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Des-Asp-1-Angiotensin II: Possible Role in Mediating the Renin–Angiotensin Response in the Rat (39169)

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In 1971, Blair-West and associates (1) reported that the heptapeptide fragment of angiotensin II, Des-Asp-1-angiotensin II, increased aldosterone secretion in sheep. In a study of the effects of inotropic agents on the myocardium, Kent *et al.* (2) realized that this heptapeptide might have physiologic or pathophysiological importance in mediating the renin–angiotensin response. From *in vitro* studies of steroidogenesis, Peach and associates (3) suggested that the heptapeptide might have physiologic significance in mediating the aldosterone response of the renin–angiotensin system. In a preliminary report, Spielman *et al.* (4) demonstrated that Des-Asp-1-angiotensin II increased aldosterone secretion in anesthetized rats, and Campbell, Brooks, and Pettinger (5) showed that it increased plasma aldosterone in conscious rats. The present study was undertaken to investigate further the possible role of Des-Asp-1-angiotensin II in mediating both steroidogenic and pressor responses of the renin–angiotensin system in the rat.

Methods. For collection of adrenal venous blood, male Sprague–Dawley rats were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally, ip). The trachea was intubated with polyethylene tubing to facilitate respiration and airway clearance. The carotid artery was cannulated, and arterial blood pressure was measured with a Statham P23Db strain gauge and Hewlett–Packard recorder. A catheter (Polyethylene No. 90) was inserted into the left femoral vein and advanced retrograde through the inferior vena cava to the left renal vein. The abdomen was opened to allow visualization and manipulation of the catheter tip into the left renal vein. At this time the rat was given 0.2 ml of aqueous heparin (1000 units/ml). The tip of the catheter was advanced as far as possible into the adrenal vein beyond the entrance of the adrenal vein tributaries. The left renal vein and kidney were not disturbed by the surgical procedure. During periods of adrenal venous blood collection, maintenance of blood pressure was achieved by infusion of whole blood from a donor rat, volume for volume, through a catheter in the external jugular vein. All rats were kept on a heating pad throughout the experiment and rectal temperature was monitored.

Following the timed collection of approximately 2.5 ml of whole blood from the adrenal vein, the exact volume was recorded, the sample was centrifuged at 4° and the plasma stored at −20°. The adrenal venous plasma concentrations of aldosterone and corticosterone were measured by the double isotope derivative method of Kliman and Peterson (6). Adrenal steroid secretion rates were calculated as the product of adrenal venous plasma flow and the concentration of the steroid in the effluent adrenal plasma. Blood samples for determination of plasma electrolytes and hematocrit were taken from the adrenal vein catheter. Plasma sodium and potassium concentrations were measured by flame photometry.

Experiment I. Effects of angiotensin II or Des-Asp-1-angiotensin II on steroid secretion in rats given dexamethasone and morphine sulfate. Two to four hours prior to anesthesia, rats were given dexamethasone phosphate (1.0 mg/kg body wt, Decadron, Merck, Sharp, and Dohme). Ten minutes after the administration of sodium pentobarbital, morphine sulfate (1.25 mg/100 g body wt) was given intramuscularly (im). A control adrenal vein sample was collected while saline was infused at 0.014 ml/min. Following the control sample, the catheter was withdrawn from the adrenal vein and the saline infusion was replaced with synthetic angiotensin II or the heptapeptide, Des-Asp-1-angiotensin II in normal saline infused at 1.0 μg/0.014 ml min^{-1}. The pep-

tide was given for 30 min prior to beginning the collection of the second steroid sample and the infusion was maintained for the duration of the sampling period (10–20 min).

Experiment II. Effects of 1-Sar-8-Ala-angiotensin II on the steroidogenic action of angiotensin II or Des-Asp-1-angiotensin II. This experiment was similar to Experiment I except that after the control period the angiotensin antagonist, 1-Sar-8-Ala-angiotensin II, was infused simultaneously with either the synthetic angiotensin II or Des-Asp-1-angiotensin II. 1-Sar-8-Ala-angiotensin II was infused at a rate of 10 μg/kg min^{-1} in the rats given angiotensin II and at rates of 10 and 50 μg/kg min^{-1} in rats infused with Des-Asp-1-angiotensin II.

Experiment III. Pressor effects of angiotensin II and Des-Asp-1-angiotensin II and inhibition with 1-Sar-8-Ala-angiotensin II. Rats were anesthetized with pentobarbital anesthesia (50 mg/kg ip) and a carotid artery cannulated for the recording of arterial blood pressure. Animals were bilaterally vagotomized and given pentolinium tartrate (2.5 mg/kg im). Bolus injections of angiotensin II or Des-Asp-1-angiotensin II were given via a catheter in the jugular vein. For studying the effects of the angiotensin antagonist, 1-Sar-8-Ala-angiotensin II, a second jugular catheter was inserted through which 1-Sar-8-Ala-angiotensin II was infused at 0.05 μg/kg min^{-1}.

