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DES-ASP[1]-Angiotensin II: Possible Role in Mediating Responses of the Renin-Angiotensin System (38840)

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Since Blair-West *et al.* (1) demonstrated that the naturally occurring heptapeptide fragment of angiotensin II (des-asp[1]-angiotensin II) stimulated aldosterone secretion in the sheep, several investigators have speculated that the conversion of angiotensin II to heptapeptide is a physiological event associated with stimulation of aldosterone secretion by the renin–angiotensin system. Recent reports by other investigators that the heptapeptide is, in fact, a potent stimulus for aldosterone biosynthesis both *in vitro* (2, 3) and *in vivo* (4, 5) are consistent with this suggestion. Further support for this hypothesis has been provided from the demonstration that sar[1]-ile[8]-angiotensin II was less effective against heptapeptide than angiotensin II in inhibiting aldosterone biosynthesis *in vitro* (2). More recently, Spielman and Davis (unpublished observations) have found that smaller doses of sar[1]-ala[8]-angiotensin II are required to block the *in vivo* steroidogenic action of angiotensin II as compared with the heptapeptide in the rat. These findings both *in vitro* and *in vivo* suggest that the heptapeptide has a greater affinity for the cellular receptors in the zona glomerulosa than does angiotensin II. In the present study, we have attempted to evaluate further the heptapeptide hypothesis by comparing the adrenal steroid secretory changes produced by angiotensin II and its heptapeptide fragment. It was reasoned that if angiotensin II stimulates steroid secretion via its conversion to heptapeptide, then these two peptides might produce identical steroid response profiles. An interesting incidental finding was the decrease in PRA with both peptides.

Materials and Methods. Nine female mongrel dogs weighing 18–25 kg were used in this study. After laparotomy, the left adrenolumbar vein was catheterized by a modification of the technique of Hume and Nelson (6). Two days after operation the animals were given 8 mg of dexamethasone (Decadron phosphate; Merck, Sharp, and Dohme) im to suppress ACTH secretion and anesthetized with sodium pentobarbital (30 mg/kg). Two polyethylene catheters were placed in the inferior vena cava by the femoral veins for infusion of peptides and replacement of blood, and another catheter was inserted into the lower abdominal aorta via the femoral artery for measurement of arterial blood pressure and sampling of peripheral blood.

Two to three hours after dexamethasone administration and anesthetization the acute study was begun. Two timed collections of adrenal venous blood were made 15 min apart before starting the infusion of either angiotensin II (Hypertensin, Ciba) or heptapeptide (des-asp[1]-angiotensin II, Schwarz-Mann). In five of the dogs, angiotensin II was infused iv at 0.075 μg/kg min^{-1} for 30 min and adrenal blood samples taken 15 and 30 min after the beginning of the infusion. After the infusion of angiotensin II, 45 min were allowed for the animal to recover from the effects of the peptide before taking two recovery blood samples from the adrenal catheter 15 min apart. Subsequently, heptapeptide was infused for 30 min at the same rate as angiotensin II and blood samples for adrenal steroids were obtained after 15 and 30 min of infusion. Again, 45 min after infusion of the peptide, two recovery blood samples were collected 15 min apart. In the other four dogs, the same protocol was followed except that the order of peptide infusion was reversed. Peripheral blood samples for determination of plasma sodium and potassium concentrations and plasma renin activity were obtained simultaneously with collection of adrenal venous blood. Mean arterial blood pressure was measured continuously by the use of a Sanborn pressure transducer (model P23Db) and a Sanborn recorder (model 7700), and the values pre-

TABLE I. EFFECTS OF ANGIOTENSIN II AND HEPTAPEPTIDE INFUSION ON ADRENAL STEROID SECRETION AND MEAN ARTERIAL BLOOD PRESSURE IN ANESTHETIZED, DEXAMETHASONE-TREATED DOGS.[a,b]

