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# Role of Converting Enzyme in the Cardiovascular and Adrenal Cortical Responses to (des-Asp[1])-Angiotensin I[1] (39456)

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The C-terminal heptapeptide homolog of angiotensin II, angiotensin III, has been demonstrated to be an active peptide of the renin-angiotensin-aldosterone system in a number of biologic systems (1). Most notable has been its steroidogenic effect on the adrenal zona glomerulosa in a variety of species (2-4) including man (5). Two pathways for angiotensin III formation can be postulated. Classically, angiotensin I, the decapeptide, is acted upon by converting enzymes to generate the octapeptide, angiotensin II. Subsequently, aminopeptidases which catalyze the degradation of angiotensin II (6) could lead to the formation of angiotensin III. Alternatively, aminopeptidases could act initially upon the decapeptide, angiotensin I, to form the C-terminal nonapeptide, (des-Asp[1])-angiotensin I. Hydrolysis of the nonapeptide by converting enzymes would yield the heptapeptide, angiotensin III (2).

The conversion of synthetic (des-Asp[1])-angiotensin I by purified converting enzymes has been demonstrated *in vitro*. The $K_m$ values and the $V_{max}/K_m$ ratios favored the nonapeptide over the decapeptide with both enzymes. Therefore, the nonapeptide was actually a better substrate for porcine plasma and pulmonary converting enzymes than was angiotensin I (7).

Angiotensin III has both steroidogenic and pressor activity in the intact rat (8). Angiotensin III has been isolated and quantified from the plasma of man, dog, and rat (9, 10). The plasma concentration of angiotensin III relative to angiotensin II is quite high in the rat (10). Hence, in the present study, the rat was utilized to compare the relative steroidogenic and pressor effects of (des-Asp[1])-angiotensin I and angiotensin III. In addition, we determined if the effects of the C-terminal nonapeptide of angiotensin I were direct or dependent on cleavage of the nonapeptide by converting enzymes and generation of the active heptapeptide.

*Materials and methods.* Male Wistar rats (200-300 g) were maintained on low sodium rat chow (4 $\mu$Eq $Na^+$/g, General Biochemicals) and 0.5% saline solution *ad libitum* for at least 5 days to insure normal sodium intake. They were housed individually in metabolic cages in a temperature controlled room (20-22°) illuminated between 6:00 AM and 6:00 PM.

*Pressor responses.* A group of 10 rats was anesthetized with ether. Following bilateral nephrectomy, the iliac artery and femoral vein were cannulated with PE-50 intramedic tubing. The animals were allowed to awaken and stabilize for 1 to 2 hr in restraining cages. Blood pressure was monitored through the arterial cannula attached to a Statham P23D pressure transducer connected to a Brush Mark 220 recorder. Peptides were administered intravenously via a Harvard infusion pump in a 50-$\mu$l volume of 5% dextrose in water ($D_5W$) and flushed with 50 $\mu$l of $D_5W$. (Des-Asp[1])-angiotensin I, angiotensin II, and angiotensin III were administered in random order. One-half hour after administration of the last peptide, a 300 $\mu$g/kg bolus injection of converting enzyme inhibitor, *Bothrops jararaca* nonapeptide (Spectrum Medical Industries Lot No. 215865), was given in a volume of 100 $\mu$l. Responses to (des-Asp[1])-angiotensin I and angiotensin II were obtained after treatment with the enzyme inhibitor. The angiotensin nona- and heptapeptides were administered only during a time period from 5

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to 20 min after treatment with the *B. jararaca* peptide.

*Steroidogenic response.* An additional 24 rats were killed by decapitation and the adrenal glands were quickly removed and cleaned. The inner zones and medulla were removed and the remaining capsular layer was minced. The minced tissue was incubated in a Dubnoff metabolic shaker for 15 min at 60 rpm under an atmosphere of 95% $O_2$/5% $CO_2$ in Krebs–Ringer bicarbonate buffer containing 0.2% D-glucose, 4% albumin, 0.008% (DNase) (KRBGAD), and 0.2% trypsin. The tissue was washed twice with KRBGAD containing 0.1% trypsin inhibitor, followed by incubation for 20 min at 120 rpm in KRBGAD with 0.1% trypsin inhibitor and 0.04% collagenase. The tissues were then pipetted 75 times with a 1-ml biopipet and reincubated for 10 min. The dissociated cells were filtered through 150-$\mu m^2$ nylon mesh and the filtrate centrifuged at 4° for 15 min at 100*g*. The pellet was washed and finally resuspended in 1 ml of KRBGA. The number of viable cells was determined by exclusion staining with nigrosin and counting with a Neubauer hemocytometer.

