Preventive Effect of Angiotensin I on Weight Reduction in the Adrenal Glands of DOCA/salt Hypertensive Rats

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Abstract

We examined the effect of angiotensin I (AI), without the effect of angiotensin II (AII) converted from AI, on the weight of the adrenal glands, adrenal corticosterone (B) and adrenal aldosterone under conditions where the renin-angiotensin system was suppressed, since a reduction in the size of the adrenal glands is often observed in DOCA/salt hypertensive rats. Sixty male Wistar rats fed on a 1% NaCl solution were divided into 6 groups as follows: a) Salt group: received sesame oil and vehicle, b) Salt+C group: received sesame oil and MK422 (0.14 mg/day), an angiotensin converting enzyme inhibitor (CEI), c) DOCA group: received DOCA (30 mg/week) and vehicle, d) DOCA+A group: received DOCA and AI (0.5 mg/kg/day), e) DOCA+A+C group: received DOCA and AI with MK422, and f) DOCA+C group: received DOCA and MK422. After 4 weeks, the rats were sacrificed to sample their blood and remove their adrenal glands. There was no significant difference in adrenal B among the groups apart from the DOCA+C group. Adrenal aldosterone was lower in the groups of DOCA/salt hypertensive rats than in the Salt group and Salt+C group. Furthermore, the DOCA+A+C group and DOCA+C group had lower adrenal aldosterone levels than the DOCA group and DOCA+A group. On the other hand, adrenal gland weight in the DOCA+A+C group was similar to that in the Salt group and Salt+C group, although it was lower in the DOCA group and DOCA+C group than in the Salt group, Salt+C group and DOCA+A+C group. These findings indicate that AI can prevent weight reduction in the adrenal glands independently of AII in DOCA/salt hypertensive rats.

We have often observed a reduction in the size of the adrenal glands in DOCA/salt hypertensive rats. It has been reported that the renin-angiotensin system is suppressed in DOCA/salt hypertensive rats (Campbell and Penttinger, 1975), and that angiotensin II (AII) is a potent stimulator of adrenal corticoid synthesis (Brown et al., 1972; Ganguly et al., 1977; Mason et al., 1977). The suppression of AII may therefore tend to inhibit the synthesis of cor-
ticoids in such rats. However, the mechanisms involved in the reduction in size of the adrenal glands in DOCA/salt hypertensive rats remain obscure. The present study was undertaken to investigate the possible role of angiotensin I (AI) in the reduction in weight of the adrenal glands in DOCA/salt hypertensive rats.

Materials and Methods

Male Wistar rats, at 10 weeks of age, were allocated to 6 groups of 10 rats each, as follows: a) Salt group: received sesame oil and vehicle administration, b) Salt+C group: received administration of sesame oil and MK422 (0.14 mg/day), an angiotensin converting enzyme inhibitor (CEI), c) DOCA group: received DOCA and vehicle administration, d) DOCA+A group: received DOCA and AI (0.5 mg/kg/day) administration, e) DOCA+A+C group: received DOCA and AI with MK422 administration, and f) DOCA+C group: received DOCA and MK422 administration. The rats were fed on a regular chow diet and 1% NaCl solution. The DOCA dissolved in sesame oil (75 mg/ml) and the same dose of sesame oil (0.2 ml) were injected subcutaneously twice a week. MK422 and AI were dissolved in 2 ml of the vehicle, saline. These drugs and the same dose of vehicle were administered intra-peritoneally with an osmotic pump. The implantation of the pump was performed on the day before commencing administration of DOCA and sesame oil under pentobarbital anesthesia (100 mg/kg). After 4 weeks, the rats were sacrificed by decapitation to sample their blood and remove their adrenal glands. Blood samples in which to measure the plasma renin activity (PRA) and the plasma AI concentration were collected into tubes containing 5 mg of EGTA. The adrenal glands were removed after being flushed free of blood with saline via the aorta. They were carefully separated from the fat tissue, homogenized in 1 ml of saline at 4°C and centrifuged at 1800 g for 20 min. The supernatant was collected to measure the adrenal corticosterone (B) and aldosterone content. The B and aldosterone in the supernatant were estimated directly by a radioimmunoassay method (Honda et al., 1977). The PRA and plasma AI concentration were measured by radioimmunoassay with a commercial assay kit (CEA-IRE-SORIN, France). Blood pressure was determined by the tail-cuff method.

MK422 was obtained from Banyu Co. Ltd., Japan. AI was purchased from the Peptide Institute, Inc., Japan. The osmotic pump was purchased from Alza Co., USA.

All results in the present study are expressed as the mean±S. E. Values for the different groups were analyzed by one way analysis of variance followed by Student’s unpaired t-test. P values less than 0.05 were considered significant.

Results

1. Comparison of body weight (B.W.) (Table 1)

There was no significant difference in B. W. in any of the groups before administration of the drugs. Four weeks after administration, the B. W. in the Salt+C group was higher than that in other groups.

2. Comparison of blood pressure (B. P.) (Table 1)

There was no significant difference in B. P. in any of the groups before administration of the drugs. Four weeks after administration, the B. P. in the 4 groups of DOCA/salt hypertensive rats was significantly higher than that in the Salt group and Salt+C group.

