Adrenal Medullary Stimulation Induced by Angiotensin I, Angiotensin II, and Analogues

By Michael J. Peach, Ph.D.

ABSTRACT
A structure-activity relationship study was attempted to determine the structural specificity of the no. 8 amino acid residue of [L-asp,5-ileu]-angiotensin II for adrenal medullary stimulation. Isolated cat adrenal glands were perfused retrograde at a flow rate of 1 ml/min with phosphate-buffered Locke's solution at 23 to 25 C. Continuous catecholamine analysis was performed using an automated trihydroxyindole procedure. All drugs were administered either by single injection (0.05 to 0.1 ml) or by perfusion. Angiotensin analogues studied were: [8-(OMe)tyr]-angiotensin II; [8-tyr]-angiotensin II; [8-ala]-angiotensin II; [5-ileu,8-(3-amino-4-phenylbutyric acid)]-angiotensin II (referred to as [8-APB]-angiotensin II); [5-ileu,8- (N-3-amino-3'-phenylisobutyric acid)]-angiotensin II (referred to as [8-APIB]-angiotensin II); [1-asp(NH 2),5-val]-angiotensin II; [1-asp,5-ileu]-angiotensin II; tetradecapeptide.

Three peptides (angiotensin II, [8-tyr]-angiotensin II, and [8-ala]-angiotensin II) were studied in vivo for stimulation of adrenal catecholamine secretion. For maximum pressor activity, angiotensin must be an octapeptide with an aromatic amino acid and free COOH group in the no. 8 position. None of these three are structural requirements in adrenal chromaffin tissue. There was cross-tachyphylaxis between angiotensin II and all the analogues studied except two ([8-APB]- and [8-APIB]-angiotensin II).

In vitro studies with 10-leu-C 14-angiotensin I and angiotensin II antibody indicated that angiotensin I has a marked, direct stimulatory effect on the adrenal medulla. Adrenal medullary catecholamine secretion induced by angiotensin I and II was greatly potentiated by DMAE perfusions. Responses to nicotine were blocked by DMAE and bradykinin-induced catecholamine release was unchanged. The cross-tachyphylaxis and DMAE studies suggest that several of the analogues and angiotensin II are interacting with a common adrenal medullary receptor.

KEY WORDS catecholamines peptides cross-tachyphylaxis DMAE receptors structure-activity studies

Angiotensin II has been reported to stimulate the adrenal medulla in vivo, in situ, and in vitro. Structure-activity relationship studies have also been reported with angiotensin II and adrenal catecholamine secretion; the activities of analogues in the adrenal were approximately equal to activities found with vasopressor assays. In two recent studies, several 8-substituted analogues of angiotensin II were found to have activities in the isolated perfused rabbit heart and isolated guinea pig ileum that were markedly different from their oxytocic and pressor activities. In view of these findings, it was of interest to expand the studies on structure-activity for angiotensin and adrenal catecholamine release.

In the present investigation, a structure-activity study was attempted in isolated, retrograde-perfused adrenals with C-terminus-substituted analogues of angiotensin II to...
determine the structural requirements for medullary stimulation. Some analogues were also studied in vivo for adrenal catecholamine release. Attempts were made to show that these analogues of angiotensin and the parent peptide interacted with the same adrenal medullary receptor.

Methods

Mongrel cats of either sex with weights ranging from 1.0 to 2.3 kg were anesthetized with intravenous pentobarbital-Na (30 mg/kg) and administered intravenous heparin-Na (500 units/kg). A midline incision was made from the base of the sternum to the pelvis and one or both adrenal glands exposed. Most experiments were performed with the left adrenal; however, some experiments were done with the right gland. Ligatures were placed around the adrenolumbar vein distal to the adrenal and between the adrenal and the inferior vena cava. A 27 G pediatric scalp vein infusion set needle (with 2 to 3 cm of cannula still attached) was inserted into the adrenolumbar vein distal to the gland and secured by ligature. The adrenal was rapidly excised and connected via the cannula to a Technicon AutoAnalyzer pump for retrograde perfusion. All extraneous tissue was dissected from the gland. Glands were perfused at room temperature (23 to 25 C) at a flow rate of 0.9 to 1.0 ml/min with phosphate-buffered Locke's solution equilibrated with oxygen.

