Chronic Inhibition of Angiotensin Converting Enzyme Decreases Ca\textsuperscript{2+}-Dependent Tone of Aorta in Hypertensive Rats

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Long-term effects of a novel angiotensin converting enzyme (ACE) inhibitor, CS-622, on Ca\textsuperscript{2+}-dependent tone in aortic smooth muscles of spontaneously hypertensive rats (SHR) were examined. CS-622 (3 or 10 mg/kg/day), when orally administered to SHR for 21 weeks, exhibited a dose-dependent antihypertensive action. In Krebs-Henseleit solution, removal of Ca\textsuperscript{2+} caused much greater relaxation in aortas excised from control SHR than those from SHR treated with CS-622. Restoration of Ca\textsuperscript{2+} from zero to 2.5 mM elicited a marked contraction in aortas from control SHR but only a small contraction in aortas from both CS-622--treated SHR and normotensive Wistar-Kyoto rats. These findings suggested that myogenic tone that resulted from increased Ca\textsuperscript{2+} permeability in aortas of SHR was suppressed by long-term treatment with CS-622. The aortic tone from the individual rats correlated well with systolic blood pressure in both CS-622--treated and control SHR. The exaggerated myogenic tone in aortas of SHR was attenuated in the medium containing nicardipine but was not altered in the presence of CS-622 diacid (active form of CS-622) at a concentration high enough to fully inhibit aortic ACE. The myogenic tone in normal Ca\textsuperscript{2+} concentration was not decreased in aortas excised from SHR treated with hydralazine (5 mg/kg/day) for 21 weeks. We conclude that after prolonged administration CS-622 reduced the high vascular tension resulting from increased Ca\textsuperscript{2+} permeability of vascular smooth muscle membrane in SHR and that the restoration of normal Ca\textsuperscript{2+} permeability of vascular smooth muscles may underlie long-term antihypertensive action of ACE inhibitors. (Hypertension 1989; 13:582-588)

It is well known that during chronic hypertension there are functional and structural changes in the vasculature that contribute to the maintenance of high peripheral resistance.\textsuperscript{1-3} The functional changes in the blood vessels may also be involved in the genesis of high peripheral resistance, and many investigators have reported abnormalities of ion transport system in vascular smooth muscle membrane of hypertensive animals.\textsuperscript{4-7} In the vascular smooth muscle of spontaneously hypertensive rats (SHR), an increase in membrane permeability to monovalent ions\textsuperscript{8-10} or to calcium ion\textsuperscript{11-13} has been observed.

Noon et al\textsuperscript{11} demonstrated that aortic strips from SHR relaxed when Ca\textsuperscript{2+} was removed from the bathing medium and contracted when Ca\textsuperscript{2+} was again added to the medium, whereas the resting tension of aortic strips from Wistar-Kyoto (WKY) rats was unaffected by alteration of Ca\textsuperscript{2+} concentration. These findings indicate that myogenic tone is increased in vascular smooth muscle of SHR, possibly because of increased permeability to Ca\textsuperscript{2+}.

It is well established that at least the acute antihypertensive effect of angiotensin converting enzyme (ACE) inhibitors is due to interference with angiotensin II generation in blood plasma, vascular tissue, and other tissues. But there may be additional antihypertensive mechanisms as well since ACE inhibitors lower blood pressure not only in high-renin patients but also in low-renin or normal renin patients, particularly during long-term therapy.\textsuperscript{14,15} Once-daily administration of captopril for a long period of time produces a long-lasting antihypertensive effect, which is never achieved by a single oral administration of the agent.\textsuperscript{16} These observations prompted us to examine the possibility that mechanisms other than the elimination of vasoconstrictor action by angiotensin II are involved in the long-term antihypertensive action of ACE inhibitors. Ito et al\textsuperscript{17} showed that a 6-week treatment with captopril altered the abnormal permeability to sodium ion of the vascular smooth muscle in...
SHR. This finding suggests that long-term treatment with ACE inhibitor changes membrane properties of blood vessels of SHR.

