EFFECTS OF CHRONIC TREATMENT WITH CAPTOPRIL (SQ 14,225), AN ORALLY ACTIVE INHIBITOR OF ANGIOTENSIN I-CONVERTING ENZYME, IN SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract—The effects of hydralazine (3 mg/kg) and the angiotensin I-converting enzyme (ACE) inhibitor captopril (SQ 14,225) (100 mg/kg) on mean arterial blood pressure, plasma renin activity, urinary volume and urinary Na, K, and aldosterone concentrations were examined in spontaneously hypertensive rats of the Okamoto and Aoki strain (SHR) after oral daily dosing for 2 weeks, 3 or 6 months. Captopril caused progressive cumulative reductions in blood pressure resulting in normalization of pressure after 6 months of dosing. Hydralazine also significantly reduced blood pressure but not to the level of normotensive rats of the Wistar-Kyoto strain (WKY). Reductions in heart size paralleled the changes in blood pressure, normalization of cardiac hypertrophy occurring after captopril but not hydralazine. Plasma renin activity increased approximately 2-3 fold after hydralazine and 15-fold after captopril. Neither hydralazine nor captopril had any consistent effects on 24-hr urine volume, urinary Na, K, or aldosterone excretion. These results indicate that chronic inhibition of ACE with captopril induces normalization of blood pressure in SHR, a normal-renin model of hypertension.

Captopril (D-3-mercaptop-2-methylpropanoyl-L-proline, SQ 14,225) is a new orally active inhibitor of angiotensin I-converting enzyme (ACE) which is effective in dogs, cats, rabbits, rats and man (1-5). In addition, captopril reduces blood pressure acutely when orally administered to 1-clip, 2-kidney renal hypertensive rats and spontaneously hypertensive rats (SHR) (1, 6). This antihypertensive effect is rapid in onset beginning within 1-3 hours after a single oral dose and lasting between 16-24 hours depending upon the dose. The mechanism(s) by which captopril decreases blood pressure may also be related to its ACE (kininase II) inhibitory actions since the compound has no other significant demonstrable autonomic effects which might be responsible for its activity (3, 4).

As of this writing, only the acute effects (2-11 daily doses) of orally administered captopril on the blood pressure of hypertensive rats have been reported (6). The purpose of the present study was to examine the effects of daily oral administration of captopril for up to 6 months on mean arterial blood pressure (MBP) of adult, conscious SHR in...
comparison with the antihypertensive agent hydralazine. In addition, the effects of captopril and hydralazine on plasma renin activity (PRA), 24-hr urine excretion, urinary Na⁺, K⁺ and aldosterone excretion were examined.

MATERIALS AND METHODS

Ten to fourteen-week old male normotensive rats of the Wistar-Kyoto strain (WKY), and spontaneously hypertensive rats of the Okamoto and Aoki strain (SHR) were obtained from Taconic Farms, Germantown, N.Y. and were placed on a normal rat chow diet with water ad lib. The rats were randomly assigned to cages according to the following oral treatment schedules:

- **Group A**—WKY, vehicle control (5 ml water/kg/day)
- **Group B**—SHR, vehicle control (5 ml water/kg/day)
- **Group C**—SHR, hydralazine-HCl (3 mg/kg/day)
- **Group D**—SHR, captopril (100 mg/kg/day)

Ten to twelve rats from each group were randomly selected after either 2 weeks, 3 or 6 months of daily dosage for the measurement of mean arterial blood pressure, PRA, urine excretion, urinary Na⁺, K⁺, and urinary aldosterone excretion.

A. Blood pressure measurements

Approximately 18 days before each of the dosage intervals described above, indwelling abdominal aortic cannulas were implanted in rats anesthetized with pentobarbital sodium according to the method of Weeks and Jones (7). After the 18-day recovery period, direct mean arterial blood pressures of conscious rats were recorded by the method of Laffan et al. (8) modified as follows. The signal from the transducer was digitized in a 10 list A/D converter with input to a PDP 11/05 computer. The program was designed to sense and store samples at a rate of 125/sec for each rat. These parameters were averaged and stored as the MBP. Data were obtained from each rat every five minutes for 8 hours. Six such sets of data were averaged to give a mean value representing a 30 min sample and this 30 min figure was stored for subsequent analysis. The data were transferred serially to a host computer (PDP 11/40) for final analysis.