Results. Experiment I. Effect of angiotensin II and Des-Asp-1-angiotensin II on adrenal steroid secretion. After pretreatment with the combination of dexamethasone and morphine, a striking increase in aldosterone secretion occurred during angiotensin II infusion from 0.53 ± 0.21 SEM to 2.62 ± 0.69 ng/min (n = 8; $P < 0.005$; see Fig. 1). This fivefold increase is in contrast to the 65% increase from 1.06 ± 0.16 to 1.70 ± 0.20 ng/min in aldosterone secretion (Fig. 1) reported previously (7) with angiotensin II when dexamethasone alone was given as a pretreatment. With dexamethasone alone, corticosterone secretion was very high which indicated failure of dexamethasone to block ACTH release. With the combination of morphine and dexamethasone, corticosterone secretion was reduced markedly from the high level characteristic of surgical stress. Under these circumstances, a fourfold increase in corticosterone production occurred with angiotensin II.

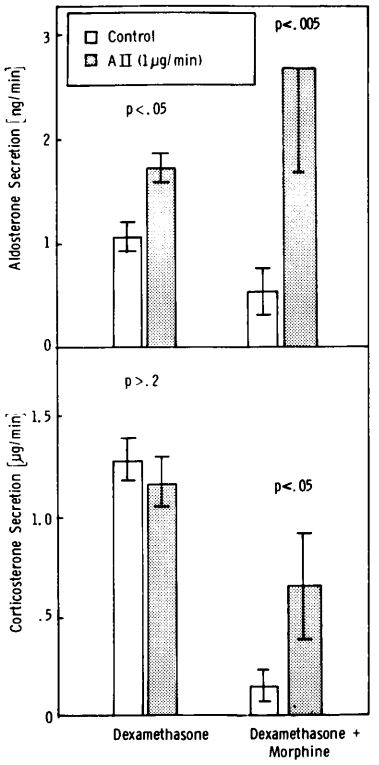

FIG. 1. Effects of angiotensin II (AII) on adrenal steroid secretion in rats given dexamethasone or dexamethasone and morphine as a pretreatment.

The aldosterone response to Des-Asp-1-angiotensin II during pretreatment with dexamethasone and morphine was also striking (Table I) but corticosterone secretion failed to change. The increase in arterial pressure with angiotensin II of 63 mm Hg was twice the increment of 32 mm Hg observed with the heptapeptide (Table I).

Experiment II. Effects of 1-Sar-8-Ala-angiotensin II on the steroidogenic action of angiotensin II or Des-Asp-1-angiotensin II. The angiotensin II antagonist, 1-Sar-8-Ala-angiotensin II, at a dose level of 10 μg/kg/min, prevented the aldosterone response to a 1 μg/min infusion of angiotensin II but corticosterone secretion increased ($P < 0.05$; Table II). In contrast, a 1 μg/min infusion of Des-Asp-1-angiotensin II pro-

TABLE I. EFFECTS OF ANGIOTENSIN II OR DES-ASP-1-ANGIOTENSIN II ON STEROID SECRETION IN RATS GIVEN DEXAMETHASONE AND MORPHINE.[a]

	Aldosterone secretion (ng/min)	Corticosterone secretion (ng/min)	Adrenal plasma flow (ml/min)	Mean arterial pressure (mm Hg)	Plasma [Na] (mEq/liter)	Plasma [K] (mEq/liter)
Angiotensin II (1.0 μg/min; (n = 8)						
Before	0.53±0.21	145±79	0.100±0.018	131±6	142±1	4.5±0.2
After	2.62±0.69**	656±271*	0.082±0.020	194±6***	141±1	4.7±0.2
Des-1-Asp-angiotensin II (1.0 μg/min; n = 6)						
Before	0.55±0.16	338±110	0.112±0.013	112±6	143±1	4.3±0.2
After	1.30±0.28***	470±103	0.116±0.018	144±4***	140±1	4.8±0.2

*** $P < 0.001$ or 0.0125
** $P < 0.005$.
* $P < 0.05$.

TABLE II. EFFECTS OF 1-SAR-8-ALA-ANGIOTENSIN II ON THE RESPONSE TO ANGIOTENSIN II OR DES-ASP-1-ANGIOTENSIN II IN RATS GIVEN DEXAMETHASONE AND MORPHINE.[a]

