Alterations in Plasma and Cerebrospinal Fluid Norepinephrine and Angiotensin II During the Development of Renal Hypertension in Conscious Dogs

HIROMICHI SUZUKI, M.D., CARLOS M. FERRARIO, M.D., ROBERT C. SPETH, PH.D., K. BRIDGET BROSNihan, PH.D., ROBERT R. SMEBY, PH.D., AND PREENIE DE SILVA, M.SC.

SUMMARY The levels of norepinephrine (NE), epinephrine (E), and angiotensin II immunoreactivity (AIIir) in plasma and in cerebrospinal fluid (CSF) were measured in eight conscious dogs before and during a 28-day period in the development of two-kidney, one clip (2K1C) hypertension produced by a two-step procedure. The early phase (< 7 days) of hypertension following partial constriction of the renal artery was accompanied by tachycardia and increases in concentrations of NE and AIIir in both plasma and CSF; E did not change. One week later blood pressure remained elevated (107 ± 2 after vs 88 ± 2 mm Hg before clipping, \( p < 0.05 \)), but other variables returned to control values. Occlusion of the partially constricted renal artery caused severe hypertension that was initially associated with a transient decrease in levels of NE in both plasma and CSF and a sustained rise in plasma and CSF concentrations of AIIir that persisted for as long as 2 weeks after the second operation. None of these effects was seen in nine sham-operated dogs. Since activation of the renal pressor system is associated with time-related changes in the concentrations of NE and AII in both plasma and CSF, these observations indicate early involvement of both sympathetic and renin-angiotensin systems in the pathogenesis of renovascular hypertension.

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KEY WORDS • renovascular hypertension • renin-angiotensin system • cerebrospinal fluid • catecholamines • brain angiotensin II • blood pressure • sympathetic nerve activity

MUCH has to be learned about the involvement of neuroendocrine factors in the pathogenesis of both renovascular and primary hypertension. It is not yet possible to ascertain whether alterations in the neurohormonal control of the circulation represent a triggering event, are subordinated to other unknown factor(s), or reflect an adaptive homeostatic response of the body to the stress imposed by hypertension.1 Emerging concepts2 indicate that many peptides may function to modulate neuronal communication both within and outside the central nervous system (CNS). Angiotensin II (AII) has now been classified as a neuropeptide because it is present in the brain and appears to play an important role in control mechanisms governing the function of the autonomic nervous system.3

Elucidation of the physiological mechanisms by which the neurohormone AII may interact with neurogenic factors requires characterization of the neurohormonal activity associated with the elevation and maintenance of high blood pressure. With this in mind, new procedures were developed to profile in both plasma and cerebrospinal fluid (CSF) the time course of the changes in the concentration of AII and catecholamines during the development phase of two-kidney, one clip (2K1C) hypertension in conscious trained dogs.

Methods

Animal Surgery

Seventeen mongrel dogs weighing between 18 and 25 kg were instrumented with a catheter placed into an iliac artery as described by Ferrario.4 Another catheter was placed in the cisterna magna to collect CSF from the spinomedullary region at regular intervals, as described by Suzuki and Ferrario.5 The device (fig. 1) was built from a short piece of PE-205 tubing (Intramedic, Becton, Dickinson and Company, Parsippany, New Jersey), shaped into a cup and fitted to one end of...
FIGURE 1. The cisterna magna catheter is shown after it has been secured to the atlantooccipital membrane with 000 silk sutures. A detailed view of the position of the catheter in relation to the upper spinal cord is shown in the insert.

a Silastic catheter (Silastic: 0.40 in. i.d., 0.085 in. o.d., Dow Corning, Midland, Michigan). At the point of attachment 1 sq in. of Dacron material (Dow Corning, Michigan) was fitted snugly at the base of the polyethylene cup. The catheter was placed in the cisterna magna by slitting the atlantooccipital membrane and positioning the flanged end into the subarachnoid space while the dog’s head was supported in a stereotaxic frame at a 45° flexing position. The free end of the catheter was tunneled through the subcutaneous tissue to the back of the neck. In this and other experiments we have been able to obtain 3 to 4 ml of CSF several times a week for as long as 10 weeks. At all times, aseptic procedures were used to prevent infection due to sampling. Only CSF samples showing less than 0.01% contamination of hemoglobin by Hemastix (Ames Company, Elkhart, Indiana) were used for the assay of catecholamines.