B. Plasma renin activity

On the day of blood pressure measurement, one-half ml of blood from the implanted aortic cannula of all rats was collected into a tube containing 50 μl of 0.25 mM EDTA, pH 7. Plasma was prepared by centrifuging the samples at 2500 × g for 30 min at 4°C. Two hundred μl of plasma was placed in a 400 μl polypropylene tube, tightly capped and stored in an ultra low temperature freezer (−70°C) until analyzed. PRA values were usually determined within 3 weeks of storage. PRA values, expressed in terms of ng of Angiotensin I (Asp₁−Ile₈-angiotensin I) generated per ml of plasma per hour of incubation at 37°C, were determined using the Squibb Angiotensin I Immutope Kit. The pH of the plasma was adjusted to 6.5 which is the pH optimum for PRA in rats (unpublished observations).
C. Urinary volume, Na⁺, K⁺, and aldosterone determinations

Twenty-four hour urine samples were collected at the dosage time intervals described above, the time of collection commencing immediately upon dosing with the stated regimen. Urine was collected in polypropylene bottles imbedded in ice within insulated containers. The urine was transferred to and measured in polycarbonate centrifuge tubes. Samples were then transferred to polycarbonate tubes and centrifuged (refrigerated) for 10 min at 10,000 rpm to provide a clear sample free of any particulate matter. Ten ml urine samples for aldosterone determinations and samples for urinary Na⁺ and K⁺ were frozen and stored at −20°C until the samples were assayed. Ten ml urine samples and distilled water blanks were extracted with 30 ml of methylene chloride and centrifuged at 1000 × g for 3 min. Five ml samples of the methylene chloride layer were transferred into centrifuge tubes and aldosterone concentrations were measured with a modified 3H-Aldosterone Assay Kit (Diagnostic Products Corp., Los Angeles, Ca). Urinary Na⁺ and K⁺ concentrations were measured with a flame photometer (Instrumentation Laboratory, Model 343). All graphical and tabular data show means ± S.E. Statistical significance was determined using an unpaired Student’s t-test with p<0.05 considered as an indication of significant difference.

RESULTS

Effects of Chronic Treatment with Captopril or Hydralazine in Conscious SHR

A. Mean arterial blood pressure

Captopril (100 mg/kg) caused significant and marked reductions of MBP in SHR when orally administered daily for periods of 2 weeks, 3 and 6 months (Fig. 1). Progressively greater reductions in MBP occurred as the duration of treatment with captopril increased such that, after 6 months of dosing, MBP was significantly lower than after 3 months of dosing (p<0.01) but not significantly different from vehicle-dosed normotensive WKY rats. Although hydralazine (3 mg/kg) also caused significant reductions in MBP when
orally administered for the same time periods, the blood pressure never reached that of the normotensive WKY (Fig. 1).

B. Plasma renin activity

Control PRA values in untreated WKY and SHR were not significantly different from each other (Fig. 2). Daily oral dosing with hydralazine (3 mg/kg) for 2 weeks, 3 or 6 months caused a consistent rise of PRA values to approximately twice their control values, whereas captopril increased PRA approximately 10–15 fold during the same dosing periods (Fig. 2).

C. Urinary volume Na⁺, K⁺ and aldosterone

Urine volumes of SHR were not significantly different from WKY after 2 weeks, 3 or

![Graph showing plasma renin activity over time for different treatments.](image)

**TABLE 1. Effects of captopril and hydralazine on 24-hr urine volume (ml)**

| RAT STRAIN | TREATMENT               | 2 weeks | 3 months | 6 months |
|------------|-------------------------|---------|----------|----------|
| SHR        | H₂O, 5 ml/kg/day        | 10.1±1.0| 17.5±1.6| 13.5±1.5 |
|            | (12)                    |         | (12)     | (8)      |
| SHR        | Captopril, 100 mg/kg/day| 16.5±2.1| 14.6±1.2| 22.3±2.0 |
|            | (11)                    |         | (8)      | (11)     |
| SHR        | Hydralazine, 3 mg/kg/day| 10.3±1.1| 13.8±0.5| 16.4±2.3 |
|            | (12)                    |         | (8)      | (10)     |
| WKY        | H₂O, 5 ml/kg/day        | 10.5±0.9| 16.0±1.1| 9.3±1.0  |
|            | (10)                    |         | (10)     | (12)     |

