Effects of Long-Term Blockade of Angiotensin Converting Enzyme with Captopril (SQ14,225) on Hemodynamics and Circulating Blood Volume in SHR

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SUMMARY  Male spontaneously hypertensive rats (SHR), 13 weeks old, received oral administration of captopril (SQ14,225) daily at a dose of 10 mg/kg or 30 mg/kg; or water at 2 ml/kg, for 6 weeks. Captopril suppressed the development of hypertension slightly at the lower dose and completely at the higher dose. At 6 weeks of dosing, hemodynamic parameters were measured by the tracer microsphere method and blood volume by the dilution method using 125I erythrocytes. Cardiac output (CO) increased and mean blood pressure decreased in a dose-dependent manner; thus, total peripheral resistance decreased. Cerebral and renal blood flows increased in a dose-dependent manner while blood flow to other organs tended to increase or remained unchanged. There was no significant difference in the circulating blood volume among the three groups. In conclusion, long-term blockade of angiotensin converting enzyme (ACE) with captopril dilated blood vessels in the whole body, especially in the brain and kidney, and lowered blood pressure without causing plasma expansion or tachycardia. (Hypertension 2: 299-303, 1980)

KEY WORDS  • captopril • SQ14,225 • converting enzyme inhibitor • chronic experiment • hemodynamics • regional blood flow • circulating blood volume • spontaneously hypertensive rat

Captopril (SQ14,225), an orally active inhibitor of angiotensin converting enzyme (ACE), has been shown to lower blood pressure of spontaneously hypertensive rats (SHR) in both acute and chronic experiments.1-3 The acute antihypertensive action of the agent is attributed to a decrease of total peripheral resistance,4 but the extent to which each vascular bed contributes to the decrease of total peripheral resistance is not known. There are no data available on hemodynamic effects of chronic blockade of ACE, despite the fact that antihypertensive drugs are clinically used on a long-term basis.

One of the most significant features of captopril seems to be, on its chronic administration, a lack of tolerance from its subjects. On the contrary, the agent has been shown to produce a progressive, cumulative decrease of blood pressure in SHR5 and to display a biphasic hypotension in renal hypertensive rats: an early, rapid hypotension and a late one associated with diuresis.4 A possible decrease of circulating blood volume may be involved in the progressive hypotension during long-term blockade of ACE, in contrast to many other antihypertensive drugs in which fluid expansion is considered to be responsible for the development of the tolerance.6

The purpose of the present study was to characterize hemodynamic changes following chronic blockade of ACE in SHR. We compared such parameters as cardiac output (CO), total peripheral resistance, organ blood flows, and circulating blood volume in SHR treated with captopril or water for 6 weeks.

Methods

Male SHR, 13 weeks old, were divided into the following three groups of 10 animals each and given either water or captopril by mouth for 6 weeks: control group, water 2 ml/kg/day; captopril low-dose group, 10 mg/kg/day; captopril high-dose group, 30 mg/kg/day.

Systolic blood pressure, heart rate, and body weight were measured 1 and 2 days before, and once a week after, initiation of dosing. Systolic blood pressure and heart rate were measured by the tail-cuff method (Narco, PE-300) 4 to 5 hours after administration of the agents. After 6 weeks of dosing, the animals were cannulated under light anesthesia with pentobarbital;
one cannula was placed in the left ventricle for injecting microspheres and the other in the abdominal aorta for drawing blood samples. Mean blood pressure was measured from the femoral artery 24 hours later, and conscious animals were subjected to the measurement of circulating blood volume and hemodynamic parameters.

Measurement of Circulating Blood Volume

Erythrocytes labelled with $^{51}$Cr according to the method of Strumia et al. were injected in a volume of 0.1 ml into the left ventricle. A blood sample of precisely 1.0 ml was drawn 10 minutes later, and radioactivity (RA) was measured in a gamma-spectrometer (Packard, Modumatic II Auto Gamma, model 5320). Circulating blood volume was calculated as follows:

$$\text{circulating blood volume} = \frac{\text{total RA injected}}{\text{RA in sampled blood}} \times \text{volume of sampled blood}.$$  

