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# Compensatory increase in adrenomedullary angiotensin-converting enzyme activity (kininase II) after unilateral adrenalectomy

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**Summary**

Angiotensin-converting enzyme (ACE, kininase II, dipeptidyl carboxypeptidase, EC 3.4.15.1) was characterized in the adrenal medulla of male Sprague-Dawley rats. Rat adrenal medulla and lung ACE were similar in their susceptibility to $Cl^-$ activation and to the inhibition by EDTA, captopril, bacitracin and thiorphan, suggesting that rat adrenal medulla and lung ACE have similar properties.

Changes in right adrenal weight and in adrenomedullary ACE activity 5 and 12 days following left unilateral adrenalectomy (UADX) were examined. Compensatory adrenocortical hypertrophy 12 days following UADX was associated with a significant increase in adrenal medullary ACE activity. This change was due not to a modified affinity of ACE for the substrate but to an alteration in ACE maximal velocity or number of available molecules. UADX had no effect on adrenocortical ACE activity. When UADX was combined with right splanchnic denervation, the increase in adrenomedullary ACE activity was blocked. The results support the existence of a functional ACE in adrenal medulla that is under neuronal control.

adrenal medulla; compensatory hypertrophy; angiotensin-converting enzyme

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## Introduction

Angiotensin-converting enzyme (ACE, kininase II, dipeptidyl carboxypeptidase, EC 3.4.15.1), is a dipeptidyl peptidase that cleaves the COOH-terminal His-Leu from angiotensin I to yield angiotensin II (ANG) [1]. Physiologically, the enzyme is a constituent of the capillary endothelial plasma membrane and it is most heavily concentrated in the lung [2]. High activity occurs also in plasma [1] and in other tissues, such as specific brain areas [4] and the adrenal gland [5].

Circulating ANG has important actions on vascular tone and aldosterone secretion [6]. However, there is some evidence that ANG may be generated locally in different tissues and act without reaching the circulation. The existence of a separate renin-angiotensin system (RAS) in the brain is now well established [7]. All of the components of the RAS have been found in neuroblastoma cells in tissue cultures [8] and cultured pheochromocytoma cells [9]. Several elements of the RAS have been detected in rat adrenal gland. ANG-immunoreactivity material has been found in rat adrenal even after bilateral nephrectomy [10,22]. In addition, renin-like activity was reported in adrenal glands of various species of animals [11,12]. Specific binding of $^{125}$I-351A – a specific ACE inhibitor – and ANG receptors, in high concentration, was shown by autoradiography in adrenal medulla and in adrenal capsule–zona glomerulosa area [5,13,14]. ANG formed in situ by adrenomedullary cells may modulate synthesis and release of catecholamine. The characterization of ACE activity and establishment of its localization within the adrenal gland will have important implications in clarifying the physiological role of adrenal ANG. The factors that regulate adrenal medulla ACE activity are unknown. The purpose of this study was to characterize the adrenomedullary ACE and to test if physiological conditions might change the enzyme activity. The response to unilateral adrenalectomy (UADX) was studied.

## Materials and Methods

*Animals*

Adult male Sprague-Dawley rats weighing 200–250 g were housed in individual cages in an artificially lighted room with an automatically controlled light cycle, with lights on from 06:00 to 18:00 h. Rat chow and water were available ad libitum. Normal rats without surgical manipulation were used for ACE characterization studies.

A left UADX, left UADX and/or right splanchnic denervation, or sham control operation (excision of a small piece of perirenal adipose tissue) was performed via lateral incision under pentobarbital anesthesia (40 mg/kg body wt.). 5 or 12 days later the animals were killed by decapitation between 09:00 and 10:00 h and adrenal glands were rapidly removed and immediately stored in a closed container on filter paper saturated with cold 0.9% NaCl until the adrenals could be cleaned of fat and capsular connective tissue and weighed to the nearest 0.1 mg. The adrenal medulla was separated from the adrenal gland with the use of fine forceps under stereomicroscopic control.