An ACE inhibitor, α-[2S,6R]-6-[(1S)-1-ethoxycarbonyl-3-phenylpropyl]amino-5-oxo-2-(2-thienyl)perhydro-1,4-thiazepin-4-yl]acetic acid hydrochloride (CS-622), has the chemical structure different from that of any existing ACE inhibitors. The agent has proven more potent than enalapril and devoid of any pharmacological actions other than ACE inhibition.¹⁸ In this paper, we examined the effects of CS-622 on Ca²⁺-dependent myogenic tone in aortas of SHR. We found that the increased Ca²⁺-dependent tone in aortas of SHR was greatly reduced by long-term administration of CS-622, and we hypothesized that the suppression of such a vascular tone contributes to the long-term antihypertensive action of ACE inhibitors.

Materials and Methods

Male SHR and WKY rats were obtained from Hoshino Laboratory Animals (Saitama, Japan). In one series of experiments, 23-week-old SHR were divided into the following three groups of 12–14 rats each. The control group received 2 ml/kg/day 0.3% carboxymethyl cellulose solution. The other groups received either 3 or 10 mg/kg/day CS-622 by oral gavage for 21 weeks. The systolic blood pressure (SBP) was determined in conscious, restrained rats by the tail-cuff plethysmographic method (PE-300, Narco-Biosystems, Houston, Texas). Measurements of SBP were done at days 3 and 7 and weeks 3, 6, 9, 15, and 21 during drug administration. In the second experiments, 40-week-old SHR and age-matched WKY rats were used. These rats did not receive any drug. In another series of experiments, vehicle or hydralazine (50 mg/1 in drinking water). Other drugs were dissolved in distilled water and the pH was adjusted to 7.0 with NaOH. Other drugs were dissolved in 0.01 HCl to make a stock solution. EGTA was dissolved in 0.25% NaHCO₃, CS-622 diacid was dissolved in 0.25% NaHCO₃, and nicardipine was dissolved in 0.01% NaHCO₃. CS-622 diacid was added to the bathing medium was reduced from 2.5 mM to zero, and after two successive contractions of an equal size had been obtained, a concentration-response curve for Ca²⁺ was constructed in the presence of CS-622 diacid (10⁻⁶ M) or its vehicle (0.001% NaHCO₃). CS-622 diacid was added to the bath 30 minutes before addition of CaCl₂. The maximum contraction induced by 60 mM K⁺ was taken as 100% contraction. The effects of nicardipine (10⁻⁴ M) and its vehicle (3×10⁻⁴ N HCl) on contraction–response curves for Ca²⁺ were examined in the same manner.

Drugs used were CS-622 (Sankyo Laboratories, Tokyo, Japan), CS-622 diacid (Sankyo Laboratories), hydralazine hydrochloride (Sigma Chemical Co., Ltd., St. Louis, Missouri), nicardipine (synthesized by Sankyo Research Laboratories), norepinephrine bitartrate (Sigma Chemical Co.), acetylcysteine chloride (Ovisit Daiichi Seiyaku Co., Ltd., Tokyo, Japan), angiotensin I (Peptide Institute Inc., Osaka, Japan), EGTA (Tokyokasei-kogyo Co., Ltd., Tokyo, Japan), and carboxymethyl cellulose (Iwaikagaku-kogyo Co., Ltd., Tokyo, Japan). CS-622 suspended in 0.3% carboxymethyl cellulose was administered to rats in a volume of 2 ml/kg. CS-622 diacid was dissolved in 0.25% NaHCO₃, and nicardipine was dissolved in 0.01 N HCl to make a 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.