	Aldosterone secretion (ng/min)	Corticosterone secretion (ng/min)	Adrenal plasma flow (ml/min)	Mean arterial pressure (mm Hg)	Plasma [Na] (mEq/liter)	Plasma [K] (mEq/liter)
Control	0.20±0.08	12±6	0.092±0.020	121±5	143±1	4.4±0.3
Angiotensin II (1.0 μg/min) + 1-Sar-8-Ala-angiotensin II (10 μg/kg/min) (N = 7)	0.46±0.19	229±130*	0.090±0.018	115±4	141±1	4.3±0.2
Control	0.08±0.03	92±28	0.102±0.019	126±8	142±1	4.5±0.2
Des-Asp-1-angiotensin II (1.0 μg/min) + 1-Sar-8-Ala-angiotensin II (10 μg/kg/min^{-1}) (N = 5)	0.74±0.20**	509±72**	0.095±0.022	120±6	141±1	4.4±0.2
Control	0.48±0.23	293±110	0.110±0.018	118±7	143±1	4.2±0.2
Des-Asp-1-angiotensin II (1.0 μg/min) + 1-Sar-8-Ala-angiotensin II (50 μg/kg/min^{-1}) (N = 7)	0.40±0.21	403±154	0.097±0.020	111±6	140±1	4.5±0.2

** $P < 0.01$.
* $P < 0.05$.

duced a 9-fold increase in aldosterone and a 5-fold increase in corticosterone secretion when the same dose of 10 μg/kg/min of the angiotensin analog was given simultaneously (Table I). An increase in the dose of 1-Sar-8-Ala-angiotensin II to 50 μg/kg/min did, however, block completely the response in both aldosterone and corticosterone to Des-Asp-1-angiotensin II (Table II).

The 10 μg/kg/min dose of 1-Sar-8-Ala-angiotensin II blocked the pressor responses to both angiotensin II and Des-Asp-1-angiotensin II (Table II).

Experiment III. Dose response curves for arterial pressure for angiotensin II and Des-Asp-1-angiotensin II and the effects of 1-Sar-8-Ala-angiotensin II. The increments in arterial pressure to single intravenous injections of angiotensin II and its heptapeptide fragment were studied before and during infusion of 0.05 μg/kg min^{-1} of 1-Sar-8-Ala-angiotensin II (Fig. 2). The response to Des-Asp-1-angiotensin II was only approximately 50% of that to angiotensin II. The

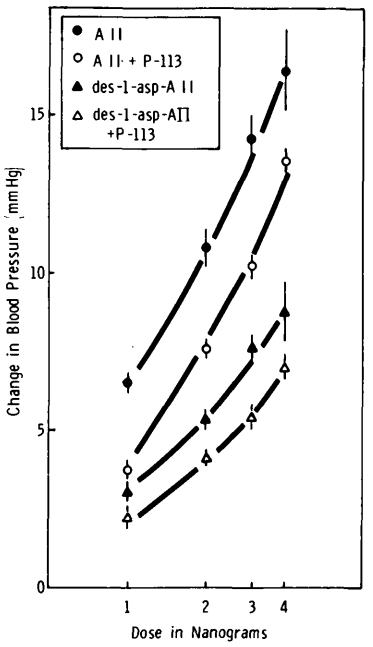

FIG. 2. Effects of 1-Sar-8-Ala-angiotensin II (P-113) given as an intravenous infusion of 0.05 μg/kg min^{-1} on the pressor response to single injections of angiotensin (AII) or Des-Asp-1-angiotensin II at four dose levels.

pressor response to both the octapeptide and the heptapeptide was partially blocked with 1-Sar-8-Ala-angiotensin II. And, in contrast to comparative blocking actions on steroidogenesis, the partial blockade of the angiotensin II pressor response appeared to be slightly greater than the antagonism of the pressor response to Des-Asp-1-angiotensin II.

Discussion. Following the discovery in 1971 (1) of the aldosterone-stimulating activity of Des-Asp-1-angiotensin II, Peach and Chiu (3) investigated the biosynthetic potency of the heptapeptide in *in vitro* studies of rabbit adrenal cell suspensions. They found that a 25-fold higher dose of the competitive antagonist, 1-Sar-8-isoleucine angiotensin II, was required to block aldosterone biosynthesis by the heptapeptide as compared with angiotensin II. This was interpreted as evidence that Des-Asp-1-angiotensin II had a much higher affinity for adrenal zona glomerulosa receptors than the octapeptide. Also, in a more recent preliminary report, Peach (8) observed that the heptapeptide, isoleucine-7-angiotensin II, was a more potent inhibitor of angiotensin II than 1-Sar-8-isoleucine-angiotensin II.

The *in vivo* studies of the steroidogenic potency of the heptapeptide in sheep by Blair-West *et al.* (1) have now been extended to the rat and the dog. In the rat, Des-Asp-1-angiotensin II increased plasma aldosterone (5). In the dog, Lohmeier *et al.* (9) have demonstrated that the heptapeptide produced the same steroid profile response for aldosterone, corticosterone, and cortisol as angiotensin II did, and the magnitude of the responses for each of the three steroids was not significantly different for the two peptides.

