Structure–Activity Relationships for the Carboxy-Terminus Truncated Analogues of Angiotension II, a New Class of Angiotensin II Antagonists†

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A series of analogues of the recently reported angiotensin II (AII) antagonist [Sar¹]AII-(1–7)-amide or des-Phe⁸[Sar¹]AII (3) have been prepared by solid-phase synthesis and purified by reverse-phase liquid chromatography. The agonist and antagonist properties of these carboxy-truncated analogues of AII were determined in the isolated rabbit aorta assay. In the analogues tested, replacement of aspartic acid in position 1 by sarcosine was found necessary to produce significant antagonist activity. At position 7 of the des-Phe⁸ analogues, prolinamide could be replaced by proline without significant change in the biological activity. However, substitution of 7-prolinamide by either glycinamide or sarcosinamide provided inactive peptides. Methylation of the 4-tyrosine in [Sar¹]AII-(1–7)-NH_2 preserved the antagonist potency in isolated rabbit aorta. Deletion of the proline at position 7 resulted in inactive hexapeptides, both in the Asp¹ and Sar¹ series. However synthesis of the *N,N*-dimethyl amide at the N-terminus afforded hexapeptide [Sar¹]AII-(1–6)-$N(CH_3)_2$ (10) with a pA_2 value of 7.05. All the antagonistic peptides synthesized were fully reversible, competitive antagonists in vitro. These findings indicate that the structural requirements for receptor blockade by these C-truncated analogues are quite stringent with respect to the nature of the amino acid at positions 1 and 6/7. The analogues **2**, **3**, **7**, **10**, **11** (saralasin), and **12** (sarmesin) were tested in vivo in the anesthetized rat and were found to inhibit the AII pressor response. In addition, **3** inhibited angiotensin II stimulated aldosterone release from isolated rat adrenal zona glomerulosa cells and had no agonist activity by itself at the doses tested. Interestingly, analogue **3**, when injected intracerebroventricularly in conscious rats, failed to antagonize the dipsogenic response to an angiotensin II icv injection and this reflects some heterogeneity in the AII receptor population. Peptide **3** is the first example of an antagonist that discriminates between peripheral and brain receptor subtypes.

Potent antagonists of the angiotensin II (AII) receptor have traditionally been obtained by a variety of alterations in position 1 (aspartic acid), 4 (tyrosine), and 8 (phenylalanine) of the AII sequence.[1] The most potent antagonists reported belong to a class of analogues obtained by a combined substitution of Phe⁸ by aliphatic amino acids and of Asp¹ by sarcosine.[2] Saralasin ([Sar¹,Val⁵,Ala⁸]AII),[3] which has blood pressure lowering activity in humans, and related analogues, e.g., [Sar¹,Ile⁸]AII and [Sar¹,Thr⁸]AII, have been extensively studied.[4,5] A second class of antagonists differs structurally from AII by modification at the Tyr⁴ residue. Sarmesin ([Sar¹,(Me)Tyr⁴]AII), the prototype of this second class, is a less potent but fully competitive, reversible antagonist.[6]

Despite the development of a significant number of related peptidic AII antagonists over the last 20 years, comparatively little information has appeared in the literature regarding their structure–activity relationships and in particular their chain-length requirements. This is perhaps due to the structure of saralasin-like compounds for which the antagonistic activity is directly linked to the presence of specific amino acids at both termini. Furthermore, the traditional knowledge of AII analogues structure–activity relationships suggests that the integrity of the carboxyl terminus of the molecule is an absolute requirement for any form of biological activity. Removal of either Phe⁸ or the dipeptide Pro⁷-Phe⁸ completely destroys the pressor and myotropic activity of AII, although Regoli reported a weak (pA_2 = 4) antagonistic effect of des[Phe⁸]AII.[7] More recently, Marshall reported that modification of the C-terminal carboxylic group results in analogues having negligible agonistic or antagonistic properties.[8] However, the report of a low affinity of [Val⁵]AII-(1–7) and [des-Phe⁸,Val⁵]AII to AII adrenal and uterine receptors (500 times less than AII by competitive binding experiments) and of a weak antagonistic effect at a high concentration (10^{-4} M) on aldosterone secretion by zona glomerulosa cells[9] stimulated our interest in the design of AII C-terminus truncated analogues as potential antagonists.