Measurement of Cardiac Output and Regional Blood Flows

After reinfusion of the same amount of blood that was drawn for the determination of blood volume, hemodynamic parameters were measured by the tracer microsphere method. Microspheres labelled with $^{113}$Ce, having a diameter of 15 ± 5 µm (3M Co., St. Paul, MN) were suspended in 10% dextran containing a small amount of Tween 80. A 0.05 ml volume of microsphere suspension (about 40,000 particles, 0.3 µCi) was placed in silastic tubing connected to the left ventricular cannula and flushed into circulation at a rate of 0.5 ml/min over a 1-minute period. Five seconds before initiation of microsphere injection, the reference blood flow was drawn from the femoral cannula at a rate of 1.3 ml/min for 1 minute. Within 15 minutes, the animal was killed and its organs excised for the measurement of RA. Total RA injected was obtained as a difference of RA in the silastic tubing before and after injections. Hemodynamic parameters were calculated as follows:

$$\text{CO} = \frac{\text{reference flow rate} \times \text{total RA injected}}{\text{RA in reference blood sample}};$$

$$\text{fractional distribution of CO} = \frac{\text{RA found in the organ}}{\text{total RA injected}} \times 100;$$

$$\text{regional blood flow} = \text{fractional distribution of CO} \times \text{CO} \times 1/100,$$

where CO and RA refer to cardiac output and radioactivity respectively.

Results

Although the experiment started with 10 animals in each group, measurements of hemodynamics and blood volume failed in some animals due to clot formation in the femoral cannula. Therefore, the time course for the changes in blood pressure and heart rate were plotted for animals in which all measurements were done successfully (figs. 1, 2). The SHR used in this study were in the developing phase of hypertension, and blood pressure in the control group increased further during the 6-week observation period. Captopril, at 10 mg/kg/day, slightly suppressed the development of hypertension (significant at the second and fifth weeks). Blood pressure in the high-dose group (captopril, 30 mg/kg/day) stayed significantly lower than that of the control group throughout the 6 weeks, and blood pressure at the end of dosing never exceeded the initial value. Heart rate in captopril groups did not differ significantly from that of the control group (fig. 2).

Table 1 shows hemodynamic parameters and circulating blood volume measured after 6 weeks' administration of captopril or water. With captopril, mean blood pressure decreased and CO increased in a dose-dependent manner; thus total peripheral resistance decreased. Differences in mean blood pressure between the control group and low- and high-dose groups were 6 and 29 mm Hg respectively (table 1), which were almost identical to those for systolic blood pressure measured by the tail plethysmographic method at 6 weeks of dosing (fig. 1). There was no significant difference in circulating blood volume between the control group and groups treated with captopril.

Chronic treatment with captopril at doses of 10 and 30 mg/kg produced only a slight change in the distribution of CO; fractional distribution of CO to the brain and kidney tended to increase, whereas that to the heart tended to decrease (fig. 3). Blood flows to the brain and kidney increased in a dose-dependent manner while that to other organs remained unchanged or tended to increase (fig. 4).

Relative organ weight as divided by body weight tended to decrease in the heart after chronic treatment with captopril, although there was no statistically significant difference between control and 30 mg groups (table 2). On the other hand, there was no difference in body weight among the three groups (table 2).

Discussion

The present study demonstrates that captopril prevents the development of hypertension in SHR. This confirms and extends previous findings that both acute and chronic administration of captopril lowers blood pressure in SHR. The antihypertensive action of chronic administration of captopril can obviously be ascribed to a decrease of total peripheral resistance (table 1), as in the case of short-term administration. Cardiac output increased significantly at a dose of 30 mg/kg/day, which may be attributable to a decrease...
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FIGURE 1. The time course of changes in systolic blood pressure during long-term treatment with SQ14,225 in spontaneously hypertensive rats (SHR). At the start of dosing, SHR were 13 weeks of age. Each point and bar indicates mean and standard error. Asterisks indicate a significant difference from controls by nonpaired t test. Number of animals are 7, 6, 7 in the control, low and high dose groups respectively.

FIGURE 2. The time course of changes in heart rate during long-term treatment with SQ14,225 in spontaneously hypertensive rats. See the legend to figure 1 for additional explanations. (Control group = seven rats; low-dose = six rats; high-dose = seven rats)

FIGURE 3. Fractional distribution of cardiac output in spontaneously hypertensive rats treated with captopril for 6 weeks.