At the completion of the control period, an externally adjustable clamp was placed around the left renal artery to reduce its lumen by about 50%. Two weeks later it was tightened to occlude the vessel completely. The procedure was performed under light sodium thiopental anesthesia (10 mg/kg, intravenously). A renal arteriogram verified that the vessel was occluded. We have shown previously that partial constriction of a renal artery without contralateral nephrectomy does not cause prolonged hypertension in the dog because of the prompt development of collateral circulation. Occlusion of the renal artery 2 weeks after constriction produced renal ischemia sufficient to elevate blood pressure permanently. Renal atrophy did not occur because of the development of collateral vessels. Measurements were resumed 24 hours after occlusion and were continued for an additional 2 weeks.

Chronology of the Experiment

Throughout the experiment, dogs were fed a pellet diet (Purina Dog Chow) containing 65 mEq Na+/day; water was given ad libitum. At the completion of the training period, arterial blood pressure was recorded for 2 hours each day for 1 week before and 28 days after clipping of the renal artery. Details of the recording procedures and criteria for establishing baseline levels are reported elsewhere. Throughout the convalescence and experimental periods the animals were repeatedly evaluated by a full-time veterinarian. Nursing included giving antibiotics to prevent infection and taking frequent records of body temperature and weight.

Neurohormonal Measurements

Twice before and several times after production of 2K1C hypertension, samples of arterial blood and CSF were taken from these dogs to measure plasma renin activity (PRA) and plasma and CSF catecholamines and AII. Additional samples were collected for the determination of blood chemistry (BUN and serum creatinine) and plasma and CSF electrolytes. Blood and CSF samples for catecholamines were collected in tubes containing 20 μl/ml of EGTA (90 mg/ml) and glutathione (60 mg/ml), centrifuged at 4°C, and frozen until assayed at −70°C. Plasma and CSF levels of norepinephrine (NE) and epinephrine (E) were measured using a radioenzymatic assay. PRA was obtained by radioimmunoassay. AII was measured as described in the Appendix. Total proteins in CSF and plasma were measured using a Technicon (Tarrytown, New York) analyzer. Serum and CSF Na+ and K+ concentrations were assayed on a flame photometer (Instrumentation Laboratory, Lexington, Massachusetts).

Data Analysis

All data are expressed as means ± SEM. Analysis of variance for repeated measurements was performed using a BMDP2V program. To assess differences within a group, a randomized blocked analysis of variance was used. This was followed by Dunnett’s test for multiple comparisons. Comparisons between groups were analyzed by the Student’s t test for either paired or unpaired data. Linear regression analyses were calculated by the least squares method. Changes were considered to be significant at p < 0.05.

Results

In the 17 trained dogs, baseline mean arterial pressure (MAP) and heart rate (HR) averaged 88 ± 1 mm Hg and 77 ± 1 beats/min respectively. Control values for plasma NE and E averaged 228 ± 16 and 117 ± 15 pg/ml respectively. During the control period, the con-
concentration of NE in the CSF averaged 214 ± 18 pg/ml while E values were below the reliable detectability limit of the radioenzymatic assay [<1 pg/50 μl of fluid]. Measurements of PRA and AII, in both the plasma and CSF were obtained in each of the 17 dogs twice during the control period. For the group as a whole, PRA averaged 1.2 ± 0.1 ng/ml/hr while AII, was 15.2 ± 2.9 pg/ml (range: from nondetectable (ND) to 61.9 pg/ml) in plasma and 6.4 ± 1.3 pg/ml (range: ND to 25.0 pg/ml) in the CSF. Blood urea nitrogen and plasma and CSF total proteins and electrolytes were within previously reported normal ranges.