In the present study, a striking increase in aldosterone secretion occurred in the rat with both Des-Asp-1-angiotensin II and the octapeptide, but this marked response to the peptides was evident only when ACTH was suppressed during pretreatment with dexamethasone and morphine. Furthermore, the aldosterone response to the two peptides was not significantly different ($P > 0.05$). As reported previously (7), in the rat the stress of laparotomy for cannulation of the adrenal vein stimulates ACTH and steroidogenesis, and this response frequently completely obscures other effects on the adrenal cortex. It should be pointed out that even the influence of angiotensin II on corticosterone secretion was quite striking when ACTH release and the control level of corticosterone output were low. It is emphasized that it is critical to measure corticosterone or ACTH itself in experiments of this type to be certain that ACTH is not producing an obscuring action on steroidogenesis (7). Also, Pettinger *et al.* (10) have reported that anesthesia increased plasma renin activity in the rat but in the present study each animal served as its own control and a slightly elevated plasma renin activity did not appear to influence the results.

The effects of angiotensin II blockade with 1-Sar-8-Ala-angiotensin II on the steroid responses to the two peptides revealed that a higher dose of the antagonist was required to block the aldosterone-stimulating activity of the heptapeptide than the octapeptide. This finding supports the earlier *in vitro* observations of Peach and Chiu (3) and the *in vivo* results of Campbell *et al.*

(5) on plasma aldosterone. The most reasonable hypothesis to explain these data is that the heptapeptide has a higher affinity for zona glomerulosa receptors than the octapeptide. The present study extends this concept to cellular receptors for the heptapeptide in its influence on corticosterone since the response to the heptapeptide for corticosterone secretion was blocked only with the higher (50 μg/kg min^{-1}) dose of 1-Sar-8-Ala-angiotensin II. Collectively, these findings support the idea that Des-Asp-1-angiotensin II mediates, at least in part, the steroidogenic response of the renin-angiotensin system in the rat. And, recent observations by Bravo *et al.* (11) with the isoleucine analog of Des-Asp-1-angiotensin II have revealed a much more potent blocking action for aldosterone secretion in the dog than with 1-Sar-8-isoleucine angiotensin II.

The other aspect of the present study deals with the effects of angiotensin II and its heptapeptide fragment on blood pressure in the rat. The pressor response to Des-Asp-1-angiotensin II was only 50% of that produced by the octapeptide and this was demonstrable over a dose range from 1–4 ng. Furthermore, 1-Sar-8-Ala-angiotensin II was as effective in partially blocking the pressor response to the octapeptide as in inhibiting the heptapeptide. These findings together with the results on steroids support the reports of Spielman and Davis (12) and Williams *et al.* (13) of dissociation of adrenal cortex and peripheral arteriolar receptors in their affinity for angiotensin. Again, these results in rats are in agreement with findings in the dog (9) which indicate a selectivity of the heptapeptide for zona glomerulosa over peripheral arteriolar receptors. Finally, however, it should be pointed out that in the dog the renal arterioles do not participate in this selective process of increased receptor affinity of the zona glomerulosa over the peripheral arterioles for the heptapeptide as compared with angiotensin II. Freeman *et al.* (14) have found a decrease in renal blood flow in response to the intrarenal arterial infusion of either the heptapeptide or octapeptide in normal dogs, and the magnitude of response was essentially the same for the two peptides.

Summary. Angiotensin II and its heptapeptide fragment, Des-Asp-1-angiotensin II, produced a striking increase in aldosterone secretion in rats pretreated with dexamethasone and morphine to reduce ACTH release. 1-Sar-8-Ala-angiotensin II (10 μg/kg min^{-1}) given simultaneously with angiotensin II (1 μg/min) blocked the aldosterone response to angiotensin II in rats pretreated to reduce ACTH release. In contrast, 1-Sar-8-Ala-angiotensin II at the same dose failed to block the steroid response to Des-Asp-1-angiotensin II (1 μg/min) but a larger dose of 50 μg/kg min^{-1} of the angiotensin II antagonist blocked completely both the aldosterone and the corticosterone responses to 1 μg/min of Des-Asp-1-angiotensin II. From these data it is suggested that the heptapeptide has a higher affinity for zona glomerulosa receptors than the octapeptide and that Des-Asp-1-angiotensin II mediates, at least in part, the steroidogenic response to the renin–angiotensin system in the rat. The pressor response to Des-Asp-1-angiotensin II was approximately 50% of that produced by the octapeptide in the rat, and 1-Sar-8-Ala-angiotensin II was as effective in partially blocking the pressor response to the octapeptide as in inhibiting the heptapeptide. The present observations indicate a dissociation of adrenal cortex and peripheral arteriolar receptors in their affinity for angiotensin.

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