Activity of [Des-Aspartyl[1]]-Angiotensin II and Angiotensin II in Man

DIFFERENCES IN BLOOD PRESSURE AND ADRENOCORTICAL RESPONSES DURING NORMAL AND LOW SODIUM INTAKE

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ABSTRACT This study was designed to compare the effect of [des-Aspartyl[1]]-angiotensin II ([des-Asp]-A II) and angiotensin II (A II) on blood pressure and aldosterone production in man under conditions of normal and low sodium (Na) intake. Seven normal male subjects in balance on constant normal Na intake ($U_{Na}V$ 160.3±5.0 meq/24 h) for 5 days received A II and [des-Asp]-A II infusions on two consecutive days; 1 mo later they were restudied after 5 days of low Na intake ($U_{Na}V$ 10.5±1.6 meq/24 h). Each dose was infused for 30 min, sequentially. During normal Na intake, [des-Asp]-A II from 2 to 18 pmol/kg per min increased mean blood pressure from 85.2±3 to 95.3±5 mm Hg and plasma aldosterone concentration from 5.2±1.1 to 14.3±1.9 ng/100 ml. During low Na intake, the same dose of [des-Asp]-A II increased mean blood pressure from 83.7±3 to 86.7±3 mm Hg and plasma aldosterone concentration from 34.4±6.0 to 51.0±8.2 ng/100 ml. In contrast, A II from 2 to 6 pmol/kg per min during normal Na intake increased mean blood pressure from 83.3±4 to 102.3±4 mm Hg and plasma aldosterone concentration from 7.0±2.2 to 26.8±2.0 ng/100 ml; during low Na intake, A II increased mean blood pressure from 83.0 ±3 to 96.0±4 mm Hg and plasma aldosterone concentration from 42.0±9.7 to 102.2±15.4 ng/100 ml. A II and [des-Asp]-A II were equally effective in suppressing renin release. Plasma cortisol and Na and K concentrations did not change.

The effects of two doses (2 and 6 pmol/kg per min) of each peptide on blood pressure and aldosterone production were evaluated. During normal Na intake, [des-Asp]-A II had 11–36% of the pressor activity and 15–30% of the steroidogenic activity of A II. Na deprivation attenuated the pressor response and sensitized the adrenal cortex to both peptides, but the increase in steroidogenesis was greater with [des-Asp]-A II than with A II. The dose-response curves for [des-Asp]-A II with respect to blood pressure and aldosterone production were not parallel, and although no maximum was established for A II, [des-Asp]-A II was less efficacious.

In summary, (*a*) [des-Asp]-A II has biologic activity in man, (*b*) [des-Asp]-A II is less efficacious than A II in stimulating aldosterone production, (*c*) Na deprivation sensitizes the adrenal cortex more markedly to [des-Asp]-A II than A II, and (*d*) dose-response curves for the two peptides differ, suggesting the possibility that they act at different receptor sites in vascular smooth muscle and the adrenal cortex.

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INTRODUCTION

In the years after the studies of Laragh (1, 2) demonstrating that angiotensin II (A II)[1] is a potent stimulator of aldosterone secretion in man, the renin-angiotensin-aldosterone cascade has become accepted as a coordinated system acting simultaneously to control blood pressure and sodium and potassium balance (3). A II is degraded into supposedly inactive fragments by the action of aminopeptidases, endopeptidases, and carboxypeptidases (4). Recently, however, the C-terminal hep-

[1] *Abbreviations used in this paper*: A II, angiotensin II; [des-Asp]-A II, [des-Aspartyl[1]]-angiotensin II; BSA, bovine serum albumin.

tapeptide fragment of A II, [des-Aspartyl[1]]-angiotensin II ([des-Asp]-A II) was found to be an agonist in that it has strong affinity for adrenal cortical receptors and stimulates aldosterone biosynthesis in vivo in the sheep (5), rat (6), rabbit (7), and dog (8) and in vitro in the rat, rabbit, cat, and dog (9, 10). On a molar basis, [des-Asp]-A II is as potent a steroidogenic agent in these species as A II. In addition, [des-Asp]-A II possesses approximately 15–25% of the pressor activity of A II (11, 12) and essentially equivalent ability to suppress renin release from the kidney (13). For these reasons, [des-Asp]-A II has been designated as "angiotensin III," a putative hormone of the renin-angiotensin system. This heptapeptide, [des-Asp]-A II, has been isolated and quantified from plasma of several species (14, 15).

Restriction of dietary sodium intake decreases the pressor response and increases the sensitivity of the adrenal zona glomerulosa to A II and [des-Asp]-A II (16). Recent studies have shown that adrenal cells from sodium-restricted rats increase maximal aldosterone production by 45% above cells from animals on normal sodium intake in response to either A II or [des-Asp]-A II. However, the increase in adrenal zona glomerulosa sensitivity to [des-Asp]-A II during sodium depletion is 10-fold greater than for A II (16). This observation suggests that [des-Asp]-A II may mediate (at least in part) the enhanced response of the adrenal zona glomerulosa to sodium depletion.

To date, in vitro data have been consistent with an action of A II and [des-Asp]-A II at the same receptor sites in the zona glomerulosa (17–19). However, a recent study (20) utilizing the rat uterus has shown nonparallel dose-response curves for A II and [des-Asp]-A II with [des-Asp]-A II displaying lower maximum efficacy. This suggests that the two peptides may act via different receptors.

The present study was undertaken to examine the effect of [des-Asp]-A II on blood pressure and aldosterone production in man. The study was designed to answer the following questions: (*a*) What is the effect of sodium deprivation on the pressor and steroidogenic responses to [des-Asp]-A II? (*b*), Do the dose-response curves of A II and [des-Asp]-A II differ from each other? To answer these questions, A II and [des-Asp]-A II were infused into normal male subjects maintained on normal and low dietary sodium.

METHODS

Human subjects and study protocol. Seven normal white male volunteer subjects, 24–30 yr, with normal arterial blood pressures and no history of renal disease were studied. The subjects were given a constant diet containing 150 meq of sodium, 60 meq of potassium, 1 g of protein/kg, and 2,860 calories/day for 5 days at the Clinical Research Center before the study. Consecutive 24-h urine samples were collected for the first 4 days of the diet and consecutive 12-h urine samples were collected for the remainder of each study and were analyzed for sodium, potassium, and creatinine. No food was given after midnight before study day 1 when the subjects assumed the supine position until completion of the study. At 6:15 a.m. on study day 1, the subjects completed their 12-h urine collections without arising. A heparin lock for obtaining blood samples was placed in the left antecubital vein and an intravenous infusion of 5% dextrose in water (D_5W) at 1 ml/min was begun in the right antecubital vein. At 6:30 a.m., blood pressure monitoring with an Arteriosonde (Medical Electronics Division, Hoffman- LaRoche Inc., Cranbury, N. J.) was begun and was continued every 2 min until completion of the study. At 7:00 and 7:30 a.m., control blood samples were obtained for the determination of plasma sodium, potassium, cortisol, and aldosterone. After completion of blood sampling at 0730 hours, an intravenous infusion of [des-Asp]-A II at either 2, 4, or 6 pmol/kg per min was begun. Thereafter, blood sampling was accomplished at 15-min intervals until completion of each study at 9:00 a.m. The heptapeptide, [des-Asp]-A II, was infused at three successively increasing dose levels ranging from 2 to 18 pmol/kg per min and maintained for 30 min at each dose level. The total duration of infusion for each subject was 90 min; blood samples for the estimation of plasma renin activity also were obtained at 0 and 90 min of infusion.

