The Importance of Residues 2 (Arginine) and 6 (Histidine) in High-Affinity Angiotensin II Antagonists[1]

J. Samanen,*† E. Brandeis,† D. Narindray,† W. Adams,† T. Cash,† T. Yellin,† and D. Regoli‡

Peptide Chemistry Department, Smith Kline and French Laboratories, Swedeland, Pennsylvania 19479, and University of Sherbrooke, Quebec, Canada J1H 5N4. *Received December 31, 1986*

The structure–antagonist activity relationship is described for analogues of [Sar[1],Ile[8]]angiotensin II substituted in position 2 (arginine) and position 6 (histidine). An extreme sensitivity of potency to alterations in these positions was observed, suggesting that both residues are important for binding. Evidence is presented suggesting that the position 6 histidine side chain in angiotensin II (AII) is not involved in receptor stimulation. The structure–activity relationship is also explored for both [des-Asp[1]]AII (AIII) and [des-Asp[1],Ile[8]]AII analogues substituted in position 2 (arginine). The substitution of D-*N*-methylalanine, D-(NMe)Ala, into position 2 of both [des-Asp[1]]AII and [des-Asp[1],Ile[8]]AII gives analogues **39** and **40** that appear to be more potent than the native [Arg[2]]peptides and that are the most potent AIII agonists and antagonists described to date.

In recent years, potent antagonists to angiotensin II (AII) have been developed[2] through a variety of alterations in positions 1 (aspartic acid) and 8 (phenylalanine). Several of these analogues have been shown to lower blood pressure in humans with high plasma renin levels, including [Sar[1],Ala[8]]AII (saralasin)[3] and [Sar[1],Ile[8]]AII.[4]

The bulk of angiotensin structure–activity relationship (SAR) studies have carefully delineated the requirements for agonist activity.[2] For example, previous studies on the structural requirements for potent pressor activity of angiotensin II analogues highlight the importance of the basic side-chain structures in positions 2 and 6. Activities of the analogues in Table I[5–8] led to the conclusions that the arginine residue is important for optimal activity.

It is readily apparent from Table II[9–14] that the imidazole moiety in histidine is important for maintaining both myotropic and pressor potency in AII. None of the AII analogues bearing substitutions in position 6 displayed antagonist activity.[10,12,13]

Very little information has appeared in the literature regarding the SAR for high-affinity AII antagonists. This paper describes the biological activities of a series of analogues of [Sar[1],Ile[8]]AII and [des-Asp[1],Ile[8]]AII with modifications of the basic amino acids in positions 2 and 6 to evaluate their contributions to antagonist affinity.

Peptide Synthesis and Purification

(*tert*-Butyloxycarbonyl)amino acids and peptide reagents were obtained from Bachem Fine Chemicals Inc., Protein Research Foundation, or Chemical Dynamics and were used without further purification. Thin-layer chromatography (TLC) was performed on Brinkman precoated silica gel plates (SIL-G-25). The compounds were visualized by ninhydrin or Pauly reagent. All peptides were prepared by the solid-phase method on Beckman 990-B peptide synthesizers.[16,17] The C-terminal residue was esterified to a chloromethylated copolymer of styrene and 2% divinylbenzene (Bio-Rad) via the cesium salt procedure.[18] The degree of substitution was determined by amino acid analysis of a hydrolysate obtained by treating the amino acid-resin with 12 N HCl–propionic acid (1:1) at 120 °C for 3 h.[19] Routine deprotection of Boc-amino protecting groups was accomplished with 30% TFA in CH_2Cl_2 and neutralization with 10% TEA in CH_2Cl_2. Coupling of each amino acid was performed with a 2.5 molar excess of (*tert*-butyloxycarbonyl)amino acid and DCC in CH_2Cl_2 with completeness of reaction monitored by the ninhydrin test.[20] In most cases, coupling was complete after 2 h. If the ninhydrin test remained positive, a recoupling cycle was performed. After the last coupling and deprotection, the peptide was cleaved from resin by treatment with anhydrous HF containing 50% (v/v) anisole at 0 °C for 60 min. After vacuum evaporation of HF, the resin was rinsed with Et_2O to remove anisole and then

† Smith Kline and French Laboratories.
‡ University of Sherbrooke.