The effluent from the gland was collected in a 4-ml reservoir and continuously analyzed for catecholamines at pH 7.0 by the automated trihydroxyindole procedure of Robinson and Watts.8 The analytical procedure for total catecholamines and differential estimation of epinephrine and norepinephrine was as described by Robinson.3 All adrenal glands were allowed to reach a constant level of spontaneous catecholamine output (usually about one hour) before any drugs were administered.

Locke's solution was the vehicle for all drugs injected in vitro, and all injection volumes were 0.05 to 0.1 ml. Injections were made with 27-28 G needles through surgical tubing connecting the perfusion line to the gland. None of the peptides administered had any effect on the fluorometric perfusion line to the gland. None of the analogues of angiotensin and the parent peptide interacted with the same adrenal medullary receptors.

To determine whether adrenal medullary stimulation induced by angiotensin I was direct or indirect (via conversion to angiotensin II), studies were performed with 10-Leu-C14-angiotensin I. Radiochemical purity of stock labeled peptide was 93%, with a specific activity of 2.5 X 106 dpm/μg. An Amicon Diaflo filter no. UM05 with a molecular weight cutoff of 500 was used to filter C14-angiotensin I from solution. Concentrations of 10-9 to 10-8 moles C14-decapeptide in 10 to 20 ml of oxygenated Locke's solution and 10-4 M ethylenediaminetetraacetate (EDTA) were placed in a 50-ml Diado chamber (23 to 25 C and 50 psi) and filtered. Filters were collected at 15 and 30 minutes and 1-ml aliquots were added to 15 ml of scintillation cocktail (POP, POPPOP, 2:1 toluene-Triton X-100) and counted (counting efficiency 95% to 96%). To establish if passage of the dipeptide (his-C14-leu) through the UM05 filter occurred, C14-decapeptide (3-amino-4-phenylbutyric acid) angiotensin II (referred to as [8-APIB]-angiotensin II); [5-Leu,8-(3-amino-4-phenylbutyric acid)]-angiotensin II (referred to as [8-APIB]-angiotensin II); [l-asp (NH),5-val]-angiotensin II; [1-asp,5-leu]-angiotensin II; [1-asp,5-ileu]-angiotensin I; tetradecapeptide (asp-arg-val-tyr-leu-ile-his-pro-phe-hisleu-leu-val-tyr-val). Nicotine (10-4 to 3 X 10-4 moles) was administered periodically throughout an experiment as a nonpeptide stimulant of adrenal catecholamine secretion.

For the in vivo experiments, cats were anesthetized with intravenous pentobarbital-Na (30 mg/kg). The left femoral artery and vein were cannulated for monitoring arterial blood pressure and drug administration, respectively. A flank incision was made about 5 cm below the rib cage on the left side. The adrenolumbar vein was cannulated distal to the adrenal gland and the vein ligated between the gland and the inferior vena cava. After cannulation of the adrenolumbar vein, heparin (1,000 units/kg) was administered intravenously. Blood from the adrenal cannula was returned to the animal via the femoral vein except when samples were obtained. Blood samples were obtained every two minutes for a total duration of eight minutes following the administration of peptides. Control adrenal blood samples were taken five minutes before a peptide injection. Peptides studied were angiotensin II (0.25 to 0.50 μg/kg), [8-tyr]-angiotensin II (0.5 μg/kg), and [8-ala]-angiotensin II (2.0 μg/kg). Adrenolumbar blood samples were centrifuged and plasma catecholamines removed by alumina column chromatography. Differential analysis of plasma catecholamine column eluates was performed by the method of Robinson and Watts.8 Column recoveries of added catecholamines were 70% to 75%. The data are not corrected for the 20% to 25% amine lost during these procedures. Column recoveries of added catecholamines were 70% to 75%.
Hypertension I (10⁻⁹ to 10⁻⁸ moles) was incubated at room temperature with 10 to 20 ml of cat plasma. Incubation was terminated after 30 minutes by addition of EDTA (final concentration 10⁻³ M) and the samples were then placed on ice. Incubates were filtered at 4°C and filtrates sampled and counted as described above. Retrograde-perfused adrenals were injected with C¹⁴-angiotensin I and the effluent from the gland collected in EDTA. The effluent was collected for 15 or 30 minutes after injection of C¹⁴-tetradecapeptide, filtered, sampled, and counted.