Design and Synthesis of C-Terminus Truncated AII Antagonists

A general strategy to design a peptidic antagonist is based on the concept that a peptide hormone is composed of a binding and an activating component.[10] Synthesizing the binding component alone should produce a fragment which occupies the receptor without activating the second-messenger system and initiating biological response.

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† Most of the analogues of angiotensin II described hereunder have the isoleucine in position 5, as found in the human, porcine, and equine hormone except for some specific cases, i.e. saralasin, which have a valine at this position as in the bovine hormone. Abbreviations are according to the IUPAC-IUB Commission on Biochemical Nomenclature, *Pure Appl. Chem.* **1974**, *40*, 317.

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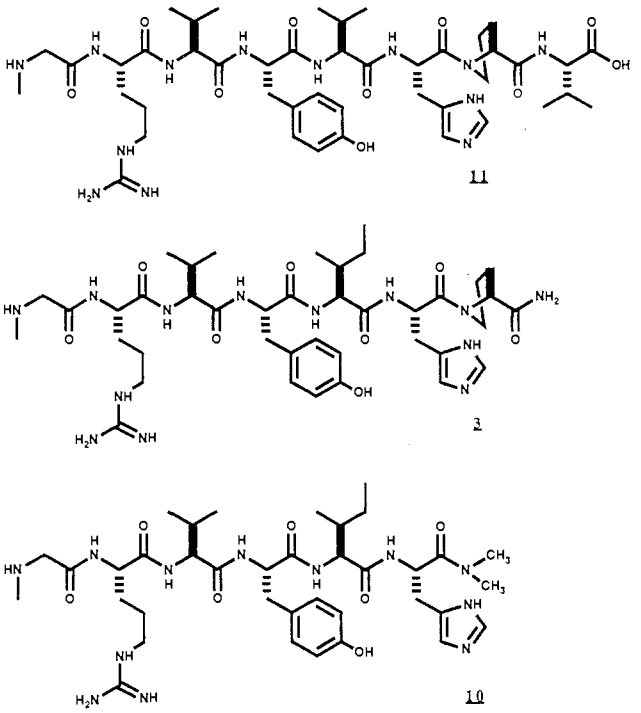

Figure 1. Comparison of the structures of [Sar^1,Val^5,Ala^8]AII (saralasin, **11**) and two of the C-truncated analogues, [Sar^1]-AII-(1–7)-amide (**3**) and [Sar^1]AII-(1–6)-*N*,*N*-dimethylamide (**10**).

Removal of one of the suspected principal activator elements from the AII sequence (Phe^8)[11–13] with preservation of the remaining residues constitutes a rational approach to the design of AII antagonists. Such a strategy often leads to weak antagonists, and affinity to the receptor has to be reinstated by subsequent alterations of the inhibitory fragment, usually inspired from modifications known to enhance affinity in the agonist series.[14] Thus, we have designed [Sar^1]AII-(1–7)-amide as the most rational probe based on the hypothesis that the residues 1–7 define the specificity, intensity, and duration of action of the biological effect, while the nature of residue 8 modulates agonist activity.[12,13] In addition sarcosine was introduced in position 1, a substitution known to increase the affinity to the receptor in other AII analogue series.[14] We have recently reported on the antagonistic activity of this compound on the myotropic and pressor actions of AII.[15]

In the present study, we address several questions pertaining to the biological effects of analogues resulting from selective modifications of the heptapeptide AII-(1–7). In particular, we looked at the effect of replacing the 7-proline residue by glycine or sarcosine. We further investigated the effect of the primary amide function versus a free carboxylic acid function at the C-terminus and the effect of sarcosine substitution over aspartic acid in position 1. Also reported are des[Pro^7,Phe^8]AII analogues and their biological evaluation. Thus, three hexapeptides (**8**–**10**) have been prepared with a carboxamide function

