), but not by chronic administration of hydralazine (Figure 3B). On the other hand, the contractile response to this agonist was not affected by CS-622 diacid at a concentration high enough to fully inhibit arterial ACE. These data indicate that, unlike dihydropyridine calcium antagonists, acute inhibition of ACE with CS-622 diacid does not block calcium channels and that chronic inhibition of ACE suppresses the abnormal contraction probably due to increased activity of dihydropyridine-sensitive calcium channels in SHR vascular smooth muscle.

The reactivity to CaCl\(_2\) of SHR aorta depolarized in high (60 mM) K\(^{+}\) solution was unaffected by long-term administration with CS-622 (Figure 2A). Because calcium channel blockers antagonize the contraction induced by Ca\(^{2+}\) in depolarized smooth muscles, this result indicates that chronically administered CS-622 does not block calcium channels.

Unlike SHR aorta, WKY rat aorta produced little contraction in response to CaCl\(_2\) in normal K\(^{+}\) solution (Figure 1C), whereas WKY rat and SHR aortas had almost the same reactivity to CaCl\(_2\) in high (60 mM) K\(^{+}\) solution (Figure 2C). CGP-28392 contracted SHR aorta but not WKY rat aorta in normal K\(^{+}\) solution (Figure 3C), whereas the agent contracted WKY rat aorta when it was slightly

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**Figure 7.** Line graphs showing dose-response relations of KCl-induced contraction (Panel A) and phenylephrine-induced contraction (Panel B) in aortas from untreated spontaneously hypertensive rats (SHR) and age-matched Wistar-Kyoto (WKY) rats. Contractile response was expressed as a percentage of maximal contraction to respective agonist. ED\(_{50}\) value and maximal contraction for each dose-response curve are described in text. Values are mean±SEM of five aortas.
depolarized (Figure 5B). The slight depolarization restored contractile response to CGP-28392 in aorta from SHR treated with CS-622 as well (Figure 5A). These results suggest that the abnormal contractility of SHR vascular smooth muscle results from the exaggerated activity of voltage-dependent calcium channels and that the increased activity of calcium channel function in SHR is reduced to a level of WKY rat aorta by long-term treatment with CS-622. Possible mechanisms underlying the increase of calcium channel activity in SHR aorta are a change in the number of calcium channels, a change in membrane potential or shift in the voltage dependence for the channel activation.

Such membrane defects of vascular smooth muscle cells in SHR are supported by reports where vascular contractile responses of SHR (or stroke-prone SHR) to some agonists were compared with those of WKY rats. Cheung measured the membrane potential of arterial smooth muscle from both SHR and WKY rats by the microelectrode technique and found that resting potential was smaller in SHR than in WKY rats, although many investigators found no difference in resting membrane potential of vascular smooth muscle cells between SHR and WKY rat in vitro measurements. A voltage-clamp study by Rusch and Hermsmeyer demonstrated that voltage-sensitive calcium channels were altered in SHR. As described already, the contractile profiles of WKY rat aorta to Ca^{2+} and to the calcium channel activator (CGP-28392) were similar to those of aorta from CS-622-treated SHR. It is conceivable that chronic inhibition of ACE could normalize the abnormal depolarization-induced contraction in SHR aorta. This abnormal contraction is most likely related to alterations of voltage-sensitive calcium channels. The results of the present study also suggest that the function of calcium channel of vascular smooth muscle cells could be controlled by the renin-angiotensin system. However, direct evidence is needed to identify the membrane defect that was corrected by ACE inhibitor therapy.

The aorta from SHR treated with CS-622 had the same reactive properties to KCl and phenylephrine contraction as those of WKY rat aorta (Figures 6 and 7). SHR aorta was much more sensitive to KCl at low concentrations than WKY rat aorta, whereas they were equally sensitive to phenylephrine (Figure 7). This was in agreement with the observation in femoral arteries by Holloway and Bohr. Long-term treatment with CS-622 lowered the increased sensitivity to KCl in SHR aorta to the same level as that of WKY rat aorta, but did not alter the sensitivity to phenylephrine in SHR aorta (Figure 6). It is well known that KCl-induced contraction is due to Ca^{2+} influx through voltage-sensitive calcium channels and that phenylephrine-induced contraction is due to both Ca^{2+} release from intracellular Ca^{2+} pools and Ca^{2+} influx through receptor-operated calcium channels. Therefore, our findings support the case for a specific defect in voltage-sensitive calcium channels, rather than intracellular calcium release or receptor-operated calcium influx. Long-term treatment with CS-622 did not alter the maximal contraction and the sensitivity (ED_{50}) of the dose-response curve for phenylephrine (Figure 6). This indicates that long-term treatment with CS-622 does not have any effect on intracellular contractile apparatus.

In experiments for constructing dose-response curves of Ca^{2+}, we used EGTA at a low concentration (0.4 mM) to remove Ca^{2+} from the surface of the tissues and the inner surface of the organ bath. Tissues were exposed to EGTA for 5 minutes and then washed by EGTA-free and calcium-free solution. Treatment with EGTA did not alter sensitivity of Ca^{2+}-induced contractions in SHR aorta under our experimental conditions (unpublished observation). However, recent studies suggest that EGTA, when present in high concentrations may have a direct effect on stability of the vascular smooth muscle cell membrane and lead to an increase in membrane permeability. In the present study, it seems that a difference in calcium contraction of aorta between SHR and WKY rats may partly be attributed to a difference in the extent to which membrane disorder was induced by calcium depletion in both rat species.

In summary, we found that SHR aortas had abnormal contractile profiles to some agonists (CaCl_{2}, KCl, and the calcium channel activator CGP-28392), probably because of functional alterations of voltage-dependent calcium channels, and that these abnormalities in SHR aortas were normalized by long-term administration of the ACE inhibitor CS-622. These facts suggest that some membrane defects in SHR vascular smooth muscle could be suppressed by long-term treatment with ACE inhibitors. Such an action may underlie their long-term antihypertensive action in SHR.

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T Sada, H Koike and M Miyamoto

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