Another method of approach utilized the specificity of antigen-antibody interaction. Antibody to angiotensin II prepared by immunizing rabbits with [1-asp,5-ileu]-angiotensin II coupled to bovine albumin by the carbodimide method of Goodfriend et al. was used.* Antibody alone, or in combination with angiotensin II or angiotensin I, was injected to the adrenal gland in vitro and secretion of catecholamines determined. The antibody had no effect on the fluorometric assay for catecholamine. It was experimentally determined by dog and rat blood pressure assay that 1 ml of antibody neutralized 2 μg of angiotensin II.

In some experiments, angiotensin was added to oxygenated Locke's solution (final concentration 10⁻² to 10⁻⁴ moles/min) and continuously perfused through the adrenal for 15 to 30 minutes to induce tachyphylaxis. If responses to nicotine were not the same before and after induction of angiotensin tachyphylaxis, the preparation was discarded. Peptides were studied for cross-tachyphylaxis with angiotensin II in the retrograde-perfused adrenal.

Studies on the specificity of the potentiation of angiotensin-induced adrenal catecholamine release produced by a,a'-bis-(dimethylammonium-acetaldehyde diethylacetal)-p,p'-diacetylbiphenyl bromide (DMEA) were performed. Peptides and nicotine were studied in the isolated adrenal before and during the administration of DMEA via perfusion (100 μg/min).* Statistical analysis of data was performed using Student's t-test.

**Results**

The effects of angiotensin II and analogues on total catecholamine secretion from the isolated adrenal are shown in Figure 1. All the analogues studied induced adrenal medullary secretion. Tetradecapeptide and [8-APIB]-angiotensin II produced an increase in catecholamine release at 10⁻⁸ moles (P<0.03). At 10⁻⁶ moles or greater doses, [8-APIB]-angiotensin II was more active than tetradecapeptide (P<0.01). The following peptides induced significant catecholamine secretion at a dose of about 5×10⁻⁷ moles (P<0.05): [8-ala]-angiotensin II; [8-APB]-angiotensin II; [8-tyr]-angiotensin II; [8-OCH₃-tyr]-angiotensin II. The amount of angiotensin I or II required for adrenal medullary stimulation was about 10⁻⁵ moles (P<0.01). Catecholamine release produced by angiotensin I was not different from responses to angiotensin II.

*Various compounds and chemicals were supplied by Schwarz BioResearch (angiotensin I, angiotensin II, 10-leu-C¹⁴-angiotensin I, and bradykinin); Eastman Organ (nicotine and EDTA); Winthrop Laboratories (epinephrine and norepinephrine bitartrate); and British Drug House (alumina).
Effects of angiotensin II and analogues on total catecholamine secretion from the adrenal medulla in vivo. Each point is the mean of five determinations. Catecholamine levels indicated at zero time represent control adrenal output prior to intravenous peptide injection and time in minutes reflects time after peptide administration. Peptides studied and concentrations injected are shown at the top of the figure.

However, the other six analogues were less active than angiotensin II at all doses studied (P < 0.01). At an injection level of 10^-8 moles or greater, [8-ala]-angiotensin II was more potent than [8-APIB]-angiotensin II (P < 0.01) and less potent than [8-APB]-angiotensin II (P < 0.01). The two tyr-substituted analogues were more active than [8-APB]-angiotensin II at doses greater than 5 x 10^-8 moles (P < 0.01). The activities of each peptide in the adrenal medulla relative to angiotensin II were: tetradecapeptide, 53%; [8-APIB]-angiotensin II, 78%; [8-ala]-angiotensin II, 22%; [8-APB]-angiotensin II, 43%; [8-tyr]-angiotensin II, 70%; [8-OCH3-tyr]-angiotensin II, 75%; angiotensin I, 98%. These percentages of relative activity can be contrasted with those for pressor activity which are shown in Figure 1 after the name of each peptide.

