Local Generation of Angiotensin II as a Mechanism of Aldosterone Secretion in Rat Adrenal Capsules (43175)

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Abstract. **Angiotensin-converting enzyme (ACE) is found in the adrenal gland, but the role of adrenal ACE in the formation of angiotensin II (AII) and subsequent stimulation of aldosterone is unclear. We examined the effect of adrenal ACE activity on aldosterone secretion by superfusing rat adrenal capsules with angiotensin I (AI) in the presence and absence of the ACE inhibitor, lisinopril. Angiotensin I (10 μM) stimulated aldosterone secretion from 914 ± 41 to 1465 ± 118 pg/min/capsule ($P < 0.05$). Simultaneous superfusion of AI plus lisinopril (100 μM) inhibited the stimulation of aldosterone by 73% ($P < 0.05$). Perfusion of the capsules with angiotensin II (1 μM) stimulated aldosterone from 893 ± 180 to 1466 ± 181 pg/min/capsule ($P < 0.01$). In contrast, simultaneous superfusion of AII plus lisinopril (100 μM) did not inhibit the AII stimulation of aldosterone. The failure of lisinopril to inhibit AII stimulation of aldosterone argues against a toxic or nonspecific action of lisinopril. The inhibition of AI stimulation of aldosterone release by lisinopril is mostly due to lisinopril inhibition of ACE and resulting decreased conversion of AI to AII. These results demonstrate that adrenal ACE may generate AII from AI in the adrenal gland, and this locally produced AII stimulates aldosterone.**

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The renin-angiotensin system consists of two main enzymes, renin and angiotensin converting enzyme (ACE) (1). ACE catalyzes the conversion of the decapeptide angiotensin I (AI) to the octapeptide angiotensin II (AII) (2), a potent stimulator of aldosterone secretion (3). ACE is found in the peripheral vasculature and functions in the local generation of AII to regulate vascular tone in the rat (4). ACE has also been reported in the rabbit (5) and rat (6, 7) adrenal gland by several investigators, but it is not clear whether adrenal ACE contributes to stimulation of aldosterone by catalyzing the formation of AII locally. We tested whether AI could stimulate aldosterone secretion from superfused rat adrenal capsules, and whether a specific ACE inhibitor could block the AI-stimulated aldosterone secretion.

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Materials and Methods

Adrenal glands were obtained from female Sprague-Dawley rats (200–250 g) after decapitation. They were cleaned of fat and the capsular portion containing the zona glomerulosa was isolated. Capsules were then cut into quarters after dividing into each group randomly, and placed into a superfusion holder maintained at 37°C and superfused at a flow rate of 0.12 ml/min. Five adrenal capsules per chamber were superfused at 37°C with oxygenated M199 media (Sigma, St. Louis, MO) containing modified Hanks' salts: 3.5 mM K^+, 1.25 mM Ca^{2+}, and 0.2% bovine serum albumin, (pH 7.4). Capsules were preequilibrated in the superfusion system for 90 min, after which 10-min fractions were collected for aldosterone assay. After 40-min baseline sampling, the capsules were superfused with AI (Sigma) or AII (Sigma) for 60 min. In other series of experiments, lisinopril (Merck Sharp & Dohme Research Laboratories, Rahway, NJ) or captopril (E. R. Squibb and Sons, Inc., Princeton, NJ) was superfused throughout the preequilibration period as well as the experimental period. Fractions were collected in 10-min samples for 200 min. Aldosterone was measured using an aldosterone radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA). The

intraassay coefficient of variation was 6.4% (n = 10) and the interassay coefficient of variation was 15.5% (n = 10).

Results are shown as the mean ± SE. When a significant effect was shown using one-way analysis of variance, Student's t test was used to determine which differences were significant. Significance was defined as $P < 0.05$.

