Effects of Chronic Administration of Captopril on Blood Pressure and the Renin-Angiotensin-Aldosterone System in Normotensive Rats

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SUMMARY

The chronic effects of oral administration of an angiotensin converting enzyme inhibitor (captopril, 63–80 mg/Kg/day) on blood pressure and the renin-angiotensin-aldosterone system were studied in normotensive male Wistar rats. Blood pressure (BP), plasma renin activity (PRA), plasma renin concentration (PRC), plasma renin substrate concentration (PRS), plasma aldosterone concentration (PAC) and renal renin content (RRC) were measured after 2, 9, 19, 29 and 58 days of captopril administration. BP was determined in awake rats and blood samples were obtained by decapitation. In addition, BP responses to bolus injections of angiotensin I (AI, 80 ng/Kg), angiotensin II (AII, 80 ng/Kg) and bradykinin (BK, 10 μ g/Kg) were examined in conscious rats which had received captopril for 20 days. The BP of the rats which had been given captopril for 9 days or longer was significantly lower than that of the control rats. PRA and PRC were markedly increased, and both PRS and PAC were decreased in the captopril-treated rats. RRC, which was reduced after 2 days of captopril administration, was substantially increased thereafter. Although the pressor responses to AII were similar in the captopril treated and the control rats, the responses to AI were reduced to 50% of those to AII in the former. The depressor responses to BK in the captopril-treated rats were 2.3 times as large as those in the control rats. Our results show that long-term use of captopril lowers BP in normotensive rats on a normal sodium intake. They also suggest that the rises in PRA, PRC and RRC in the captopril-treated rats may be related to the effects of both the BP reduction and interruption of the negative feedback mechanism of AII on renin

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release and synthesis, and that the decline in PRS is due to its increased consumption and probably also to the decreased rate of hepatic substrate synthesis induced by the fall in plasma AII.

Additional Indexing Words:

Plasma renin activity	Plasma renin concentration	Plasma
renin substrate concentration	Renal renin content	Plasma
aldosterone concentration	Bradykinin	

THE role of the renin-angiotensin-aldosterone (R-A-A) system in the normal regulation of blood pressure and the pathogenesis of hypertension remains a subject of active investigation and debate. In normotensive subjects, the R-A-A system has generally been regarded as a defence mechanism that maintains blood pressure only during volume or sodium depletion.¹⁾ Until recently, saralasin, a competitive inhibitor of angiotensin II (AII), and teprotide, an inhibitor of angiotensin converting enzyme, have been used to study the role of the R-A-A system in the regulation of blood pressure. However, both saralasin and teprotide have to be given intravenously, and thus inhibit only the short-term effects of circulating AII. Saralasin is an antagonist which only inhibits the action of circulating AII when its level is high.

Recently developed orally active angiotensin converting enzyme inhibitors have enabled investigators for the first time to study the long-term effects of direct angiotensin blockade. With such converting enzyme inhibitors, captopril or MK 421, MacGregor et al^{2),3)} showed that the R-A-A system contributes to the maintenance of blood pressure in normotensive subjects receiving a normal sodium intake. These converting enzyme inhibitors have been proved to be effective antihypertensive agents and are now commonly used for the long-term treatment of hypertension. Converting enzyme inhibitors *per se* can by their nature induce complicated changes in each component of the R-A-A system. For example, Rasmussen et al⁴⁾ reported that captopril combined with thiazide markedly lowered renin substrate concentration, thus making it necessary to measure plasma renin concentration instead of plasma renin activity in this particular situation.

The present study was designed to investigate the chronic effects of captopril administration on blood pressure and the R-A-A system, including renal renin content, in normotensive rats on a normal sodium intake.

MATERIALS AND METHODS

Experiment 1

Male Wistar rats weighing 160–250 Gm were maintained on a normal

rat chow diet containing 104 mEq/Kg of sodium and 177 mEq/Kg of potassium. Captopril was added to the experimental animals' drinking water at a concentration of 0.5 mg/ml. Control rats drank tap water. Groups of 10 rats each were killed by decapitation at 2, 9, 19, 29 and 58 days after commencement of captopril administration. Blood pressure was measured by the tail-microphone method in unanesthetized rats a day before sacrifice. Blood samples were collected in ice-cold tubes containing disodium EDTA (4%) and o-phenanthroline (0.33%) immediately after decapitation and centrifuged at 4°C. Plasma was separated and frozen at -20°C until assay. Both kidneys were removed, weighed and stored at -70°C. Plasma renin activity (PRA) was measured by radioimmunoassay (RIA) of angiotensin I (AI) generated during 6 hours of incubation of 0.5 ml of plasma at 37°C, pH 6.0, using a Becton-Dickinson AI RIA kit and expressed as ng AI/ml/hr.

