EVALUATION OF HYPOTENSIVE MECHANISMS OF CAPTOPRIL IN
ADDITION TO ITS INHIBITION OF THE CONVERTING ENZYME
IN SPONTANEOUSLY HYPERTENSIVE RATS

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In spontaneously hypertensive rats (SHR), the hypotensive effect of captopril (30 mg/kg/day p.o. for 4 days), which inhibits the converting enzyme when given orally, was significantly potentiated rather than suppressed by aprotinin (100,000 KIU/day s.c. for 7 days), but was not affected by indomethacin. These findings suggest that neither the kallikrein-kinin system nor the prostaglandin system is involved in any of the hypotensive actions of captopril in SHR other than the inhibition of the converting enzyme.

CAPTOPRIL (SQ 14,225), an orally active and potent converting enzyme (EC 3.4.15.1) inhibitor (CEI), has been demonstrated to have an excellent hypotensive effect in various animal models of hypertension as well as in human hypertension, especially in the renin-dependent type. Its hypotensive effect in spontaneously hypertensive rats (SHR) has been fully examined. Captopril in a single dose caused a significant decrease in blood pressure (BP), and its chronic administration normalized the BP in these animals. Moreover, development of a high BP in these animals was prevented by administration of captopril from before the hypertensive stage. However, in previous studies no definite renin-dependency of the elevated BP of SHR was found, judging from the plasma renin activity (PRA) and the BP response to angiotensin II antagonists. Therefore, captopril was suspected to have other hypotensive mechanisms in SHR in addition to an inhibition of the converting enzyme and its action in relation to kinin potentiation has been investigated.

In the present study we administered indomethacin and aprotinin with captopril to assess the participation of the prostaglandin system and the kallikrein-kinin system in the hypotensive mechanism of captopril in SHR.

MATERIALS AND METHODS

Male SHR were housed 2 rats in one cage in metabolic cages in a room illuminated from 6:00 to 20:00. They were given food pellets containing sodium 0.20 mEq/g and tap water ad lib. After a period of adaptation to the metabolic cages, the rats were divided into 8 weight-matched groups of 8 rats each. They were 16 weeks old and weighed 276 ± 3 g (mean ± SE) (range: 230–315 g) at the beginning of the experiment.

The experiment consisted of 3 periods: a baseline period of 3 days, a treatment period of 7 days and a recovery period of 3 days. Aprotinin and indomethacin were given throughout the
treatment period and captopril was given in the last 4 days of the treatment period (captopril period). The rats were divided into 8 groups depending on the treatments they received in the treatment period. Group 1 received indomethacin (Sumitomo Chemical Co., 2.5 mg/kg per os daily) and the vehicle of aprotinin (0.2 ml of 5% dextrose in water, s.c. twice daily) during the treatment period, and the vehicle of captopril (0.3 ml/100 g body weight of distilled water per os) in the captopril period. Group 2 was given aprotinin [50,000 kallikrein inhibitory units (KIU), s.c. twice daily] (Bayer, Japan) and the vehicle of indomethacin (0.5 ml/100 g body weight of 5% CM-cellulose sodium suspended in water per os daily) in the treatment period and the vehicle of captopril in the captopril period. Group 3 received both indomethacin and aprotinin as groups 1 and 2, respectively, and the vehicle of captopril. Group 4 received oral captopril (Sankyo Co.) 30 mg/kg per os daily in the captopril period and vehicles of indomethacin and aprotinin in the treatment period. Group 5 received indomethacin as group 1 and the vehicle of aprotinin with captopril as group 4. Group 6 received aprotinin as group 2 and the vehicle of indomethacin with captopril as group 4. Group 7 was given all these 3 drugs during the treatment periods. Group 8 as a negative control receiving only vehicles of the drugs. The drugs and their vehicles given per os were administered by gavage. The doses of captopril and indomethacin used were based on a report by Antonacchio et al., and that of aprotinin on a report by Nasletti et al.

The systolic BP was measured by tail plethysmography (USM-10SR, Ueda Kosan, Tokyo, Japan) after keeping the rats at 37°C for 10 min. The BP was determined on the last day of the baseline period, the 3rd day of the treatment period, the 2nd and 4th days of the captopril period and the 3rd day of the recovery period. The drugs were given about 2 hours before measurement of the BP during the treatment period.