(1) The abbreviations for natural amino acids and nomenclature for peptide structures follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 977). Unnatural amino acids used in this study have been given the following abbreviations: (αMe)Ala, α-methyl-L-alanine; hArg, homoarginine, or L-2-amino-6-guanidinocaproic acid; D-Abu, D-2-aminobutyric acid; Thi, L-β-(3-thienyl)alanine; Pza, L-β-(3-pyrazolyl)alanine; (γ-im)Abu, L-2-amino-4-(2-imidazolyl)butyric acid; Ima, β-(2-imidazolyl)-alanine; (4-NH_2)Phe, 4-aminophenylalanine.

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Table I. Position 2 Analogues of AII in the Literature

	primary structure								biological activities		
									in vitro rabbit aorta[a]		in vivo rat blood pressure,[b]
	1 Asp	2 Arg	3 Val	4 Tyr	5 Ile	6 His	7 Pro	8 Phe	AII-like[c]	pA_2	AII-like[c]
1[f]		Ala[2]									7.7
2[g]	Asn[1]	D-Arg[2]									4.0
3[h]	Sar[1]	Sar[2]						Thr[8]	0	8.78	
4[h]	Sar[1]							Thr[8]	0.6	8.79	
5[i]	des-Asp[1]								47.4 (RI)[d]	*e*	15.0
6[i]	des-Asp[1]	D-Arg[2]							43.3 (RI)		33.3
7[i]	des-Asp[1]	Lys[2]							10.9 (RI)		3.0
8[i]	des-Asp[1]	D-Lys[2]							20.6 (RI)		23.0
9[i]	des-Asp[1]	hArg[2]							25.2 (RI)		2.07
10[i]	des-Asp[1]	D-hArg[2]							26.8 (RI)		11.0
11[i]	des-Asp[1]	Orn[2]							6.0 (RI)		3.0
12[i]	des-Asp[1]	D-Orn[2]							20.0 (RI)		23.0
13[j]	des-Asp[1]	Abu[2]							2.3 (RI)		0.92
14[j]	des-Asp[1]	D-Abu[2]							20.6 (RI)		22.0
15[j]	des-Asp[1]	Ala[2]							2.5 (RI)		3.0
16[j]	des-Asp[1]	D-Ala[2]							20.6 (RI)		48.0

[a] Agonist "AII-like" activity and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay unless noted otherwise. [b] Residual agonist "AII-like" activity was measured in vivo in the rat blood pressure assay. [c] AII-like agonist activity is expressed as percent activity relative to AII. [d] (RI) = rat terminal ileum. [e] Not tested. [f] Reference 5. [g] Reference 6. [h] Reference 7. [i] Reference 8. [j] Reference 9.

Table II. Position 6 Analogues of AII in the Literature

	primary structure								biological activities		
									in vitro rabbit aorta[a]		in vivo rat blood pressure,[b]
	1 Asp	2 Arg	3 Val	4 Tyr	5 Ile	6 His	7 Pro	8 Phe	AII-like[c]	pA_2	AII-like[d]
17[a]						(γ-Im)Abu[6]					0.05
18[e]						Phe[6]			1.9 (RU)[j]	0 (RU)	5.1
19[f]						Ala[6]			0.1 (GI)[k]		0.8
20[g]						Thi[6]			4.2 (RU)		19.3
21[h]						(N-3Me)His[6]			10.0 (RU)		5.0
22[h]						(N-1Me)His[6]			0.1 (RU)		0.05
23[g]						Ima[6]					0.40
24[e]						Pza[6]			20.7 (RU)	0 (RU)	49.6
25[g]						(4-NH_2)Phe[6]					0.05
26[g]						Lys[6]					0.10
27[e]						Arg[6]			0 (RU)	0 (RU)	0.012
28[i]						D-His[6]					4.0

[a] Agonist "AII-like" activity and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay unless noted otherwise. [b] Residual agonist "AII-like" activity was measured in vivo in the rat blood pressure assay. [c] AII-like agonist activity is expressed as percent activity relative to AII. [d] Reference 9. [e] Reference 10. [f] Reference 11. [g] Reference 12. [h] Reference 13 [i] Reference 14.

rinsed with glacial HOAc and filtered. The filtrate was diluted with water and lyophilized to a powder of crude peptide material.