Data were expressed as mean±SEM. Statistical difference was calculated by Student’s t test or Cochran-Cox test for single comparison and by Duncan’s multiple range test for comparison of three groups whose variances were uniform.
Results

Effects of Chronic Administration of CS-622 on Blood Pressure in Spontaneously Hypertensive Rats

Oral administration of CS-622 (3 or 10 mg/kg/day) for 21 weeks lowered systolic blood pressure of SHR in a dose-dependent manner. The time courses of changes in SBP during CS-622 dosing are shown in Figure 1. In each point, measurement of systolic blood pressure was performed 24 hours after the last administration of the drug. Points and vertical bars represent mean±SEM. *Significant difference from the vehicle-treated group (p<0.05).

Effects of Chronic Administration of CS-622 on Resting Tension in Aortas of Spontaneously Hypertensive Rats

Twenty-four hours after the final administration of CS-622, the rat was killed, and the aorta was isolated. In the isolated aorta, changes in tension were examined when Ca²⁺ concentration of the bathing medium was changed. The aortic strip from vehicle-treated SHR exhibited a pronounced decrease of the resting tone when Ca²⁺ in the medium was decreased from 2.5 mM to zero (Figure 2). Addition of Ca²⁺ (from zero to 2.5 mM) yielded a concentration-related increase in the aortic tension (Figure 2). These results confirm the findings of Noon et al.11 and Fitzpatrick and Szentivanyi.12

On the other hand, changes in tension caused by decreasing or increasing Ca²⁺ concentration were much smaller in the aortas from CS-622-treated SHR than from control SHR (Figure 2). Figure 3 summarizes changes in tension induced by the vehicle-treated SHR exhibited a pronounced decrease of the resting tone when Ca²⁺ in the medium was decreased from 2.5 mM to zero (Figure 2). Addition of Ca²⁺ (from zero to 2.5 mM) yielded a concentration-related increase in the aortic tension (Figure 2). These results confirm the findings of Noon et al.11 and Fitzpatrick and Szentivanyi.12

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FIGURE 1. Plot of time course of changes in systolic blood pressure during long-term treatment with CS-622 in spontaneously hypertensive rats. At the start of dosing, rats were 23 weeks of age. In each point, measurement of systolic blood pressure was performed 24 hours after the last administration of the drug. Points and vertical bars represent mean±SEM. *Significant difference from the vehicle-treated group (p<0.05).

FIGURE 2. An example of tracings of tension in aortas of spontaneously hypertensive rats (SHR) when Ca²⁺ concentration of bathing medium was changed. Removal of Ca²⁺ induced a clear relaxation, and restoration of Ca²⁺ to normal level (2.5 mM) produced a marked contraction in aortas from control SHR (upper tracing). But the resting tension was little affected by manipulation of Ca²⁺ concentration in aortas from SHR treated with CS-622 at dose of 10 mg/kg/day for 21 weeks (lower tracing).

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FIGURE 3. Plot of changes in tension in aortas of spontaneously hypertensive rats when Ca²⁺ concentration in the bathing medium was decreased from 2.5 mM to zero and was increased from zero to 2.5 mM. This figure summarizes data obtained from experiments as presented in Figure 2. Resting tension obtained in Ca²⁺-free medium was taken as 0%, and the maximum tension induced by norepinephrine (NE) was taken as 100% contraction. Points and bars represent mean±SEM. *Significant difference from vehicle-treated group (p<0.01).
alteration of Ca$^{2+}$ concentration in aortas from the three groups. The tension was expressed as percent of the maximal contraction induced by NE ($10^{-6}$ M) in 2.5 mM Ca$^{2+}$. The resting tension achieved when Ca$^{2+}$ was increased from zero to 2.5 mM averaged 44.1±4.2, 17.9±3.2, and 11.1±3.0% for the vehicle-treated group, the CS-622 3 mg/kg/day group, and the CS-622 10 mg/kg/day group, respectively. These values indicated that chronic treatments with CS-622 reduced the resting tone of aortas of SHR in a dose-dependent manner. But there was no difference in contractile responses to NE among the three groups; thus, chronic dosing with CS-622 does not decrease nonselectively the contraction of aortas of SHR.