3. Comparison of weight of adrenal glands (Fig. 1)

The weight of the adrenal glands was expressed as the weight (mg) per g B. W. No significant difference between adrenal weight in the Salt group (0.137±0.005 mg/g) and the Salt+C group (0.138±0.007) was observed. The adrenal gland weight was significantly (p<0.05) lower in the DOCA group (0.118±0.005 mg/g) than in the Salt group and Salt+C group. The weight was also significantly (p<0.01) lower in the DOCA+C group (0.111±0.006 mg/
g) than in the Salt group and Salt+C group. In contrast to these findings, the weight of the adrenal glands in the DOCA + A+C group (0.143±0.07 mg/g) was similar to that in the Salt group and Salt + C group, and was significantly (p<0.01) higher than in the DOCA group and DOCA + C group. No significant difference between adrenal gland weight in DOCA+A group and other groups was observed.

4. Comparison of adrenal corticosterone (B) (Fig. 1)

Adrenal B was significantly (p<0.05) lower in the DOCA+C group (1570±252 ng/mg protein) than in the other groups (Salt group: 3719±526, Salt+C group: 3241±321, DOCA group: 3141±316, DOCA+A group: 2681±272, and DOCA +A+C group: 2811±145 ng/mg protein). There were no significant differences among the groups apart from the DOCA+C group.

5. Comparison of adrenal aldosterone (Fig. 1)

Adrenal aldosterone was lower in the Salt+C group (701±101 pg/mg protein) than in the Salt group (1031±192), but the difference was not statistically significant. The level was significantly (p<0.01) lower in the DOCA group (403±63 pg/mg protein) than in the Salt group and Salt+C group. Adrenal aldosterone was also significantly (p<0.05) lower in the DOCA+A group (436±126 pg/mg protein) than in the Salt group, but no statistically significant difference was observed between the DOCA+A group and Salt+C group. The DOCA+A+C group showed a greater decrease in adrenal aldosterone (231±15 pg/mg protein) than the Salt group (p<0.01), Salt+C group (p<0.01), and the DOCA group (p<0.05), but there was no significant difference between the DOCA+A group and DOCA+A+C group. The lowest adrenal aldosterone level (134±29 pg/mg protein) was observed in the DOCA +C group (vs. Salt group: p<0.01, vs. Salt+C group: p<0.01, vs. DOCA group: p<0.01, vs. DOCA+A group: p<0.05, and vs. DOCA+A+C group: p<0.05).

6. Comparison of plasma renin activity (PRA) (Fig. 2)

PRA was higher in the Salt+C group (5.45±1.66 ng/ml/hr) than in the Salt group (4.27±1.41), but the difference was not statistically significant. The PRA in both

Table 1. Comparison of body weight and blood pressure before and after drug administration in various groups of rats

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<th>DOCA+A</th>
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Salt: received vehicle (n=10), Salt+C: received MK422 (n=8), DOCA: received DOCA and vehicle (n=9), DOCA+A: received DOCA and A1 (n=9), DOCA+A+C: received DOCA and A1 with MK422 (n=10), and DOCA+C: received DOCA and MK422 (n=10). Each group was fed on 1% NaCl solution. *: p<0.01 vs. Salt group, †: p<0.05, ††: p<0.01 vs. Salt+C group, and #: p<0.01 vs. DOCA group.
the Salt group and Salt+C group was less than one half of that in Wistar rats in our previous reports (13.7±0.6 ng/ml/hr: Honda et al., 1988; 11.4±1.8: Izumi et al., 1988). The PRA was significantly (p<0.01) suppressed in the groups of DOCA/salt hypertensive rats as compared to the Salt group and Salt+C group. There were no significant differences among these groups of rats.

Fig. 1. Comparison of adrenal corticosterone, adrenal aldosterone and weight of adrenal glands in various groups of rats. Salt: received vehicle (n=10), Salt+C: received MK422 (n=8), DOCA: received DOCA and vehicle (n=9), DOCA+A: received DOCA and Al(n=9), DOCA+A+C: received DOCA and AI with MK422 (n=10), and DOCA+C: received DOCA and MK422 (n=10). Each group was fed on 1% NaCl solution. Each bar indicates the mean±S.E. **: p<0.01, +: p<0.05, ++: p<0.01 vs. Salt group; and #: p<0.05, #: p<0.01 vs. Salt+C group.
7. **Comparison of plasma angiotensin I (AI) (Fig. 2)**

Plasma AI was higher in the Salt+C group (1277 ± 605 pg/ml) than in the Salt group (636 ± 227), but the difference was not statistically significant. The level was significantly (p<0.05) suppressed in the groups of DOCA/salt hypertensive rats as compared to the Salt group and Salt+C group. The plasma AI was significantly (p<0.05) higher in the DOCA+A+C group (92 ± 21 pg/ml) than in the DOCA+A group (44 ± 4). Values for the DOCA group and DOCA+C group were not detected.

