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Angiotensin II Antagonism. Structure–Activity Relationships of 8-Substituted Angiotensin II Analogs

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Six analogs of angiotensin II were synthesized by the solid-phase method: [Asn^1,Val^5,Gly^8]-, [Asn^1,-Val^5,Ala^8]-, [Asn^1,Val^5,β-Ala^8]-, [Asn^1,Val^5,Abu^8]-, [Asn^1,Val^5,Val^8]-, and [Asn^1,Val^5,Leu^8]angiotensin II. These analogs were potent inhibitors of the vascular action of angiotensin II.

Many analogs of the pressor peptide hormone angiotensin II, Asp-Arg-Val-Tyr-Ile(or Val-)-His-Pro-Phe,[1] have been synthesized to delineate the relationship between chemical structure and biologic activity. Although these studies have provided a great wealth of information on the structural requirements for pressor activity, little is known about the role angiotensin II plays in hypertension. Elucidation of this role has been hampered by the lack of a specific, competitive inhibitor of the pressor activity of angiotensin II. With such an inhibitor the structural requirements for binding to the receptor sites could be differentiated from those necessary for biologic activity.

Recently specific inhibitors of the action of angiotensin II on smooth muscle have been reported. Khairallah, *et al.*,[2] and Türker, *et al.*,[3] found that [Ile^5,Ala^8]angiotensin II antagonized the action of angiotensin II on strips of guinea pig ileum and rabbit aorta, but found no *in vivo* activity. Marshall, *et al.*,[4] reported that [Phe^4,Tyr^8]angiotensin II inhibited angiotensin II activity on isolated rat uterus and also inhibited its pressor activity in rats. Park and Regoli[5] found that [D-Phe^8]angiotensir II and [8-(1-aminocyclopentanecarboxylic acid)]angiotensin inhibited the pressor and myotropic action of angiotensin I and angiotensin II. Pals, *et al.*,[6] have reported that [Asn^1,Val^5,Ala^8]angiotensin II[6a] and [Sar^1,Val^5,Ala^8]angiotensin II[6b] are specific, competitive antagonists of the action of angiotensin II on vascular smooth muscle both *in vitro* (rabbit aorta) and *in vivo* (pithed rats). Further investigation of analogs with aliphatic amino acids in the 8 position resulted in a series of inhibitors (Table I) which is the subject of this report.

Results and Discussion

The octapeptides in Table I were prepared by the basic solid-phase procedure described by Marshall and Merrifield[7] for the synthesis of [Ile^5]angiotensin II and were evaluated as antagonists of the action of angiotensin II on vascular smooth muscle as described in the Experimental Section.

This series of angiotensin II antagonists provides information on the role of the 8-phenylalanine residue of angiotensin II and allows some speculation on the molecular requirements for competitive antagonistic activity of analogs of the parent hormone. The aromatic ring in the 8 position is necessary for the pressor effect of the parent hormone.[5] This effect could be brought about either by π bonding of the aromatic ring to the receptor or by hydrophobic interaction of the ring with a specific part of the receptor. Further work will be necessary to elucidate the mechanism of the pressor effect caused by the phenylalanine residue. The fact that [Phe^4,Tyr^8]angiotensin II was also an antagonist would seem to contradict this theory. Its lack of pressor activity cannot be attributed to the substitution of tyrosine for phenylalanine, since [Tyr^8]angiotensin II is a potent pressor agent. However, Regoli and Park[5] have reported that [Phe^4,Tyr^8]angiotensin II, when given in relatively high doses, evoked pressor effects similar to angiotension II. In addition, the dose-response curve for [Ala^8]-angiotensin II was not parallel to the curves for either angiotensin II or [Phe^4,Tyr^8]angiotensin II. Thus it appears that the interaction of [Phe^4,Tyr^8]angiotensin II with the receptor may be more like that of angiotensin II than the 8-aliphatic substituted analogs.

Secondly, it is apparent from the pA_2 values for compounds **1–6** that the side chain of the 8 substituent does participate in binding to the receptor, since a steady increase in potency was observed as the length of the side chain was increased. The most dramatic increase was observed between peptide **2** with 8-alanine and peptide **4** with 8-α-aminobutyric acid. The ethyl side chain in **4** caused 20-fold greater binding than the methyl group in peptide **2**.

The free carboxyl group on the residue in position 8 has been shown *via* esterification or amide formation to be an essential feature for pressor activity.[8] From comparison of peptide **3** (8-β-alanine) to peptide **2** (8-alanine) it is apparent

Table I. Activity of 8-Substituted Angiotensin II Analogs
Asn-Arg-Val-Tyr-Val-His-Pro-R

No.	R	pA_2[a]
1	Gly	6.54 ± 0.01
2	Ala	6.84 ± 0.03
3	β-Ala	5.08 ± 0.08
4	Abu	7.93 ± 0.02
5	Val	8.31 ± 0.03
6	Leu	8.26 ± 0.07

[a] Values = mean ± standard error.

that changing the position of the carboxyl group with respect to the remainder of the molecule decreased its affinity for the receptor but did not completely destroy the capacity of the molecule to bind to the receptor.