Plasma renin concentration (PRC) measurement was performed by means of substrate enrichment. Renin substrate was prepared from 24-hour bilaterally nephrectomized rats. Plasma was incubated with renin substrate for 6 hours at 37°C, pH 6.0. After incubation, the AI generated was measured by the method outlined above. For the measurement of plasma renin substrate concentration (PRS), plasma was incubated with hog renin (Biochemical, 1 Goldblatt unit/ml) for 30 min, at 37°C, pH 6.0. Following incubation, the AI generated was measured similarly and PRS was expressed as ng of AI generated per ml of plasma.

Renal renin content (RRC) was measured according to the method of Orth et al.⁵⁾ Briefly, the whole kidneys were frozen and thawed 5 times. They were then homogenized in 50 ml of ice-cold 0.9% saline containing disodium EDTA and chlorhexidine digluconate with a micronizer (Nihon Seiki). All renal homogenate samples were centrifuged twice at 5,000 rpm for 15 min. The supernatant was used for renin assay. Aliquots of supernatant were incubated with 24-hour bilaterally nephrectomized rat plasma for 30 min at 37°C. Generated AI was measured and RRC was expressed as ng AI/mg of tissue. Plasma aldosterone concentration (PAC) was determined by the RIA method following the extraction of plasma with dichlormethane using a CIS aldosterone RIA kit.

Experiment 2

Male Wistar rats weighing 160–250 Gm were also used. They were similarly fed a normal rat chow as in experiment 1. Eight rats received captopril in drinking water at a concentration of 0.5 mg/ml. Another 8 rats drank tap water and served as controls. After 20 days of captopril administration, all rats were anesthetized with sodium pentobarbital injected

intraperitoneally. A venous catheter was placed in the jugular vein and an arterial catheter was introduced into the carotid artery. Both catheters, filled previously with 0.9% saline containing heparin 10 U/ml, were tunnelled subcutaneously to exit from the skin between the scapulae. Following the surgical procedure, all rats were allowed to recover for about 5 hours. The arterial catheter was connected to a pressure transducer for continuous blood pressure monitoring on a Nihon-Koden multipurpose recorder. After stabilization of blood pressure, the blood pressure responses to norepinephrine (NE), angiotensin I (AI), angiotensin II (AII), 1-Sar, 8-Ile, angiotensin II (ATA) and bradykinin (BK) injected through the venous catheter were determined. The dose used for NE was 800 ng/Kg, for AI 80 ng/Kg, for AII 80 ng/Kg, for ATA 50 μ g/Kg and for BK 10 μ g/Kg. Each was diluted with 5% dextrose so as to make the injection volume 1 ml/Kg. Between injections, blood pressure was allowed to return to baseline for at least 5 min.

Results are expressed as mean $\pm$ SEM. All data were analyzed by Student's t-test and a p-value of less than 0.05 was considered significant.

RESULTS

Experiment 1

During the experimental period, the rats which received captopril consumed 63–80 mg/Kg per day of captopril. Table I depicts blood pressure, PRA, PRC, PRS, RRC and PAC in the control and the captopril-treated rats. The blood pressure in the rats treated with captopril for at least 9 days was significantly lower by 16–26 mmHg than that in the control rats.

In response to captopril administration, the experimental rats had markedly increased PRA throughout the observation period. Similarly, chronic use of captopril caused a substantial and continuous rise in PRC. In contrast, a marked decline in PRS was continuously present in the captopril-treated rats.

Following 2 days of captopril administration, RRC in the experimental rats was decreased compared to that in the control rats, while there was no difference in RRC between the 2 groups after 9 days. Thereafter significant and sustained increases in RRC were observed in the captopril-treated rats. PAC which was measured at 9 and 58 days after the start of captopril treatment was significantly reduced in the experimental rats compared to the control rats.

