Role of Mineralocorticoid in the Chronic Antihypertensive Effect of Converting Enzyme Inhibitor

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SUMMARY The chronic antihypertensive effect of converting enzyme inhibitor (CEI) may be due to a decrease in aldosterone secretion secondary to blockade of angiotensin II formation. To study this hypothesis, changes in blood pressure (BP), in response to chronic administration of the CEI, captopril, were measured in spontaneously hypertensive (SHR) and in chronic two-kidney, one clip hypertensive (2K-1C) rats. To avoid a decrease in mineralocorticoid activity, half of the rats in these two models of hypertension were adrenalec-tomized and maintained with daily administrations of deoxy cortisol acetate and hydrocortisone (steroid replacement) while the other half had the adrenal gland left in situ and no exogenous steroids administered. The doses of steroids used were devoid of hypertensive effect in Wistar Kyoto (WKY), SHR, 2K-1C, and sham-clipped rats. Chronic administration of the CEI decreased the BP to normotensive levels in the SHR with intact adrenals and no steroid replacement. However, the antihypertensive effect of the CEI was almost completely blocked in those SHR with steroid replacement. In contrast, the antihypertensive effect of the CEI in 2K-1C was similar in the rats with steroid replacement and in the rats with intact adrenals (no steroid replacement). These results suggest that the chronic antihypertensive effect of CEI in SHR is due partially to a decrease in aldosterone activity secondary to the blockade of angiotensin II formation, whereas in 2K-1C it is due to mechanisms other than lower mineralocorticoid activity. (Hypertension 3: 205-210, 1981)

KEY WORDS • aldosterone • angiotensin • systolic blood pressure • plasma renin activity • SHR • two-kidney, one clip hypertension • kinin

THE orally active angiotensin converting enzyme inhibitor (CEI), captopril, has been found to decrease the blood pressure (BP) in human beings1,2 and in different models of experimental hypertension3,4 with either normal or high plasma renin activity (PRA). A significant correlation has been reported between the decrease in BP and the decrease in plasma aldosterone concentration in response to chronic administration of CEI.5,6 Thus, it is possible that part of the antihypertensive effect of the CEI is due to a chronic decrease in mineralocorticoid secretion secondary to the blockade of angiotensin II formation. To study this hypothesis, the antihypertensive effect of CEI was examined in spontaneously hypertensive rats (SHR) and two-kidney, one-clip renovascular hypertensive rats (2K-1C). To avoid a decrease in mineralocorticoid activity, half of the rats in these two models of hypertension were adrenalec-
tomized and maintained on a daily dose of steroid replacement. In the remaining half, the adrenals were left in situ and no steroid replacement was given. It was postulated that, if a decrease in aldosterone activity is partially responsible for the chronic antihypertensive effect of the CEI, then the decrease in BP in the rats with steroid replacement should be less than in those rats with intact adrenals in which mineralocorticoid secretion is allowed to decrease during treatment with the converting enzyme inhibitor. The doses of steroid replacement used were devoid of hypertensive effect in adrenalectomized SHR and 2K-1C and in Wistar Kyoto (WKY) and Sprague-Dawley control rats.

The SHR and 2K-1C models were selected for this study because, in the SHR, angiotensin II is not generally implicated in the pathogenesis of hypertension7 whereas it is in the 2K-1C model. 8 Evaluation of the effect of CEI in these two models of hypertension, with and without steroid replacement, should therefore help to elucidate the antihypertensive mechanism of this drug.

Methods

Male SHR and WKY 10-12 weeks of age were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. Two-kidney, one clip hypertension was induced in male Sprague-Dawley rats...
rats weighing 150–180 g by placing a U-shaped silver clip with an internal gap of 0.2 mm around the left renal artery. The contralateral kidney was left untouched. Control rats were sham-clipped. The studies with the CEI were performed 10 weeks after clipping or sham-clipping. Adrenalectomy was performed through a flank incision. All surgical procedures were performed under ether anesthesia. All rats were placed in separate cages and had free access to rat chow containing 0.4% sodium chloride (Ralston Purina Company) and tap water, unless otherwise specified.

Systolic BP was measured three times a week by tail-cuff method, 2 hours after administration of either the CEI or 0.9% NaCl (vehicle). Control BP measurements were conducted in all rats for 1 week before administration of the CEI or its vehicle.