The cell suspensions were divided into aliquots containing $5 \times 10^4$ cells, diluted to 1 ml with KRBGA, and equilibrated for 30 min at 60 rpm in a Dubnoff bath (37°, 95% $O_2$, 5% $CO_2$). Steroidogenic compounds to be tested were added in 10-$\mu$l volumes after the equilibration period. The same amount of vehicle was added to controls. When utilized, *B. jararaca* nonapeptide was administered 5 min prior to the addition of the angiotensins. The experimental incubation time was 1 hr after addition of steroidogenic peptides.

The LH-20 column purification and aldosterone assay were performed according to the method of Sarstedt *et al.* (11).

*Results. Pressor effects.* The cardiovascular responses to angiotensin II, (des-Asp[1])-angiotensin I, and angiotensin III are shown in Fig. 1. Both the nonapeptide and the heptapeptide exhibited about one-third of the pressor activity of angiotensin II. There was no statistical difference between the pressor responses to the nonapeptide and angiotensin III.

Pretreatment with the converting enzyme inhibitor (CEI), *B. jararaca* nonapeptide, at a fixed dose of 300 $\mu$g/kg (dose required to block the response to an $ED_{50}$ dose of angiotensin I) markedly reduced ($p < 0.01$) the pressor activity of (des-Asp[1])-angiotensin I at all doses administered (Fig. 2). Inhibition of converting enzyme did not alter the pressor response to angiotensin III. Since higher concentrations of CEI were not utilized, residual pressor activity of the nonapeptide may represent either uninhibited converting enzyme activity or the direct intrinsic activity of the angiotensin nonapeptide (or both).

To clearly quantify the intrinsic activity of (des-Asp[1])-angiotensin I on aldosterone

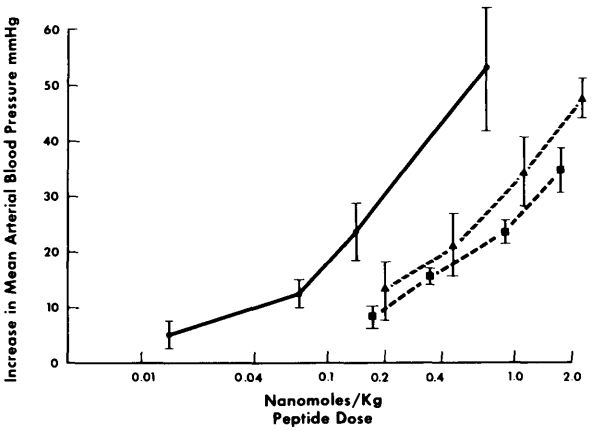


FIG. 1. Increase in mean arterial blood pressure induced by angiotensin II (●——●), angiotensin III (▲- - -▲), and [des-Asp[1]]-angiotensin I (■- - -■). Each point represents the mean ± SEM of eight experimental observations.

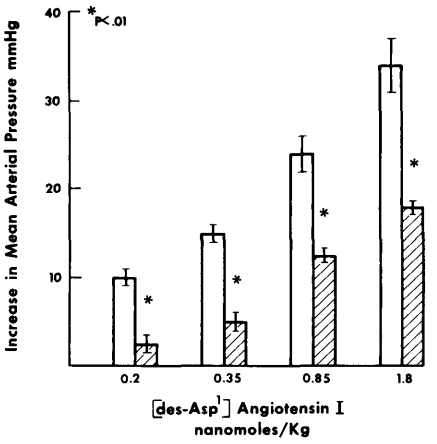


FIG. 2. Inhibition of [des-Asp[1]]-angiotensin I-induced (□) pressor responses with 300 μg/kg of *B. jararaca* nonapeptide (▨). Each bar represents the mean ± SEM of eight observations. The * indicates $p < 0.01$.

biosynthesis, the *in vitro* zona glomerulosa cell suspensions were studied. In the rat adrenal zona glomerulosa, (des-Asp[1])-angiotensin I had only 0.1% of the steroidogenic effect of angiotensin III (Fig. 3). After treatment with CEI, the concentration of (des-ASP[1])-angiotensin I had to be increased to $1 \times 10^{-6}$ *M* before a significant rise in aldosterone ($p < 0.01$) occurred. In these studies with rat adrenal cell suspension, angiotensin I was found to be about 1% as potent as angiotensin III, and the response to the decapeptide was not altered significantly by treatment with the *B. jararaca* nonapeptide (data not shown). In contrast, a comparable response was obtained with $1 \times 10^{-9}$ *M* angiotensin III. The weak steroidogenic response to (des-Asp[1])-angiotensin I reflected direct activity since the response was not significantly inhibited ($p < 0.5$) by $1 \times 10^{-6}$ *M* or higher concentrations of CEI. Converting enzyme inhibitor had no effect on basal steroidogenesis or on angiotensin III-induced aldosterone biosynthesis.