WKY = Normotensive rats of the Wistar-Kyoto strain.  
SHR = Spontaneously hypertensive rats of the Okamoto and Aoki strain.  
( ) = No. of rats/group.
Table 2. Effect of captopril and hydralazine on 24-hr urinary Na⁺ and K⁺ concentration

| RAT STRAIN | TREATMENT     | 0.5 Months | 3.0 Months | 6.0 Months |
|------------|---------------|------------|------------|------------|
|            |               | Na⁺ (Meq/liter) | K⁺ (Meq/liter) | Na⁺/K⁺ | Na⁺ (Meq/liter) | K⁺ (Meq/liter) | Na⁺/K⁺ | Na⁺ (Meq/liter) | K⁺ (Meq/liter) | Na⁺/K⁺ |
| SHR        | H₂O 5 ml/kg/day | 89±15 | 160±22 | 0.54±0.04 (10) | 124±13 | 182±17 | 0.67±0.02 (12) | 104±10 | 180±11 | 0.58±0.03 (8) |
| SHR        | Captopril 100 mg/kg/day | 78±10 | 134±14 | 0.59±0.05 (11) | 152±8 | 214±16 | 0.73±0.04 (8) | 84±7 | 142±12 | 0.59±0.01 (11) |
| SHR        | Hydralazine 3 mg/kg/day | 137±19 | 212±17 | 0.64±0.04 (12) | 150±4 | 223±9 | 0.68±0.02 (8) | 103±8 | 178±14 | 0.58±0.03 (10) |
| WKY        | H₂O 5 ml/kg/day | 109±14 | 163±20 | 0.66±0.05 (10) | 127±16 | 210±17 | 0.59±0.04 (10) | 120±9 | 220±12 | 0.54±0.03 (12) |
Neither captopril nor hydralazine produced consistently significant changes in 24 hr urine volumes of SHR in comparison with vehicle treated animals at any examined interval of dosing (Table 1). Similarly, 24 hr urinary Na⁺, K⁺ was not significantly different between vehicle treated SHR and WKY, nor did dosing with captopril or hydralazine significantly change these concentrations or ratios after 2 weeks, 3 or 6 months of dosing (Table 2). WKY showed a slightly higher 24 hr urinary aldosterone level in comparison with SHR but treatment of SHR with either SQ 14,225 or hydralazine for 3 or 6 months caused no significant change in urinary aldosterone secretion in comparison with vehicle-treated SHR (Table 3).

D. Relative heart weights

Relative heart weights (heart weight/body weight × 100) of the various treatment groups are shown in Fig. 3A. After two weeks of dosing, relative heart weights of rats treated with either hydralazine or captopril were slightly but not significantly lower than water-treated SHR, which in turn, were significantly (p<0.02) higher than the water-treated normotensive group. The hydralazine-treated group had relative heart weights which were lower than water-treated SHR after 3 months of daily dosing but higher than those of normotensive rats; this pattern was unchanged after 6 months of dosing, although the captopril-treated group had a marked reduction in relative heart weights after 3 months of daily dosing and, unlike the hydralazine-treated group, actually had normal relative heart weights after 6 months of dosing (Fig. 3A). Thus, the relative heart weights were directly correlated to the level of blood pressure in the various groups; the heart weight changes were not a result of body weight changes since there were no differences in the SHR groups (Fig. 3B).

**DISCUSSION**

Previous studies have demonstrated that captopril effectively reduced blood pressure in 1-clip, 2-kidney renal hypertensive rats and SHR when orally administered daily for up to 11 days (1, 6). The present study demonstrates that captopril maintains its antihypertensive effect in SHR after daily oral administration for at least 6 months. No tolerance was observed. Rather, MBP of conscious SHR was reduced to the levels of WKY with apparent
accumulation of effect, the reductions in pressure being progressively increased with duration of treatment with captopril. Hydralazine also significantly reduced blood pressure of SHR but, in contrast to captopril, the magnitude of the fall in blood pressure was the same at each time period tested. These differences in antihypertensive activity between captopril and hydralazine were surprising since the doses selected were based on equal efficacy after single oral doses.

Treatment with either hydralazine or captopril decreased the heart weights of treated rats, an effect which was directly correlated with the level of blood pressure. Hence, captopril was found to normalize blood pressure as well as heart size whereas hydralazine had an intermediate effect on both parameters. The reduction in cardiac hypertrophy with antihypertensives supports the well-documented reversal of hypertrophy which occurs by removing the cause of hypertension as in DOCA, renal, aortic-coarctated and SHR (9-12). However, it remains to be determined whether the incomplete reversal of cardiac hypertrophy was due simply to a relatively higher blood pressure than those treated with captopril or whether another factor is involved. Increased renin levels after hydralazine are obviously not the answer (11) since captopril caused much higher levels of renin to be released than did hydralazine. Perhaps the tachycardia caused by hydralazine and not captopril (not shown) or the prevention of angiotensin II (AII) formation by captopril might account

Fig. 3. A. Relative heart weights (heart weight/body weight × 100) of spontaneously hypertensive rats of the Okamoto and Aoki strain (SHR) after 0.5, 3 and 6 months daily treatment with H2O, captopril or hydralazine and in normotensive rats of the Wistar-Kyoto strain (WKY) treated with H2O for the same time period. Values shown are mean ± S.E. Numbers in parenthesis indicate number of rats per group. B. Actual body weights of same animals used in A.
for the differences in heart weights observed in this study.