Interrelations among the parameters of the R-A-A system were examined. A positive relationship was found between PRA and PRC in the control rats ($r=0.68$, $p<0.01$), but not in the captopril-treated rats. As shown in Fig. 1,

Table I. Blood Pressure (BP), Plasma Renin Activity (PRA), Plasma Renin Concentration (PRC), Plasma Renin Substrate Concentration (PRS), Renal Renin Content (RRC), Plasma Aldosterone Concentration (PAC) in Control and Captopril-treated Rats

	No.	BP (mmHg)	PRA (ng/ml/hr)	PRC (ng/ml/hr)	PRS (ng/ml)	RRC (ng/mg)	PAC (ng/dl)
control	11	114±3	5.5±1.1	8.7±1.3	511±25	419±57	not measured
captopril-treated (2 days)	10	107±3	11.9±2.8**	51.4±12.6*	348±32*	256±13†	
control	10	116±3	3.5±0.4	4.6±0.8	578±20	385±28	83.3±3.0
captopril-treated (9 days)	10	100±5	7.8±0.8*	94.3±22.3*	201±29*	420±40	66.3±6.4*
control	9	121±2	4.0±1.0	6.6±1.3	533±26	377±30	not measured
captopril-treated (19 days)	10	101±3*	8.5±0.9*	79.6±25.8*	238±38*	515±40**	
control	10	129±3	3.1±0.5	6.0±0.7	534±19	336±23	not measured
captopril-treated (29 days)	10	103±3*	12.4±1.4*	40.2±3.2†	175±26*	599±63*	
control	9	127±3	3.4±0.5	7.7±1.8	664±31	280±24	97.3±8.9
captopril-treated (58 days)	9	103±2*	6.9±0.5*	36.3±4.3*	283±34*	536±25*	53.5±4.8*

Results are expressed as mean±SEM.

* $p<0.01$, † $p<0.02$, ** $p<0.05$ vs the control rats.

Fig. 1. A significant negative correlation between plasma renin concentration (PRC) and plasma renin substrate concentration (PRS) in captopril-treated rats.

a negative correlation was identified between PRC and PRS in the rats which received captopril ($r=-0.43$, $p<0.01$). RRC positively correlated with PRC in the control rats ($r=0.44$, $p<0.01$), but not in the experimental rats.

The relationships between blood pressure and the parameters of the R-A-A system were also examined. There was an inverse relationship only

Fig. 2. A significant negative correlation between blood pressure (BP) and plasma renin concentration (PRC) in captopril-treated rats.

between blood pressure and PRC in the captopril-treated rats ($r = -0.60$, $p = 0.01$, Fig. 2).

Experiment 2

During the 20-day experimental period, captopril intake averaged 76 mg/Kg per day. Table II shows the mean blood pressure and absolute changes in blood pressure in response to bolus injections of NE, AI, AII, ATA and BK. The directly measured blood pressure in the captopril-treated rats was significantly lower than that in the control rats. The blood pressure lowering effect of captopril in normotensive rats was also confirmed in this experiment.

We could not find any differences in the pressor responses to NE, AII and ATA between the 2 groups. The sensitivity to exogenous AI and BK was tested in order to evaluate the efficacy of converting enzyme inhibition. The pressor response to AI was markedly blunted, whereas the depressor response to BK was greatly augmented in the captopril-treated rats. The rise

Table II. Directly Measured Mean Blood Pressure and Its Alterations in Response to Various Vasoactive Agents

	MBP	Δ MBP to NE	Δ MBP to AII	Δ MBP to AI	Δ MBP to ATA	Δ MBP to BK
Control (n=8)	130 $\pm$ 3	36 $\pm$ 3	35 $\pm$ 3	33 $\pm$ 3	40 $\pm$ 3	-23 $\pm$ 6
Captopril-treated (n=8)	109 $\pm$ 2	35 $\pm$ 3	36 $\pm$ 2	18 $\pm$ 2	34 $\pm$ 4	-46 $\pm$ 7
p-value	<0.001	NS	NS	<0.001	NS	<0.05

All values are in mmHg. Results are expressed as mean $\pm$ SEM.