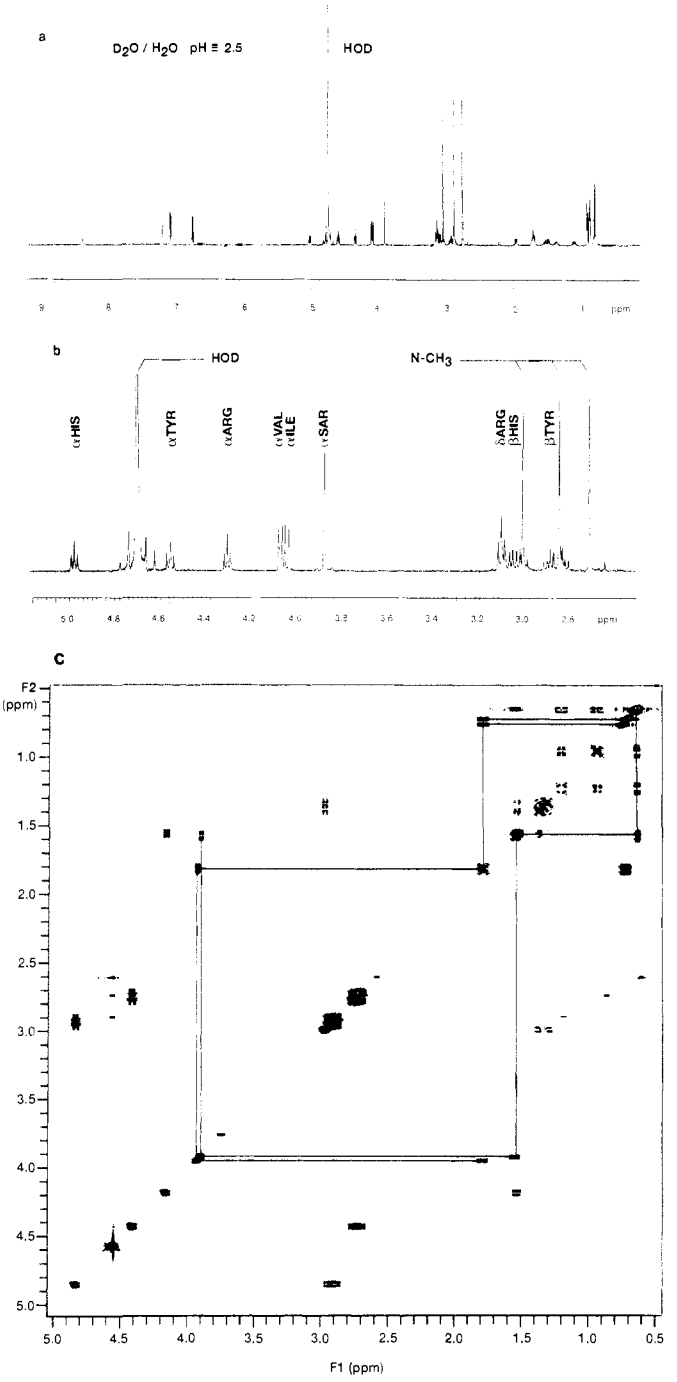

Figure 2. (a) 500-MHz ^{1}H NMR spectrum of [Sar^1]AII-(1–6)-$N(CH_3)_2$, (**10**; 5 mg/mL), in D_2O/H_2O at pH 2.5 and 20 °C. The three singlets are attributed to the *N*-methyl group from sarcosine (**1**) and to the conformationally nonequivalent methyls from the C-terminal *N*,*N*-dimethyl amide function due to restricted rotation around the CO–N bond. (b) Expansion of the 2.5–5.5 ppm region showing all signals from the methine protons is shown. (c) 2D-COSY spectrum was used to facilitate attribution of each individual signal.

at the C-terminus. One of them, compound **10**, was synthesized as the *N*,*N*-dimethyl carboxamide at its C-terminal histidine, a structural modification intended to mimic the tertiary amide peptidic bond His^6-Pro^7 (Figure 1).

We will try to answer the following questions: (i) what effect do such structural modifications have on biological properties of AII-(1–7) analogues, (ii) what is their influence on potency and affinity, (iii) what type of antagonistic activity (competitive or uncompetitive) is present, and (iv)