The reason(s) for the difference between captopril and hydralazine on blood pressure, or for the antihypertensive effect itself of captopril, is unknown. Whereas hydralazine is a direct vasodilator (13), captopril possesses no such activity (3-4). Furthermore, captopril possesses no other apparent acute peripheral autonomic blocking or central activities which could account for its antihypertensive action (2-4). Thus, the effects of captopril are most likely related to its ability to inhibit angiotensin-I converting enzyme (ACE) (1, 4, 6). The dose of captopril used in this study was several-fold greater than that required to completely inhibit ACE in rats (4). Moreover, the marked increase in PRA values after captopril is good indirect evidence that AII levels were reduced as a result of ACE inhibition. AII inhibits renin release through a negative feedback mechanism by stimulating AII receptors, most likely on the juxtaglomerular apparatus (14). Reductions of circulating AII or AII receptor blockade cause a compensatory increase in renin release in an attempt to restore AII levels. Although some of the rise in PRA caused by captopril is probably a result of compensatory reflex sympathetic stimulation caused by the reduction in blood pressure, this probably contributed minimally since hydralazine caused only relatively small increases in PRA despite significant reductions in blood pressure.

As shown in this and other studies (15-18), PRA values of adult SHR are either no different or lower than normotensive adult rat models. Thus, it seems paradoxical that captopril should be able to normalize the blood pressure of SHR simply by inhibiting the renin-angiotensin system (RAS), especially since administration of AII receptor antagonists or AII antibodies have no effect on the blood pressure of SHR (19-20). Miyazaki and Yamamoto (21) recently demonstrated that a synthetic phosphatidylethanolamine inhibitor of renin was capable of reducing blood pressure of both renal hypertensive rats and SHR. Thus, the possibility must be considered that PRA activity per se is not a good determinant of the significance of the RAS in maintaining blood pressure.

Since ACE is probably the same as kininase II, the enzyme responsible for the degradation of bradykinin (22), it is possible that the antihypertensive actions of captopril might be a result of bradykinin potentiation. Acute intravenous administration of captopril to rabbits and rats enhances the magnitude and, in particular, the duration of the hypotensive action of bradykinin, possibly mediated through the release of prostaglandins (3-4). However, no direct evidence for any accumulation of kinin activity, either at the organ level or in plasma, has been presented to date.

Captopril has been shown to be diuretic and natriuretic in dogs maintained on a low-salt diet (23). In addition, blood pressure and plasma aldosterone levels were reduced while PRA was increased. Therefore, it is possible that captopril might have decreased blood pressure by a diuretic-natriuretic action and/or a reduction in aldosterone secretion. However, in the present study, captopril had no diuretic or natriuretic effect during the 6-month dosing period, the difference between dog and rat probably being due to the normal salt diet of the rats. Moreover, no reduction in urinary aldosterone excretion was observed in SHR after chronic captopril treatment despite marked increases in PRA. This lack of
effect of captopril on aldosterone excretion is puzzling since AII has been demonstrated to be a powerful stimulus to aldosterone secretion, and AII levels, as reflected by the high PRA values, were probably reduced in the captopril-treated rats. It is possible that aldosterone secretion in SHR is suppressed to a minimal level and no further reduction can occur after the AII levels were reduced in the captopril treated rats. An important difference between SHR and WKY has been demonstrated by Freeman et al. (18) who reported that, while the RAS may not be critically involved in the hypertensive process of SHR, the RAS system—especially aldosterone secretion—is suppressed in these animals. Also it is very possible that urinary levels of aldosterone do not reflect plasma or local organ levels. Whatever the reason for the lack of effect on aldosterone levels, it is apparent that the antihypertensive action of captopril was not the result of diuresis or naturesis caused by inhibition of aldosterone secretion.

Thus, the present study demonstrates that captopril reduces mean arterial blood pressure of SHR. The effect is cumulative with blood pressure reaching normotensive levels after 6 months of daily oral administration. The mechanism of action is probably due to ACE inhibition but the precise locus, either inhibition of the renin-angiotensin system or enhancement of the kallikrein-kinin system, remains speculative.

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