The crude peptides were purified to homogeneity either by (a) partitioning through 200 transfers of countercurrent distribution in *n*-BuOH–HOAc–H_2O (4:1:5), (b) by partition chromatography[21] on Sephadex G-15 in *n*-BuOH–HOAc–H_2O (4:1:5), or (c) reversed-phase semipreparative HPLC[22] on a Whatman C_{18} column using the appropriate solvent mixture of CH_3CN–0.1 N NH_4OAc, pH 4. The volumes of chromatographic fractions containing pure peptide were reduced by partial rotary evaporation and dried to powders by lyophilization to constant weight.

Homogeneity of each peptide was determined by the following methods; the results are shown in Table III. (a) Amino acid analysis of 72-h (for complete hydrolysis) acid hydrolysate (6 N HCl, 110 C) performed on a Beckman Model 120C analyzer. (b) Analytical TLC on silica gel plates with solvent systems A, *n*-BuOH–AcOH–H_2O (4:1:5); B, *n*-BuOH–AcOH–H_2O–EtOAc (1:1:1:1); C, *n*-BuOH–AcOH–H_2O–pyridine (15:3:12:10), visualizing spots with Pauly reagent.[17] (c) Analytical reversed-phase HPLC on a C_{18} silica gel column using the appropriate CH_3CN–0.1 N NH_4OAc (pH 4) mixture, following elution by UV (250-nm detection). Analytical data for all peptides are listed in Table III.

Bioassays

All compounds were tested for agonistic and antagonistic activities on an in vitro preparation, on the rabbit aorta[23] as described by Rioux et al.,[24] and on rat blood pressure (in vivo), according to the technique described by Regoli et al.[25]

The pharmacological parameter of apparent affinity (pA_2), as defined and used by Ariens[26] and by Van Rossum,[27] was utilized to characterize the in vitro activities

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Table III. Peptide Analytical Data[a]