After completion of the infusion at 9:00 a.m., the subjects continued on the constant diet and 12-h urine collections. On study day 2, an identical protocol as for study day 1 was followed except that instead of [des-Asp]-A II, A II at 2, 4, and 6 pmol/kg per min (30 min at each dose level) was administered. The diet was discontinued, and the subjects were discharged from the Clinical Research Center after completion of the final 12-h urine collection at 6:30 p.m. on study day 2. The total volume of blood obtained from each subject during the two study days was 220 ml.

After an interval of 4 wk on an ad lib. diet, the same subjects were placed on a constant diet containing 10 meq of sodium, 60 meq of potassium, 1 mg of protein/kg, and 2,860 calories/day for 5 days at the Clinical Research Center before study. After sodium balance had been achieved, an identical protocol was carried out again at the Clinical Research Center with infusion of the same dose levels of [des-Asp]-A II and A II on the two consecutive study days.

Before and during angiotensin peptide infusions, blood pressure was monitored with an Arteriosonde automatic ultrasonic blood pressure recorder. A blood pressure cuff (with a width approximately two-thirds the width of the arm and a length such that the bladder completely encircled the arm) was wrapped snugly around the left arm. The Arteriosonde was calibrated daily against a random-zero mercury sphygmomanometer. Control blood pressures are expressed as the mean of 15 readings between −60 and −30 min (6:30–7:00 a.m.) and between −30 to 0 min (7:00–7:30 a.m. = zero control value) before initiation of the peptide infusions. During peptide infusions, blood pressure is expressed as the mean of seven readings for each 15-min period from 0 to 90 min of infusions (7:30–9:00 a.m.). The change in blood pressure is the difference between the zero control value and each 15-min infusion period. Written informed consent for these studies was obtained from all subjects. If the mean blood pressure increase during any peptide infusion was 20 mm Hg, the infusion was terminated.

Synthetic [des-Asp, Ile[5]]-A II was provided by Beckman Instruments, Inc., Fullerton, Calif. Synthetic [Asn[1], Val[5]]-A II (Hypertensin, Ciba) was purchased from Ciba Pharmaceutical Co., Div. CIBA-GEIGY Corp., Summit, N. J. Previous studies have shown no differences between val[5] and ile[5] analogues of [des-Asp]-A II and A II with respect to blood pressure, adrenal cortical aldosterone production, or

binding to adrenal cortical cells (5, 6, 12, 17). The [Asn^1, Val^5]-A II was not contaminated with [des-Asp]-A II as demonstrated by thin-layer chromatography in a system of butanol, acetic acid, water, and puridine. These peptides were diluted in sterile H_2O, filtered with 0.22-μm millipore filter (Millipore Corp., Bedford, Mass.), and stored at 4°C in sterile vials for 1–6 wk at a concentration of 50 μg/ml.

Animal in vivo and in vitro studies. Random aliquots of the sterilized preparations of the peptides infused into man were assayed subsequently for pressor activity in the anesthetized rat and steroidogenesis in adrenal cortical cell suspensions from rabbits and rats.

Male Wistar rats (200–300 g) were housed individually in metabolic cages in a temperature-controlled room (20–22°C) illuminated between 8 a.m. and 8 p.m. and were maintained on low sodium rat chow (General Biochemicals, Grand Island Biologicals, Chagrin Falls, Ohio) and 0.5% saline solution ad lib. to insure normal sodium intake.

A group of rats on normal sodium diet were anesthetized with intraperitoneal pentobarbital sodium (60 mg/kg). The iliac artery and femoral vein were cannulated with PE-50 intramedic tubing. A bilateral nephrectomy was performed, and the animals were allowed a 3- to 4-h equilibration period so that endogenous levels of renin and angiotensin were as low as possible without risking postnephrectomy changes in vascular reactivity. Arterial blood pressures were monitored through the arterial cannula via a Statham P23D pressure transducer (Statham Instruments Div. Gould Inc., Oxnard, Calif.) connected to a Brush Mark 220 recorder (Instrument Systems Division, Gould Inc., Cleveland, Ohio). Peptides were administered via the femoral vein in a 10-μl vol of 5% dextrose in water and flushed with 20 μl of 5% dextrose in water. A II, [des-Asp]-A II (Schwarz/Mann Div. Becton, Dickinson & Co., Orangeburg, N. Y.), sterilized [des-Asp]-A II (Beckman Instruments), and vehicle were administered in random order.

10 rats per group (five groups) were decapitated, and the adrenal glands removed rapidly. The medulla and inner cortex were removed from each gland, and the cells of the capsular layers were dispersed. The capsular tissue was minced and incubated in Krebs-Ringer bicarbonate buffer containing 0.2% D-glucose, 0.025% trypsin, 4% bovine serum albumin (BSA), and 0.005% deoxyribonuclease (1 ml buffer/20 mg tissue wet wt). After incubation for 15 min in a Dubnoff metabolic shaker under an atmosphere of 95% O_2–5% CO_2 at 60 rpm, the tissue was washed twice with Krebs-Ringer bicarbonate buffer containing 0.05% trypsin inhibitor, 0.2% D-glucose, 0.005% deoxyribonuclease, and 4% BSA (0.5 ml/20 mg tissue). Next, the tissue was dissociated by incubation in Krebs-Ringer bicarbonate buffer with 0.2% D-glucose, 0.02% collagenase, 0.05% trypsin inhibitor, 4% BSA, and 0.005% deoxyribonuclease (0.5 ml buffer/20 mg tissue) for 20 min in a Dubnoff metabolic shaker (10 rpm) under a 95% O_2–5% CO_2 atmosphere. The enzyme-treated tissue was dispersed by pipetting. The preparation was then incubated for 10 min, and the suspension was filtered through 150 μm^2 nylon mesh. The filtrate was centrifuged, and the pellet was washed three times and resuspended in Krebs-Ringer bicarbonate buffer with 0.2% D-glucose and 4% BSA. The number of viable cells present in the suspension was determined by exclusion staining with nigrosin and counting, using a Neubauer hemocytometer.

The cell suspensions were preincubated in the Dubnoff metabolic shaker for 30 min at 37°C in an atmosphere of 95% O_2–5% CO_2. All compounds were added to a 10-μl vol, and 10 μl of vehicle was administered to control. Incubations lasting 60 min were used to determine dose-dependent steroidogenic responses.

The adrenal glands of male New Zealand white rabbits (2–3 kg) that were killed by cervical dislocation also were used to prepare capsular adrenal zona glomerulosa cell suspensions in an identical manner as used for rat suspensions.