In vivo adrenal catecholamine secretion induced by angiotensin II and two of the analogues studied in vivo are presented in Figure 2. Mean plasma catecholamine levels were significantly elevated for five minutes postinjection (P < 0.01). The greatest increase in adrenolumbar venous catecholamine levels occurred at two minutes after intravenous administration of the peptides. By six minutes after injections, the induced elevation of plasma catecholamine levels had returned to control levels. The analogues ([8-tyr]-angiotensin II and [8-ala]-angiotensin II) were less potent than angiotensin II in vivo, and their relative activities were 75% and 25%, respectively.

A differential analysis of epinephrine and norepinephrine secretion from the isolated adrenal in response to [L-asp, 5-ileu]-angiotensin I or II and [L-asp(NH2), 5-val]-angiotensin II is shown in Figure 3. The ratio of epinephrine:norepinephrine in the spontaneous secretion from the glands was 78 ± 4.3% ± 3.8% and did not differ between the left and right adrenal glands. This ratio of epinephrine:norepinephrine was approximately 75%: 25% during an induced secretory response with any of the peptides studied regardless of the dose administered or whether the left or right gland was studied. Over the range of doses shown in the figure, there was no difference in induced secretion of catecholamines obtained in response to injections of angiotensin I, angiotensin II, or angiotensin II-amide.

Studies with 10-leu-C14-angiotensin I are summarized in Figure 4. Diaflo UM05 filters were studied for effectiveness in filtering C14-angiotensin I from Locke's solution. This filter effectively blocked the passage of decapeptide at all three concentrations studied (10^-9 to 10^-5 mmoles). About 10% of the C14 activity added passed through the filter. After 30 minutes' incubation with plasma converting enzyme at 25 C to split off the dipeptide (his-C14-leu), radioactivity readily filtered through the 500-molecular-weight filter. Approximately 90% of the C14 activity added to plasma was recovered in the filtrate from the 30-minute incubate. Effluents collected from the adrenal
after injection of C<sup>14</sup>-angiotensin I were also filtered. Filterable C<sup>14</sup>-peptide in these effluents was not greater than in control experiments. About 85% to 90% of the C<sup>14</sup>-angiotensin I administered to the perfused adrenal was present in the effluent collected. The remaining 10% was sequestered by the gland. Even if one assumes that all the radioactivity retained in the adrenal represents histidyl-C<sup>14</sup>-leucine, this would indicate minimal intra-adrenal conversion of angiotensin I. A 10% conversion could not possibly explain the adrenal medullary stimulation induced by angiotensin I.

A representative AutoAnalyzer record from an experiment with antibody for [1-asp,5-ile]-angiotensin II is presented in Figure 5. All injections were given at 20-minute intervals in a volume of 0.1 ml. The doses of peptides administered to the perfused adrenal were 10<sup>-8</sup> moles of angiotensin I and 10<sup>-6</sup> moles of angiotensin II. The slight response shown with the administration of antiserum alone represents an effect of antibody on fluorescence and not the release of adrenal catecholamines. Angiotensin II or angiotensin I and antibody were mixed in a syringe and the mixture injected to the adrenal. Release of

**Figure 3**

In vitro release of epinephrine and norepinephrine (NE) from the adrenal in response to injection of [1-asp,5-ile]-angiotensin I and II and [1-asp(NH<sub>2</sub>),5-ile]-angiotensin II. Each point represents the mean of 8 to 10 observations. The solid line depicts total catecholamine secretion; the broken lines, norepinephrine secreted. The difference between total catecholamines and norepinephrine equals epinephrine released.
of 10-Leu-C14-angiotensin II in Locke's solution, plasma incubates, and perfused adrenal effluents. The solid bars at the top of the figure represent activity in dpm for C14-decapeptide (10^-2 to 10^-4 moles) added to the filtration system. The dotted bars at the top represent C14-angiotensin I administered to the adrenal gland. The bottom part of the figure depicts radioactivity that passed through the 500-molecular-weight cutoff (Diaflo UM05) filter. The hatched bars at the bottom represent filterable C14 in adrenal perfusion effluents. Each bar represents the mean of 8 to 10 observations.