Experimental Protocols

Protocol 1

After a control period of 7 days, 10 SHR and nine 2K-1C were adrenalectomized, and endogenous steroids were replaced by daily subcutaneous injections of 0.2 mg of DOCA and 1.0 mg of hydrocortisone in 0.2 ml of cottonseed oil. Converting enzyme inhibitor was administered by gavage at a dosage of 50 mg/kg twice a day. The CEI was dissolved in 1 ml of 0.9% NaCl.

Protocol 2

Ten SHR and nine 2K-1C were used for this protocol, which was similar to Protocol 1 except that these rats were sham-adrenalectomized and instead of steroids received a daily subcutaneous injection of 0.2 ml cottonseed oil from the day of sham operation.

Protocol 3

Nine SHR, eight WKY, nine 2K-1C, and seven sham-clipped rats were used in this protocol, which was similar to Protocol 1, except that these rats were sham-adrenalectomized and instead of steroids received a daily subcutaneous injection of 0.2 ml cottonseed oil from the day of sham operation.

Protocol 4

Ten SHR, nine WKY, nine 2K-1C, and seven sham-clipped rats were used in this protocol, which was similar to Protocol 2 with the exception that the rats received 1 ml of saline twice a day.

Results

The effect of the CEI treatment on the BP of the SHR with and without steroid replacement is shown in figure 1. The average BP of the sham-adrenalectomized SHR was 160 ± 2 mm Hg prior to treatment with the CEI and decreased to 130 ± 2 mm Hg on the fourth day after the treatment was initiated. This decrease was maintained through the 20th day (137 ± 2 mm Hg) and was highly significant (p < 0.001) when compared to the initial BP. In the SHR with steroid replacement, however, the BPs on the fourth and 20th days of treatment with the CEI were 150 ± 5 and 165 ± 3 mm Hg, respectively, not significantly different from those prior to the treatment (157 ± 3 mm Hg). Withdrawal of hydrocortisone on the 13th day of treatment or withdrawal of DOCA (replaced with 1% saline in the drinking water) on the 25th day had no effect on BP.

The BP of the SHR with and without steroid replacement, but without CEI treatment, is shown in figure 2. No significant differences were found in the BP of these two groups. The average BP of the SHR with and without steroid replacement gradually in-
creased, reaching 179 ± 3 and 172 ± 3 mm Hg respectively on the 20th day of the experiment (fig. 2). The BPs of these two groups were significantly higher than the BP of the corresponding groups treated with the CEI (compare BP measurements in figs. 1 and 2). The withdrawal of hydrocortisone on the 13th day of treatment and the replacement of DOCA with 1% saline as drinking fluid on the 25th day had no effect on the BP of the adrenalectomized SHR. The BPs of the WKY with and without steroid replacement were similar and did not change significantly throughout the experiment (fig. 2).

The PRA of the SHR and the WKY on the 21st day of the experiment is shown in figure 3.

The effect of CEI treatment on the BP of the hypertensive 2K-1C rats with and without steroid replacement is shown in figure 4. In both groups of rats, the BP after CEI treatment decreased significantly (p < 0.001) from 193 ± 13 and 196 ± 15 mm Hg to 142 ± 5 and 135 ± 6 mm Hg respectively. At no time during the experimental period did the BPs of these two groups differ significantly from each other.

The BP of the 2K-1C with and without steroid replacement, but without CEI treatment, is shown in figure 5. No significant differences were found in the BP of these two groups. The BP of the two groups without CEI treatment was significantly higher (p < 0.001) than the BP of the two groups treated with CEI (compare BP measurements in figs. 4 and 5). The BP of sham-clipped rats with and without steroid replacement remained in the normotensive range and did not differ significantly from each other (fig. 5).

**Figure 2.** Blood pressure in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) with (open circles) and without (black circles) steroid replacement (hydrocortisone and DOCA). Negative numbers on the abscissa indicate control periods. During experimental periods, the rats received only the vehicle used for administering the CEI. Hydrocortisone and DOCA administration were stopped on Days 13 and 25 respectively. DOCA was replaced by 1% saline as drinking fluid. Vertical bars indicate SE, numbers in parenthesis indicate number of rats.

**Figure 3.** Plasma renin activity in spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY). CEI indicates rats treated with the CEI, captopril. Roman numerals indicate protocol, for details see text. Numbers in parenthesis indicate number of rats. Statistical significance between the different groups was: SHR = I vs II, p < 0.001, I vs III, p > 0.05, II vs IV, p < 0.001, III vs IV, p < 0.001. WKY = III vs IV, p > 0.05.