The incubations were terminated by adding 2 ml of 99% acetone to the experimental samples. The supernatant fraction was decanted and retained, and the pellet was resuspended in 80% acetone and centrifuged. The two supernatant fractions were pooled and evaporated to aqueous phase under a stream of N_2 at 40°C. Aldosterone was purified by extraction into dichloromethane and by Sephadex (Pharmacia Fine Chemicals, Piscataway, N. J.) LH-20 column chromatography and was estimated by radioimmunoassay.

Analytical methods. All blood samples were collected on ice, centrifuged immediately, and the plasma was separated and frozen until the time for assay. Samples for plasma renin activity and aldosterone utilized EDTA as the anticoagulant; heparin was used in the samples for cortisol.

Plasma sodium and potassium were measured by flame photometry (model 143, Instrumentation Laboratory, Inc., Lexington, Mass.). Aldosterone was measured by the radioimmunoassay method of Bühler et al. (21). After incubation, plasma renin activity was determined by radioimmunoassay of angiotensin I generated as described by Sealey et al. (22). Plasma cortisol was measured by the fluorometric method of Mattingly (23).

Statistical analysis. The results are expressed as mean±1 SE. Statistical analysis was carried out with the double-tailed Student's t test for paired data, and P values of <0.05 are considered significant.

RESULTS

[des-Asp]-A II and A II infusion studies in man

Characteristics of subjects on normal and low sodium intake. The characteristics of the subjects in sodium balance on the fifth day of normal (150 meq/day) and low (10 meq/day) sodium intake are summarized in Table I. Sodium restriction with iso-

TABLE I

Characteristics of the Subjects on Normal and Low Sodium Intake on the 5th Day of Constant Sodium Diet

	Sodium intake/24 h		
	150 meq	10 meq	*P* value
Weight, *kg*	75.5±2.2	75.3±1.8	NS
24 h urinary sodium, *meq*	160.3±5.0	10.5±2.5	<0.000001
24 h urinary potassium, *meq*	61.4±1.9	59.0±2.5	NS
Serum sodium, *meq/liter*	152.3±2.1	153.3±3.1	NS
Blood pressure, *mm Hg*			
Systolic	109.6±4.1	105.2±3.6	NS
Diastolic	73.4±3.1	73.0±2.1	NS
Plasma renin activity, *ng/ml/h*	1.84±.22	8.22±1.20	<0.001
Plasma aldosterone, *ng/100 ml*	5.3±1.2	34.4±6.6	<0.005

caloric intake was not associated with significant weight reduction. On each diet, 24-h urinary sodium output closely matched intake, and 24-h urinary potassium excretion was unchanged in the presence of a constant 60-meq/day potassium intake. In the process of achieving sodium balance on the low sodium intake, the subjects acquired a cumulative sodium deficit of 187±15 meq. Serum sodium and potassium concentrations were unchanged by dietary sodium intake. Systolic blood pressure tended to be slightly lower when the subjects were on low sodium intake. Restriction of dietary sodium intake was associated with a highly significant increase in plasma renin activity and aldosterone concentration ($P < 0.001$ and $P < 0.005$, respectively). A 4.5-fold rise in plasma renin activity was associated with a 6.5-fold increase in plasma aldosterone concentration.

Responses to [des-Asp]-A II infusion during normal sodium intake (Fig. 1). The [des-Asp]-A II infusion was associated with a series of reproducible responses. In the first 30 min of the control period, blood pressure was 111.0±4.5 mm Hg systolic and 72.4±2.6 mm Hg diastolic and was unchanged in the second 30 min of the control period, 109.6±4.1 mm Hg systolic and 73.4±3.1 mm Hg diastolic. [des-Asp]-A II infusion resulted in a rise in systolic blood pressure to 116.7 ±3.9 mm Hg ($P < 0.02$) within 15 min at 2 pmol/kg per min. However, further increase in the dose of [des-Asp]-A II to 6, 12, and 18 pmol/kg per min did not result in a statistically significant increase in systolic blood pressure from the initial (2 pmol/kg per min) dose. Diastolic blood pressure also increased to 76.7±3.0 mm Hg at 15 min of infusion, but did not achieve statistical significance until the dose was in-

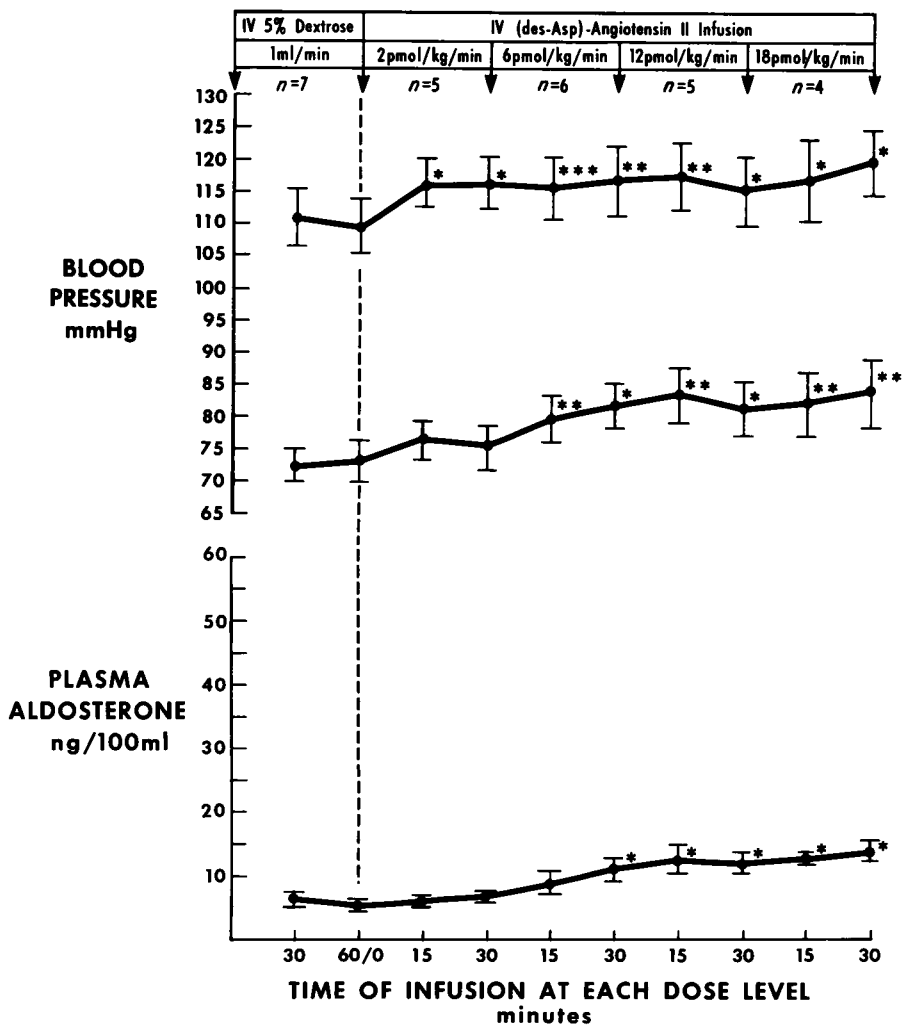

FIGURE 1 Blood pressure and aldosterone responses of subjects to [des-Asp]-A II infusion during normal sodium intake. Each subject received three cumulative doses of heptapeptide. *, values significantly different from zero control values ($P < 0.05$); **, $P < 0.01$); ***, $P < 0.001$.

creased to 6 pmol/kg per min at 45 min of infusion, when the diastolic blood pressure was 79.8±3.5 mm Hg ($P < 0.005$). At 60 min of infusion, when the subjects still were receiving 6 pmol/kg per min, the diastolic blood pressure was 82.2±3.6 mm Hg; additional increases in the dose of [des-Asp]-A II did not result in a further significant rise in diastolic blood pressure.