Two-kidney, one clip hypertension was produced in eight of these 17 dogs at the completion of a 1-week control period. In nine remaining dogs (sham, time control experiments), the clamp was placed around the renal artery (RA) but the vessel was neither constricted nor occluded. The time course of the changes in MAP, HR, and PRA during the first 14 days after partial constriction of the renal artery and for the 2 weeks that followed occlusion of the vessels are shown in figure 2. Renal artery constriction caused moderate hypertension (MAP: 107 ± 2 mm Hg, p < 0.05, 2nd week average) and a significant (p < 0.01) but temporary increase in HR. These hemodynamic changes were

![Graph showing changes in mean arterial pressure, heart rate, and plasma renin activity over time.](http://hyper.ahajournals.org/)

**Figure 2.** Time course of the changes in mean arterial pressure (MAP), heart rate (HR), and plasma renin activity (PRA) for 14 days after clipping of the renal artery (first stage) and 2 weeks following occlusion of the previously constricted vessel (second stage). Values are means ± 1 se from eight dogs developing 2K1C hypertension (broken lines and hatched bars) and nine sham-operated controls (solid lines and clear bars). Prior to constriction of the renal artery (RA), baseline values for MAP, HR, and PRA averaged 88 ± 3 mm Hg, 76 ± 2 beats/min, and 1.1 ± 0.2 ng/ml/hr respectively. Corresponding values for nine sham-operated controls are: MAP: 87 ± 2 mm Hg; HR: 81 ± 1 beats/min; PRA: 1.2 ± 0.2 ng/ml/hr. **= p < 0.01; *= p < 0.05.
accompanied by increases in PRA persisting for 7 days (fig. 2). A more pronounced increase in arterial pressure followed occlusion of the partially constricted renal artery (fig. 2). During the first 3 days after occlusion, MAP averaged 138 ± 2 mm Hg ($p < 0.02$); during the next week MAP stabilized at about 124 ± 3 mm Hg ($p < 0.02$). The abrupt and sustained hypertensive response was accompanied by significant bradycardia; 3 days after operation, HR averaged 58 ± 3 beats/min ($p < 0.01$). Toward the end of the experiment (24 to 28 days after clipping, fig. 2) HR became normal. None of these changes was seen in sham-operated animals. Development of 2K1C hypertension was associated with unaltered BUN, serum creatinine, and plasma and CSF proteins and electrolytes. However, 28 days after clipping of the renal artery (2nd week after occlusion) the CSF concentration of Na$^+$ was slightly increased (154 ± 2 vs 152 ± 2 mEq/liter in the control period, $p < 0.05$ by paired comparison) with no changes in the CSF content of K$^+$ (2.9 ± 0.1 after clipping vs 3.0 ± 0.1 mEq/liter before clipping, $p > 0.05$).

**Plasma and CSF Catecholamines**

Development of 2K1C hypertension produced significant changes in the concentration of NE but not E in

![Graph showing changes in plasma and CSF catecholamines](image)

**Figure 3.** Development of 2K1C hypertension was associated with transient changes in the concentration of NE in both the plasma and cerebrospinal fluid (CSF) compartments. Clear bars are means ± 1 SE of values determined in the sham operated group; hatched bars are values for the group of dogs developing renovascular hypertension. For the group subjected to renal hypertension, control values for plasma and CSF NE averaged 223 ± 23 and 220 ± 24 pg/ml respectively. In the sham group, baseline plasma and CSF norepinephrine (NE) was 219 ± 42 and 151 ± 34 pg/ml respectively. Comparisons of control baseline values between the two groups of dogs showed no significant differences at $p > 0.05$. Other $p$ values as in figure 2.
FIGURE 4. In the group of dogs developing 2K1C hypertension, the concentration of norepinephrine (NE) in plasma correlated weakly ($r = 0.40, p < 0.05$) with the corresponding values in the cerebrospinal fluid (CSF) collected from the cisterna magna. (First stage refers to clipping period; second stage includes measurement after occlusion of the renal artery).

Both plasma and the CSF obtained from the cisterna magna. Figure 3 shows that plasma and CSF NE levels were significantly increased 3 to 7 days after clipping of the renal artery and then markedly reduced on the 3rd day after renal artery occlusion. At this time the dogs displayed a combination of severe hypertension and marked bradycardia (fig. 2). Similar changes were not observed in sham-operated animals (figs. 2 and 3).