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Table I. Biological Activities of C-Terminus Truncated Angiotensin II Analogues in Rabbit Isolated Aorta and the Anesthetized Rat

	primary sequence										in vivo	
	1	2	3	4	5	6	7	8		rabbit		
no.	Asp	Arg	Val	Tyr	Ile	His	Pro	Phe	mol wt	aorta pA_2[a]	agonist[b]	antagonist[c]
1	Asp	Arg	Val	Tyr	Ile	His	Pro		899.02	<6	–	>300
2	Sar	Arg	Val	Tyr	Ile	His	Pro		855.01	7.5	45	>100
3	Sar	Arg	Val	Tyr	Ile	His	$ProNH_2$		854.03	7.6	60	60 (10)
4	Asp	Arg	Val	Tyr	Ile	His	$SarNH_2$		872.02	<6		
5	Sar	Arg	Val	Tyr	Ile	His	$GlyNH_2$		813.96	<6		
6	Sar	Arg	Val	Tyr	Ile	His	$SarNH_2$		827.99	<6		
7	Sar	Arg	Val	(MeO)Tyr	Ile	His	$ProNH_2$		868.05	7.7	65	>100 (5)
8	Asp	Arg	Val	Tyr	Ile	$HisNH_2$			800.97	<6		
9	Sar	Arg	Val	Tyr	Ile	$HisNH_2$			756.91	<6		
10	Sar	Arg	Val	Tyr	Ile	$HisN(CH_3)_2$			784.97	7.05	50	100 (25)
11	Sar	Arg	Val	Tyr	Val	His	Pro	Ala	926.05	(8.3)[e]		10 (10)
12	Sar	Arg	Val	(MeO)Tyr	Ile	His	Pro	Phe	1016.22	7.6[d]	60	30 (10)

[a] Mean of two through six paired determinations (see the Experimental Section). [b] Maximum agonistic pressor response in vivo (expressed as the percentage of the pressor response to a dose 30 ng/kg AII bolus) observed during the first minutes of infusion at 30 μg/kg per min; this transient response subsides about 5 min after the beginning of infusion. [c] The in vivo activity is expressed as the smallest dose (expressed in μg/kg per min) observed to completely antagonize the effect of a bolus injection of AII (30 ng/kg) during infusion of the antagonist; the number in parantheses is the time in min to recover ~50% of the effect of AII bolus injection after stopping the infusion of the antagonist. [d] For sarmesin, values of 7.5–8.1 have been reported in the rat isolated uterus assay.[14] [e] pA_2 value obtained with the isomeric technique in the rat stomach as reported previously.[7]

what effect do these modifications have on duration of action in vivo? In addition, do any of the new antagonists described demonstrate tissue specificity?

The peptides reported have been synthesized by the solid-phase method as reported in the Experimental Section. Purification was by high-pressure liquid chromatography on C_{18} bonded silica gel. Characterization was by amino acid analysis, fast atom bombardment (FAB) mass spectroscopy, and in some cases by ^1H NMR. The 500-MHz ^1H NMR spectrum of peptide **10** depicted in Figure 2 was recorded to characterize the *N,N*-dimethyl amide in this unusual compound. This *N,N*-dimethyl carboxamide in compound **10** was generated by transamidification with *N,N*-dimethylamine of the protected peptide from the resin followed by anhydrous HF deprotection and purification of the peptide. The desired product was obtained in low yield due to the presence of an unidentified side product (about a 50:50 mixture in the crude reaction mixture as estimated on the basis of the integrated area under the peak versus the total integrated area recorded at 220 mm). In the case of **10**, we also recorded the 2D-COSY spectrum which helped us in the complete assignment.

Bioassays

Myotropic properties in vitro were determined on the isolated rabbit aorta by using a protocol described previously.[15,16] The in vivo pressor responses were evaluated in ganglion-blocked, inactin-anesthetized rats. The aldosterone release by zona glomerulosa cells from rat adrenals stimulated by angiotensin II was measured by a procedure similar to that described by Douglas et al.[24] The dipsogenic effects upon intracerebroventricular injections were determined in the conscious rat by using the protocol described by Tonnair and co-workers.[17]