*Determination of angiotensin-converting enzyme activity*

Adrenal medullae were homogenized in 50 $\mu$l of cold 0.1 M potassium phosphate buffer, pH 8.0, containing 1 mM *p*-chloromercuriphenylsulphonic acid and 0.2% Triton X-100. Duplicate 10-$\mu$l aliquots of homogenate were used for protein determination [17]. ACE activity was assayed in duplicated 10-$\mu$l aliquots of the homogenate, with 0.88 mM of Hippuryl-His-Leu-[1-$^{14}$C]glycine (2.9 Ci/mol, New England Nuclear, Boston, MA) as substrate [18]. Lung and plasma ACE activity was determined under similar conditions. Lung homogenates were prepared in 200 volumes of 0.1 M potassium phosphate buffer, pH 8.0.

*Characterization of adrenal medulla ACE activity*

Adrenal medulla and lung homogenates from non-operated rats were assayed simultaneously for $Cl^-$ activation (0–600 mM NaCl) and EDTA (0.1 and 1.0 mM), bacitracin (0.125-25 mM), thiorphan (0.05–2.0 $\mu$M) and captopril (0.05–2.0 $\mu$M) inhibition.

*Determination of kinetic parameters*

The kinetic parameters of ACE activity were determined by measuring the hydrolysis of HHL[1-$^{14}$C]glycine hy 10 $\mu$l of adrenal medulla homogenates in concentrations ranging from 0.25 to 4.0 mM.

*Statistical analysis*

Significance between groups was determined using the Student's *t*-test or the one-way analysis of variance (ANOVA) with unequal cell sizes, followed by comparison of individual means with the Student Newman Keul's test.

## Results

Adrenal gland ACE activity was found located in the adrenal medulla (32.16 $\pm$ 2.5 pmol/h per $\mu$g protein, $n = 23$) with lower activity in the adrenal cortex (10.3 $\pm$ 0.98 pmol/h per $\mu$g protein, $n = 12$). Lung and plasma ACE activity were 1306 $\pm$ 98 pmol/h per $\mu$g protein and 1090.4 $\pm$ 119 nmol/h per ml plasma, respectively ($n = 12$). Rat adrenal medulla ACE activity closely resembled classical lung ACE in its susceptibility to $Cl^-$ activation (Fig. 1) and to inhibition by captopril, bacitracin, thiorphan (Fig. 2) and EDTA. Maximal inhibition of both lung and adrenal medulla ACE was achieved at 1.0 mM EDTA (95 and 100%), 2 $\mu$M captopril (85 and 70%), 10 mM bacitracin (22 and 40%) and 2 $\mu$M thiorphan (30 and 35%), respectively (Fig. 2).

*Effect of left unilateral adrenalectomy on adrenal medulla ACE activity*

The comparison of the mean right adrenal weight in unilateral adrenalectomized animals with that in sham-operated controls shows a significant increase in right adrenal weight: 57.8% at 5 days and 41.4% at 12 days after UADX (Fig. 3). There was no significant difference in adrenal medulla ACE activity between sham-operated

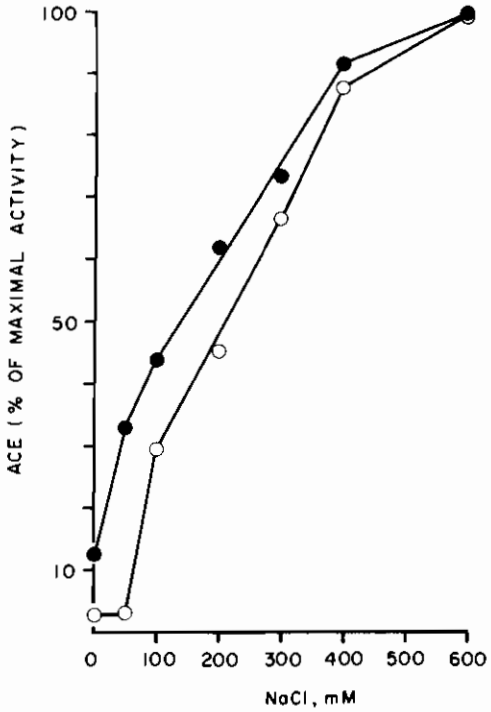


Fig. 1. Effect of $Cl^-$ activation on angiotensin-converting enzyme activity in rat lung and adrenal medulla. Values represent means of duplicate determinations from one typical experiment which was replicated three times. ○, lung; ●, adrenal medulla.