Plasma aldosterone concentration was 6.4±1.1 ng/100 ml at the end of the second 30-min control period. There was no significant increase in plasma aldosterone concentration until 60 min of infusion at a dose of 6 pmol/kg per min when plasma aldosterone concentration rose to 11.0±1.8 ng/100 ml ($P < 0.05$). Thereafter, plasma aldosterone concentration remained elevated, but did not increase significantly from 11.0 ng/100 ml with additional doses of [des-Asp]-A II.

Infusion of [des-Asp]-A II did not result in any change in plasma cortisol levels, which were 21.1 ±2.5 μg/100 ml and 18.6±2.4 μg/100 ml (P = NS) at the end of the two consecutive 30-min control periods, respectively. Plasma potassium concentration was not changed significantly by the administration of [des-Asp]-A II.

Plasma renin activity was 1.84±0.22 ng/ml per h at the end of the control period and was decreased to 0.97±0.19 ng/ml per h ($P < 0.001$) at 90 min of [des-Asp]-A II infusion.

Responses to [des-Asp]-A II infusion during low sodium intake (Fig. 2). In the first 30 min of the control period, blood pressure was 106.0±4.0 mm Hg

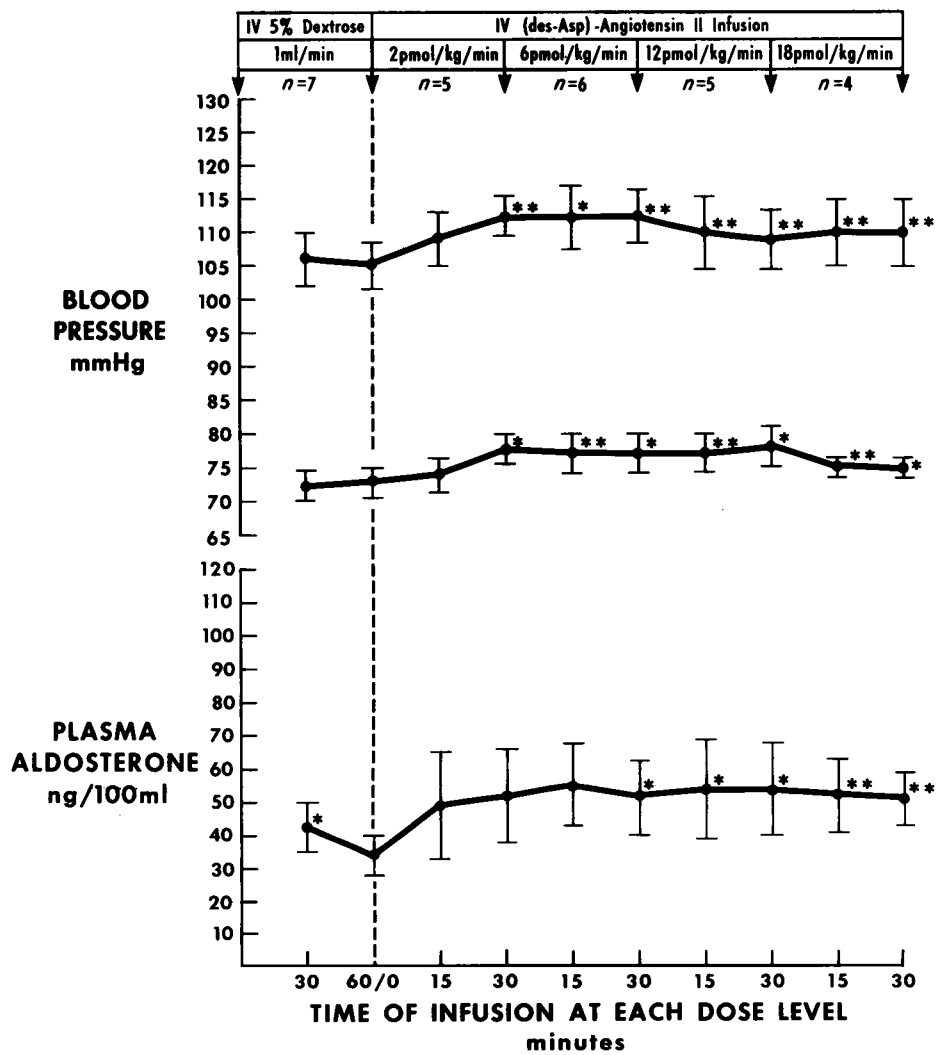

FIGURE 2 Blood pressure and aldosterone responses of subjects to [des-Asp]-A II infusion during low sodium intake. Each subject received three cumulative doses of heptapeptide. *, values significantly different from zero control values ($P < 0.05$); **, $P < 0.01$.

systolic and 72.4±2.2 mm Hg diastolic, and in the second 30 min of the control period was unchanged at 105.2±3.6 mm Hg systolic and 73.0±2.1 mm Hg diastolic. Infusion of [des-Asp]-A II again resulted in a rise in systolic blood pressure. This became statistically significant (112.4±3.3 mm Hg, $P < 0.005$) by 30 min of the 2-pmol/kg per min dose. Thereafter, with increasing doses and duration of infusion the increase in systolic blood pressure was sustained, but a distinct plateau occurred. Similarly, diastolic blood pressure increased to 77.8±2.5 mm Hg ($P < 0.02$) by the end of infusion of 2 pmol/kg per min and reached a plateau with time and increasing doses of [des-Asp]-A II. In spite of continued infusion with escalating doses of [des-Asp]-A II, neither systolic nor diastolic blood pressure increased significantly above the value obtained during the last 15 min of the 2-pmol/kg per min dose. The pattern of an initial rise in mean blood pressure followed by a plateau in response to [des-Asp]-A II was more apparent with sodium restriction than with normal sodium intake. In addition, with low sodium intake, the plateau of the elevated diastolic blood pressure occurred earlier than with the normal sodium diet.

Plasma aldosterone concentration was 42.7±7.6 ng/100 ml in the first 30 min of the control period and fell to 34.4±6.0 ng/100 ml ($P < 0.02$) in the second 30 min of the control period. With [des-Asp]-A II infusion, plasma aldosterone concentration increased within 15 min with an infusion of 2 pmol/kg per min to 48.7±16.5 ng/100 ml, and within 30 min to 52.1 ±14.0 ng/100 ml, when it reached a plateau. Because of the wide individual variation in control and infusion values, the increase in plasma aldosterone concentration became statistically significant only at 30 min of the 6-pmol/kg per min dose, and was statistically different from the control throughout the remainder of the infusion. There was no significant difference between the plasma aldosterone concentration at 30 min of the 2-pmol/kg per min dose and any value that was obtained subsequently.