Results and Discussion

Table I compares the contractile properties of the C-truncated AII analogues **1–10** to those of known antagonists saralasin (**11**) and sarmesin (**12**). None of the reported compounds showed any agonist activity in the rabbit aorta assay. As previously reported, [Sar^1]AII-(1–7)-NH_2 (**3**) shows antagonist activity characterized by a potency similar to that of sarmesin and significantly lower than that reported for saralasin in the same assay (pA_2 value of 8.3).[7] In contrast to the apparent nonreversible, noncompetitive behavior of the latter, however, all the other antagonists reported in the table behave as competitive, fully reversible inhibitors. Examination of the pA_2 values of compounds **1–10** suggests some conclusions about the structure–activity relations in this new structural type of antagonists. As previously reported the des[Phe^8]AII heptapeptide **1** has little or no activity compared to its analogue in which Sar is substituted for Asp in position 1. Compound **2** differs from **3** only by the function at the C-terminus. It appears that this modification has little or no influence on the affinity for the receptor as judged from its pA_2 value. On the other hand, Gly and Sar substitution at position 7 resulted in inactive analogues; peptides **5** and **6** have no activity despite the presence of Sar at position 1. Compound **7** represents an attempt to combine methylation of the Tyr^4 hydroxyl, the logic that led to the discovery of sarmesin with the C-terminus deletion. Neither enhancement nor mitigation in the biological effect was observed: **3**, **7**, and **12** have virtually identical pA_2 values. With compounds **8–10**, we have examined the effect of further truncation at the C-terminus; the two hexapeptide amides **8** and **9** differ by the nature of the amino acid at position 1 and have no antagonistic activity. In compound **10**, the C-terminus has been converted to an *N,N*-dimethyl amide which minimally mimics the proline ring. This structural change proved very effective since it provided an hexapeptide with a pA_2 value of 7.05 in the isolated rabbit aorta.

Table II reports the effect of the peptides **1–3**, **7**, and **10** on the increase in arterial pressure produced by angiotensin II in anesthetized rats in comparison to the effect of sarmesin (**12**) and salarasin (**11**). Heptapeptide **1** had no effect up to 300 μg/kg/per min infusion rate; that the analogues **2**, **3**, **7**, and **10** displayed an antagonistic effect to pressure response to bolus injection of AII is apparent. The potency of the antagonistic effect in vivo is somewhat lower than that of sarmesin or saralasin and the duration of action (at the dose tested) is generally similar (as estimated from the time to recover full effect of the AII bolus injection after cessation of the infusion of the antagonist). All four peptides **2**, **3**, **7**, and **10** also produced a transient agonistic response similar to that observed with other peptidic antagonists.[7]

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Table II. Effect of Saralasin (11), Sarmesin (12), and Analogues **2**, **3**, **7**, and **10** on the Increase in Arterial Pressure Produced by Angiotensin II in the Anesthetized Rat

AII antagonist	dose, μg/kg per min	variation in mean arterial pressure (% of control)[b]						
		control	during antagonist[a]	time after antagonist infusion, min				
				5	10	20	40	60
saralasin (n = 4)	10[c]	100	0	29 ± 5	50 ± 5	78 ± 5	96 ± 4	94 ± 6
sarmesin (n = 4)	30[d]	100	0	35 ± 1	63 ± 3	87 ± 4	96 ± 4	97 ± 3
analogue **2** (n = 2)	100[f]	100	9 ± 1	24 ± 3	36 ± 0	60 ± 2	93 ± 2	100
analogue **3** (n = 6)	60[e]	100	6 ± 3	50 ± 5	61 ± 5	78 ± 5	91 ± 4	91 ± 5
analogue **7** (n = 2)	100[g]	100	19 ± 1	70 ± 4	86 ± 4	89 ± 1	91 ± 7	100
analogue **10** (n = 4)	100[h]	100	5 ± 3	10 ± 5	13 ± 5	28 ± 5	67 ± 4	82 ± 5

[a] Variation of MAP (% of control) induced by a bolus injection of 30 ng/kg of AII during the infusion of the test compound. [b] Values are mean ± SEM. [c] $1.1\ 10^{-8}$ mol/kg per min. [d] $2.5\ 10^{-8}$ mol/kg per min. [e] $7.1\ 10^{-8}$ mol/kg per min. [f] $1.7\ 10^{-7}$ mol/kg per min. [g] $1.15\ 10^{-7}$ mol/kg per min. [h] $1.2\ 10^{-7}$ mol/kg per min.