	Control	Angiotensin II infusion[b]	Recovery	Control	Heptapeptide infusion[b]	Recovery
Aldosterone secretion (ng/min)	11.6 ± 5.4 (9)	41.4[c] ± 7.6 (9)	13.9 ± 6.4 (9)	15.7 ± 6.4 (9)	37.4[c] ± 7.9 (9)	11.4 ± 5.7 (9)
Corticosterone secretion (μg/min)	0.34 ± 0.19 (8)	1.60[d] ± 0.50 (8)	0.18 ± 0.10 (8)	0.14 ± 0.07 (8)	0.48[c] ± 0.13 (8)	0.13 ± 0.06 (8)
Cortisol secretion (μg/min)	1.29 ± 0.56 (8)	3.21 ± 1.25 (8)	0.64 ± 0.23 (8)	0.91 ± 0.33 (8)	1.19 ± 0.29 (8)	0.56 ± 0.16 (8)
	1.11 ± 0.61 (7)	3.57[d] ± 1.38 (7)	0.69 ± 0.26 (7)	0.65 ± 0.23 (7)	1.26[d] ± 0.34 (7)	0.62 ± 0.17 (7)
Adrenal Plasma flow (ml/min)	2.6 ± 0.3 (9)	2.0[e] ± 0.2 (9)	2.5 ± 0.4 (9)	2.7 ± 0.4 (9)	2.1[e] ± 0.3 (9)	2.6 ± 0.4 (9)
Mean arterial pressure (mm Hg)	156 ± 4 (9)	175[c] ± 4 (9)	152 ± 3 (9)	154 ± 3 (9)	162[c] ± 3 (9)	153 ± 5 (9)

[a] Values are means ± SEM; numbers in parentheses represent the number of dogs studied.
[b] Peptides infused at 0.075 μg/kg min^{-1}.
[c] Significantly different from both control and recovery values ($P < 0.01$).
[d] Significantly different from both control and recovery values ($P < 0.05$).
[e] Significantly different from control ($P < 0.01$) and recovery ($P < 0.05$) values.

sented in the results were those recorded just prior to blood sampling. All blood removed during each sampling period was replaced by fresh blood from a donor dog.

The concentrations of adrenal steroids in adrenal venous plasma were determined by the double-isotope derivative method of Kliman and Peterson (7). The simultaneous secretion rates of aldosterone, corticosterone, and cortisol were calculated from the concentration of each steroid and the adrenal plasma flow. Plasma samples for renin activity were processed for generation of angiotensin I by the method described by Schneider *et al.* (8) and assayed by the pressor response in the pentobarbital-anesthetized, pentolinium-blocked rat. The concentrations of sodium and potassium in the plasma were determined by flame photometry. The Student's *t* test for paired observations was used to compare the average of the experimental period with the average of both the control and recovery periods, as well as to compare the magnitude of the steroid response to each peptide.

Results. The results are presented in Table I. The pressor potency of angiotensin II was about double that of heptapeptide, while adrenal plasma flow was reduced significantly and to the same degree by both peptides. Both angiotensin II and the heptapeptide produced statistically significant increases in the secretion rates of aldosterone and corticosterone and by 45 min after infusion of the peptides, the enhanced secretion rates of these steroids had returned to the control levels or below. This return of steroid secretion to a level below the control reflects an elevated control level in some dogs because of incomplete suppression of ACTH by dexamethasone. The elevation in cortisol secretion induced by these peptides was not statistically significant because of the excessively elevated control secretion rates of this steroid due to incomplete ACTH suppression preceding the infusion of angiotensin II in one dog and heptapeptide in another; the data for these dogs are presented in the first set of values for cortisol secretion (Table I). In these two dogs with the high control secretion for cortisol, the initial peptide infusion was associated with a fall, rather than an increase, in cortisol secretion and an even more dramatic decline in steroid secretion during the subsequent recovery period. Excluding these two instances, the augmented secretion rates of cortisol associated with infusion of both peptides were statistically significant ($P < 0.05$); these data are shown in the second series for cortisol secretion where $N = 7$. The comparative steroidogenic responses to angiotensin II and the heptapeptide were not significantly different. It should be emphasized that qualitatively the responses to the two peptides appear to be very similar. Plasma renin activity was de-

pressed significantly ($P < 0.05$) and to essentially the same degree by both peptides. For the control, angiotensin II infusion, and recovery periods, PRA was 18.1 ± 5.1, 8.8 ± 2.1, and 18.5 ± 5.1 SEM ng angiotensin/ml plasma, respectively. The corresponding values for heptapeptide infusion were 20.3 ± 4.7, 12.5 ± 3.1, and 16.5 ± 4.9. There were no significant changes in plasma electrolyte concentrations throughout the experiment.