Although in the group of dogs subjected to renal hypertension plasma and CSF NE changed in the same direction, only a weak correlation ($r = 0.40, p < 0.05$), was found between plasma and CSF NE (fig. 4). On the other hand, the percentage change in plasma NE showed a stronger correlation with the percentage change in HR during both the clipping and occlusion period ($r = 0.61, p < 0.02$, fig. 5).

FIGURE 5. In dogs developing renovascular hypertension, percentage changes in heart rate (HR) proved to be related ($r = 0.61, p < 0.02$) to the percentage change in plasma NE. Labels are as in figure 4.
Plasma and CSF Angiotensin II Immunoreactivity

Figure 6 shows that the plasma and CSF concentrations of AII$_p$ increased transiently after clipping of the renal artery. Following occlusion of the vessel, concentrations of AII$_p$ were again increased, and the increases were sustained for the remainder of the experiment. After occlusion of the renal artery, the increases in MAP correlated significantly ($p < 0.01$) with the AII$_p$ measured in plasma ($r = 0.55$) and CSF ($r = 0.55$). Blood pressure also correlated with PRA ($r = 0.53$, $p < 0.01$). In addition, PRA correlated with the levels of plasma AII$_p$ ($r = 0.62$, $p < 0.01$) in both periods. Although PRA and CSF AII$_p$ were correlated only after occlusion of the vessel ($r = 0.48$, $p < 0.05$), there was no significant correlation between plasma and CSF AII$_p$ in either of the two periods.

Discussion

Measurements of AII$_p$ and catecholamines in plasma and CSF of conscious 2K1C hypertensive dogs provide a new insight into the contributions of sympathetic and renin-angiotensin systems to the pathogenesis of renal hypertension. The changes in arterial pres-

![Figure 6](http://hyper.ahajournals.org/)
NEUROHORMONAL CORRELATES OF RENOVASCULAR HYPERTENSION/Suzuki et al. I-145

sure, HR, and PRA shown in this study are similar to those described by Masaki et al.6-7 In addition, the present findings suggest exciting possibilities regarding the complex relationship between the sympathetic and renin-angiotensin systems.3,12

The multiplicity of investigative techniques we have used allows us to suggest that the onset of hypertension after clipping of a renal artery is accompanied by increased activity of the sympathetic nervous system as reflected by the occurrence of tachycardia and increases in plasma NE. The more abrupt rise in arterial pressure which accompanies occlusion of the previously constricted renal artery triggers a reflex decrease in HR and a reduction in sympathetic activity, as reflected by a marked decrease in plasma NE. As the heart rate normalizes in spite of sustained elevations in blood pressure, the concentration of neurotransmitter in both CSF and plasma returns to baseline values. The tachycardia that accompanies the early increases in blood pressure suggests that a central factor opposes the natural response of the baroreceptor reflex. A similar phenomenon has been observed by Machado et al.13 in rats developing one-kidney, one clip hypertension. On the other hand, the temporary fall in heart rate observed following occlusion of the renal artery suggests that there is an initial activation of the baroreceptor reflex with progressive resetting due to the elevated pressure.14 Circulating AIIr remained elevated, indicating that throughout the time course of these events the renin-angiotensin system is involved in the development of this form of hypertension. Levels of AIIr in blood rose, first transiently after clipping of the renal artery and remained elevated, for 2 weeks following its occlusion. As hypertension became chronic, we again6-7 observed a return of PRA to normal values even through plasma levels of AIIr were still elevated above baseline values. These data, therefore, provide additional support for the contention that measurements of PRA may be of limited value in estimating the actions of AII within the circulation.15 The increases in the concentration of plasma AIIr were accompanied by similar changes in the CSF; an observation that has not been reported previously, but is consonant with previous concepts regarding the cardiovascular actions of this peptide within the CNS.3,12