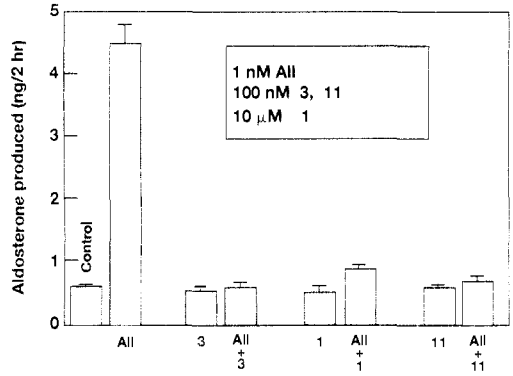

Figure 3. Effect of AII-(1–7) (**1**, at 10 μM), [Sar[1]]AII-(1–7)-NH_2 (**3**, at 0.1 μM), and saralasin (**11**, at 0.1 μM) on aldosterone release from isolated rat adrenal zona glomerulosa cells in the absence and the presence of AII (1 nM).

Figure 3 compares the effect of angiotensin II, analogue **1**, and analogue **3** on aldosterone release from rat adrenal zona glomerulosa cells. Only angiotensin II was able to stimulate aldoterone release (EC_{50} = 0.6 nM). When tested for inhibition of aldosterone release in response to a 1 nM angiotensin II, analogue **3** (at 100 nM) completely inhibited the AII response while analogue **1** required 100 times this dose (10 μM) to produce a similar inhibitory effect. The weak inhibitory effect of AII-(1–7) (**1**) in this test is consistent with a similar observation by Capponi and Catt in dog glomerulosa cells.[9] Thus, in terms of angiotensin II stimulated aldosterone release, [Sar[1]]AII-(1–7)-NH_2 is a robust antagonist, at least 100-fold more potent than AII-(1–7), which is in good agreement with the results observed in the myotropic assay.

Table III reports the results of attempts to inhibit the drinking response to intracerebroventricular (icv) injections of AII in conscious Sprague–Dawley rats with sarmesin and analogues **1** and **3**. The AII receptor antagonist sarmesin (**12**; 500 pmol icv) significantly attenuated AII-stimulated water intake (100 pmol icv). The two other analogues (500 pmol icv) did not affect water drinking induced by icv administration of 100 pmol of AII. Even at 2500 pmol icv (5 times the dose of sarmesin that effectively blocked water intake), analogue **3** did not show antagonist effect in the brain. Interestingly, analogue **3** and sarmesin have very similar potencies in peripheral tissues as antagonists to AII pressor effect. Injection of sarmesin, **1**, or **3** alone had no effect on water intake.

With respect to the questions asked in the introduction, the following answers are offered. First, none of the new peptides examined in this study showed any agonist activity in vitro, in agreement with the premises that led to the design of these C-truncated peptides. A low residual agonistic effect was observed at the high doses used in vivo, indicating that Phe[8] alone or Phe[8] in conjunction with a free hydroxyl on Tyr[4] (as shown by **7**) are not the only determinants for agonistic activity.

Table III. Effect of Sarmesin (**12**), AII-(1–7) (**1**), and [Sar1]AII-(1–7)-NH_2 (**3**) on Angiotensin II Drinking Response in the Conscious Rat

AII + compound[b] (mL/20 min)[a]	dose antagonist, pmol icv	water intake
(vehicule)		7.6 ± 0.5 (n = 22[c])
sarmesin (**12**)	500	3.1 ± 1.6 (n = 5)*
AII-(1–7) (**1**)	500	6.1 ± 1.9 (n = 4)
[Sar[1]]AII-(1–7)-NH_2 (**3**)	500	10.0 ± 0.7 (n = 4)
[Sar[1]]AII-(1–7)-NH_2 (**3**)	2500	10.8 ± 1.06 (n = 5)

[a] Data are mean ± SE. *$p < 0.05$ compared to vehicule alone. [b] AII (100 pmol) was given icv immediately after the test compound. [c] Includes the verification carried on each rat the day before the actual experiment was performed.