Plasma cortisol concentration was 22.6±1.6 μg/100 ml at −30 min and 19.1±1.9 μg/100 ml ($P < 0.05$) at 0 min. During the [des-Asp]-A II infusion, plasma cortisol concentration did not change significantly. Plasma potassium concentration was 3.8±0.1 meq/l at −30 min and 3.7±0.1 meq/l (P = NS) at 0 min and did not change during the [des-Asp]-A II infusion period.

Plasma renin activity was 8.42±1.26 ng/ml per h in the control period and was decreased to 5.90±1.32 ng/ml per h ($P < 0.005$) by 90 min of [des-Asp]-A II infusion.

Responses to A II infusion during normal sodium intake (Fig. 3). In the first 30 min of the control period, blood pressure was 109.1±5.6 mm Hg systolic and 69.8±3.3 mm Hg diastolic, and in the second 30 min of the control period was 109.5±5.0 mm Hg systolic and 70.3±2.9 mm Hg diastolic (P = NS). Infusion of A II resulted in a rise in blood pressure at 15 min of the 2-pmol/kg per min dose to 117.2 ±2.8 mm Hg ($P < 0.01$). In contrast to the blood pressure response to [des-Asp]-A II, A II produced a stepwise increase in both systolic and diastolic blood pressure with time and with each increase in dose up to 6 pmol/kg per min when an increase of 15.2 mm Hg systolic and 20.6 mm Hg diastolic from base line was obtained.

Plasma aldosterone concentration was 8.7±2.5 ng/100 ml at −30 min and 7.0±2.2 ng/100 ml (P = NS) at 0 min. A II infusion resulted in an increase in plasma aldosterone to 11.7±1.7 ng/100 ml by 15 min of the 2-pmol/kg per min dose. Thereafter with time and higher dose of A II, plasma aldosterone concentration increased in a step-wise fashion to 26.8 ±2.0 ng/100 ml ($P < 0.005$) with 6 pmol/kg per min at the end of the infusion.

Plasma cortisol concentration was 20.7±3.6 ng/100 ml at −30 min and 18.3±3.4 ng/100 ml (P = NS) at 0 min. A II infusion did not alter plasma cortisol levels significantly at any time or dose. Similarly, plasma potassium concentration remained constant during the A II infusion.

Plasma renin activity was 2.06±0.28 ng/ml per h in the control period and was decreased to 1.08±0.09 ng/ml per h ($P < 0.01$) by the termination of the A II infusion.

Responses to A II infusion during low sodium intake (Fig. 4). In the control period, blood pressure was 106.1±4.4 mm Hg systolic and 70.8±2.9 mm Hg diastolic in the first 30 min and 107.5±4.3 mm Hg systolic and 70.9±2.3 mm Hg diastolic (P = NS) in the second 30 min. A II administration resulted in a rise in blood pressure to 113.5±4.8 mm Hg systolic and 78.0±2.7 mm Hg diastolic ($P < 0.05$) in the first 15 min of the 2-pmol/kg per min dose. Thereafter, with increasing time and peptide dose, blood pressure rose in a stepwise fashion. The maximum dose of 6 pmol/kg per min produced an increase from base line of 12.1 mm Hg systolic and 13.3 mm Hg diastolic at the end of the infusion.

Plasma aldosterone concentration was 43.3±7.6 ng/100 ml at −30 min and 42.0±9.7 ng/100 ml (P = NS) at 0 min. A II infusion resulted in a rise in plasma aldosterone concentration to 62.9±11.0 ng/100 ml at 15 min and 70.4±11.6 ng/100 ml ($P < 0.02$) at 30 min of the 2-pmol/kg per min infusion. Increasing doses of A II resulted in a dose-dependent increase of plasma aldosterone to 102.2±15.4 ng/100 ml ($P < 0.005$) at the end of the infusion.

Plasma cortisol concentration was 28.6±2.7 μg/100 ml at −30 min and 18.1 μg/100 ml ($P < 0.05$) at 0

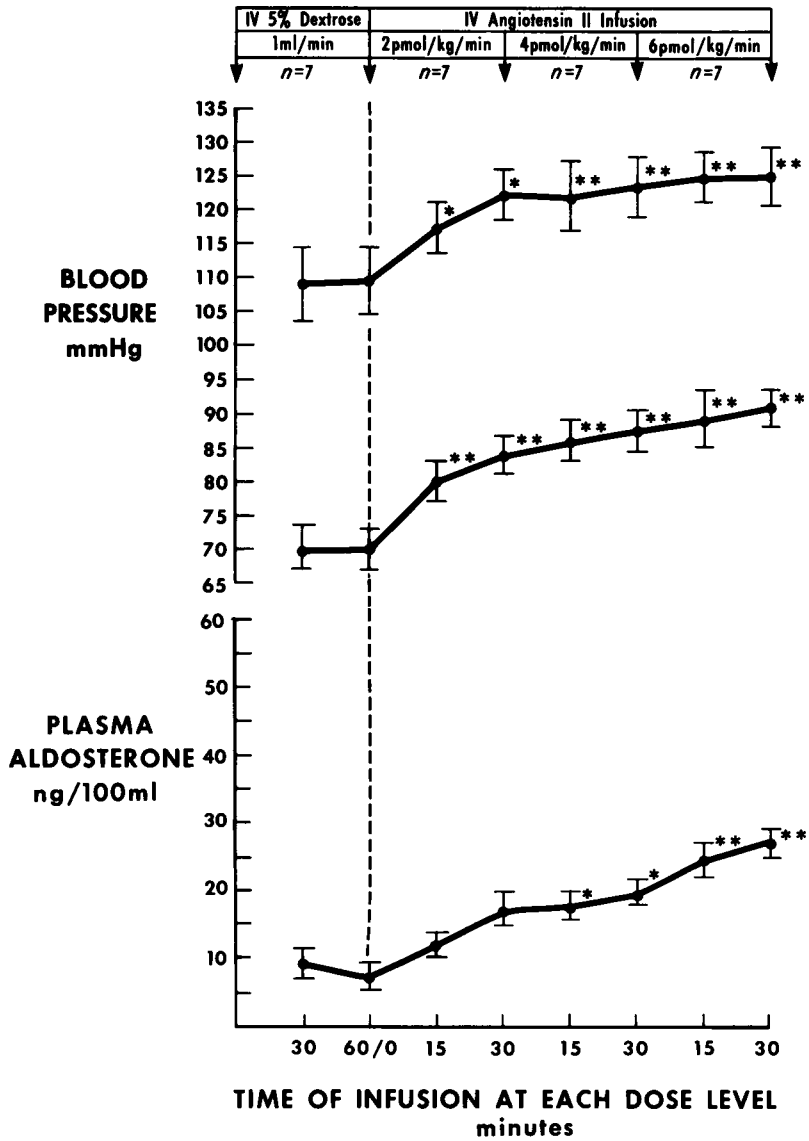

FIGURE 3 Blood pressure and aldosterone responses to A II infusion of subjects during normal sodium intake. Each subject received three cumulative doses of octapeptide. *, values significantly different from zero control values ($P < 0.05$); **, $P < 0.01$.

min. A II infusion produced no change (from base line) in plasma cortisol or potassium levels in sodium-depleted subjects.