NE=norepinephrine; AII=angiotensin II; AI=angiotensin I; ATA=1-Sar, 8-Ile, angiotensin II; BK=bradykinin.

in blood pressure in response to AI was about 50% of that to AII in the rats which received captopril (Table II).

DISCUSSION

We found that chronic captopril administration reduced blood pressure in normotensive rats on a normal sodium intake. Although the dose of captopril used in this study was greater than that usually prescribed in clinical practice, our results are not inconsistent with others. Spertini et al⁶⁾ and Clough et al,⁷⁾ independently, showed that captopril lowered blood pressure in normotensive rats on a normal sodium intake. Captopril reportedly reduces blood pressure in sodium-depleted normotensive, spontaneously hypertensive, one-kidney one-clip hypertensive and two-kidney one-clip hypertensive rats. There is a controversy about the way in which captopril lowers blood pressure. The only established action of captopril is its inhibition of angiotensin converting enzyme. This was shown indirectly in our study by the fall in PAC.

Inhibition of angiotensin converting enzyme can lead not only to a fall in AII levels, but also to an accumulation of BK, a potential vasodilator. Our results show that captopril actually enhances the depressor response to exogenous BK and partly inhibits the inactivation of BK. However, a recent report⁸⁾ has demonstrated that there is no increase in plasma BK with captopril. It has also been suggested that prostaglandins may be involved in the blood pressure lowering effect of captopril.⁹⁾ At present, whether BK or prostaglandins are significant factors in the antihypertensive actions of captopril still remains unsettled. If, as seems likely, captopril lowers blood pressure through a fall in plasma AII, our observations strongly indicate that the R-A-A system is a factor in maintaining blood pressure in normotensive rats on a normal sodium intake.

PRC as well as PRA were markedly increased in rats which received captopril. This increase in PRC was presumably related to the reduction in blood pressure, taking into consideration the inverse relationship between PRC and blood pressure. Furthermore, plasma AII directly suppresses renin release through the short loop negative feedback mechanism.¹⁰⁾ The interruption of this negative feedback mechanism by captopril might also be responsible for the rise in PRC and PRA.

On the other hand, captopril administration substantially reduced PRS. This finding is consistent with Rasmussen's report.⁴⁾ Moreover, our data clearly indicate that captopril alone could induce a considerable decrease in PRS. The negative correlation between PRS and PRC observed in

captopril-treated rats suggests that the substantial reduction of PRS is due to a consumption of substrate caused by enhanced renin release. Further, the positive feedback of AII on hepatic renin substrate synthesis¹¹⁾ might be profoundly disturbed by captopril treatment.

To our knowledge this is the first observation of the sequential changes in RRC after captopril administration. It is noteworthy that while RRC was reduced after 2 days of captopril treatment, it was increased thereafter throughout the experiment. Gavras et al¹²⁾ pointed to the possibility of "exhaustion" of the juxtaglomerular cells after continuous stimulation of renin release by captopril. Our results clearly show that this is not the case. It has generally been recognized that the kidney secretes only a small fraction of its total renin content and a large storage pool of renin exists.¹³⁾ However, our data could be interpreted as follows: (1) Captopril administration for 2 days stimulated renin release so rapidly and profoundly that the renin secretory rate was much greater than the concurrent rate of renin synthesis. (2) Thereafter, captopril, which continued to increase renin release, ultimately caused a comparable increase in renin synthesis. Except in acute experimental conditions, RRC and PRC often show parallel changes.¹⁴⁾ In fact we found a direct relation between PRC and RRC in control rats. In contrast, there was no clearcut relation between these parameters in captopril-treated rats. This finding suggests that captopril somehow modifies the interrelationship which is normally present between renin secretion and renin synthesis. Chronic captopril administration markedly diminished the sensitivity to exogenous AI. But the pressor response to AI was still present and reached 50% of that to AII. This finding confirms the observation that the blood pressure lowering effect of captopril does not require the complete blockade of converting enzyme for its expression.¹⁵⁾

In summary, sustained blockade of angiotensin converting enzyme by captopril lowered blood pressure in normotensive rats on a normal sodium intake. Furthermore, long-term use of captopril caused a continuous alteration in each component of the R-A-A system in normotensive rats.

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