Figure 6 is a representative record from a cross-tachyphylaxis study. Stimulation of adrenal catecholamine secretion was induced by injections of angiotensin II, angiotensin I, angiotensin II-amide (10^-9 moles), and nicotine (10^-4 moles). Tachyphylaxis was produced by exposing the gland to perfusions of angiotensin in high concentrations (10^-2 to 10^-4 moles/min) for 15 to 30 minutes. Tachyphylaxis could be induced by perfusion of either of these peptides. After a peptide perfusion there was cross-tachyphylaxis be-

catecholamines induced by angiotensin II was blocked by angiotensin II antibody. Adrenal medullary responses to angiotensin I were identical in the presence or absence of angiotensin II antiserum. All glands still responded to angiotensin I and II administration after washout of antibody or antibody-peptide complexes. In 12 experiments the antibody totally blocked responses to angiotensin II (10^-11 to 10^-8 moles), but had no effect on responses to angiotensin I (10^-11 to 10^-8 moles).
AutoAnalyzer record of fluorometric estimations of total catecholamines secreted from the retrograde-perfused adrenal in response to injections of angiotensin. The dose of angiotensin I administered was $10^{-9}$ moles, angiotensin II, $10^{-8}$ moles. Antiserum used was for [(I-asp,5-due)]-angiotensin II, and 1 ml of antiserum blocked pressor responses to $2 \times 10^{-6}$ moles of angiotensin II. Antiserum and peptides were mixed in a microsyringe (total volume, 0.1 ml) and injected to the gland; the interval between injections was 20 minutes.

Effects of adrenal medullary tachyphylaxis induced by [(I-asp,5-due)]-angiotensin II on responses to [(I-asp,5-deu)]-angiotensin II and nicotine. Total catecholamine secretion is shown on the ordinate. Injections were given at 20-minute intervals. The peak response occurred in 30 to 60 seconds and total response durations were 3 to 4 minutes. The first four responses are before production of tachyphylaxis and the last four after a 30-minute perfusion of $10^{-7}$ moles/min [(I-asp,5-deu)]-angiotensin II.
Effects of DMAE Perfusion on Adrenal Medullary Catecholamine Secretion Induced by Nicotine, Bradykinin, and [L-asp,6-ileu]-Angiotensin I and II

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration injected</th>
<th>Locke's solution total catecholamines (ng)</th>
<th>DMAE (100 µg/tube) total catecholamines (ng)</th>
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</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td></td>
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<tr>
<td>0.5 µg</td>
<td>128 ± 18</td>
<td>0*</td>
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<tr>
<td>1.0 µg</td>
<td>400 ± 25</td>
<td>0*</td>
<td></td>
</tr>
<tr>
<td>10.0 µg</td>
<td>990 ± 55</td>
<td>0*</td>
<td></td>
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<tr>
<td>Bradykinin</td>
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<tr>
<td>0.5 µg</td>
<td>475 ± 20</td>
<td>450 ± 26</td>
<td></td>
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<tr>
<td>1.0 µg</td>
<td>825 ± 70</td>
<td>775 ± 85</td>
<td></td>
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<tr>
<td>Angiotensin II</td>
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<tr>
<td>0.0 ng</td>
<td>450 ± 25</td>
<td>600 ± 105*</td>
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<tr>
<td>10.0 ng</td>
<td>850 ± 55</td>
<td>3150 ± 200*</td>
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<tr>
<td>50.0 ng</td>
<td>1500 ± 100</td>
<td>1380 ± 95</td>
<td></td>
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<tr>
<td>Angiotensin I</td>
<td></td>
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<tr>
<td>5.0 ng</td>
<td>330 ± 20</td>
<td>830 ± 105*</td>
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<tr>
<td>10.0 ng</td>
<td>790 ± 40</td>
<td>2820 ± 170*</td>
<td></td>
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<tr>
<td>50.0 ng</td>
<td>1380 ± 95</td>
<td>1380 ± 95</td>
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*P < 0.01.

Angiotensin I has been reported to have very little biological activity on vascular smooth muscle,10 uterus,11 perfused kidney,12 or norepinephrine uptake.13 Data presented here showed a marked release of adrenal catecholamines following the administration of angiotensin I. Studies with 10-leu-C14-angiotensin I and/or octapeptide antiserum indicated that no significant intra-adrenal conversion of angiotensin I occurs, and that angiotensin I has a direct stimulatory effect on adrenal chromaffin tissue. Renin administration has been shown to be accompanied by catecholamine release,4 but any contribution of angiotensin I to this response has not been reported previously.