Plasma renin activity was 9.40±1.05 ng/ml per h in the control period and was decreased to 4.87±0.68 ng/ml per h ($P < 0.001$) by the end of the A II infusion.

Comparison of the effects of the angiotensin peptides on blood pressure of subjects during normal and low sodium intake (Fig. 5). The effects of two doses (2 and 6 pmol/kg per min) of each peptide on blood pressure were evaluated. With normal sodium intake [des-Asp]-A II increased mean blood pressure by 1.1±1.9 mm Hg at the 2-pmol/kg per min dose and by 7.4±1.7 mm Hg at the 6-pmol/kg per min dose. In contrast, A II induced an increase in mean blood pressure of 10.3±1.8 mm Hg at 2 pmol/kg per min and of 20.4±1.0 at 6 pmol/kg per min. At the 2-pmol/kg per min dose of peptide, [des-Asp]-A II had 11% of the pressor activity of A II and at the 6-pmol/kg per min dose 36% of the pressor activity of A II.

With low sodium intake, [des-Asp]-A II increased mean blood pressure by 3.8±0.3 mm Hg at the 2-pmol/kg per min dose and by 4.1±0.5 mm Hg at the 6-pmol/kg per min dose. By comparison, A II induced

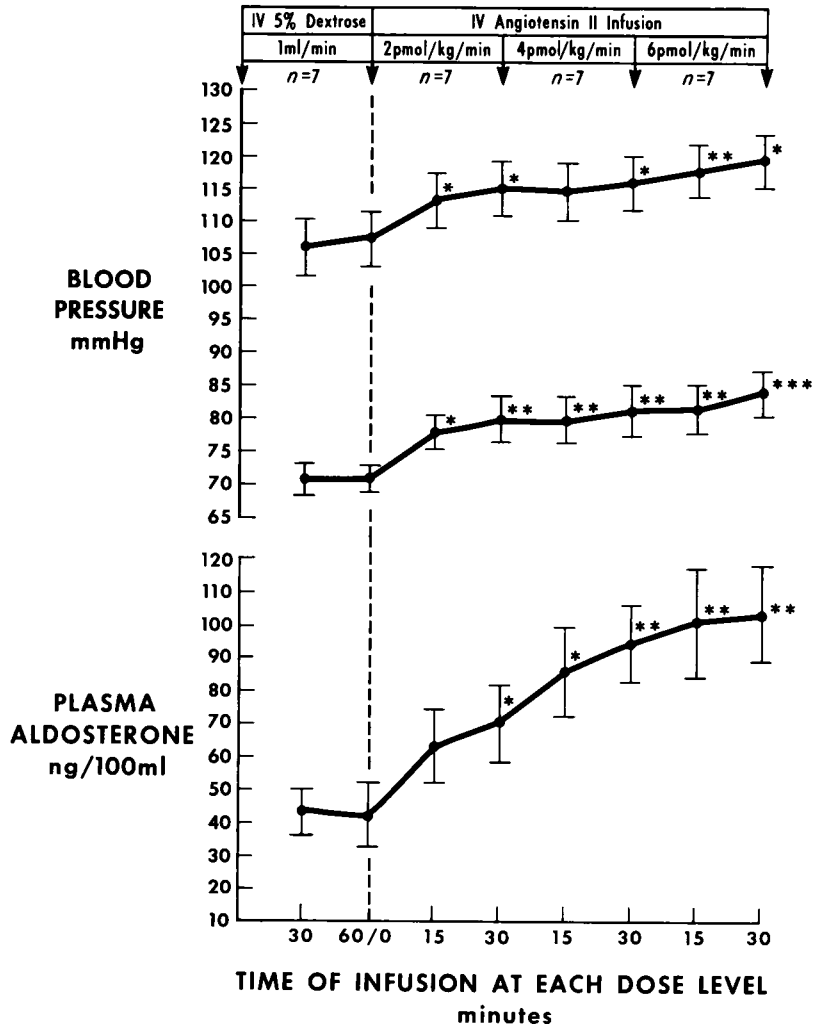

FIGURE 4 Blood pressure and aldosterone responses to A II infusion of subjects during low sodium intake. Each subject received three cumulative doses of octapeptide. *, values significantly different from zero control values ($P < 0.05$); **, $P < 0.01$, ***, $P < 0.001$.

a pressor response of 6.9±0.9 mm Hg at 2 pmol/kg per min and of 12.8±1.6 mm Hg at 6 pmol/kg per min. The dose-response curves at 2 pmol/kg per min of [des-Asp]-A II had 55% of the pressor activity of A II, whereas, at the 6-pmol/kg per min dose, [des-Asp]-A II had 32% of the pressor activity of A II.

At 2 pmol/kg per min of A II, low sodium intake decreased the pressor response induced by A II from 10.3±1.9 to 6.9±0.9 mm Hg ($P < 0.05$). At 6 pmol/kg per min with low sodium intake, the pressor activity of A II decreased from 20.4±1.0 to 12.8±1.6 mm Hg ($P < 0.005$). Low sodium intake also decreased pressor responsiveness to [des-Asp]-A II at the 6-pmol/kg per min dose level ($P < 0.05$) but did not attenuate pressor responsiveness to the 2-pmol/kg per min dose of [des-Asp]-A II.

Comparison of the effect of the angiotensin peptides on aldosterone production of subjects during normal and low sodium intake (Fig. 6). With normal sodium intake, [des-Asp]-A II increased plasma aldosterone concentration by 1.5±1.1 ng/100 ml at 2 pmol/kg per min and 5.8±2.0 ng/100 ml at 6 pmol/kg per min. A II increased the aldosterone concentration by 8.3 ±3.4 and 19.0±2.6 ng/100 ml at 2 and 6 pmol/kg per min, respectively. The heptapeptide was less efficacious than the octapeptide. At the 2-pmol/kg per min dose, [des-Asp]-A II had 15% of the steroidogenic activity of A II and at 6 pmol/kg per min [des-Asp]-A II had 30% of the aldosterone-stimulating activity of A II.

With low sodium intake, [des-Asp]-A II increased plasma aldosterone concentration by 17.7±8.6 and 17.8

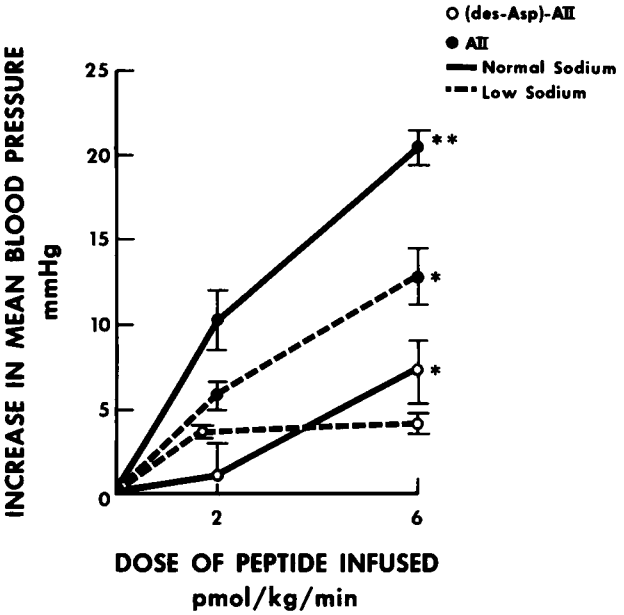

FIGURE 5 Comparison of the effect of the angiotensin peptides on blood pressure of subjects during normal and low sodium intake. *, values significantly different from values at 2 pmol/kg per min ($P < 0.05$); **, $P < 0.01$. During normal sodium intake, [des-Asp]-A II had significantly less pressor activity than A II at 2 and 6 pmol/kg per min ($P < 0.001$). During low sodium intake, [des-Asp]-A II also had less pressor activity and A II at each peptide dose ($P < 0.01$).