	amino acid analysis[b]								TLC, R_f			HPLC		
no.	1	2	3	4	5	6	7	8	A	B	C	solvent, % CH_3CN	K'	% purity
29	Sar	Lys	Val	**Tyr**	Ile*	His	Pro	Ile*	0.17	0.30	0.43	20	2.6	90
	(+)	(1.04)	(1.01)	(1.00)	(0.99)	(1.00)	(0.98)	(0.99)						
30	Sar	Cit	Val	Tyr	Ile*	His	Pro	Ile*	0.24	0.60	0.43	20	10.3	90
	(+)	(+)	(1.00)	(1.00)	(0.96)	(1.00)	(1.07)	(0.96)						
31	Sar	hArg	Val	Tyr	Ile*	His	Pro	Ile*	0.19	0.25	0.58	20	2.9	>98
	(+)	(0.96)	(1.01)	(0.98)	(0.99)	(0.99)	(0.96)	(0.99)						
3	Sar	Sar	Val	Tyr	Ile	His	Pro	Thr	0.11	0.40	0.50	17	3.89	98
	(+)	(+)	(1.02)	(1.01)	(0.97)	(0.99)	(1.04)	(0.96)						
32	Sar	Sar	Val	Tyr	Ile*	His	Pro	Ile*	0.25	0.67	0.57	15	3.5	>98
	(+)	(+)	(1.00)	(1.04)	(0.96)	(1.07)	(0.98)	(0.96)						
33	Sar	D-Arg	Val	Tyr	Ile*	His	Pro	Ile*	0.23	0.45	0.57	20	3.48	>98
	(+)	(1.00)	(1.01)	(1.02)	(0.97)	(1.02)	(1.01)	(0.97)						
6		D-Arg	Val	Tyr	Ile	His	Pro	Phe	0.20	0.52	0.55	20	4.54	>98
		(1.02)	(1.03)	(1.01)	(0.96)	(0.98)	(1.00)	(1.00)						
34		D-Arg	Val	Tyr	Ile*	His	Pro	Ile*	0.19	0.60	0.61	30	1.99	>98
		(1.00)	(1.02)	(1.01)	(0.98)	(1.01)	(0.98)	(0.98)						
16		D-Ala	Val	Tyr	Ile	His	Pro	Phe	0.31	0.53	0.69	35	3.42	>98
		(1.00)	(1.02)	(1.01)	(0.95)	(1.01)	(1.02)	(1.00)						
35		D-Ala	Val	Tyr	Ile*	His	Pro	Ile*	0.37	0.75	0.80	23	2.10	>98
		(1.02)	(1.01)	(1.01)	(0.98)	(1.01)	(1.00)	(0.98)						
15		Ala	Val	Tyr	Ile	His	Pro	Phe	0.29	0.74	0.60	30	1.41	>98
		(1.00)	(1.02)	(1.01)	(0.97)	(0.99)	(1.00)	(1.00)						
36		Ala	Val	Tyr	Ile*	His	Pro	Ile*	0.32	0.68	0.68	30	2.15	>98
		(1.00)	(1.02)	(1.02)	(0.98)	(1.00)	(0.98)	(0.98)						
37		(αMe)Ala	Val	Tyr	Ile	His	Pro	Phe	0.28	0.72	0.59	25	2.27	>98
		(+)	(1.01)	(1.00)	(0.98)	(1.00)	(1.02)	(1.00)						
38		(αMe)Ala	Val	Tyr	Ile*	His	Pro	Ile*	0.38	0.68	0.70	25	3.50	>98
		(+)	(1.06)	(1.04)	(1.00)	(1.05)	(1.00)	(1.00)						
39		(NMe)D-Ala	Val	Tyr	Ile	His	Pro	Phe	0.30	0.72	0.61	20	7.00	>98
		(+)	(1.00)	(1.02)	(0.97)	(1.04)	(0.97)	(0.99)						
40		(NMe)D-Ala	Val	Tyr	Ile*	His	Pro	Ile*	0.28	0.64	0.71	35	4.16	>98
		(+)	(1.07)	(0.93)	(1.00)	(0.96)	(1.03)	(1.00)						
5		Arg	Val	Tyr	Ile	His	Pro	Phe	0.22	0.61	0.47	20	2.73	>98
		(1.09)	(0.98)	(1.03)	(0.97)	(1.00)	(0.94)	(1.00)						
42	Sar	Arg	Val	Tyr	Ile*	His	Pro	Ile*	0.19	0.67	0.75	20	2.80	>98
	(+)	(0.99)	(0.97)	(0.97)	(1.01)	(1.07)	(0.99)	(1.01)						
43	Sar	Arg	Val	Tyr	Ile*	Phe	Pro	Ile*	0.33	0.60	0.77	35	3.8	>98
	(+)	(0.97)	(1.02)	(1.01)	(0.99)	(1.02)	(0.98)							
44	Sar	Arg	Val	Tyr	Ile*	Thi	Pro	Ile*	0.26	0.64	0.60	30	2.67	>95
	(+)	(1.01)	(1.02)	(1.00)	(098)	(+)	(1.01)	(0.98)						
45	Sar	Arg	Val	Tyr	Ile*	Trp	Pro	Ile*	0.26	0.47	0.55	45	5.65	93
	(+)	(0.99)	(1.07)	(0.97)	(0.96)	(0.80)	(1.00)	(0.96)						
46	Sar	Arg	Val	Tyr	Ile*	(NMe)His	Pro	Ile*	0.14	0.48	0.56	19	2.15	>94
	(+)	(1.01)	(1.01)	(1.00)	(0.98)	(+)	(1.01)	(0.98)						
47	Sar	Arg	Val	Tyr	Ile*	Lys	Pro	Ile*	0.14	0.50	0.50	15	3.55	>97
	(+)	(1.01)	(1.02)	(0.99)	(0.98)	(1.01)	(1.01)	(0.98)						
48	Sar	Arg	Val	Tyr	Ile	Asp	Pro	Thr	0.14	0.43	0.54	20	1.74	>98
	(+)	(1.01)	(1.00)	(1.02)	(0.99)	(1.04)	(1.00)	(0.94)						
49	Sar	Arg	Val	Tyr	Ile*	D-His	Pro	Ile*	0.31	0.63	0.76	15	3.25	>98
	(+)	(1.01)	(1.00)	(1.01)	(0.99)	(1.03)	(0.98)	(0.99)						

[a] See text for details of analytical procedures. [b] Amino acid analysis expressed in molar ratios of the D,L amino acids in the peptides. (+) = amino acid present in roughly 1 molar equiv (in cases where quantitation is difficult). * = amino acid present in two positions. Value expressed is half the experimental value.