**Discussion**

The present findings indicated that intraperitoneal AI administration prevented reduction in the weight of the adrenal glands in DOCA/salt hypertensive rats. Braley et al. (1981), Douglas et al. (1979) and Hepp et al. (1977) have reported that AI can stimulate aldosterone secretion from isolated rat glomerulosa and bovine fasciculata cells, while Mendelsohn and Kachel (1980) have reported that the stimulatory action of AI on aldosterone and B depended on conversion in vitro to AII, and that AII has only low intrinsic activity in corticoid production.
In the present study, AI administration in vivo did not stimulate adrenal aldosterone in DOCA/salt hypertensive rats in which the renin-angiotensin system was suppressed. One explanation for this could be that the dose of AI administered was so small that AI was unable to stimulate adrenal aldosterone production, since the plasma AI concentrations in the DOCA/salt hypertensive rats were lower than $10^{-10}$ M in this study. It has been reported that the threshold for the stimulation of aldosterone and B by AI in vitro is $10^{-10}$ M (Braley et al., 1981; Douglas et al., 1979; Mendelsohn and Kachel, 1980). However, it remains uncertain whether the stimulatory action of AI on steroidogenesis is dependent on conversion to AII or not. Although no significant difference was observed in the PRA and plasma AI in the Salt group and Salt+C group, MK422 was considered to inhibit the conversion from AI to AII, since adrenal aldosterone was significantly reduced by MK422 with and without AI administration in DOCA/salt hypertensive rats (DOCA+A+C group and DOCA+C group). Shier et al. (1989) have reported that the CEI, lisinopril, can reduce the production of aldosterone by adrenal cell cultures by inhibiting adrenal AII. We suspected that chronic feeding on a 1% NaCl solution might have suppressed renin production in both the Salt group and Salt+C group, so accounting for the disappearance of a significant difference between these groups. The observation that no significant difference in adrenal B occurred except in the DOCA+C group suggests that adrenocorticotropic hormone (ACTH) may not be a factor contributing to the reduction in adrenal aldosterone and the weight of the adrenal glands in DOCA/salt hypertensive rats. Eguchi et al. (1980) found that the CEI, captopril, did not alter the effect of ACTH on the control of aldosterone. Adrenal aldosterone was therefore considered to depend on the plasma and/or adrenal AII in the present study. It is difficult to explain why a decrease in adrenal B occurred in the DOCA+C group. Angeli et al. (1981) reported that the CEI, captopril, did not interfere with ACTH and cortisol in human subjects with hypertension. On the other hand, Petkova et al. (1986) found that captopril caused a significant decrease in adrenal B in old spontaneously hypertensive rats (SHR) at 8 months of age, while no decrease was observed in young SHR at 1 month of age. However, they did not discuss the underlying mechanisms involved. The above experimental report did not match with our study in terms of the age and kind of animal used. We have previously reported that chronic MK422 administration did not alter the level of plasma B in Wistar rats (Fukuda et al., 1987). We suspected therefore that adrenal B could have been reduced when AII was suppressed to below some particular concentration, since plasma and/or adrenal AII was considered to be suppressed more strongly in the DOCA+C group than in the other groups. In contrast to the reduction in adrenal aldosterone, AI administration attenuated the reduction in weight of the adrenal glands in DOCA/salt hypertensive rats (DOCA+A group). Furthermore, AI with MK422 administration in DOCA/salt hypertensive rats (DOCA+A+C group) gave a similar adrenal gland weight to that in the Salt group and Salt+C group. If AII contributed to the prevention of a reduction in the weight of the adrenal glands, the weight of the adrenal glands in the DOCA+A group could have been higher than that in the DOCA+A+C group in which the plasma AI level was significantly higher than in the other groups of DOCA/salt hypertensive rats. We did not examine the effect of AI on the adrenal medulla, or pathological changes in the adrenal glands following the administration of the drugs to DOCA/salt hypertensive rats. However, it seems unlikely that the...
adrenal medulla was suppressed by the administration of DOCA, since we have previously reported an increase in urinary adrenaline excretion in DOCA/salt hypertensive rats, suggesting an increase in adrenal adrenaline production in this animal model (Minato et al., 1990).

A hypotensive action of MK422 was found in DOCA/salt hypertensive rats, although there have been various observations indicating that the CEI, CV-3317 (Inada et al., 1986), and saralasin, an antagonist of All (Morton et al., 1979), did not reduce the B. P. in renin-angiotensin independent hypertensive models such as DOCA/salt hypertensive rats. It has been reported, however, that the hypotensive effect of CEI is due not only to the blockade of angiotensin converting enzyme but also to stimulation of prostaglandin synthesis (Barr et al., 1980), an increase in kinin (Marks et al., 1980) and a reduction of vasopressin (Igarashi et al., 1985). Thus, such mechanisms might have induced a decrease in B. P. in the present study.

The above findings suggest that All can prevent a reduction in the weight of the adrenal glands independently of All in DOCA/salt hypertensive rats. However, details of the mechanisms involved remain obscure. Further investigations are clearly needed.

References


Igarashi, Y., H. Suzuki, Y. Itaya, K. Kondo