We examined correlation between SBP of the individual rats and their aortic tone in 2.5 mM Ca$^{2+}$ in 39 animals of the three groups. There was a positive correlation between SBP and aortic tone with a linear equation: $y=0.61x-90$ ($r=0.74, p<0.001$) (Figure 4). In 13 animals of the vehicle-treated group, there was also a significant correlation between the two parameters ($y=0.81x-126, r=0.69, p<0.01$) (Figure 4).

To examine whether the decreased myogenic tone could be simply attributed to long-lasting hypotension, we administered hydralazine at 5 mg/kg p.o. or water to 23-week-old SHR for 21 weeks. At the end of the dosing, SBP values were 142±7 and 211±12 mm Hg, respectively, in the hydralazine and control groups. These data indicated that prolonged hypotension per se did not decrease myogenic tone of the aorta at physiological Ca$^{2+}$ concentration.

Changes in aortic tone induced by altering Ca$^{2+}$ concentration in the bathing medium were examined in 40-week-old SHR (SBP=199±5 mm Hg) and age-matched WKY rats (SBP=142±4 mm Hg). As already shown in an example (Figure 2), aortas of SHR exhibited a pronounced change in the tension, whereas aortas of WKY rats did not (Figure 6). Resting tone achieved when Ca$^{2+}$ was increased from zero to 2.5 mM averaged 58.6±6.0% in SHR and 5.4±2.8% in WKY rats. These values are expressed as percentage of the contraction induced by $10^{-6}$ M NE. Although data are not shown, there were differences in the tension of Ca$^{2+}$ (2.5 mM) between the two groups. These data indicated that prolonged hypotension per se did not decrease myogenic tone of the aorta at physiological Ca$^{2+}$ concentration.
was a significant correlation between SBP and aortic tone in 17 rats (both SHR and WKY rats) used in this experiment ($y=0.78x-100$, $r=0.81$, $p<0.001$).

Effects of Acute Inhibition of Angiotensin Converting Enzyme or Ca$^{2+}$ Channel Blockade on the Ca$^{2+}$-Dependent Myogenic Tone in Aortas of Spontaneously Hypertensive Rats

We examined whether acute inhibition of aortic ACE could suppress the myogenic tone due to Ca$^{2+}$ in aortas of SHR. CS-622 diacid was used in this study since CS-622 is rapidly converted to the active diacid in vivo, and almost all molecules of CS-622 are present as diacid forms in the bloodstream. As described elsewhere, CS-622 diacid at a concentration of $3\times10^{-8}$ M completely abolished the contraction evoked by angiotensin I ($3\times10^{-8}$ M) in rat aorta. $10^{-6}$ M CS-622 diacid was thus considered to be sufficient to inhibit ACE of the aortic smooth muscle. At this concentration, CS-622 diacid did not affect the concentration–response curve for Ca$^{2+}$ in aortas of SHR (Figure 7). This indicates that acute inhibition of ACE in vitro could not reproduce the effects of chronic ACE inhibition on the increased myogenic tone in aortas of SHR.

The effects of a calcium antagonist, nicardipine, on aortic tone of SHR were examined. Nicardipine ($10^{-8}$ M) inhibited contraction in aortas of SHR induced by increasing Ca$^{2+}$ concentration (Figure 8). This effect suggests that the increased Ca$^{2+}$ influx through Ca$^{2+}$ channels underlies the increased myogenic tone in aortas of SHR.

Discussion

The cell membrane of vascular smooth muscle from hypertensive animals has been shown to have some abnormalities in its ion transport system. A large body of evidence supports the contention that the vascular cell membrane of SHR is more permeable to monovalent ions or divalent ions than that of normotensive WKY rats.