In a previous structure-activity study4 of angiotensin and the adrenal medulla, fragments of the peptide chain (hexa- and heptapeptides), substitutions of amino acid positions 1 or 4, and sequence variations among positions 5 through 9 (nonapeptide) or 5 through 10 (decapeptide) were investigated. The nonapeptides and decapeptides studied were not naturally occurring decapetide or fragments of angiotensin I. A study was also reported by Staszewska-Barczak and Konopka-Rogatk in which they studied substitutions of amino acid residues 1, 2, 4, and 6. Both these reports4 showed good correlation between pressor activity of each

Discussion

In the present study, seven angiotensin II analogues with alterations in the no. 8 position were studied. All of these compounds had identical peptide structures at positions 1 through 7. Of these analogues, five had much greater activities with adrenal medullary than with vascular smooth muscle assay. The effects of angiotensin II and two of these peptides ([8-tyr]-angiotensin II and [8-ala]-angiotensin II) on in vivo adrenal catecholamine release agreed very well with results obtained from in vitro adrenal studies. No evidence was obtained in vivo or in vitro for peptide-induced preferential catecholamine release.
analogue and the release of catecholamines from the adrenal. On the basis of these studies, the conclusion was made that the structural requirements of angiotensin II in vascular smooth muscle and the adrenal medulla were identical.

The structure-activity relationships of angiotensin II on vascular or uterine smooth muscle have recently been reviewed. The following structural requirements have been established: (1) there must be at least 8 amino acids in the peptide chain (residues 3 to 8 of angiotensin II); (2) 8 amino acids are required for maximum activity; (3) a tyrosine residue must be present in position 4; (4) the imidazole ring of histidine may be required in position 6; (5) proline must be adjacent to the no. 8 amino acid; and (6) an aromatic amino acid and free carboxyl (COOH) are required in the no. 8 position.

With regard to the adrenal medulla, requirements 1, 3, and 4 listed above for pressor or oxytocic activity have been shown to correlate very well. The requirement of the no. 8 amino acid (no. 6 of the requirements enumerated above) for adrenal catecholamine release has not previously been reported. The data presented here have shown the following not to be structural requirements for adrenal catecholamine release: (1) the octapeptide chain; (2) aromatic nucleus in no. 8 position; and (3) free COOH in no. 8 position. The marked activity of angiotensin I on chromaffin tissue obviates the necessity of 8 amino acid residues for maximum activity. The no. 8 position aromatic amino acid is not an absolute structural requirement since [8-ala]-angiotensin II (0.1% pressor activity) was 25% as active as angiotensin II. A free COOH (no. 8 position) is not needed in the adrenal medulla based on the activities displayed by angiotensin I and tetradecapeptide. Both angiotensin I and tetradecapeptide have C-terminus free COOHs, however, on leucine (2 amino acid residues removed from the no. 8 position) or on serine (6 residues removed).

To suggest receptor analysis based on a structure-activity relationship study, one must assume or establish that the parent compound and its congeners produce a common response via interaction with an identical receptor. If the postulate that angiotensin tachyphylaxis is due to partial or complete receptor saturation is accepted and the angiotensin-receptor schematic proposed by Khairallah et al. is followed, the conclusion can be made that the occurrence of cross-tachyphylaxis indicates the same specific receptor site is involved in initiating responses to angiotensin II and its analogues. On the other hand, the absence of cross-tachyphylaxis between an analogue and a parent compound is difficult to evaluate. Additional proof that structural analogues are interacting with a common receptor might be based on the specificity of pharmacological antagonism or potentiation of responses. In the present structure-activity study, cross-tachyphylaxis was one criterion established to evaluate the specificity of angiotensin receptors in adrenal chromaffin tissue. The use of DMAE to potentiate selectively adrenal medullary responses to angiotensin was examined. Greenberg and Long have recently reported potentiation by DMAE of the sympathetic component of in vivo pressor responses to angiotensin II. In the isolated, retrograde-perfused adrenal, DMAE blocked responses to nicotine and had no effect on bradykinin-induced catecholamine secretion. Adrenal catecholamine release in response to angiotensin I or II was markedly potentiated by DMAE. This hemicholinium congener (DMAE) may well be a very useful tool in determining the specificity of chromaffin tissue receptors that respond to angiotensin analogues. All the 8-substituted analogues used in this investigation, with the exception of [8-APB]- and [8-APF]-angiotensin II, exhibited cross-tachyphylaxis with angiotensin II.