Urine was collected for 24 hours before each BP measurement except on the 2nd day of the captopril period. Urinary kallikrein was measured by the method of Morita et al. with the fluorogenic peptide substrate prolylphenylalanylarginine-4-methylcoumaryl-7-amide (Pro-Phe-Arg-MCA). The assay method was as follows: 100 μl of urine and 20 μl of 10 mM Pro-Phe-Arg-MCA solution dissolved in dimethyl-formamide were added to 2.0 ml of 0.1 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl. The amount of fluorogenic amino-4-methylcoumarine (AMC) liberated was measured in a Shimazu fluorescence spectrophotometer (Model 503, Kyoto, Japan) with excitation and emission wave lengths of...
380 nm and 460 nm. The fluorescence was measured relative to that of 10 μM AMC in 0.1% dimethylsulfoxide. Thus, urinary kallikrein was expressed as nmol AMC/min.

One-way analysis of variance was used to examine the effects of the 8 treatments on the BP and urinary kallikrein excretion. The significance of differences between the means for the control and treated groups at various periods during the experiment were examined by Tukey's method.

RESULTS

Groups 1, 2 and 3 showed no appreciable difference in BP from the control group throughout the experimental period. Group 4, which received captopril alone, had a significantly lower BP than the control group on the 2nd day (143 ± 7 mmHg, p < 0.01) and the 4th day (140 ± 5 mmHg, p < 0.01) of captopril treatment, but returned to the baseline value at the end of the recovery period. Group 5 showed a similar change in BP to group 4. On the other hand, group 6 treated with aprotinin and captopril showed a decrease in BP like that in group 4 (137 ± 4 mmHg, p < 0.01) on the 2nd day of the captopril period and a further reduction in BP (119 ± 3 mmHg, p < 0.01) on the 4th day, which was significantly greater than that of group 4. In this group the BP rose to as high as 152 ± 5 mmHg at the end of the recovery period, but this value was still significantly lower than that of the control group (p < 0.05). Group 7 showed almost the same BP change as group 6 (Fig. 1).

The urinary excretions of kallikrein (U_kallV) in groups 2, 3, 6 and 7 throughout the treatment period were significantly lower than those of the control group and other groups not receiving aprotinin, and were elevated in the recovery period, although they were still significantly lower than that of the controls. Groups 1 and 5, which received indomethacin (2.5 mg/kg per os) had a significantly low U_kallV on the 3rd day of the treatment period (147 ± 26 and 137 ± 13 nmol AMC/min/day, p < 0.05 and p < 0.01, respectively). Thereafter, U_kallV in this group increased to a level that was not significantly different from that of the control group (Fig. 2).

DISCUSSION

Recently Overlack et al. found that the hypotensive effect of captopril was abolished in patients with normal or low-renin essential hypertension by infusion of aprotinin, presumably by suppression of kinin formation through inhibition of kallikrein. On the contrary, marked reduction in the BP was seen in our study after administration of captopril to animals pretreated with aprotinin, and the reduction was found to

