Pharmacology of Antagonists of Angiotensin I and II

By Philip Needleman, Eugene M. Johnson, Jr., William Vine, Everett Flanigan, and Garland R. Marshall

ABSTRACT

The octapeptide analogues 8-Cys-AII, 8-Ile-AII, 4-Phe-8-Tyr-AII, and p-fluoro-4-Phe-AII were potent competitive antagonists of angiotensin I and II (AI and AII) in rat uterus strips, whereas the decapeptide analogues 4-Phe-8-Tyr-AI and 8-Ile-AI were weak antagonists. 8-Cys-AII and 8-Ile-AII were potent antagonists of the rise in blood pressure induced by angiotensin, although 4-Phe-8-Tyr-AII and p-fluoro-4-Phe-AII were less potent antagonists. AI was rapidly converted and was equipotent to AII in vivo, but 8-Ile-AI was a hundred times less potent than 8-Ile-AII. However, some conversion of 8-Ile-AI did occur, since SQ 20881 (an inhibitor of angiotensin-converting enzyme) markedly diminished the AII antagonistic action of 8-Ile-AI. The decapeptides AI, 8-Ile-AI, and 4-Phe-8-Tyr-AI were potent competitive antagonists of the hydrolysis of hippurylhistidylleucine by converting enzyme in vitro. Infusion of the octapeptide and the decapeptide angiotensin analogues resulted in a significant lowering of the blood pressure in anesthetized rats treated with phenoxybenzamine and propranolol and made acutely hypertensive by unclamping a renal pedicle that had been temporarily occluded. The decapeptide analogues, therefore, can function as inhibitors of angiotensin-converting enzyme and simultaneously generate octapeptide antagonists in vivo.

KEY WORDS renal hypertension p-fluoro-4-Phe-angiotensin II 8-Cys-angiotensin II 8-Ile-angiotensin II converting enzyme 4-Phe-8-Tyr-angiotensin II p-fluoro-8-Phe-angiotensin II 4-Phe-8-Tyr-angiotensin I 8-Ile-angiotensin I SQ 20881 venom peptide rat uterus rabbit lung

The decapeptide angiotensin I (AI) possesses minimal biological activity, but the octapeptide angiotensin II (AII) is an extremely potent substance with a wide range of effects. AI is converted to AII in vivo primarily in the pulmonary circulation (1-3). AII administration causes profound vasoconstriction and stimulates aldosterone, catecholamine, and prostaglandin secretion (4). Furthermore, the renin-angiotensin system has been implicated in renal hypertension (4).

In principle, antagonists of the renin-angiotensin system could oppose AII directly, or they could oppose it indirectly by inhibiting either renin or angiotensin-converting enzyme. 4-Phe-8-Tyr-AII was the first reported specific antagonist of AII (5), but there have since been a number of other AII antagonists reported (6, 7). Also, peptides isolated from snake venom have proven to be potent inhibitors of angiotensin-converting enzyme that have little or no effect at AII receptor sites (8).
ANGIOTENSIN ANTAGONISTS

In this investigation, the ability of a number of peptide analogues to antagonize angiotensin in vitro and in vivo and to lower the blood pressure of rats with renal hypertension was measured. In addition, decapeptide analogues of known All antagonists were prepared and similarly tested. The structure of the decapetides was such that they could compete with AI for angiotensin-converting enzyme and also be converted into octapeptides capable of antagonizing All.

Methods

Isolated Uterus Preparation.—The uterus was removed from a decapitated albino rat (Zivic Miller, 150–200 g), freed of adhering fat, and divided into four strips (2 cm each). The tissue was then suspended in a 5-ml tissue bath at room temperature in de Jalon's solution (9) and aerated with 95% O₂-5% CO₂ (pH 6.8). The quiescent uterus preparation was equilibrated for 15 minutes under 1 g of initial tension before drugs were added. Isotonic contractions were measured in grams of tension with a myograph (Physiograph F-50 linear-core transducer, E & M Instrument Company). Angiotensin dose-response curves were determined before and after exposure to antagonists so that each uterus strip served as its own control.

Blood Pressure Determinations.—Blood pressure was measured in albino rats (Zivic Miller, 150–200 g) anesthetized with sodium pentobarbital (30 mg/kg, iv) and treated with phenoxybenzamine (30 mg/kg, iv) and propranolol (15 mg/kg, iv). The pressure was recorded from the carotid artery (Physiograph P-1000 linear-core pressure transducer). Both jugular veins were cannulated: one vein was used for infusions of antagonists (25 μliters/min) and the other for injections of a bolus (50 μliters) of AI or AII. A dose-response curve for AII was determined before each test of the antagonists so that each rat served as its own control.