FIGURE 4. Organ blood flows in spontaneously hypertensive rats treated with captopril for 6 weeks. Values for skeletal muscle represent blood flow per 100 of the thigh muscle, while other regional blood flow is expressed per kg body weight.
of cardiac after-load. The measurement of CO by the tracer microsphere technique has been recently validated by Tsuchiya et al. 7

In the face of decreased blood pressure, there was no organ in which blood flow decreased, indicating that vasodilatation occurred in the whole body. This general vasodilatation following chronic treatment with captopril most likely results from the inhibition of ACE or kininase II, as the agent does not have direct vasodilator and autonomic actions. 8, 9 The selective increase of cerebral and renal blood flows is difficult to interpret. The only clue to explain the increase of cerebral blood flow is a finding by Brecher et al. 10 that the microvessels in the brain showed an unusually high ACE activity that is comparable to that of the lung tissue.

A possible reason for the specific increase of renal blood flow is: the vasoconstrictor action of angiotensin II (AII) has been demonstrated to be particularly strong in the renal vascular bed; 11 therefore, a decreased formation of AII in the blood stream due to blockade of ACE would lead to a preferential vasodilatation in the kidney. This speculation is based on the assumption that the renin-angiotensin system plays a role in the maintenance of blood pressure in SHR, which is still a matter of controversy. Recent demonstration by Bean et al. 12 seems to have established the concept that AII has slow pressor effects distinct from acute vasoconstrictor action. According to this, a slight difference in plasma AII concentration can produce a great difference in blood pressure in the chronic stage. Therefore, the renin-angiotensin system can play a role in SHR even if there is no appreciable difference in plasma renin activity between SHR and normotensive rats.

Gavras et al. 13 reported that acute blockade of ACE with SQ20,881 increased cerebral, renal, and myocardial blood flows in salt-deficient dogs in which the blood pressure is supported by the renin-angiotensin system. In our chronic experiment, cerebral and renal blood flows increased, but myocardial blood flow did not. The decrease of the heart weight after chronic blockade of ACE 2 (table 2), may account for this difference of myocardial flow response in acute and chronic experiments. If so, our results are rather similar to those of Gavras et al., suggesting that the renin-angiotensin system is involved in the maintenance of blood pressure in SHR. Recently, Hollenberg et al. 14 have suggested a possible involvement of the renin-angiotensin system in the reduced renal blood flow and glomerular filtration rate frequently seen in essential hypertension.

Apart from its action on the renin-angiotensin system, captopril has been shown to increase plasma kinin level 16 and urinary excretion of kinin, 17 which reflects an increase of renal kinin concentration. Since bradykinin is a potent stimulant of prostaglandin biosynthesis, 18 a rise of renal prostaglandin level is also expected, although there is no direct evidence for it. These possible increases in renal levels of both kinin and prostaglandins may account for the specific increase of renal blood flow. Whatever the mechanisms, the specific vasodilatation in the kidney appears to be characteristic for this agent and may be of great clinical importance, particularly in connection with lack of plasma expansion after long-term administration. Indeed, SQ20,881, another ACE inhibitor, has been reported to improve renal excretory function in hypertensive subjects, presumably by a counter effect on renal vasoconstriction. 19

Many antihypertensive agents produce tolerance associated with plasma volume expansion. 6 This volume expansion is brought about by activation of the renin-angiotensin-aldosterone system and through hemodynamic mechanisms, e.g., decrease of transcapillary pressure favoring inward movement of extravascular fluid and decrease of renal perfusion pressure leading to a fluid retention. Chronic treatment with...
captopril did not alter blood volume, however, which suggests that a hemodynamic consequence of prolonged hypotension, i.e., the tendency toward plasma expansion, was balanced out by the indirect diuretic action of captopril. The lack of weight gain (table 2) also suggests that there was no increase in extracellular fluid volume.

In conclusion, chronic blockade of ACE with captopril dilates blood vessels in the whole body — especially in the kidney and brain — and lowers blood pressure without causing either plasma expansion or tachycardia. These effects seem to result from the inhibition of ACE or kininase II.

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References

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