Discussion. It is well established that angiotensin II acts at an early biosynthetic step in adrenocortical steroidogenesis (9, 10). Furthermore, infusion of angiotensin II in small doses stimulates specifically aldosterone biosynthesis, whereas larger doses of the peptide also stimulate production of the major glucocorticoids of the adrenal cortex, corticosterone, and cortisol (11). Angiotensin II infusion at the dose level employed in this study produced a significant increase in secretion rates of aldosterone, corticosterone, and cortisol. Similarly, and most importantly, the heptapeptide fragment of angiotensin II, des-asp^1-angiotensin II, produced a qualitatively identical steroid response profile to the octapeptide. This finding strongly suggests that adrenocortical steroidogenesis induced by these two peptides is mediated via the same biosynthetic pathways and is consistent with reports suggesting a common receptor site for angiotensin II and its heptapeptide metabolite. By employing isotopically labeled angiotensin II, receptor sites for angiotensin II have been described in adrenal glomerulosa cell suspensions (12) or adrenal membrane fragments (13). The heptapeptide, as well as pharmacological antagonists of angiotensin II, displace the as pointed out above, there is evidence to suggest that the heptapeptide has a much higher affinity for binding sites in zona glomerulosa cells (2–4, Spielman and Davis, unpublished observations). In two very recent studies, Peach (14) and Bravo *et al.* (15) have shown *in vitro* and *in vivo*, respectively, that the heptapeptide antagonist, des-asp^1-ile^8-angiotensin II is more potent than sar^1-ile^8-angiotensin II in inhibiting steroidogenesis induced by angiotensin II. One interpretation of this finding is that the response to angiotensin II was mediated by the heptapeptide.

An alternative hypothesis has been proposed by Goodfriend (16). He suggested the possibility that angiotensin II unites with a membrane receptor site in zona glomerulosa cells and that the heptapeptide might mediate the intracellular response. This suggestion is consistent with reports that there apparently is little heptapeptide in arterial plasma, in contrast to venous plasma, in at least humans (17) and sheep (18). Also, Peach and Chiu (2) have found in incubated capsular zona glomerulosa cell suspensions that there is, in fact, considerable degradation of angiotensin II to heptapeptide and that the Sar^1- and Arg^1-angiotensin II analogs, which are poorly metabolized by aminopeptidase, do not readily stimulate aldosterone biosynthesis.

Thus, the evidence in the literature, along with the present finding that angiotensin II and heptapeptide produce qualitatively identical steroid-response profiles, support the hypothesis that the heptapeptide is an active steroidogenic peptide at the zona glomerulosa cell. Further studies are required, however, before one can unequivocally attribute a physiological role to heptapeptide in the control of aldosterone secretion.

Although incidental to the primary objective of this study, the nature of the experimental design allowed for a cursory evaluation of the renin-inhibitory activity of heptapeptide, relative to angiotensin II. That heptapeptide, like its octapeptide progenitor, did depress PRA raises the possibility that heptapeptide might mediate not only the adrenal steroidogenic effects of angiotensin II, but also the direct renin-inhibitory activity of angiotensin II at the kidney. It is interesting that the depressions in PRA produced by angiotensin II and heptapeptide were essentially the same magnitude although the rise in arterial pressure with heptapeptide was only half that produced by angiotensin II. This finding suggests a possible dissociation between the peripheral vascular effects of these two peptides and their renin-inhibitory activity. The present study, therefore, is consistent with the hypothesis that the heptapeptide fragment of angiotensin II mediates the action of angiotensin II on

renin release by the kidney and on steroid secretion by the adrenal.

Summary. In dexamethasone-treated dogs, both antiogensin II and its heptapeptide fragment (des-asp[1]-angiotensin II) stimulated the secretion of the adrenal steroids aldosterone, corticosterone, and cortisol. Further, there was no statistically significant difference in the steroidogenic potency of the two peptides. An interesting incidental finding was the decrease in PRA with both peptides, again with no demonstrable difference in the magnitude of the responses. The present data are consistent with the hypothesis that the heptapeptide mediates responses produced by the renin–angiotensin system in both the adrenals and the kidneys.

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