Production of Renal Hypertension.—Acute renal hypertension was produced by unclamping the left renal pedicle after it had been occluded for 4.5 hours (10). The contralateral kidney was left intact.

Converting Enzyme Assay.—The activity of angiotensin-converting enzyme was measured in acetone-powder extracts of rabbit lung by a modification (11) of the method of Piquilloud et al. (12), using hippurylhistidylleucine as the substrate.

Materials.—The angiotensin analogues were synthesized by the solid-phase procedure of Marshall and Merrifield (13), purified by Sephadex chromatography, and characterized by amino acid analysis (5, 14). Hippurylhistidylleucine and SQ 20881 (Pyr-Tyr-Arg-Pro-Cl-Ile-Pro-Pro) were not synthesized in our laboratory.¹

Results

OXYTIC EFFECTS OF PEPTIDE ANALOGUES

p-Fluoro-8-Phe-AII was equipotent to All as an oxytocic agent, but AI had only 2% of the oxytocic activity of All (Table 1). The low AI activity is consistent with the finding that rat uterus has minimal angiotensin-converting enzyme activity (16). The octapeptide analogues 8-Ile-AII, 8-Cys-AII, 4-Phe-8-Tyr-AII, and p-fluoro-4-Phe-AII were potent competitive antagonists of All and AI (Table 1). 8-Ile-AII, 8-Cys-AII, All, and p-fluoro-8-Phe-AII all appeared to have a similar affinity for the uterine receptor sites.

The decapeptide analogues 4-Phe-8-Tyr-AI and 8-Ile-AI were weak antagonists which required very high concentrations to produce 10–20-fold shifts in the dose-response curves of either All or AI (Table 2). Thus, 8-Ile-AI was 2000-fold less effective than 8-Ile-AII against All. If significant conversion of the decapeptide analogues to the octapeptide analogues had occurred, the AI analogues would have been much more potent antagonists.

¹These drugs were supplied by D. W. Cushman of the Squibb Research Institute.

TABLE 1

<table>
<thead>
<tr>
<th>Peptide</th>
<th>ED₅₀ (ng/ml)</th>
<th>Peptide</th>
<th>ID₅₀ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>5</td>
<td>8-Ile-AII</td>
<td>5</td>
</tr>
<tr>
<td>AI</td>
<td>200</td>
<td>4-Phe-8-Tyr-AII</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-fluoro-4-Phe-AII</td>
<td>500</td>
</tr>
<tr>
<td>p-fluoro-8-Phe-AII</td>
<td>5</td>
<td>8-Cys-AII</td>
<td>6</td>
</tr>
</tbody>
</table>

ED₅₀ is the dose required to produce a half-maximal response, and ID₅₀ is the dose required to produce a half-maximal inhibition. Each peptide was tested on 4–8 uterus strips.
TABLE 2
Effectiveness of Decapeptide Analogues as AII and AI Antagonists on Isolated Rat Uterus Strips

<table>
<thead>
<tr>
<th>Peptide</th>
<th>AII Antagonist concentration (ng/ml)</th>
<th>AII ED₅₀ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AII</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>AII + 8-Ile-AI</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>AII + 4-Phe-8-Tyr-AI</td>
<td>25</td>
<td>50-100</td>
</tr>
<tr>
<td>AI</td>
<td>0</td>
<td>250</td>
</tr>
<tr>
<td>AI + 8-Ile-AI</td>
<td>100</td>
<td>2500</td>
</tr>
<tr>
<td>AI + 4-Phe-8-Tyr-AI</td>
<td>25</td>
<td>2500-5000</td>
</tr>
</tbody>
</table>

ED₅₀ is the dose required to produce a half-maximal response. Each peptide was tested on 4-8 uterus strips.

The effects of the angiotensin analogues on uterus strips were partially dependent on the conditions of the experiment. When de Jalon’s solution was bubbled with 100% O₂ (instead of 95% O₂-5% CO₂), the pH rose from 6.8 to 8.6. Uterus strips bathed in the more basic solution possessed some spontaneous contractility and were more sensitive to AII (ED₅₀ 3.3 ng/ml), but 8-Ile-AII and 8-Cys-AII were about ten times less potent angiotensin antagonists (ID₅₀ 35 and 50 ng/ml, respectively). 4-Phe-8-Tyr-AII, p-fluoro-4-Phe-AII, and 8-Ile-AI were antagonists at pH 6.8 and agonists at pH 8.6.

IN VIVO ANGIOTENSIN ANTAGONISM IN NORMOTENSIVE RATS

Initial blood pressure experiments were performed in rats anesthetized with sodium pentobarbital and treated with phenoxybenzamine, an alpha blocking agent. Injection of AII (<100 ng/kg) caused an increase in blood pressure followed by a decrease to pressures below those initially observed. The fall in blood pressure before the initial level was abolished by administration of propranolol, a beta blocker, and, therefore, was apparently the result of catecholamine release. All subsequent blood pressure experiments were performed in rats that were treated with both phenoxybenzamine and propranolol.

