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

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 adrenalectomized and maintained with daily administrations of deoxycorticosterone 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

From the Hypertension Research Laboratory, Department of Medicine, Henry Ford Hospital, Detroit, Michigan. This work was done during Dr. Carretero's tenure as an Established Investigator of the American Heart Association. Supported in part by National Institutes of Health Grants HL 21092 and HL 15839.

Address for reprints Oscar A. Carretero, M.D., Hypertension Research Laboratory, Henry Ford Hospital, 2799 W Grand Boulevard, Detroit, Michigan 48202.

Received March 11, 1980; revision accepted August 13, 1980.
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, but instead of CEI they received 1 ml of saline twice a day.

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 instead of the converting enzyme inhibitor.

Blood was drawn from all rats for PRA determinations after 3 weeks of administration of the CEI or its vehicle. For this, an arterial catheter made from PE 10 tubing was chronically implanted into the abdominal aorta through the femoral artery. The catheters were exteriorized on the back of the neck and filled with heparinized solution (100 U/ml). Forty-eight hours later, blood (0.7 ml) from conscious rats was drawn into a tube containing 0.01 ml of 3.8% Na₂EDTA.

The PRA was determined by using a modification of the radioimmunoassay method of Haber et al.,* as previously described; the main modification consisted of adjusting the pH of the plasma to 7.4 with 4 M Tris-HCl buffer, pH 7.3. The PRA was expressed as nanograms (ng) of angiotensin I generated per milliliter (ml) of plasma per hour of incubation.

The BP among the different groups was compared by the two-way analysis of variance and the Hotelling's $t$ square. A $p > 0.05$ was considered not significant. All data are expressed as mean ± SE.

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).
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,\textsuperscript{7,11} while in the 2K-1C, renin is accepted as being an important pathogenetic factor.\textsuperscript{11,13} As previously reported,\textsuperscript{18} 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.\textsuperscript{1,3,6} McCaa et al.\textsuperscript{19} 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.\textsuperscript{10} 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\textsuperscript{21},\textsuperscript{22} 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 metacorticoid 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 those 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. 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 endothelial 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 qualitatively, 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 were 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 mineralocorticoid activity were 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. In the 2K–1C, the action of captopril does not appear to be related to the suppression of aldosterone secretion. 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.

References

21. Ingle DJ, Young S. Effect of cortisone acetate and of certain stressors on blood pressure and on pathology of heart and kidney in uninephrectomized male rats Endocrinology 70: 806, 1962
24. Bagby SP, McDonald WJ, Mass RD. Serial renin-angiotensin studies in spontaneous hypertensive and Wistar-Kyoto normotensive rats. Transition from normal to high-renin status during the established phase of spontaneous hypertension Hypertension 1: 347, 1979
35. Carretero OA, Scollo AG, Maitra SR. Role of kinins in the pharmacological effects of converting enzyme inhibitors. In Angiotensin Converting Enzyme Inhibitors: Mechanisms of Actions and Clinical Implications Urban and Schwarzenberg, in press
Role of mineralocorticoid in the chronic antihypertensive effect of converting enzyme inhibitor.
S R Maitra, A G Scicli, S Miyazaki and O A Carretero

doi: 10.1161/01.HYP.3.2.205

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/3/2/205

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/