The average changes in the concentration of NE and AIIr within the CSF sampled from the cisterna magna were always in the same direction as those observed in the plasma. It has been shown before that these two substances may not cross the blood-brain barrier (BBB).16,17 Therefore their presence in the CSF has been attributed to be of brain tissue origin.18 In the present experiments, blood contamination of CSF samples due to either chronic seepage of blood at the site of catheter insertion or exudation of proteins as a result of an aseptic inflammatory response to the presence of the cisterna magna catheter was shown to be unlikely. Processed samples of CSF contained no hemoglobin as assessed by Hemastix. In both renal hypertensive and sham-operated animals, the CSF protein content remained within the low range described previously by Brosnihan et al.10 and also showed no tendency to increase with time. In addition, the values of CSF NE obtained in sham dogs throughout the course of the experiment and in the renal group during the control period are similar to those observed using a procedure that did not involve the insertion of a catheter into the cisterna magna.10

The composition of the CSF reflects the extracellular environment of the brain and may also provide a route for transport of neurohumoral factors to target sites within the CNS.19 It is possible that the CSF concentration of NE reflects the activity of brain-stem noradrenergic systems.10,20 Increases in CSF NE have been observed in hypertensive subjects20 but the interpretation of these findings is still open to question, because an increase in the permeability of the BBB secondary to the elevation in blood pressure could account for the increased NE in CSF. In this and other experiments,10 CSF E was present in trace amounts relative to plasma levels; moreover, the correlation between plasma and CSF NE was weak. These data militate against an increase in permeability of the BBB as the source for the transient changes in CSF NE documented in the current study. If we assume that CSF NE is not derived from the plasma pool, the rise in the CSF concentration of the neurotransmitter may reflect participation of CNS noradrenergic mechanisms in the pathogenesis of renal hypertension.

Clear evidence of the presence of AIIr in the CSF of animals developing experimental renal hypertension has long been awaited, partly because few laboratories have as yet employed a direct assay for AIIr and a relatively easy method to collect CSF from conscious dogs has just become available.3 Our experiments have shown that AIIr is not always detectable in the CSF obtained from the cisterna magna of conscious normal dogs. When AIIr was detected in CSF, the concentration was substantially less than that found in plasma. On the other hand, CSF AIIr was markedly elevated in dogs with renal hypertension. These experiments do not show what factor, or factors, account for the presence of AIIr in the CSF of the dog during both the initiation and established phases of renal hypertension. Frankmann et al.21 investigated whether CSF AIIr originated from the blood. In anesthetized rats AIIr was infused in doses as large as 1 μg/min for 30 minutes and plasma and CSF levels of the peptide measured by radioimmunoassay. Their data indicate that AII does not penetrate the CSF even when pressor doses of exogenous hormone produced enormous elevations in plasma values (AIIr 2,652 pg/ml). Cerebrospinal fluid contains significant amounts of angiotensinogen;22 moreover, in our laboratory Husain and Bumpus (unpublished observations) have shown that a peptide immunologically and pharmacologically indistinguishable from [Ile8] AII is generated by incubation of dog CSF with kidney renin. Similar findings were obtained by Hermann et al.23 in the rat. Since both angiotensin forming and converting enzymes have been shown to exist in brain tissue, choroid plexus and hypophysis,22 the CSF content of AIIr may represent tissue overflow,
CSF formation, or a combination of both. Although these findings are of significance with regard to the possibility that the brain renin-angiotensin system may have a causative role in the pathogenesis of hypertension, more detailed studies are needed before conclusions can be drawn. The lack of correlation between plasma and CSF AI\textsubscript{I} suggests that plasma is not the source of the peptide in the CSF. It is not known, however, whether functional or structural alterations in the BBB during the evolution of hypertension may account for the increase in CSF AI\textsubscript{I}. Even if we assume that the increased quantities of the peptide in the CSF are contributed by the plasma pool, it should not be forgotten that exogenous administration of AI\textsubscript{I} into brain ventricles triggers a variety of cardiovascular responses including relatively long term elevations in blood pressure. Conversely, intraventricular administration of either inhibitors or antagonists of the renin-angiotensin system evokes antihypertensive effects. Therefore the demonstration of elevated levels of endogenous AI\textsubscript{I} in the CSF of renovascular hypertensive dogs suggests evidence for the participation of this hormone in the evolution of this form of hypertension. In summary, these experiments have strengthened the hypothesis that AI\textsubscript{I} plays a role in the pathogenesis of renovascular hypertension by acting within the brain. This is the first demonstration of significant increases in the concentration of AI\textsubscript{I} in the CSF of conscious 2K1C hypertensive dogs. Moreover, these experiments have shed further light on the importance of neurogenic factors in the production of renal hypertension by demonstrating that the tachycardia that accompanies the early rises in blood pressure after clipping of the renal artery is associated with striking increases in the levels of NE and AI\textsubscript{I} in both plasma and CSF. On the other hand, the transient reduction in sympathetic nerve activity that followed the more severe elevation in arterial pressure after occlusion of the partially constricted vessel was reflected not only by the appearance of bradycardia but by a concurrent fall in levels of NE in both plasma and CSF. At this time, however, the levels of AI\textsubscript{I} were markedly elevated in both compartments. The latter finding indicates that the relationship between sympathetic activity and AI\textsubscript{I} may be more complex than has been previously thought.