8-Ile-AII and 8-Cys-AII were potent antagonists of the AII-induced increase in blood pressure. Infusion of these two analogues (100 μg/kg min⁻¹) caused a 200-250-fold shift in the dose-response curve for AII (Table 3). 4-Phe-8-Tyr-AII and p-fluoro-4-Phe-AII were less potent antagonists, and they caused a 10-25-fold shift in the dose-response curve at the same concentration. 4-Phe-8-Tyr-AII and p-fluoro-4-Phe-AII possess some agonistic activity, and, when infused at high dose levels (100 μg/kg min⁻¹ or more) they caused a transient rise in blood pressure which returned to baseline within 5 minutes after the start of the infusion. Park and Regoli (6) demonstrated that 4-Phe-8-Tyr-AII was equipotent with AII and AI antagonists in vivo.

AI was equipotent with AII in elevating rat blood pressure, indicating the rapid in vivo conversion of the decapeptide to the octapeptide. 8-Ile-AI proved to be much less potent (100-fold) as an AII antagonist than was 8-Ile-AII (Table 3); thus this decapptide analogue appeared to be a less efficient substrate in vivo for the rat angiotensin-converting enzyme than was AI (Table 3). The antagonism of 8-Ile-AI was substantially decreased (but not completely abolished) by the nonapeptide SQ 20881, an inhibitor of angiotensin-converting enzyme (Table 3). SQ 20881 at a dose of 50 μg/kg min⁻¹ did not affect the increase in blood pressure caused by AII but strongly inhibited the pressor effects of AI (dose-response curve shifted 200-fold).

EFFECT OF PEPTIDE ANALOGUES ON RENAL HYPERTENSION

The analogues were tested in models of acute renal hypertension following release of a clamp that had been placed on one renal artery and vein for 4.5 hours. On release, the blood pressure rose 40% over a period of 3-10 minutes and remained elevated for hours (Fig. 1). Infusion of 100 μg/kg min⁻¹ of 8-Cys-AII (Fig. 1), 8-Ile-AII, p-fluoro-4-Phe-AII, or 4-Phe-8-Tyr-AII as well as infusion of 50 μg/kg min⁻¹ of SQ 20881 or 1 mg/kg min⁻¹ of 8-Ile-AI or 4-Phe-8-Tyr-AII caused a 15-30% reduction in mean blood pressure, which returned to the level that existed before infusion 10 minutes after completion of the infusion (Table 3). Infusion of SQ 20881 together with 8-Ile-AI caused no greater
ANGIOTENSIN ANTAGONISTS

TABLE 3

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Analogue dose (µg/kg min)</th>
<th>Normotensive – ED₅₀ of AII* (µg/kg)</th>
<th>Hypotensive – fall in blood pressure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AII</td>
<td>0.1 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-Cys-AII</td>
<td>10</td>
<td>1.2 (4)</td>
<td>29 ± 2 (5)</td>
</tr>
<tr>
<td>p-Fluoro-4-Phe-AII</td>
<td>100</td>
<td>2.5 (3)</td>
<td>17 (4)</td>
</tr>
<tr>
<td>4-Phe-8-Tyr-AII</td>
<td>100</td>
<td>1.0 (5)</td>
<td>14 ± 4 (8)</td>
</tr>
<tr>
<td>4-Phe-8-Tyr-AI</td>
<td>1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-Ile-AII</td>
<td>1</td>
<td>0.1 (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.5 (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>22 ± 3 (9)</td>
<td></td>
</tr>
<tr>
<td>8-Ile-AI</td>
<td>10</td>
<td>0.1 (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.2 (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>4.5 (6)</td>
<td>20 ± 3 (7)</td>
</tr>
<tr>
<td>SQ 20881</td>
<td>50</td>
<td>0.1 (3)</td>
<td>30 ± 2 (4)</td>
</tr>
<tr>
<td>SQ 20881 + 8-Ile-AI</td>
<td>50 + 1000</td>
<td>0.6 (3)</td>
<td>33 ± 5 (4)</td>
</tr>
</tbody>
</table>

Normotensive rats were anesthetized with sodium pentobarbital and treated with phenoxybenzamine and propranolol. The analogues were infused for 15 minutes prior to and throughout the determination of the dose-response curves for AII (AII was given as pulse injections in the other jugular vein). In the rats with renal hypertension, the mean blood pressure after administration of phenoxybenzamine, propranolol, and sodium pentobarbital but before clamp removal was 79 ± 3 mm Hg (17); after clamp removal, it was 112 ± 4 mm Hg. The values represent the means ± se of blood pressure measured after 10 minutes of analogue infusion. Number of rats tested is given in parentheses.