Results

Mean basal aldosterone release during 0–40 min was 914 ± 41 pg/min/capsule for capsules treated without lisinopril (n = 3), and 723 ± 18 pg/min/capsule for capsules with lisinopril (n = 3). The former was higher than the latter ($P < 0.05$). Aldosterone release was significantly ($P < 0.05$) stimulated by 10 μM AI from 914 ± 41 to 1465 ± 118 pg/min/capsule. AI stimulation of aldosterone release was inhibited 73% by the simultaneous superfusion of 100 μM lisinopril ($P < 0.05$, Fig. 1). The maximum AI-induced increase in aldosterone secretion was 61.3 ± 16.2% in the absence and 16.4 ± 2.6% in the presence of lisinopril.

To investigate the possibility of nonspecific inhibition of aldosterone secretion by lisinopril, the adrenal capsules were superfused with 1 μM AII in the absence and presence of 100 μM lisinopril (Fig. 2). Mean basal aldosterone release during 0–40 min was 893 ± 180 pg/min/capsule in the absence of lisinopril (n = 3), and 912 ± 155 pg/min/capsule in the presence of lisinopril (n = 3). Aldosterone release in both groups was significantly increased by AII stimulation ($P < 0.01$). There

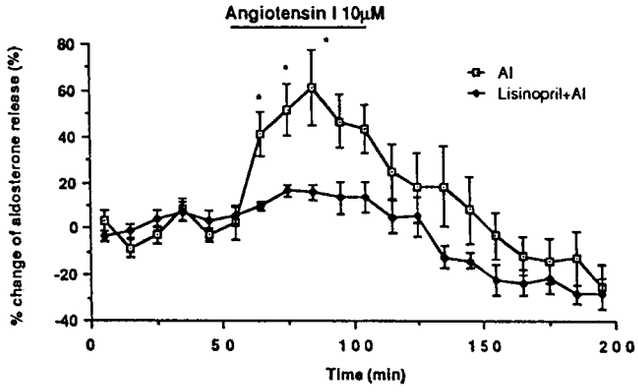

Figure 1. The effect of 10 μM AI on aldosterone secretion from rat adrenal capsules continuously superfused. Open squares, capsules with AI alone; closed diamonds, capsules superfused with AI and 100 μM lisinopril. The superfusion was collected continuously into 10-min samples. Aldosterone was measured in these samples. The results are expressed as the percentage of change in each 10-min sample compared with the mean basal aldosterone release. The mean basal aldosterone release was calculated from the aldosterone measured in the 10-min samples collected during the 40-min control period. The mean basal aldosterone release was 914 ± 41 pg/min/capsule without lisinopril (n = 3) and 723 ± 18 pg/min/capsule for capsules treated with lisinopril (n = 3). The control part of curve demonstrates the stability of the aldosterone release during the control period. *P < 0.05 compared with lisinopril.

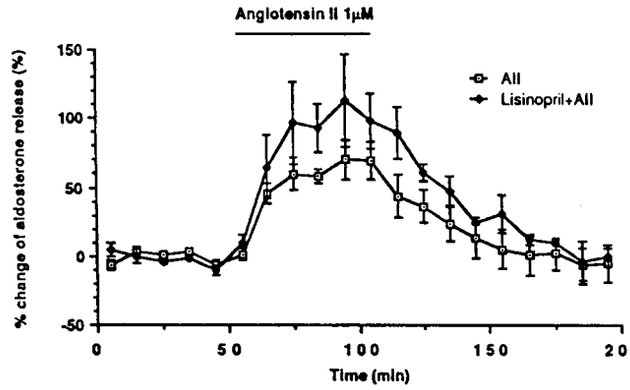

Figure 2. The effect of 100 μM lisinopril on AII stimulation of aldosterone secretion from superfused rat adrenal capsules. The results are expressed as in Figure 1. One micromolar AII stimulated aldosterone release in the absence (open squares) and presence (closed diamonds) of lisinopril. The mean basal aldosterone secretion was 893 ± 180 pg/min/capsule without lisinopril (n = 3) and 912 ± 155 pg/min/capsule for capsules treated with lisinopril (n = 3).

was no significant difference of aldosterone release between both groups, but there was a trend toward potentiation of AII at each time period. The maximum AII-induced increase in aldosterone release was 69.9 ± 14.4% in the absence of lisinopril, and 113.0 ± 33.5% in the presence of lisinopril. Thus, lisinopril did not inhibit AII-induced aldosterone secretion.