In the present study, we observed that removal of Ca$^{2+}$ from the bathing solution induced a clear relaxation and that restoration of Ca$^{2+}$ to normal levels produced a marked contraction in aortas of SHR (Figure 6). On the other hand, the resting tension in aortas of WKY rats was unaffected by alteration of Ca$^{2+}$ concentration (Figure 6). These results confirm the findings of Noon et al. and Fitzpatrick and Szentivanyi. As described by them, these phenomena suggest that the vascular smooth muscle of SHR possesses a high resting tension (myogenic tone) possibly due to an increased permeability to Ca$^{2+}$. In the present study, the myogenic tone was decreased by a calcium antagonist, nicardipine (Figure 8). This indicates that the high resting tension in aortas of SHR results from the increased Ca$^{2+}$ influx through Ca$^{2+}$ channels.

We found that this Ca$^{2+}$-dependent myogenic tone in aortas of SHR was suppressed by long-term administration of an ACE inhibitor, CS-622 (Figures 2 and 3). But, tension development evoked by increasing Ca$^{2+}$ concentration was not affected in the presence of CS-622 diacid at a concentration high enough to completely inhibit aortic ACE (Figure 7). These findings indicate that the myogenic tone...
due to exaggerated Ca\(^{2+}\) permeability in aortas of SHR is suppressed by chronic inhibition of ACE in vivo but not by acute inhibition in vitro.

CS-622 is a selective ACE inhibitor that is devoid of any other pharmacological actions, not to mention Ca\(^{2+}\) blocking action.\(^{18}\) Why does chronic treatment with CS-622 suppress the abnormal tone in aortas of SHR? This is not clear from data obtained in the present study, but some speculation can be presented.

Shibata et al\(^{21}\) demonstrated that some Ca\(^{2+}\)-like cations such as Mn\(^{2+}\), Co\(^{2+}\), La\(^{3+}\), and Sr\(^{2+}\) contracted aortas of SHR but not aortas of WKY rats and that such a contraction was already seen in young SHR whose blood pressure was not elevated. Along the same line, increased Ca\(^{2+}\)-dependent myogenic tone in aortas of SHR seen in the present study may reflect a genetic alteration of membrane properties rather than a secondary response to high blood pressure. Thus, it is unlikely that the decrease of the myogenic tone produced by chronic treatment with CS-622 resulted from prolonged hypotension. Indeed, long-term administration of hydralazine did not decrease myogenic tone in aortas of SHR at physiological Ca\(^{2+}\) concentration (Figure 5). A slight decrease of myogenic tone at lower Ca\(^{2+}\) concentrations may have resulted from a small amount of hydralazine that remained in the vascular tissue. At any rate, the decreased aortic tone at physiological concentration of Ca\(^{2+}\) in CS-622-treated SHR would not be explained on the basis of prolonged hypotension.

Lamb et al\(^{22}\) and Myers et al\(^{23}\) reported another aspect of abnormal Ca\(^{2+}\) entry in the artery of hypertensive rats; the tail artery isolated from stroke-prone SHR (SHRSP), but not from WKY rats, exhibited an oscillatory contractile response to NE. This abnormal behavior of the tail artery was also not corrected by prolonged administration of hydralazine and hydrochlorothiazide; this behavior suggests a genetic defect in the artery of SHRSP.\(^{24}\)

If this phenomenon involves the same Ca\(^{2+}\) channels as those in the present study, the oscillatory contraction in the arteries of SHRSP would be abolished after prolonged administration of CS-622.

It is unlikely that CS-622 produced its action via a change in intracellular contractile apparatus since the aortas from CS-622-treated SHR showed the same contractile response to NE (10\(^{-6}\) M) and KCl (60 mM) as the aortas from control SHR. The target site of CS-622 is more likely the cell membrane of vascular smooth muscles.

One possibility is that chronic dosing with CS-622 lowers angiotensin II concentration in the aortic tissue for a long period of time, and this in turn would decrease activity of Ca\(^{2+}\) channels. Villamil et al\(^{25}\) showed that infusion of angiotensin II to dogs for 5–6 weeks increased Na\(^{+}\) and Ca\(^{2+}\) permeability in vascular smooth muscles of the carotid artery and altered the ionic composition of this artery. Ito et al\(^{19–17}\) reported that long-term inhibition of ACE with captopril decreased the abnormal permeability to Na\(^{+}\) in the aortic smooth muscle membrane of SHR. These observations suggest that permeability to Na\(^{+}\) in the cell membrane of vascular smooth muscles is accelerated by chronic exposure to angiotensin II.