**Figure 4.** Effect of CEI on the blood pressure (BP) of two-kidney, one clip hypertensive rats with (open circles) and without (closed circles) steroid replacement (hydrocortisone and DOCA). Negative numbers on the abscissa indicate control periods. Vertical bars indicate SE, numbers in parenthesis indicate number of rats.
The PRA of the 2K-1C hypertensive and sham-clipped control rats is shown in figure 6.

Discussion
The antihypertensive effect of chronic treatment with CEI was compared in nonadrenalectomized hypertensive rats receiving no exogenous steroids and in adrenalectomized hypertensive rats in which a decrease in mineralocorticoid activity was avoided by maintenance on fixed daily doses of DOCA and hydrocortisone. Two experimental models of hypertension, in which renin plays different pathogenetic roles, were selected for this study. In the SHR, it has been reported that renin does not play an important role during the development and maintenance of hypertension, while in the 2K-1C, renin is accepted as being an important pathogenetic factor. As previously reported, the BP of intact SHR and 2K-1C was reduced to or near normotensive levels by chronic treatment with converting enzyme inhibitor. In the hypertensive rats receiving exogenous steroids and CEI, two different patterns in the BP response were observed. In the SHR, the antihypertensive effect of the CEI was markedly lessened, whereas it was virtually unaffected in the 2K-1C model. This suggests that a decrease in mineralocorticoid activity is important in the antihypertensive effect of the CEI in the SHR, but not in the 2K-1C, model.

Although in this study we did not measure sodium balance, it has been previously reported that CEI not only decreases the plasma concentration of angiotensin II and aldosterone, but also produces an increase in natriuresis and diuresis. McCaa et al. have proposed that part of the antihypertensive effect of the CEI is due to natriuresis secondary to the decrease in aldosterone secretion. This hypothesis is also supported partially by the lack of an antihypertensive effect of the CEI in the bilaterally nephrectomized spontaneously hypertensive rats. In the SHR, replacement of DOCA with 1% saline as drinking fluid was also effective in decreasing the antihypertensive effect of the CEI. This suggests that both DOCA and high sodium intake may have impaired a negative sodium balance secondary to the blockade of angiotensin II formation and to the low aldosterone secretion. The blockade of angiotensin II formation followed by a decrease in mineralocorticoid activity and natriuresis and diuresis may be important in mediating the chronic antihypertensive effect of the CEI in this model of hypertension.

An excess of glucocorticoids and mineralocorticoids is known to produce hypertension, and accordingly, it could be argued that the failure of the CEI to decrease the BP was not due to a replacement of the endogenous steroid activity, but was possibly due to a hypertensive effect produced by the administration of
an excessive amount of steroids. Yet, removal of hydrocortisone on the 13th day and replacement of DOCA with 0.9% NaCl as drinking fluid on the 25th day did not result in a decrease in BP. It could be argued further that these rats had already developed metacorticotoid hypertension; however, this was not the case, since the BP of the adrenalectomized SHR and WKY rats receiving the same doses of steroids and no CEI were no different than the BP of the non-adrenalectomized SHR and WKY rats receiving no steroid and no CEI, respectively (fig. 2). Therefore, the dose of steroids used did not have any hypertensive effects on the rats receiving no converting enzyme inhibitor.

In the 2K–1C model, the effect of steroid replacement was different than in the SHR model. In this model of renovascular hypertension, chronic administration of the CEI resulted in an antihypertensive effect of similar magnitude, independent of steroid replacement. These data suggest that the antihypertensive effect of the CEI in the 2K–1C hypertensive model is caused by a mechanism(s) other than a decrease in aldosterone secretion. The BP of the 2K–1C and sham-clipped groups receiving steroids and no CEI were similar to the corresponding untreated nonadrenalectomized control groups (fig. 5). Again, this suggests that the dose of steroids used had no hypertensive effect. Furthermore, these data indicate that the dose of steroids used was sufficient to maintain the BP of the adrenalectomized rats at the same level as in the nonadrenalectomized rats. This also indicates that these rats were not in overt adrenal insufficiency, which could have produced a decrease in the BP independent of CEI administration.