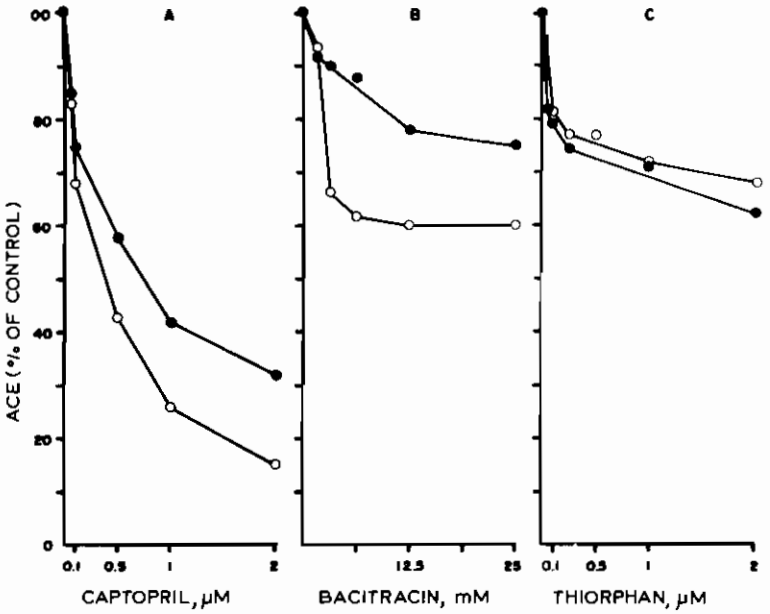


Fig. 2. Inhibition curves for the effects of (A) captopril, (B) bacitracin and (C) thiorphan on lung and adrenal medulla ACE activity. Values represent a typical experiment which was replicated three times. ○, lung; ●, adrenal medulla.

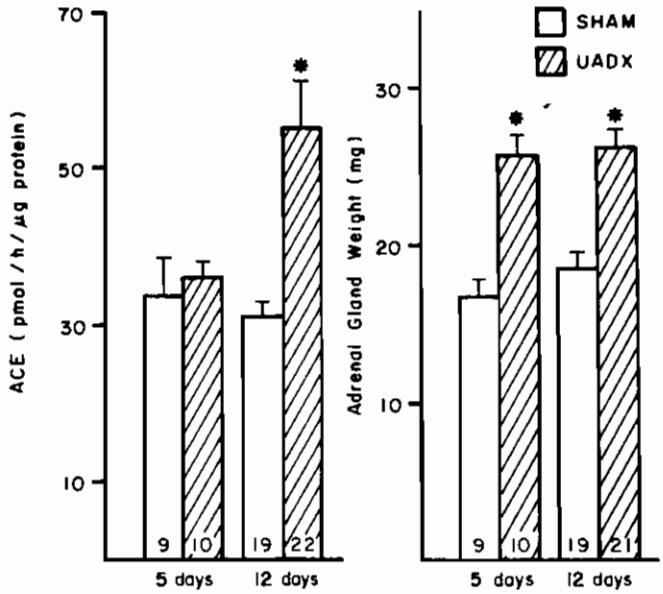


Fig. 3. Angiotensin-eonverting enzyme activity (left panel) and adrenal weight (right panel) of sham-operated, 5 days UADX and 12 days UADX rats. Values represent mean ± S.E.M. of $n$ individual determinations run in duplicate. Significance between groups for $^{*}P < 0.001$ (Student's $t$-test). Numbers in the bottom of the bars represent animals per group.

