Synthesis and Specific Pressor Activity of [1-Aspartic acid,5-valine,9-serine]angiotensin I ("Fowl Angiotensin I")

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[Asp[1],Val[5],Ser[9]]angiotensin I was synthesized by Merrifield's solid-phase procedure. The dansylated derivative of this angiotensin was cochromatographed on the TLC with the dansylated angiotensin decapeptide isolated from white leghorn fowl. Either angiotensin showed identical behavior. The per mole pressor activity of the synthetic decapeptide (in rats anesthetized with pentobarbital and treated with pentolinium) as compared to mammalian angiotensins, namely, [Ile[5]]angiotensin I, [Val[5]]angiotensin I, [Ile[5]]angiotensin II, and [Val[5]]angiotensin II, was 157, 181, 114, and 85%, respectively.

Native angiotensin I has been characterized as a decapeptide[1] and the only species variation reported hitherto is in position 5 (Chart I).[2] However, recently fowl (white leghorn) angiotensin has been found to be different from [Asp[1],Ile[5]]angiotensins I or II or [Asn[1],Val[5]]angiotensin II by its SE-Sephadex chromatographic behavior, ratio of oxytocic to pressor activity, and susceptibility to carboxypeptidase A.[3a] On the basis of its amino acid composition and characteristics of its 1-dimethylaminonaphthalene-5-sulfonyl (DNS) derivative, fowl angiotensin has been identified as a decapeptide, Asp-Arg-Val-Tyr-Val-His-Pro-Phe-Ser-Leu ([Asp[1],Val[5],Ser[9]]angiotensin I).[3b] Although this is the first example of species variation in position 9, a recent report indicates that kangaroo angiotensin I also differs from the known sequence near the C terminus.[4]

At present, the active form of the pressor substance (angiotensin I or II or both) in the fowl has not been established nor that of its converting enzyme system. However, the high pressor activity obtained with "fowl angiotensin I" in rats, which is presumably due to conversion into [Asp[1],Val[5]]angiotensin II, corroborates the finding that dipeptidylcarboxypeptidase is not specific for the phenylalanine–histidine bond.[5] Enzymatic degradation with carboxypeptidase A indicates that this enzyme splits leucine from the "fowl angiotensin I" but not serine from the nonapeptide thus generated. The latter nonapeptide loses approximately 80% of the pressor activity of fowl angiotensin, probably due to poor conversion into the octapeptide.[3] In view of the important bearing these results have on the functioning of this prohormone or on the specificity of dipeptidylcarboxypeptidase ("converting enzyme"), it was thought of interest to synthesize this decapeptide and to study its specific pressor activity.

[Asp[1],Val[5],Ser[9]]angiotensin I was synthesized by the solid-phase procedure[6] (cf. Experimental Section). A four-point assay was performed on rats anesthetized with pentobarbital (50 mg/kg, ip) and treated with pentolinium (5 mg/kg, iv);[7] the femoral artery and vein were cannulated for blood pressure registration and intravenous injection, respectively. Assay was performed in six rats to determine the specific pressor activity against each synthetic angiotensin I or II (Table I). The weight of the peptide on a 1-mg sample was determined by optical density of tyrosine (OD 275).

Results and Discussion

The native fowl angiotensin, after dansylation, was cochromatographed with the dansylated derivative of [Asp[1],Val[5],Ser[9]]angiotensin I. Either angiotensins showed an identical behavior on thin-layer chromatogram.[8] Table I compares the rat pressor activity of [Asp[1],Val[5],Ser[9]]-angiotensin I ("fowl angiotensin I") with that of mammalian angiotensin I and II, while Table II compares the pressor activities of angiotensins relative to [Ile[5]]- or [Val[5]]angiotensin II = 100. The results indicate that the pressor activity of [Asp[1],Val[5],Ser[9]]angiotensin I is higher than the mammalian angiotensin I, viz., [Ile[5]]- or [Val[5]]angiotensin I. However, when compared to the corresponding angiotensins II, the activity of [Asp[1],-Val[5],Ser[9]]angiotensin I has been found to be approximately equal to that of [Ile[5]]angiotensin II but less than that of [Val[5]]angiotensin II. This latter difference is debatable since, except for one report,[9] the pressor activity of [Val[5]]- and [Ile[5]]angiotensin II has been found to be equal.[2] When assayed at the Cleveland Clinic, on ganglion-blocked

Table I. Pressor Activity of Fowl Angiotensin I[a]

Mammalian angiotensin[b] used as standard = 100	% pressor act. of fowl angiotensin I in rats (n = 6)[c]	
	Weight basis	Molar basis
[Ile[5]]angiotensin I	160.4 (147-175.1)	157.0 (143.8-171.3)
[Val[5]]angiotensin I	182.8 (173.4-192.7)	180.8 (171.5-190.6)
[Ile[5]]angiotensin II	94.10 (87.7-101.1)	114.4 (106.4-122.5)
[Val[5]]angiotensin II	69.20 (65.8-72.80)	85.10 (80.90-89.50)

[a] Relative to mammalian angiotensin I or II = 100. [b] [Val[5]]angiotensin I and II were synthesized by I. Honda and S. Sakakibara (Protein Research Foundation, Minoh) and their specific pressor activities against [Ile[5]]angiotensin II were approximately 60 and 110 per mol, respectively.[9] [c] The 95% confidence interval is in parentheses.