The data presented here (plus angiotensin structure-activity studies of isotonic contractions and stimulation of parasympathetic neurons in isolated intestine, inhibition of myocardial norepinephrine uptake, vasopressor and oxytocic responses) indicate that the angiotensin receptor sites in vascular (or uterine) smooth muscle, in the sympathe-
adrenal system, and in parasympathetic nerves are different.

Acknowledgment
The author would like to acknowledge the technical assistance of Mrs. Brenda Graham, Miss Susan Van Lear, and Mr. Alan Blumberg. He would also like to express appreciation to Dr. F. M. Bumpus and Dr. F. A. Khairallah, Research Division of the Cleveland Clinic, for generous supplies of angiotensin I, angiotensin II, angiotensin analogues, peptides, and angiotensin II antibody; to Dr. A. J. Flumner, Ciba Pharmaceuticals, for supplying angiotensin II-ante; and to Dr. J. P. Long, Department of Pharmacology, University of Iowa, for supplying DMAE.

References

Discussion

Dr. William M. Manger, New York, New York: Dr. Peach, have you any evidence regarding the mechanism of action of angiotensin and analogues which cause release of catecholamines from the isolated adrenal gland? Do you think this is a direct action on the adrenal medullary cells or an indirect action such as via fragments of nerve endings still connected to the isolated gland?

Dr. Peach: We have tried to block the response to angiotensin, following the protocol that was established by Feldberg and Lewis. Nicotine will not block angiotensin, nor will standard ganglionic blockers, chloridronsamine, TEA, or hexamethonium. We have extended this through a series of KCl, methacholine, histamine, and a long list of standard drugs that have been studied by Douglas' group. None have effect on the activities of angiotensin, either angiotensin
I, II, or the analogues. They are dependent on calcium, as had been reported earlier. If the gland is deprived of calcium for 15 to 30 minutes it will no longer respond to the parent compound or any of the analogues. So I would guess, if I were going to build a model, that the peptides are in some way influencing chromaffin cell permeability to calcium and then calcium is completing the secretion link. It does not appear to have anything to do with acetylcholine or remaining fibers, preganglionic fibers, that might be there.

Dr. Theodore Goodfriend, Madison, Wisconsin: Dr. Peach, when we consider other hormone systems such as the steroids or the catecholamines, the precursors and products of the first hormone studied in each system have turned out to have biological activities. It would be nearly unique to the angiotensin system if its precursors and products were totally inert. In fact, you have demonstrated in the adrenal medulla that one of the precursors, angiotensin I, formerly thought to be inert, is quite potent. We showed that angiotensin I binds very avidly to the adrenal cortex. We have also been able to show that one of angiotensin's products, the heptapeptide 2-8-(des-l-angiotensin II), binds very avidly to myocardium, and that it exerts a positive inotropic effect. The inotropic effect can be seen in papillary muscle from denervated or blocked hearts. This was studied by the group directed by Dr. Theodore Cooper. So we are beginning to see a spectrum of activities of the precursors and products of the first object of our attention, angiotensin II. The entire system appears to have more generalized physiological effects.

Dr. Peach: We have looked at fragments of angiotensin II, the heptapeptide and the hexapeptide. Thesedo have activities in the adrenal medulla and their activities correspond very well to the activities of these fragments in oxytocic or pressor assays. In order to show that the smaller peptides are really not physiologically significant we would have to know their concentrations in the circulation. One peptide fragment of angiotensin II with 20% to 30% of the activity of angiotensin II may well circulate at high enough concentrations to be quite significant. We don't know at this point. Interestingly, the precursor angiotensin I in the adrenal medulla is equal to angiotensin II.

References
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Circ Res. 1971;28:II-107-II-117
doi: 10.1161/01.RES.28.5_Suppl_2.II-107

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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