*Discussion.* It has been established in the rat that angiotensin III induces a steroidogenic response comparable to that of angiotensin II and possesses about one-third of the pressor potency of angiotensin II (8). Since angiotensin III is the product of the sequential actions of both converting enzymes and aminopeptidases, it is theoretically possible that (des-Asp[1])-angiotensin I could be a precursor of angiotensin III. However, this would necessitate the rapid hydrolysis of the nonapeptide by converting enzymes *in vivo*. (Des-Asp[1])-angiotensin I has been shown to be a substrate for porcine-converting enzymes *in vitro* (7). Administration of the nonapeptide in the conscious rat produced pressor changes indistinguishable from those obtained with the heptapeptide. In addition, this pressor response could be significantly attentuated by pretreatment with the CEI, indicative of rapid conversion. Residual pressor effects could be due to intrinsic activity of the nonapeptide or incomplete inhibition of conversion. The latter explanation is more plausible since (des-Asp[1])-angiotensin I has very little direct biologic activity on the isolated rat uterus (12). In the rat, if (des-Asp[1])-angiotensin I is present in the circulation, it will serve as a substrate for coverting enzymes and therefore is a potential precursor of angiotensin III.

These *in vivo* studies provided evidence for the potential alternate pathway for the generation of angiotensin III and stimulated evaluation of (des-Asp[1])-angiotensin I on aldosterone biosynthesis. Sodium deprivation results in renin release, activation of aminopeptidases (6), and increased aldos-

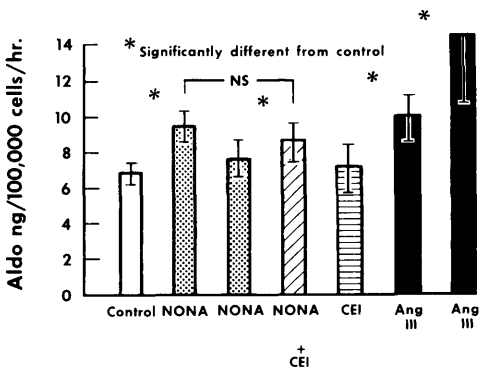


FIG. 3. Steroidogenic actions of [des-Asp[1]]-angiotensin I and angiotensin III in rat adrenal cell suspensions. Bars from left to right represent control (□); [des-Asp[1]]-angiotensin I $1 \times 10^{-6}$ and $1 \times 10^{-7}$ *M* (▨); [des-Asp[1]]-angiotensin I $1 \times 10^{-6}$ *M* plus *B. jararaca* nonapeptide $1 \times 10^{-6}$ *M* (▨); *B. jararaca* nonapeptide $1 \times 10^{-6}$ *M* (▤); angiotensin III $1 \times 10^{-9}$ and $1 \times 10^{-8}$ *M* (■). Each bar represents the mean ± SEM of 5-6 observations in different cell preparations. The * indicate $p < 0.05$.

terone biosynthesis which is resistant to blockade by angiotensin II antagonists (11). A role for angiotensin III-induced aldosterone biosynthesis in sodium deficient states is suggested by the finding that subcutaneous administration of (Ile[7])-angiotensin III partially decreased aldosterone biosynthesis (11). The present studies on steroidogenesis were performed *in vitro* so that accurate assessment of the direct steroidogenic activity of (des-Asp[1])-angiotensin I could be obtained. The aldosterone responses of cell suspensions show that (des-Asp[1])-angiotensin I has little capacity to stimulate steroid biosynthesis. However, the increase in aldosterone synthesis obtained with micromolar concentrations of angiotensin nonapeptide (0.1% of the steroidogenic effect of angiotensin III) represents direct activity since it was not inhibited by CEI.

The results of the present investigation support the proposed alternate pathway for the formation of angiotensin III from (des-Asp[1])-angiotensin I. Therefore, angiotensin III may be formed by the C-terminal hydrolysis of (des-Asp[1])-angiotensin I and/or the N-terminal degradation of angiotensin II.

*Summary.* (Des-Asp[1])-angiotensin I, angiotensin II and III were evaluated for pressor activities in conscious nephrectomized rats and for steroidogenic actions in rat adrenal zona glomerulosa. The pressor effect of this angiotensin nonapeptide was similar to that found with mole-equivalent doses of angiotensin III (one-third as active as angiotensin II) and was significantly attenuated by pretreatment with the *B. jararaca* nonapeptide converting enzyme inhibitor. Hence, (des-Asp[1])-angiotensin I is a substrate for converting enzyme *in vivo*, and the rapid conversion indicates that an alternate pathway for the formation of angiotensin III could exist. (Des-Asp[1])-angiotensin I possessed only 0.1% of the activity of angiotensin III as a steroidogenic agent in cell suspensions of rat adrenal zona glomerulosa. Angiotensin I was a weak steroidogenic agent *in vitro* (1%) and was not blocked by an inhibitor of converting enzyme. Adrenal cells dispersed from the outer zone of the cortex would appear to be devoid of significant converting enzyme activity.

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