Table II. Comparative Pressor Activities of Mammalian and Fowl Angiotensins[a]

Angiotensin	% pressor act. (wt basis)	
	A	B
[Ile[5]]angiotensin II	100	73.5
[Val[5]]angiotensin II	136	100
[Ile[5]]angiotensin I	58.6	43.1
[Val[5]]angiotensin I	51.5	37.8
[Val[5],Ser[9]]angiotensin I	94.1	69.2

[a] Calculated from Table I, relative to [Ile[5]]angiotensin II = 100 (A) or [Val[5]]angiotensin II = 100 (B).

Chart I. Species Variations in Angiotensin I[2] [a]

	1	2	3	4	5	6	7	8	9	10
human, pig, rat, and horse	Asp	Arg	Val	Tyr	Ile	His	Pro	Phe	His	Leu
sheep and ox	Asp	Arg	Val	Tyr	Val	His	Pro	Phe	His	Leu
fowl (white leghorn)	Asp	Arg	Val	Tyr	Val	His	Pro	Phe	Ser	Leu

[a] For a definition of native angiotensin, see ref 1.

vagotomized rats,[10] the pressor activity of [Asp[1],Val[5],-Ser[9]]angiotensin I has been found to be 91.2% of that of [Ile[5]]angiotensin II on weight basis, which is in good agreement with the Japanese assay (Table I).

High pressor activity obtained with fowl angiotensin I indicates that [Ser[9]] decapeptide is being easily converted to the octapeptide in the rat. However, with the present data it is difficult to understand as to why the activity is higher than either of the mammalian angiotensins I. One may speculate that either "fowl angiotensin" is more stable or is more easily converted to [Val[5]]angiotensin II.

Experimental Section

[Val[5],Ser[9]]angiotensin I was synthesized by the solid-phase procedure of Merrifield.[6] The protocol used for the synthesis was similar to the one previously described by Khosla et al.[11-13] for the synthesis of analogues of angiotensin II. Precautions were taken to avoid racemization by using 1-hydroxybenzotriazole as an additive during the coupling of *tert*-butyloxycarbonyl-*N*-imidazolebenzylhistidine.[14] The crude decapeptide was purified on a column of AG-1X2 (AcO^-) using NH_4OAc buffer (pH 4.8) by the procedure reported earlier.[13] Fractions giving Pauly-positive reaction were chromatographed on cellulose TLC plates using 1-butanol–pyridine–water (65:35:65, upper phase) as the solvent. Components with the same R_f values were pooled, evaporated to a small volume, and lyophilized; peptides giving correct amino acid analysis for the desired decapeptide were then chromatographed on a column of Sephadex G-25 using 1-butanol–pyridine–water (10:1:5, upper phase). The fractions containing the desired product were pooled, evaporated, and rechromatographed on an AG-1X2 (AcO^-) column using NH_4OAc buffer (pH 4.8). The fractions giving homogeneous spots on TLC were pooled, evaporated, and lyophilized.

Fractions in the column chromatography were cut with emphasis on purity rather than on yield and no attempt was made to rechromatograph the minor fractions for identification purposes.

The homogeneity of the peptide was determined by (a) thin-layer chromatography in solvents of different pH; (b) electrophoresis at pH 1.95 and 8.6; and (c) amino acid analysis.

TLC (cellulose) showed R_f 0.66 (1-butanol–acetic acid–water, 4:1:5); R_f 0.60 (1-butanol–acetic acid–water, 4:1:1); R_f 0.41 (1-butanol–pyridine–water, 65:35:35); R_f 0.68 (1-butanol–acetic acid–water–pyridine, 30:6:24:20); R_f 0.53 (1-butanol–pyridine–water, 65:35:65).

Ionophoresis, carried on filter paper strips (S & S 2043A) in a Beckman electrophoresis cell at 400 V using formic acid–acetic acid [prepared by diluting 60 ml of formic acid and 240 ml of acetic acid to 2 l. with water (pH 1.9)], gave 0.83-fold relative electrophoretic mobility of [Val[5],Ser[9]]angiotensin I, as compared to histidine. The corresponding electrophoretic mobility of the compound in Beckman barbiturate buffer B_2 (pH 8.6) was 1.2 as compared to histidine. Detection of the compound on TLC plates and on electrophoresis filter papers was carried out by spraying with Pauly's and chlorine–toluidine reagents.

Amino acid ratio in the acid hydrolysate (6 N HCl at 110 °C for 24 h in a sealed tube in the presence of phenol) gave Asp 1.01, Arg 1.02, Val 1.95, Tyr 1.01, His 1.00, Pro 1.02, Phe 1.01, Ser 0.95, Leu 1.15.

Specific optical rotation as determined on a Perkin-Elmer polarimeter Model 141, equipped with a digital readout, was $[\alpha]^{23}D$ −13.9° (*c* 0.2, 6 N acetic acid).

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References and Notes

(1) Native angiotensin for a species has been defined as naturally occurring forms produced by endogenous renin and angiotensinogen in vivo or as products resulting from incubation of homologous renin with angiotensinogen in vitro. Native hog, human, and rat angiotensin I is [Asp[1],Ile[5]], but the so-called equine ([Asp[1],Ile[5]]) or bovine ([Asp[1],Val[5]]) angiotensin I is not native for the horse or the ox by this definition. Cf. H. Sokabe and M. Ogawa in *Int. Rev. Cytol.*, **37**, 271–327 (1974).

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