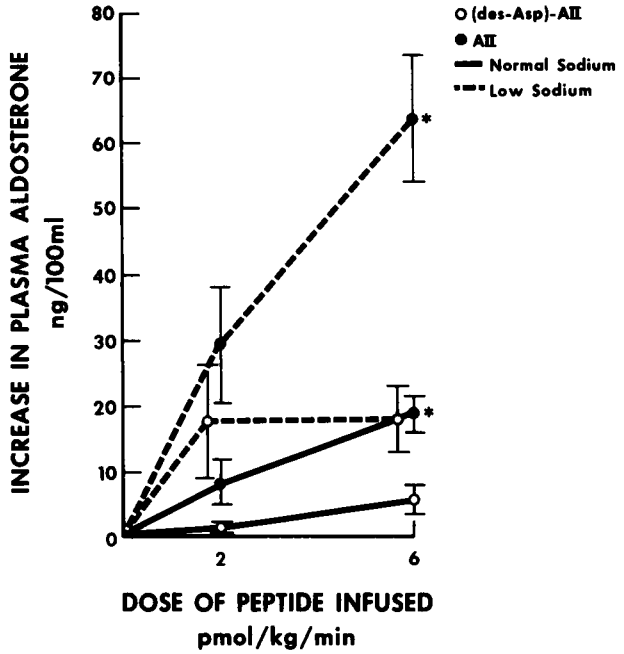

FIGURE 6 Comparison of the effect of the angiotensin peptides on aldosterone production of subjects during normal and low sodium intake. *, values significantly different from values at 2 pmol/kg per min ($P < 0.05$). During normal sodium intake, [des-Asp]-A II had significantly less aldosterone-stimulating activity than A II at both peptide doses ($P < 0.05$). During low sodium intake, the difference in aldosterone-stimulating activity in response to the 2-pmol/kg per min dose of the two peptides was not significantly different, but at 6 pmol/kg per min, A II had significantly greater activity ($P < 0.005$).

±5.1 ng/100 at the 2- and 6-pmol/kg per min dose levels, respectively. On the other hand, A II produced an increase in the aldosterone level of 31.7±8.7 and 63.5 ±9.7 ng/100 ml with the two doses, respectively. Low sodium intake increased the increment in aldosterone production induced by [des-Asp]-A II at both dose levels: 0.5±1.1 vs. 16.8±8.6 ng/100 ml ($P < 0.05$) at 2 pmol/kg per min and 5.8±2.0 vs. 15.9±5.1 ng/100 ml ($P < 0.05$) at 6 pmol/kg per min.

Sodium deprivation sensitized the adrenal zona glomerulosa to [des-Asp]-A II more than to A II at the 2-pmol/kg per min dose, when [des-Asp]-A II had 62% of the aldosterone-stimulating activity of A II. However, there was a plateau in the dose-response curve of the heptapeptide, so that [des-Asp]-A II had only 29% of the steroidogenic activity of the octapeptide at 6 pmol/kg per min. Low sodium intake increased the increment in aldosterone production in response to A II at the 2-pmol/kg per min dose level (8.3±3.4 vs. 29.6±9.3 ng/100 ml, $P < 0.05$) and at the 6-pmol/kg per min dose level (19.0±2.6 vs. 63.5±9.7 ng/100 ml, $P < 0.005$).

Animal in vivo and in vitro studies

Blood pressure responses to [des-Asp]-A II and A II preparations in nephrectomized rats. In rats maintained on a normal sodium diet, A II from 10 to 200 pmol increased blood pressure from 4 to 50 mm Hg. [des-Asp]-A II (Schwarz/Mann) and the sterilized preparation of [des-Asp]-A II (Beckman) used in the human studies displayed one-third of the pressor activity relative to A II. A II-induced blood pressure responses were significantly different from [des-Asp]-A II responses ($P < 0.05$). There was no significant difference in the blood pressure dose-response curves for [des-Asp]-A II (Schwarz/Mann) and the nonsterilized or sterilized preparations of [des-Asp]-A II (Beckman Instruments, Inc.).

Aldosterone responses to [des-Asp]-A II and A II preparations in adrenal cell suspensions. The threshold concentrations of A II and the Schwarz/Mann and Beckman preparations of [des-Asp]-A II in adrenal cell suspensions were identical at 50 pM. The maximal steroidogenic responses to [des-Asp]-A II and A II occurred at approximately 10 nM, respectively. Responses of adrenal cells to [des-Asp]-A II were greater than those to A II ($P < 0.05$) at 0.1, 0.5, and 1 nM concentrations of the peptides. There was no significant difference in the aldosterone dose-response

curves for [des-Asp]-A II (Schwarz/Mann) and the nonsterilized or sterilized preparations of [des-Asp]-A II (Beckman).

DISCUSSION

The present experiments demonstrate that [des-Asp]-A II has biologic activity in man. In normal subjects on normal sodium intake, [des-Asp]-A II in the dose range of 2–18 pmol/kg per min produced an increment of 30–250% in plasma aldosterone concentration and a rise in mean arterial pressure of 4.1–10.1 mm Hg.

In man, as in experimental animals, the present data indicate that [des-Asp]-A II has 20–30% of the pressor activity of A II. This corresponds with the results of a preliminary investigation by Kono et al. (24), who infused an approximately 10-fold higher single dose of the two peptides in man. We were surprised to find, however, that when cumulative doses were administered [des-Asp]-A II had only 15–20% of the steroidogenic activity of A II in subjects on normal sodium intake. This relationship of the aldosterone-stimulating activity of the two peptides differs from experimental animal studies (5–9) and from the results of Kono and co-workers, who found that [des-Asp]-A II was approximately equipotent with A II in the human adrenal cortex. With similar baseline plasma aldosterone concentrations in Kono's and our study, we achieved about the same increment in plasma aldosterone with 18 pmol/kg per min of [des-Asp]-A II as Kono et al. did with the administration of 22 pmol/kg per min. However, Kono used a much larger dose of A II (20 pmol/kg per min) than we did, producing a striking pressor effect which markedly exceeded our preset upper limit for the rise in blood pressure. His single sampling for plasma aldosterone was at 30 min, which may have been too early to observe maximum steroidogenesis (25). Inasmuch as we achieved about the same increase in aldosterone with only 6 pmol/kg per min of A II by 60 min of infusion as Kono obtained with 22 pmol/kg per min for 30 min, the most likely explanation for the discrepancy in results between the two studies is that aldosterone production in the response to A II was still increasing at the time of Kono's sampling but may have risen close to peak at that time with [des-Asp]-A II. Other factors that may also have contributed to the difference in results include differences in subjects or their ages, differences in sodium balance, which is not stated for Kono's subjects, differences in the methods of measuring aldosterone, or differences in the chemical purity of the heptapeptide preparation. This disparity between in vivo human and in vitro animal tests of relative potency in the adrenal zona glomerulosa which we have demonstrated may be a consequence of species differences or of more rapid in vivo degradation of [des-Asp]-A II than A II (26). The results suggest that in man under conditions of normal sodium balance, [des-Asp]-A II possesses appreciable aldosterone-stimulating activity, but less than that observed in other species such as the rat or rabbit. Further studies, including infusion of higher doses of both peptides initially and for a longer period of time, and comparison of the inhibitory effects of hepta- and octapeptide analogues on the cardiovascular system and adrenal cortex, will be required to amplify the results of this study.