Experimental Section

Homogeneity of the peptides was demonstrated by tlc on Merck silica gel H plates in two solvent systems: BuOH–AcOH–H_2O (6:2:3) and BuOH–AcOH–H_2O–pyridine (9:2:6:7). Amino acid analyses were run on acid hydrolysates prepared in 6 *N* HCl at 110° for 24 hr under N_2. The analyses were performed on a Jeol JLC-5AH Amino Acid Analyzer. Solvents employed in solid phase synthesis were AR grade or spectroquality (DMF) and were used without further purification. Boc amino acids were prepared by the method described by Schnabel.[9]

Solid-Phase Synthesis. The resin esters were prepared from Bio Beads SX-2 by standard procedures. The synthesis was started with approximately 2 mequiv of Boc amino acid resin ester. The Boc-protecting group was removed at each stage with 0.8 *N* HCl in HOAc for 40 min. The resin hydrochloride was neutralized with 10% Et_3N in DMF for 10 min. The Boc-amino acids were coupled to the peptide resin with DCI in CH_2Cl_2 employing a 3-molar excess of both reagents for 18 hr. Because of their poor solubility, Boc-N^{im}-benzylhistidine and Boc-nitroarginine were coupled in DMF. Asparagine was coupled using the Boc-*p*-nitrophenyl ester derivative in DMF for 48 hr. After the synthesis was complete, the peptide resin was washed with HOAc and EtOH and dried *in vacuo*. The peptides were removed from the resin by treating a mixture of the resin and trifluoroacetic acid with dry HBr (2 × 30 min). The filtrates from the cleavage reaction were evaporated to an oil which solidified upon trituration with ether. The protecting groups were removed by hydrogenation of the protected peptide over a mixture of 10% Pd/C (150 mg) and 5% Pd/$BaSO_4$ (150 mg) in 50% HOAc at atmospheric pressure for 72 hr.

The peptides were purified by gel filtration in 1% HOAc on Sephadex G-25 (5 × 90 cm) (method A). The fractions containing product by tlc were combined and lyophilized. When method A failed to provide a product which was homogeneous by tlc in the described systems or by amino acid analysis, further purification was accomplished by ion-exchange chromatography (method B) on Sephadex-SE-C25 (2.5 × 60 cm) with a gradient of NH_4OAc in 1 *N* acetic acid (0.05 → 0.75 *M*).

The buffer salts were removed by gel filtration and lyophilization.

Asn-Arg-Val-Tyr-Val-His-Pro-Gly was purified by method B: $[\alpha]^{20}D$ −63° (*c* 0.5, 1 *N* HOAc); amino acid analysis: Gly, 1.00; Pro, 1.00; His, 0.97; Val, 2.15; Tyr, 1.03; Arg, 0.99; Asp, 1.10.

Asn-Arg-Val-Tyr-Val-His-Pro-Ala was purified by method B: $[\alpha]^{20}D$ −71° (*c* 0.50, 1 *N* HOAc); amino acid analysis: Ala, 1.00; Pro, 0.97; His, 1.03; Val, 2.05; Tyr, 0.89; Arg, 1.02; Asn, 1.05.

Asn-Arg-Val-Tyr-Val-His-Pro-β-Ala was purified by method A: $[\alpha]^{22}D$ −53° (*c* 0.53, 1 *N* HOAc); amino acid analysis: β-Ala, 1.00; Pro, 0.97; His, 1.05; Tyr, 1.04; Val, 2.13; Arg, 1.00; Asn, 0.97.

Asn-Arg-Val-Tyr-Val-His-Pro-Abu was purified by method B: $[\alpha]^{21}D$ −70° (*c* 0.59, 1 *N* HOAc); amino acid analysis: Abu, 1.00; Pro, 0.89; His, 0.89; Val, 1.93; Tyr, 0.88; Arg, 0.89; Asp, 0.98.

Asn-Arg-Val-Tyr-Val-His-Pro-Val was purified by method B: $[\alpha]^{20}D$ −64° (*c* 0.52, 1 *N* HOAc); amino acid analysis: Val, 3.35; Pro, 1.00; His, 0.94; Tyr, 0.97; Arg, 0.96; Asp, 0.98.

Asn-Arg-Val-Tyr-Val-His-Pro-Leu was purified by method A: $[\alpha]^{23}D$ −70° (*c* 1, 1 *N* HOAc); amino acid analysis: Leu, 1.14; Pro, 1.10; His, 1.00; Val, 2.08; Tyr, 0.91; Arg, 0.85; Asp, 0.81.

Bioassay. Paired rabbit aortic strips were prepared from one rabbit according to the method of Furchgott and Bhadrakom[10] and mounted as described in ref 6a. All pairs of strips were exposed to an initial concentration of angiotensin II in the absence of the antagonist to determine whether both strips possessed the same degree of sensitivity. Responses to two consecutive doses of angiotensin II† (9×10^{-9} *M*) which caused a contraction approximately half of the maximum attainable were recorded. Following each contraction, the strips were washed with fresh Krebs solution until the strip relaxed to base line. A concentration of antagonist was then added to the bath and allowed to remain for 3 min, and the response to a double dose of angiotensin II recorded. Following washing, the responses to two more single doses of angiotensin II were recorded. A second concentration of antagonist was added to the bath and allowed a 3-min contact time, a double dose of angiotensin II was added, and the response was recorded. The two concentrations of antagonist were such that the initial concentration reduced the effect of a double dose of angiotensin II to just slightly less than, and the second concentration to just slightly greater than, the effect of a single dose of angiotensin II. Following the responses to two more single doses of angiotensin II, one strip was exposed to the antagonist while the other served as a control. The responses of the aortic strips to a large dose of angiotensin II, which caused a maximum contraction, were then recorded.

The pA_2 value (the negative logarithm of the molar concentration of a competitive antagonist which reduces the effect of a double concentration of agonist to that of a single one) was obtained by a modification of the method of Schild and calculated by linear interpolation on a log scale as described by Schild.[11]

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†Hypertensin, Ciba.