**Appendix**

Several radioimmunoassay (RIA) procedures for the measurement of AI\textsubscript{I} in plasma have been described. In most cases it has been found necessary to separate the peptide from plasma proteins to avoid nonspecific interference with the antigen-antibody reaction. Recently, octadeclsilane reverse phase cartridges (C\textsubscript{18} Sep-Pak cartridges, Waters Associates, Massachusetts) have been used for the separation of vasopressin (VP) from plasma proteins prior to RIA of that peptide. Since this technique is simpler, faster, and possibly more reproducible for separation of the peptide from plasma proteins, we have applied it to the RIA of AI\textsubscript{I}.

For extraction of AI\textsubscript{I} from plasma with the Sep-Pak cartridge, we modified the method used for VP. Blood was collected in a prechilled syringe containing 0.05 ml of a solution containing 15% ammonium EDTA and 9.25 mM o-phenanthroline/ml blood. The sample was centrifuged at 3000 rpm for 20 minutes at 4°C and the plasma collected. If not assayed immediately the plasma was stored at -20°C. Before assay, 0.05 ml of an ethanol solution of phenyl methylsulfonyl fluoride (1 mg/ml) was added per ml plasma. The C\textsubscript{18} Sep-Pak cartridge was attached to a Luer Lock (Becton, Dickinson and Company, Rutherford, New Jersey) syringe and washed through with methanol (10 ml), tetrahydrofuran (10 ml), methanol (10 ml), water (10 ml), and 0.1 M Tris buffer (10 ml) with 15 mM EDTA and 0.1% lysozyme, pH 7.4. The plasma was then applied to the Sep-Pak, and washed through with distilled water (10 ml), and then 4% acetic acid (10 ml). The AI\textsubscript{I} was then eluted from the Sep Pak with 10 ml of ethanol-acetic acid-water (80:4:20). The eluate was adjusted to an apparent pH of 6.0 to 6.5 with ammonium hydroxide, the ethanol evaporated under a stream of nitrogen, and the remaining sample lyophilized. For RIA, the sample was redissolved in 0.1 M Tris buffer with 15 mM EDTA and 0.1% lysozyme, pH 7.4 (0.5 ml buffer/ml original plasma); then 0.2 ml was used for RIA. Since the concentration of protein is lower in CSF than in plasma, CSF samples can be assayed directly without extraction of peptide with the Sep-Pak. For CSF, 0.2 ml was directly assayed as though it were a processed plasma sample.