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be significantly greater than that caused by captopril alone. No significant BP reduction was observed in group 2, treated with aprotinin alone. It is unlikely that aprotinin itself inhibited the converting enzyme, even nonspecifically. Aprotinin is a serine protease inhibitor and is unlikely to potentiate the inhibitory activity of captopril on the converting enzyme. It appears, therefore, that aprotinin may have an action through another mechanism(s) in potentiating the hypotensive effect of captopril when given concomitantly with the latter. Therefore, two hypotheses may be given to explain this apparently paradoxical phenomenon. 1) The elevation of PRA in response to captopril prevents further reduction in the BP due to massive accumulation of angiotensin I (AI), a small part of which may be converted to angiotensin II (AII). This may result in maintenance of equilibrium in BP. 2) Renal kallikrein is believed to contribute to the activation and release of renin from the kidney. Our results might be interpreted as follows: suppression of kallikrein by aprotinin may have abolished, or at least diminished, the increase in activation and release of renin in response to captopril. This might have contributed to further reduction of the BP. The results of Thurston and Swales\(^6\) seem to the first hypothesis: they observed that teprotide (SQ 20,881), a nonapeptide CEI from snake venom, had a marked hypotensive effect in anesthetized, sodium depleted normotensive rats pretreated by continuous infusion of saralasin; the reduction in the BP was much more than that by teprotide alone. On reversing the order of administration of drugs, i.e., saralasin before teprotide, no further change in BP was observed after infusion of saralasin. A similar phenomenon was reported by Marx et al., who administered captopril intravenously to anesthetized normotensive rats pretreated with saralasin.\(^7\) The same phenomenon may have been observed in our study under different conditions: aprotinin may have blocked the responsive renin increase mediated by kallikrein, whereas saralasin may have blocked the action of the small amount of AII generated by massive accumulation of AI. Thus, both drugs may have augmented the action of CEI. However, we believed that this difference was related to bradykinin potentiation by CEI. Our hypothesis is supported by a report that captopril is ineffective in nullifying the plasma AII level in rats.\(^8\)

The following findings may support our second hypothesis: Suzuki et al. found that urinary kallikrein of rats stimulated the release and activation of renin from renal cortical slices in rats, and that this release was abolished by aprotinin.\(^9\) Lijnen et al. reported that captopril caused significant increases in PRA and the total and the active plasma renin concentration without any change in the inactive plasma renin concentration in hypertensive patients.\(^10\) We may infer from these two reports that the increases in activation and release of renin from the kidney in response to captopril administration are mediated by a renal kallikrein system. It may be argued that the significantly low urinary kallikrein excretion may be due to an apparent suppression of urinary kallikrein activity by aprotinin, which is excreted in the urine, and that urinary kallikrein excretion cannot be used as an indicator of the activity of the renal kallikrein-kinin system when aprotinin is given. However, Trautschold et al.\(^11\) demonstrated that aprotinin is fixed in the kidney without losing its inhibitory activity and is excreted exclusively via the kidney after being metabolized to an inactive form, and that only a small part of the inhibitor is excreted in a biologically active form over several days. Furthermore, Nasjletti et al.\(^3\) observed that urinary excretion of PGE, a mediator of the action of the renal kallikrein-kinin system, decreased in rats as the renal and urinary kallikrein levels were lowered by aprotinin, suggesting that the renal kallikrein-kinin system was suppressed indirectly by administration of aprotinin. Therefore, it appears that the present study the renal kallikrein-kinin system was suppressed sufficiently, though it is still possible that aprotinin excreted in the urine may have suppressed urinary kallikrein activity because the dose of the inhibitor was very large.

However, Antonaccio et al. showed that during captopril therapy of SHR, propranolol had no significant effect on the reduction in BP, although it significantly decreased the responsive elevation of PRA, suggesting that the high PRA and AI level did not participate in maintenance of the BP after captopril administration? They claimed that the increase in PRA caused by captopril in SHR was probably mediated reflexly by compensatory sympathetic activation. The validity of our hypothesis should be tested by determination of PRA during administration of captopril with and without aprotinin. The urinary kallikrein excretions in groups 2, 3, 6 and 7,

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which were given aprotinin, remained significantly lower than that of the control in the recovery period. This may be due to the presence of aprotinin fixed in the kidney without being metabolized to an inactive form. This, in turn, may have resulted in delayed recovery of the BP in groups 6 and 7 given aprotinin with captopril, which must have remained in the body because it was given in a large dose.

As Antonaccio et al.2 have reported, we found that addition of indomethacin to captopril had no detectable effect on the BP change caused by captopril. PGE1 is known to increase urinary kallikrein excretion when injected into the renal artery of dogs12 and indomethacin decreased urinary kallikrein excretion when given to patients with Bartter's syndrome13. Thus, the renal kallikrein-kinin and prostaglandin systems are closely related and the one can stimulate the other. Indomethacin may have suppressed urinary kallikrein excretion in this way, but its effect appears to have been transient.

Thus, in this work we could not demonstrate the participation of either the kallikrein-kinin system or the prostaglandin system in the hypotensive mechanism of captopril in SHR.

REFERENCES

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