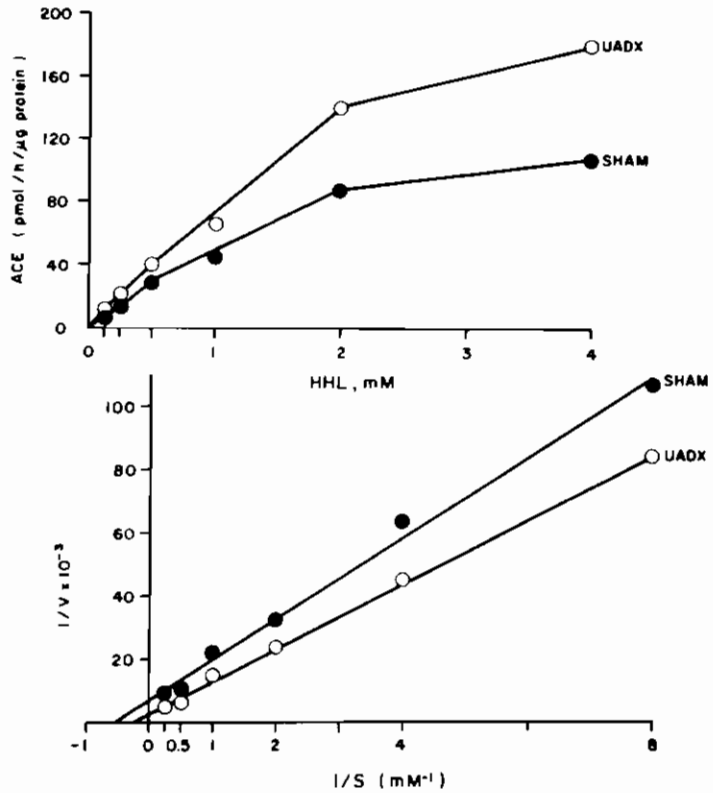


Fig. 4. Michaelis-Menten and Lineweaver-Burke plots of metabolism of Hippuryl-His-Leu-[1-$^{14}$C]glycine by homogenates of adrenal medulla from sham-operated and 12 days UADX rats. Values represent means of duplicate determinations from one typical experiment which was replicated four times ($V_{max}$ 123.8 pmol/h per µg protein, $K_m$ 1.58 mM in sham-operated; $V_{max}$ 274.4 pmol/h per µg protein, $K_m$ 2.76 mM in UADX).

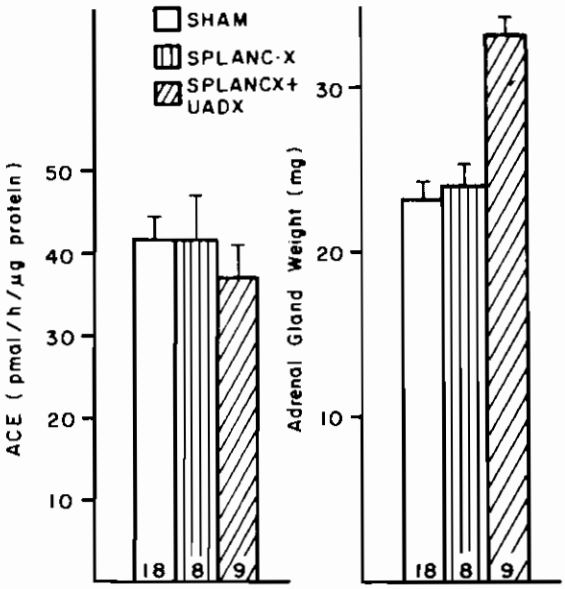


Fig. 5. Angiotensin-converting enzyme activity (left panel) and adrenal weight (right panel) in the adrenal medulla of sham-operated, right splanchnic denervated and both right splanchnic denervated and left UADX rats. Values represent mean ± S.E.M. of $n$ individual determinations run in duplicate. Significant $F$ ratio from ANOVA was for adrenal weight $F(2,33) = 29.6$ *$P < 0.001$. Numbers at the bottom of the bars represent animals per group.

and 5-day unilateral adrenalectomized rats. However, 12 days after UADX, total medullary and enzyme specific activity in the right adrenal medulla was significantly increased (+58%) (Fig. 3). Total medullary ACE activity in pmol/h per µg protein was: 3294 ± 323 in sham-operated and 5315 ± 370 in UADX rats ($P < 0.01$). Medullary proteins, in mg, were 19.57 ± 1.4 and 20.7 ± 2 in sham-operated and UADX rats, respectively. No changes in adrenal cortex, lung and plasma ACE activity were observed.

Kinetic analysis of ACE activity in adrenal medulla homogenates over substrate concentrations of 0.25–4.0 mM revealed that the observed changes were due not to a modified affinity of the substrate HHL but to an alteration in ACE maximal velocity and/or number of available enzyme molecules ($V_{max}$ 142.2 ± 34 pmol/h per µg protein, $K_m$ 2.07 ± 0.44 mM in sham-operated; $V_{max}$ 244.4 ± 12 pmol/h per µg protein, $K_m$ 2.68 ± 0.17 mM in UADX, $n = 4$) (Fig. 4).

*Effect of simultaneous right splanchnic denervation and left UADX on ACE activity*

When the animals were both UADX and splanchnicotomized, normal compensatory growth of the remaining adrenal gland occurred. However, UADX-induced increase in medullary ACE activity was no longer evident. Right splanchnie denervation alone had no effect on adrenal weight and medullary ACE activity (sham = splancX < splancX + UADX, Newman Keul's test) (Fig. 5).