For RIA, standard and unknown samples were assayed in triplicate with two types of blank included in every assay. The first blank (a method blank) consisted of tubes containing 0.3 ml Tris buffer and 0.1 ml 125\textsuperscript{I}-AI\textsubscript{I} to measure nonspecific binding or incomplete charcoal adsorption. The second blank consisted of 0.1 ml Tris buffer, 0.1 ml of All-free processed plasma and 0.1 ml of 125\textsuperscript{I}-AI\textsubscript{I} to measure nonspecific binding due to processed plasma. The 125\textsuperscript{I}-AI\textsubscript{I} (New England Nuclear Corp., Boston, Massachusetts) was diluted with Tris buffer to give 6000 cpm per 0.1 ml. Each RIA assay tube contained either 0.2 ml of standard AI\textsubscript{I} in Tris buffer (0–200 pg) or 0.2 ml of unknown processed plasma sample, 0.1 ml of 125\textsuperscript{I}-AI\textsubscript{I}, and 0.1 ml of diluted antibody. The solution was incubated for 20 hours at 4°C; at the end of this incubation, 1 ml of dextran coated charcoal was added to each tube. The dextran coated charcoal suspension contained 250 mg charcoal and 25 mg dextran in 100 ml of 0.1 M Tris buffer, pH 7.4. The tubes were mixed with a vortex mixer, centrifuged at 4°C for 20 minutes, and the supernatant separated for counting of radioactivity.

Dr. Subha Sen obtained the antibody for this assay. The antiserum was prepared by immunizing a goat with AI\textsubscript{I} coupled to human IgG. This coupling was accomplished by adding 5 mg of AI\textsubscript{I} to 5 ml of human IgG (1 mg/ml), pH 5.0. Then 5 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide was added and stirred for 1 hour at room temperature. An additional 5 mg carbodiimide was added four times; stirring continued 1 hour after each addition. The solution
was dialyzed against 0.05 M phosphate buffer pH 7.4 in 0.9% NaCl for 24 hours. Each goat was immunized with 150 μg of coupled AII mixed with Freund’s adjuvant (total volume, 0.6 ml) intradermally at 3 week intervals. The first three immunizations were done with complete Freund’s adjuvant and the remainder with incomplete Freund’s adjuvant. Blood samples were taken at 3-week intervals for the determination of antibody titer. The antibody was used at a final dilution of 1:40,000 with an affinity constant of 0.9 × 10^11 liter/mol.

Both AII (heptapeptide) and AII (3-8 hexapeptide) showed 100% cross reactivity with AII antiserum, while the pentapeptide showed 35% cross reactivity. The following other substances were tested and showed no cross reactivity with the antibody: A1, renin substrate, [Ala^6] AII, Ile-His-Pro-Phe, His-Pro-Phe, Des Asp^1 Al nonapeptide, [Sar^1, Ile^8] AII, VP, desglycinamide deamino [Lys^8] VP, oxytocin, gastrin (human), substance P, methionine enkephalin, bradykinin, neurotensin, somatostatin, pressinon acid, captopril, and VP antagonist. An unexpected 0.01% cross reactivity was found when glucagon was tested. To study the linearity of the assay, two plasma samples (32.9 ± 1.8 pg/ml and 63.0 ± 1.6 pg/ml) were assayed at three different dilutions. No differences were seen at the various dilutions. Figure A-1 shows the standard curve for this assay, with AII dissolved in both buffer and a Sep-Pak extracted plasma sample. It is clear from this figure that interfering substances that may have been present in plasma have been removed by the Sep-Pak extraction procedure. The lower limit of measurement of AII is 1 pg. The addition of AII to plasma in the range of 10 to 100 pg/ml gave an average recovery of 98% (SD 10%), while the addition of a tracer quantity of [3H]-AII to plasma gave a recovery of 95% (SD 6%). The within-assay coefficient of variation averaged 5.13% and the between-assay coefficient of variation averaged 8.63% for more than 600 individual assay samples. With the inhibitors used, AII was found to be stable in whole blood for 60 minutes at 4°C, but after that time loss of peptide was noted.

The concentration of AII was found to be unaffected by hemolysis when the hemoglobin concentration was less than 0.1 mg/ml.

Since the antibody used in this work cross-reacts with AIII, the hexapeptide, and the pentapeptide, this assay measures all AII-like peptides in plasma which cross-react with the antibody. Thus, the results have been given as AU^1, free plasma.

The use of the adsorbant represents a significant improvement over previous methods.

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