As expected, blocking the formation of angiotensin II with the CEI resulted in an increase in the PRA in both the SHR and the 2K–1C hypertensive rats (figs. 3 and 6). This PRA increase was probably due to blockade of angiotensin II-renin negative feedback. The effect of DOCA on the PRA was dissimilar in the different groups of rats. In the SHR, DOCA suppressed the PRA even when they were receiving the CEI, while in the WKY receiving DOCA, the PRA was similar to that of those WKY receiving no steroids (fig. 3). These results suggest that the mechanism regulating renin release in the SHR is more sensitive to the suppressor effect of mineralocorticoid than that of the Wistar-Kyoto rats.

In previous studies with 40-week-old SHR, treatment with DOCA resulted in an incomplete suppression of PRA. However, it appears that suppression of the PRA, at least in the SHR, is age-dependent, since the younger rats are more sensitive to the suppressor effect of DOCA than the older rats.

In the 2K–1C treated with the CEI, administration of DOCA did not result in suppression of the PRA, which remained very high. DOCA treatment in the 2K–1C and in the normotensive sham-clipped control rats receiving no CEI resulted in a suppression of the PRA, which was statistically significant in only the sham-clipped control rats (fig. 6). This suggests that, after constriction of one renal artery, renin release becomes less sensitive to the suppressor effect of mineralocorticoids. Similarly, incomplete suppression of PRA with DOCA or with high sodium intake has been observed in patients with either malignant or renovascular hypertension and in dogs with renovascular hypertension. The mechanism by which DOCA suppresses the PRA was not studied; however, it is probably secondary to sodium retention and volume expansion.

The mechanisms by which the CEI decreases BP are still largely unsettled. Our experiments indicate that only part of the antihypertensive action of captopril in the SHR could be due to lower mineralocorticoid activity. The BP of the adrenalectomized rats treated with steroids and CEI was still significantly lower than the BP of either the SHR with or without steroid replacement but receiving no converting enzyme inhibitor. Our studies do not clarify the nature of this non-mineralocorticoid-dependent mechanism by which the CEI decreases BP. Nevertheless, it is recognized that the mechanisms of action of the CEI may be of a remarkable complexity, since converting enzyme not only converts angiotensin I to angiotensin II, a potent vasoconstrictor, but also degrades bradykinin, a potent endogenous vasodilator. Thus, there is a simultaneous drop in plasma angiotensin II and an increase in plasma bradykinin concentration following the administration of the CEI, captopril and teprotide. A decrease in angiotensin II concentration could cause a decrease in the BP, not only by directly decreasing vasoconstrictor activity, but also through indirect mechanisms, since it is known that angiotensin II could influence the BP by peripheral or central increase of sympathetic activity, by stimulation of ACTH and vasopressin release and by its direct and aldosterone-mediated antinatriuretic effects, among others.

The role of kinins in the chronic antihypertensive effect of CEI is controversial. An increase in the concentration of the peptides could decrease the BP because of their potent vasodilator activity and also because of their effect on the prostaglandin system and its natriuretic and diuretic effects. That an increase in kinins may play a role in mediating part of the antihypertensive effect of CEI has been suggested in a recent report, in which CEI (captopril) induced a marked decrease in the BP of DOCA-salt hypertensive rats which had suppressed PRA and are known to have an increased excretion of urinary kallikrein. Furthermore, we have recently shown that treatment with kinin antibodies blocked part of the antihypertensive effect of the CEI (captopril) in SHR, and in 2K–1C chronic hypertensive rats, while it did not affect the hypotensive response observed in sodium-depleted rats.

The data from the present study and those of others seem to indicate that the mechanism by which CEI decreases the BP is not uniform. The mechanisms that lower the BP during CEI administration are probably quantitatively, and may be qualitatively, different in distinct experimental and clinical situations. This would explain some of the contradictory results found.
in the literature regarding the mechanisms of the action of the converting enzyme inhibitor.

In conclusion, in the SHR, in which variations in mineralocorticoid activity are avoided by adrenalectomy and by giving a fixed daily dose of steroids, the antihypertensive action of the CEI, captopril, was partially blocked. In the 2K-1C rats, the same doses of steroids did not affect the antihypertensive effect of captopril. It is proposed that, in the SHR, a decrease of aldosterone secretion secondary to blockade of aldosterone secretion, potassium retention, and natriuresis Hypertension 1: 274, 1979

Perhaps in this model, the chronic antihypertensive effect of the CEI, captopril, could be due mainly to the absence of the vasoconstrictor effect of angiotensin II.

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