Now, we present the following hypothesis regarding the suppression by CS-622 of increased aortic tone in SHR. In aortas of SHR, the increased permeability to Na\(^{+}\) may cause a decrease in transmembrane Na\(^{+}\) gradient, a slight depolarization of membrane, and hence, an activation of voltage-dependent Ca\(^{2+}\) channels. Long-term administration of CS-622 lowers angiotensin II levels in aortic tissue, and this in turn would suppress the abnormally high permeability to Na\(^{+}\). This would restore normal membrane potential and normalize the activity of Ca\(^{2+}\) channels.

In this hypothesis, vascular angiotensin II concentration should be increased in SHR compared with WKY rats. This occurrence is not certain at present, but quite possible. The existence of the complete renin-angiotensin system in local tissues, including vascular tissues, has been well established.\(^{26}\) Several investigators have reported that vascular renin activity is higher in SHR than WKY rats.\(^{27,28}\) Furthermore, long-term administration of captopril lowers blood pressure in SHR in which vascular renin activity, but not plasma renin activity, is elevated.\(^{27}\) These observations suggest that vascular angiotensin II production is increased in SHR compared with WKY rats.

Dzau\(^{26}\) hypothesized that the local renin-angiotensin system has an important role in long-term regulation of vascular tone whereas the humoral renin-angiotensin system plays a role in short-term cardio renal homeostasis. The hypothesis underlying the present study was similar to his: The increased local angiotensin II production in vascular tissue may have a role in the maintenance of high blood pressure in SHR. What we wanted to stress was the mechanism by which long-lasting increase of angiotensin II concentration raises blood pressure. The present results suggest that in SHR this was, at least partly, brought about through the activation of Ca\(^{2+}\) channels in vascular smooth muscle cells.

We found a positive correlation between the Ca\(^{2+}\)-dependent myogenic tone of aorta and the systolic blood pressure in both CS-622–treated and untreated SHR (Figure 4). This finding indicates that the suppression of Ca\(^{2+}\)-dependent myogenic tone of vascular smooth muscle underlies the chronic antihypertensive action of CS-622 in SHR. This can most probably be generalized to all ACE inhibitors because CS-622 does not have any pharmacological action other than ACE inhibition. Our previous report\(^{29}\) demonstrated that the antihypertensive action of captopril after long-term administration in SHR is ascribed to a decrease of total peripheral resistance as a result of vasodilatation occurring in
the whole body. But we did not answer the question in the previous paper: Why does chronic inhibition of ACE dilate blood vessels in SHR whose plasma renin-angiotensin system is not activated? The results of the present study appear to answer this question. We showed that chronic inhibition of ACE decreased Ca\(^{2+}\)-dependent myogenic tone of aorta that was elevated in SHR. If this decrease in tone occurs in other blood vessels in the whole body as well, this would lead to a decrease of total peripheral resistance.

Further studies are needed to clarify the exact mechanisms underlying this phenomenon: the measurement of membrane potential in vascular smooth muscles, the measurement of Ca\(^{2+}\) influx in vascular smooth muscles, and the pharmacological analysis of Ca\(^{2+}\) channels that were affected by chronic inhibition of ACE. Some of those studies are now under way in our laboratories.

In summary, we found that long-term treatment with a novel ACE inhibitor, CS-622, normalized the exaggerated myogenic tone due to increased Ca\(^{2+}\) permeability in aortas of SHR. Since the vascular tone correlated well with blood pressure of individual rats, it is conceivable that reduction of exaggerated vascular tone due to increased Ca\(^{2+}\) influx underlies the chronic antihypertensive action of ACE inhibitors.

References


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