In a pilot experiment, we used captopril instead of lisinopril using the same protocol. AI stimulated aldosterone from 690 pg/min/capsule to 1357 pg/min/capsule (196%). Simultaneous superfusions of AI with 100 μM captopril stimulated aldosterone from 1045 pg/min/capsule to 1216 pg/min/capsule (116%), an 80% reduction in the response to AI.

Discussion

ACE converts angiotensin I to angiotensin II, a potent stimulator of aldosterone secretion (3) and vasoconstriction. It is reported that AI is converted rapidly to AII in the pulmonary circulation, but other tissues may also generate AII (2). Immunohistologic studies have demonstrated the presence of ACE in blood vessels of the rabbit adrenal cortex (5), and autoradiography using the ACE inhibitor, [^{3}H]captopril (6), or ^{125}I-lisinopril (7) demonstrated binding sites in the rat adrenal capsules outer zona glomerulosa (6), zona glomerulosa (7), and adrenal medulla (6, 7). Previous works have shown that the ACE inhibitor, captopril, inhibits AI-induced aldosterone secretion *in vivo* (8) and *in vitro* using zona glomerulosa cells (9, 10). However, Braley *et al.* (10) found only a 50% inhibition of AI stimulation of aldosterone production using separated zona glomerulosa cells in a static incubation system. They suggested that a part of the AI stimulation may be a direct action of AI on the adrenal glomerulosa cell. We reexamined this question in view of the presence of an adrenal renin-angiotensin system and used

a continuous superfusion system to avoid the possible pool action of metabolic peptide fragments that may occur in static incubation and that might influence the results. We investigated the adrenal ACE activity on aldosterone secretion by superfusing rat adrenal capsules with AI in the presence and absence of the ACE inhibitor, lisinopril. In previous studies, we have shown that 100 μM lisinopril does not appear to alter tissue viability in adrenal explant cultures by microscopic examination and measuring [^{3}H]thymidine (11). Therefore, we used 100 μM lisinopril in this experiment. The results of the present study demonstrated that ACE in adrenal capsules most likely generates AII in response to exogenous AI, and that this locally produced AII can stimulate aldosterone secretion. These conclusions are supported by the observations that the ACE inhibitor, lisinopril, blocked AI-induced aldosterone secretion by more than 70% without inhibiting AII-induced aldosterone secretion. Thus, our findings suggest that the main effect of exogenous AI on aldosterone secretion is not via a direct stimulation but rather by ACE conversion of AI to AII. Our findings are more in keeping with those of Mendelsohn and Kachel (9) who reported a 20-fold decrease in AI stimulation by captopril.

The adrenal glands appear to contain all of the components of the renin-angiotensin system. However, the functions of this local adrenal renin-angiotensin system remain to be determined. Renin has been reported in the adrenal gland (12), and renin mRNA is found in rat adrenal glomerulosa cells (13, 14). Angiotensinogen mRNA is also found in rat adrenal gland (15), and immunoreactive AII is reported in adrenal capsules (16–18). We have reported that adrenal renin in the adrenal capsules is higher than in the other portions (19), and that a low sodium or high potassium diet increases both adrenal renin and aldosterone, whereas a high sodium diet decreases both (20). In addition, we have demonstrated a positive correlation *in vivo* between adrenal renin and adrenal aldosterone in rats on various levels of sodium intake (19, 20). These results suggest the presence of a local renin-angiotensin system in the adrenal gland, and this system might have a role in aldosterone secretion. The results of the present study are in agreement with this hypothesis.

We have shown that exogenous AI stimulates aldosterone secretion in the superfused rat adrenal capsule, and the ACE inhibitor, lisinopril, blocks AI stimulation of aldosterone. These results suggest that adrenal ACE may locally generate AII from exogenous AI, resulting in stimulation of aldosterone secretion.

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