Dietary sodium deprivation in man is followed by enhanced activity of the renin-angiotensin-aldosterone system as manifested by increased plasma renin activity and aldosterone secretion and excretion (1, 3, 27). In the present study, as reported by others (25, 27), vascular sensitivity to A II decreased significantly when sodium intake was restricted. The pressor response to infused A II in subjects on low sodium intake was reduced to 62–68% of the pressor response obtained on normal sodium intake. In contrast, however, low sodium intake increased the pressor activity of [des-Asp]-A II at the initial and lowest dose of 2 pmol/kg per min. However, with progressively higher doses of [des-Asp]-A II, we observed a progressive and significant decrease in vascular sensitivity during sodium depletion. At 18 pmol/kg per min of [des-Asp]-A II, the pressor response was reduced to 29% of the response in subjects on a normal sodium diet. These results, with the exception of the lowest dose, agree with previous descriptions of vascular responsiveness to [des-Asp]-A II in the presence of sodium depletion in the rat (16).

Recent studies suggest that the octapeptide and heptapeptide differ somewhat in their selectivity for vascular smooth muscle and adrenal cortex. Aldosterone biosynthesis induced by A II or [des-Asp]-A II is inhibited more readily by the heptapeptide analogue, [des-Asp^1, Ile^8]-A II, than by the octapeptide analogue, [Sar^1, Ile^8]-A II in vitro and in vivo (28). The octapeptide analogue, however, effectively inhibits vascular smooth muscle and blood pressure responses to both peptides (16, 29). In the present study, [des-Asp]-A II and A II administration under normal conditions of sodium balance resulted in predominance of vascular smooth muscle and adrenal cortical responses to the octapeptide. These results differ from those of animal studies showing that the heptapeptide displays greater affinity for the adrenal zona glomerulosa and, thus, for stimulation of aldosterone secretion. During sodium restriction in man, however, the adrenal cortex was sensitized more to the action of the heptapeptide than to the octapeptide at the initial dose of 2 pmol/kg per min, resulting

in approximately equivalent potency of the two peptides. These results are consistent with the hypothesis derived from animal studies that [des-Asp]-A II may mediate, at least in part, the aldosterone response to sodium restriction.

In our study, both A II and [des-Asp]-A II produced equivalent suppression of renin release in sodium-repleted and sodium-depleted normal subjects. This confirms the results of Freeman et al. (13, 30) in the dog and Steele and colleagues (7) in the rabbit. The mechanism of the inhibitory effect on renin secretion is not understood completely, but the data are consistent with feedback inhibition of the peptide at the juxtaglomerular apparatus (31, 32). A renal hemodynamic mechanism also is possible, but the marked differences in renal vasoconstrictor effects of the octapeptide and heptapeptide in the rabbit, dog, and isolated, perfused rat kidney (33) do not account for equivalent suppression of renin secretion by the two peptides. As well, it is unlikely that renin suppression is due to an effect of the peptides on sodium delivery to the distal tubule or sodium transport in the ascending limb of the loop of Henle because the degree of renin suppression is independent of changes in renal sodium excretion (7, 30). Because we did not assess renal hemodynamics and sodium excretion in response to [des-Asp]-A II, the contribution of these mechanisms to the observed renin suppression in man remains to be clarified.

The most striking finding of the present study is the difference in dose-response curves to A II and [des-Asp]-A II in man. In the sodium-depleted subjects, increasing doses of A II produce a stepwise increase in blood pressure and aldosterone production. The initial dose of [des-Asp]-A II results in a rise in blood pressure and aldosterone production. However, with progressively higher doses of [des-Asp]-A II, a plateau of blood pressure and aldosterone production occurs. A similar, less dramatic difference in dose-response curves with the two peptides also was observed in the sodium-repleted subjects. The dose-response curves are not parallel and although no maximum was established for A II, [des-Asp]-A II was clearly less efficacious. This difference in dose-response curves to A II and [des-Asp]-A II both in vascular smooth muscle and adrenal cortex suggests that the receptors for A II and [des-Asp]-A II in these tissues may be either anatomically or functionally different.

The in vivo difference in dose-response curves to A II and [des-Asp]-A II in man is consistent with dose-response relationships in certain in vitro preparations. For example, in the normal cat papillary muscle, the dose-response curves of [des-Asp^1, Val^5]-A II and [des-Asp^1, Ile^5]-A II for tension (T) and dT/dt are flat compared with the dose-response curves for A II (34). Recent studies in rat uterine smooth muscle also have shown nonparallel dose-response curves for A II and [des-Asp]-A II (20). The fact that [des-Asp^1, Ile^8]-A II and [Sar^1, Ile^8]-A II displayed different PA_2 values (negative log of the molar concentration of the antagonist which requires twice the concentration of agonist to induce the same response as in its absence) against A II than against the heptapeptide was taken as evidence that A II and the heptapeptide act upon different uterine receptors. This conclusion was supported by the absence of complete cross-tachyphylaxis: tachyphylaxis to A II blocked the response to the heptapeptide but tachyphylaxis to the heptapeptide did not attenuate the response to A II. However, another possible explanation for these findings is conversion of A II to heptapeptide by tissue aminopeptidases resulting in blockade of further stimulation by exogenous heptapeptide. Other evidence also has accumulated that A II and [des-Asp]-A II may act via different receptors in some tissues. Cycloheximide or aminoglutethimide, in concentrations that totally block ACTH- and A II-induced steroidogenesis in the rabbit adrenal cortex, have no appreciable effect upon [des-Asp]-A II-induced aldosterone biosynthesis (M. J. Peach, unpublished observations). However, A II and [des-Asp]-A II have been shown to act at the same receptor in the renal vascular bed of the dog (35). The availability of two angiotensin agonists, [des-Asp]-A II and A II, clearly demonstrating different blood pressure and steroidogenic dose-response relationships, offers a new approach to clarify whether or not a family of angiotensin receptors exists in man. Cross-tachyphylaxis experiments and comparison of the degree of inhibition of A II and [des-Asp]-A II by their respective analogues, when available, should provide fruitful avenues for future investigation.

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