of the compounds. AII antagonists bearing sarcosine in position 1 are slowly reversible antagonists depressing both the slope and maximum of the AII dose–response curve in vitro at high doses but not at low doses.[28] The "pA_2" values reported here for comparative purposes were determined at low doses in the range of competitive inhibition and consequently may be overestimated.[29] The residual angiotensin-like activity of the compounds in vivo was evaluated by measuring the pressor effects of bolus intravenous injections of 1 μg, and the antagonistic effect was calculated from dose–response curves measured before and during the infusions of each compound. Antagonism in vivo is expressed by the ID_{50} in nanograms/rat per min with 250-g rats according to Regoli et al.[24]

Results and Discussion

Position 2 Arginine. The results displayed in Table IV indicate that arginine is critical for maintaining antagonist activity in [Sar¹,Ile⁸]AII. Replacement of the cationic side chain of arginine with the cationic side chain of lysine results in analogue **29** with considerably diminished activity. Citrulline contains a urea group, which is isosteric with the guanidine group of arginine but nonionic. The citrulline analogue **30** is a weaker antagonist. Simple extension of the trimethylene chain of arginine to the tetramethylene chain in homoarginine gives an analogue **31** with lower activity.

Due to this sensitivity of antagonist activity to side-chain structure, we were surprised by the report of considerable activity for [Sar¹,Sar²,Thr⁸]AII[7] (analogue **3**, Table I),

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Table IV. Position 2 Analogues of AII in This Study

	primary structure								biological activities			
									in vitro rabbit aorta[a]		in vivo rat blood pressure[b]	
	1 Asp	2 Arg	3 Val	4 Tyr	5 Ile	6 His	7 Pro	8 Phe	AII-like[c]	pA_2	AII-like[d]	ID_{50}
29	Sar	Lys^2						Ile^8	0	7.75	12.50 ± 1.5[e]	50 ± 6.1
30	Sar	Cit^2						Ile^8	0	6.2	25.0 ± 3.2	
31	Sar	$hArg^2$						Ile^8	0	8.25	7.5 ± 1.0	50.0 ± 8.2
3	Sar	Sar^2						Thr^8	0	<6.0	0	0
32	Sar	Sar^2						Ile^8	0	7.5	8.5 ± 0.9	150 ± 12.5
33	Sar	D-Arg^2						Ile^8	0	7.5	10.0 ± 2.1	100 ± 14.0
6	des-Asp	D-Arg^2							4.5	≪6.0	65.0 ± 8.5	
34	des-Asp	D-Arg^2						Ile^8	0	7.0	5.0 ± 1.0	100 ± 13.2
16	des-Asp	D-Ala^2							5.0		75.0 ± 11.2	
35	des-Asp	D-Ala^2						Ile^8	0	7.0	5.0 ± 0.8	100 ± 9.0
15	des-Asp	Ala^2							0	≪6.0	40.0 ± 6.3	
36	des-Asp	Ala^2						Ile^8	0	6.0		
37	des-Asp	(αMe)Ala^2							0	≪6.0	60.0 ± 8.0	
38	des-Asp	(αMe)Ala^2						Ile^8	0	≪6.0		
39	des-Asp	D-(NMe)Ala^2							1.4	≪6.0	85.0 ± 12.0	
40	des-Asp	D-(NMe)Ala^2						Ile^8	0	7.1	10.0 ± 1.3	25 ± 3.2
compared to:												
5	des-Asp^1								2.4		75.0 ± 6.3	
41	des-Asp^1							Ile^8	0	7.75	5.0 ± 3.2	200 ± 25.0
42	Sar^1							Ile^8	0	9.1	10.2 ± 1.4	10 ± 1.2

[a] Agonist "AII-like" activity and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay according to the method of Rioux et al.[28] [b] Residual agonist "AII-like" activity and antagonist activity, ID_{50} (ng/rat per min with 250-g rats), were measured in vivo in the rat blood pressure assay described by Regoli et al.[29] [c] AII-like activity in vitro is expressed as percent activity relative to AII. [d] AII-like activity is expressed by the blood pressure increase (millimeters of mercury) produced by a 1-μg bolus intravenous injection of compound. The dose-response curves for these peptides were too disimilar for a comparison of ED_{50}s. [e] Mean standard error of at least five tests.