## Discussion

Angiotensin-converting enzyme activity was readily detected in the adrenal medulla. The fact that the enzyme activity was chlorine dependent and was inhibited by EDTA, captopril and poorly by bacitracin and thiorphan (both enkephalinase inhibitors [23,27]) confirms that the activity measured was indeed angiotensin-converting enzyme. Enkephalinase A, another dicarboxypeptidase, would not be inhibited by this range of concentration of captopril [24,27] and is inhibited by the chloride ion [23].

The presence of ACE in adrenal medullary tissue may be physiologically important. A peptide closely resembling ANG has been detected in rapidly frozen rat adrenal tissue; the levels found greatly exceed that which can be accounted for by blood contamination and persist even after nephrectomy [22,10]. Renin-like activity in the adrenal glands of several species has been reported [11,12]. ANG receptors and specific binding of $^{125}$I-351A – a specific ACE inhibitor – were shown by autoradiography to exist in high concentration in adrenal cortex–zona glomerulosa and in adrenal medulla cells [5,13,14]. ANG has been shown to stimulate adrenal catecholamine release from chromaffin cells [25]. Although it may be premature to attempt to integrate these findings with the present characterization of the ACE in the adrenal medulla, it may be proposed that adrenal ACE may exert its effect by generating ANG through an intracellular mechanism in a manner similar to that proposed for cultured neuroblastoma cells [8] and cultured pheochromocytoma cells [9]. It is likely that the ANG thus formed may participate in the regulation of the synthesis and release of catecholamines.

It has been known for a long time that UADX leads to compensatory hypertrophy of the remaining adrenal gland [26]. Our data confirm this, but also provide some important additional insight concerning the role of the splanchnic nerve. UADX, as expected, resulted in a significant increase in adrenal weight. A major new finding in the present work is that UADX-induced compensatory hypertrophy of the adrenal gland was associated with significant increase in adrenal medulla ACE activity. It is interesting to note that earlier reports showed that the activity of other adrenomedullary enzymes (phenylethanolamine *N*-methyl transferase (PMNT) and dopamine-beta-hydroxylase (DBH)) are increased following UADX [20,21]. Furthermore, 12 days of right splanchnic denervation did not produce any change in adrenal medulla ACE activity, however, splanchnicotomy combined with UADX led to significant inhibition of the increase in ACE activity observed in the UADX group. These observations suggest a direct involvement of the splanchnic nerve in adrenal enzyme UADX-induced increase of activity. The increase in ACE activity, prevented by splanchnic denervation, presumably occurs as a result of an increased reflex in splanchnic nerve activity after UADX.

Compensatory adrenal hypertrophy is thought to be a result of a hypocortical signal with subsequent increased secretion of pituitary ACTH [26]. However, evidence has accumulated recently suggesting a neuronal rather than hormonal regulation of compensatory adrenal growth [3]. In support of this concept it has been demonstrated that an intact pituitary is not necessary for compensatory adrenal

growth to occur following UADX [15], that it can be prevented by spinal cord hemisection at T2–T3 contralateral to the adrenalectomy [16], and by lesions in the ventromedial hypothalamus ipsilateral to the adrenalectomy [19]. These results strongly suggested that compensatory adrenal growth is a neurally mediated reflex that comprises afferent nerves from one adrenal gland, interneurons in, or passing through the hypothalamus, and efferent nerves from the hypothalamus to the contralateral adrenal gland [3]. Our present observations that, in the UADX-splanchnic denervated group, the UADX-induced increase of adrenal ACE activity was significantly inhibited, but not the compensatory adrenal growth, may suggest that the splanchnic nerve contributes to the mechanism of neural mediation of compensatory hypertrophy whereby the compensatory increase in adrenal ACE activity occurs, but not in that involving the increase in adrenal weight. In addition, evidence from peripheral denervation studies of Dallman et al. [3] suggests that the coeliac ganglion and the splanchnic nerve are not involved in mediating compensatory adrenal growth.

In summary, our results indicate the existence of a functional angiotensin-converting enzyme activity in rat adrenomedullary tissue, and therefore, a possible new regulatory mechanism for the adrenal function. The participation of a local renin-angiotensin system in the regulation of the synthesis and release of catecholamines, however, remains to be determined.

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