*ED₅₀ is the dose of AII necessary to cause a 25% increase in blood pressure in the presence of peptides.

reduction in renal hypertension than did administration of either agent alone.

CONVERSION OF PEPTIDE ANALOGUES BY RABBIT LUNG EXTRACTS

Hippurylhistidylleucine (HHL) was used as a substrate to characterize angiotensin-converting enzyme activity, because it is not hydrolyzed by most other tissue peptidases. The Km for HHL with the extract of rabbit lung acetone powder was 2.5 mM. The decapetides AI, 8-Ile-AI, and 4-Phe-8-Tyr-AI were equipotent competitive inhibitors of the hydrolysis of HHL with a Kᵢ of 1–4 µM. The decapetides AI, 8-Ile-AI, and 4-Phe-8-Tyr-AI were equipotent competitive inhibitors of the hydrolysis of HHL with a Kᵢ of 1–4 µM. Incubation of the decapetides with the extract of rabbit lung acetone powder (at concentrations [5 µM] comparable to those needed for HHL inhibition) did not result in the appearance of assayable His-Leu; therefore, conversion was less than 25%. Similarly, Yang and Neff (17) demonstrated that 4-Phe-8-Tyr-AI inhibited angiotensin-converting enzyme from brain but was not itself a substrate for the enzyme. The Kᵢ (2.5 mM) of HHL agrees with previously reported data (18).

Discussion

Several new angiotensin antagonists were reported in this paper. Two analogues were previously prepared (14) with p-fluoro-Phe substituted in either the 4 or the 8 position of AII so that the conformation of the peptides in solution could be analyzed by ¹⁹F nuclear magnetic resonance spectroscopy. p-Fluoro-8-Phe-AII was an agonist and equipotent to AII, whereas p-fluoro-4-Phe-AII was a competitive antagonist. p-Fluoro-4-Phe-AII was the first antagonist of AII not modified in the 8 position. 8-Cys-AII and 8-Ile-AII were potent...
antagonists and had the same affinity for uterine receptors as did AII. 8-Cys-AII allows a radioisotope label to be introduced into an angiotensin analogue with a high receptor affinity and could, therefore, be very useful for binding and receptor studies.

The decapptide analogues of angiotensin differed from angiotensin-converting enzyme inhibitors (e.g., SQ 20881) in that they were also AI antagonists (Table 2). The fact that 8-Ile-AI and 4-Phe-8-Tyr-AI caused a 10-20-fold shift in the dose-response curves for both AI and AII in uterus supports the conclusion that AI and AII must act at the same receptor sites. Aiken and Vane (16) demonstrated that a pentapeptide inhibitor of angiotensin-converting enzyme (SQ 20475) reduced but did not abolish the response of isolated blood vessels, rat colon, rat ileum, and rat uterus to AI.

AI and the decapptide analogues were equipotent as competitive inhibitors of HHL for the rabbit lung angiotensin-converting enzyme preparation in vitro but were apparently not comparable substrates for the rat enzymes in vivo. This finding implies a much greater specificity for the conversion than had previously been assumed. However, a proportion of 8-Ile-AI was definitely converted in vivo to the AII antagonist 8-Ile-AII, since the angiotensin-converting enzyme inhibitor SQ 20881 greatly diminished the effectiveness of 8-Ile-AI (Table 3). 8-Ile-AI must have some AII inhibitory activity in vivo, otherwise SQ 20881 would have abolished all the antagonistic activity of the decapptide. Aiken and Vane (16) noted that a poor correlation existed between angiotensin-converting enzyme activity in isolated rat organs and that in homogenates of those tissues. Tissue homogenization could possibly lead to the release of nonspecific peptidases capable of degrading angiotensin. Although HHL and AI are mutually competitive for angiotensin-converting enzyme in vitro (19), Barrett and Sambhi (20) demonstrated that HHL injected simultaneously with AI in rats did not alter AI conversion to AII, probably because AI was a much better substrate for the enzyme than was HHL. 8-Ile-AI is apparently capable of competing with AI for conversion and of concurrently generating a specific AII antagonist. The ability of the decapptides to lower blood pressure in rats with renal hypertension indicates that angiotensin-converting enzyme catalyzes a rate-limiting step in the maintenance of AII levels and is consistent with the high ratios of AI to AII (45:1) noted in renal hypertension (21).

The use of specific antagonists of AII and AI and of angiotensin-converting enzyme inhibitors in biological systems should provide means for evaluating the contribution of the renin-angiotensin system to the maintenance of blood pressure. These agents should also
have potential clinical utility for confirmatory diagnosis in renal hypertension.

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References
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