Table V. Position 6 Analogues of AII in This Study

	primary structure								biological activities			
									in vitro rabbit aorta[a]		in vivo rat blood pressure[b]	
	1 Asp	2 Arg	3 Val	4 Tyr	5 Ile	6 His	7 Pro	8 Phe	AII-like[c]	pA_2	AII-like[d]	ID_{50}
43	Sar^1					Phe^6		Ile^8	0	6.75	10.0 ± 1.2[e]	100 ± 12.5
44	Sar^1					Thi^6		Ile^8	0	7.5	12.5 ± 1.7	100 ± 14.5
45	Sar^1					Trp^6		Ile^8	0	7.4	7.5 ± 1.0	100 ± 15.0
46	Sar^1					(N-3Me)His^6		Ile^8	0	6.75	2.5 ± 0.5	100 ± 12.8
47	Sar^1					Lys^6		Ile^8	0	≪6.0		
48	Sar^1					Asp^6		Thr^8	0	≪6.0		
49	Sar^1					D-His^6		Ile^8	0	≪6.0		
compared to:												
42	Sar^1							Ile^8	0	9.0	10.0 ± 2.0	10 ± 1.3

[a] Agonist "AII-like" activity and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay according to the method of Rioux et al.[28] [b] Residual agonist "AII-like" activity and antagonist activity, ID_{50} (ng/rat per min, with 250-g rats), were measured in vivo in the rat blood pressure assay described by Regoli et al.[29] [c] AII-like activity in vitro is expressed as percent activity relative to AII. [d] AII-like activity is expressed by the of blood pressure increase (millimeters of mercury) produced by a 1-μg bolus intravenous injection of compound. [e] Mean standard error of at least five tests.

which lacks the guanidinium side chain. In our hands, analogue **3** was practically inactive (Table IV), and the corresponding analogue [Sar^1,Sar^2,Ile^8]AII (**32**) as only a weak antagonist.

We were intrigued by the elegant study by Lehmann[8] on arginine modifications in [des-Asp^1]AII (**5**, AIII). As seen in Table I, a number of D-amino acids could be substituted for arginine in AIII to give analogues with in vivo activities that were equal to or greater than AIII itself. The most potent analogue in vivo was obtained with D-alanine substitution (analogue **16**). Jorgensen et al.[30] were the first to propose that D-amino acid substitution for arginine in AIII would offer aminopeptidase resistance. As seen in Table I, analogues of AIII bearing D-amino acids display activities in the rat ileum that are lower than [des-Asp^1]AII (**5**) in vitro but are comparable to or more active than **5** in vivo.[8,9] In the rabbit aorta, we found that the D-Arg analogue **6** and D-Ala analogue **16** were comparable to the L-Arg analogue **5** (Table IV).

With the hope that these substitutions would increase the activity of [des-Asp^1,Ile^8]AII, **41**, the analogues **34** and **35** were prepared containing D-Arg^2 and D-Ala^2, respectively. As seen in Table IV, the in vitro activities (pA_2) of these analogues for the rabbit aorta are not greater than that of [des-Asp^1,Ile^8]AII (**41**) but are somewhat lower. The analogues **34** and **35**, however, display twice the antagonist activity of **41** in vivo, suggesting that these D-amino acid substitutions protect against aminopeptidase degradation of [des-Asp^1, Ile^8]AII.

The SAR of [des-Asp^1]AII reported by Lehmann[8,9] indicated that D-amino acids in position 2 with small side chains, even D-alanine, could be substituted for L-Arg. The preference of D-Ala in AIII over L-Ala suggested that there might be rather specific three-dimensional requirements for this N-terminal amino acid regardless of its N-terminal position and simple structure. We chose to pursue these requirements with analogues bearing alanine derivatives, namely (αMe)Ala and D-(NMe)Ala.

The (αMe)Ala^2 agonist analogue **37** was inactive at 1 μg

(30) Jorgensen, E. C.; Windridge, G. C.; Lee, T. C. *J. Med. Chem.* **1970**, *13*, 352.

in vitro but relatively potent in vivo. The corresponding (αMe)Ala^2 antagonist analogue **38** was also inactive in vitro. The (NMe)Ala^2 agonist analogue **39** displayed reduced in vitro activity but was marginally superior in vivo to the (αMe)Ala^2 agonist. In contrast to the agonist analogues, D-(NMe)Ala^2 substitution, in the corresponding antagonist analogue, is distinctly superior to (αMe)Ala^2 substitution. Although the D-(NMe)Ala^2 antagonist analogue **40** is somewhat less active in vitro than the native L-Arg^2 antagonist analogue **41**, analogue **40** is clearly more potent than the (αMe)Ala^2 antagonist analogue **38**. Analogue **40** was 4 times more potent in vivo than the D-Arg^2 and D-Ala^2 antagonist analogues **34** and **35** and 9 times more potent in vivo than [des-Asp^1,Ile^8]AII (**41**). As seen in Table IV, the in vivo potency of **40** approaches the activity of [Sar^1,Ile^8]AII, **42**. Analogues **39** and **40** are the most potent AIII agonist and antagonists described to date. These results suggest that the D-(NMe)Ala peptides are more resistant to amino peptidase degradation than (αMe)Ala or other substitutions.

The partial agonist activity of **40** (10.0-mmHg blood pressure elevation upon 1-μg bolus injection) is identical with that of [Sar^1,Ile^8]AII, suggesting that the arginine group is only involved in receptor binding, not in receptor stimulation.

As with AII itself, the N-terminal amino acid and the side chain in position 2 can be eliminated from AII antagonists like [Sar^1,Ile^{8}1AII when the position-2 amino acid is D-(NMe)Ala. Greater in vitro and in vivo potency is still obtained, however, by retaining both the position 1 sarcosine and position-2 arginine.

Position 6 Histidine. As seen in Table V our results indicate that histidine is critical for maintaining antagonist affinity in [Sar^1,Ile^8]AII.

The rank ordering of position 6 analogues in the AII agonist series (Table II), i.e., His^6 $\gg$ Thi^6 > Phe^6, (N-3Me)His^6 $\gg$ Lys^6, is maintained in the [Sar^1,Ile^8]AII antagonist series (Table V).

The fact that antagonist activity was not detected in previous position 6 analogues of AII[11,13,14] suggests that the histidine residue may be involved in receptor binding but not receptor stimulation. It is well known that positions 4 (tyrosine) and 8 (phenylalanine) are residues that are involved in receptor stimulation since modification to these residues results in antagonistic activity.[2,31]

This postulation of histidine involvement in receptor binding but not receptor stimulation is supported in that analogues **43–45** display partial agonist activities (10-mmHg blood pressure increase upon 1-μg bolus injection), comparable to the His^6 analogue **42**. This would not be expected if the histidine side chain was involved in receptor stimulation. The histidine side chain is clearly not responsible for the partial agonist activity of AII antagonists such as [Sar^1,Ile^8]AII and saralasin.

The histidine residue could serve one or both of two roles in agonist and antagonist receptor binding to receptor: (a) direct interaction with receptor ligands or (b) intramolecular interactions to maintain a proper conformation for receptor binding. Bioassay results cannot distinguish between the two roles. Conformational studies of angiotensin II in solution have suggested possible interactions between either the tyrosine side chain[32] or the C-terminal carboxyl group with the histidine side chain.[33] Solution studies of analogues in Table V are in progress and may shed light on the possible involvement of the histidine side chain in maintaining a receptor-binding conformation.

Registry No. **1**, 22684-01-1; **2**, 98391-24-3; **3**, 63146-93-0; **4**, 53632-49-8; **5**, 13602-53-4; **6**, 32738-16-2; **7**, 33054-46-5; **8**, 32738-20-8; **9**, 32738-18-4; **10**, 32738-19-5; **11**, 33033-46-4; **12**, 13758-31-1; **13**, 25849-91-6; **14**, 25857-43-6; **15**, 51988-70-6; **16**, 51988-71-7; **17**, 57667-80-8; **18**, 51006-12-3; **19**, 25119-43-1; **20**, 50765-40-7; **21**, 43210-75-9; **22**, 43157-22-8; **23**, 112195-86-5; **24**, 71239-93-5; **25**, 90937-05-6; **26**, 71239-94-6; **27**, 47917-67-9; **28**, 49707-73-5; **29**, 112173-39-4; **30**, 112173-40-7; **31**, 112173-41-8; **32**, 98641-03-3; **33**, 111771-38-1; **34**, 112173-42-9; **35**, 112173-43-0; **36**, 112173-44-1; **37**, 112173-45-2; **38**, 112173-46-3; **39**, 112173-47-4; **40**, 112195-87-6; **41**, 52498-25-6; **42**, 37827-06-8; **43**, 112195-88-7; **44**, 112195-89-8; **45**, 112195-90-1; **46**, 112195-91-2; **47**, 112195-92-3; **48**, 112195-93-4; **49**, 111821-41-1.

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