Second, the potency of the antagonist effect of the analogues examined is sensitive to the structural modifications at position 1 and 7 that we have examined. Thus, sarcosine substitution over aspartic acid in position 1 is required to produce the desired effect and the C-terminal proline could not be replaced by glycine or sarcosine. However, the proline residue could be deleted with small loss of activity when a tertiary *N,N*-dimethyl amide function is used at the C-terminus of 6-histidine. The simple hexapeptides **8** and **9** were inactive. This may be interpreted as the existence of some lipophilic interaction between the proline backbone and the receptor or a conformational effect on the preceding fragment Ile[5]-His[6]. The interaction with the receptor, however, appears similar whether a free carboxylic (negatively charged at pH 7) or a primary amide (neutral) group is present at the C-terminus of the heptapeptide (both groups can participate in a hydrogen bond). Methylation of the phenolic function of 4-tyrosine did not modify significantly the affinity of the heptapeptide for the receptor, as judged from the pA_2 value of analogue **7**. Concurrent modification of position 4 and truncation of position 8 did not give rise to any increase in potency nor did this change suppress the residual agonistic activity observed in vivo. A similar observation has been reported for simultaneous modifications at positions 4 and 8.[18] It is noteworthy that in these truncated peptides, the increase in affinity due to the replacement of aspartic acid by sarcosine in position 1 is much more important (2 orders of magnitude) than in the agonist or antagonist octapeptides.[3,7]

Third, in the isolated rabbit aorta assay, all the C-truncated antagonists described are competitive, fully reversible antagonists in contrast with the behavior of saralasin and related antagonists containing an aliphatic side chain substituted amino acid at position 8. The an-

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tagonist effect was also observed for AII-stimulated steroidogenesis in the two cases examined (1 and 3) with conservation of their relative potencies in the myotropic assay.

Fourth, the duration of action of the peptides examined in vivo was similar to that of other peptidic antagonists (20–30 minutes following cessation of infusion most of the antagonistic effect had subsided) although compound 10 showed a tendency to have a somewhat longer duration of action, perhaps the result of both modified termini being more resistant to amino- and carboxy-peptidases.

The finding that no antagonistic activity for 3 was observed in the brain at doses similar or higher than those at which sarmesin antagonized the dypsogenic effect of AII is exciting and in agreement with other literature report. Indeed, in binding studies with 3, several hundred fold differences between affinity for the brain and the uterus receptor have been reported.[19] These observations, perhaps to consider in parallel with the recent report on the presence of the heptapeptide AII-(1–7) in various brain tissues at concentrations similar or higher than those of AII itself,[20] may reflect some differences between the central and peripheral AII receptor populations.

In conclusion, the strategy used to design these peptides has been successful in producing competitive, fully reversible antagonists which inhibit angiotensin II myotropic and pressor responses in vitro and in vivo. The reduced size of these compounds with respect to traditional AII antagonists provide interesting information about the interactions prevalent in the hormone-receptor complex. Last but not least, [Sar1]AII-(1–7)-amide holds the promise to uncover some of the molecular determinants for discrimination between receptor subtypes. Such information should prove useful for the modeling and design of further modified peptides.

Experimental Section

Chemistry. All peptides were prepared by solid-phase synthesis[21] with the aid of an Applied Biosystems Inc. Model 430A peptide synthesizer. The synthesis was performed with 4-methylbenzhydrylamine resin or Merrifield resin serving as an insoluble support that generated respectively a carboxamide or a carboxylic acid C-terminal in the final product. Boc-*N*-π-[(benzyloxy)methyl]-L-histidine was esterified to a chloromethyl resin by the Gisin method.[22] Most protected amino acids (free or on resin) were purchased from Peninsula Laboratories or Peptides International Inc. The *tert*-butyloxycarbonyl group (Boc) was used for amino protection during the coupling procedure. Reactive side chain protections were as follows: Arg, N^g-tosyl; Asp, *O*-benzyl; Tyr, *O*-[[(2-bromophenyl)methoxy]carbonyl]; His, *N*-π-[(phenylmethoxy)methyl]. The peptides were assembled by using the standard protocols of the Applied Biosystems Inc. System Software version 1.30 except for the coupling of histidine, which required a customized activation cycle using dimethylformamide (DMF) as the solvent. Removal of the peptides 1–9 from the resin and simultaneous deprotection of the side-chain functions were achieved by treatment with anhydrous hydrogen fluoride (0 °C, 60–90 min). In the case of peptide 10, the dried resin obtained after assembling the amino acid sequence was suspended in dry dimethylamine and stirred for 96 h at 25 °C. After evaporation of the dimethylamine, the residue was extracted with methanol. Removal of the methanol left a yellow powder, which was treated with dry HF in the presence of anisole and 2-mercaptopyridine. Removal of HF was followed by repetitive washing of the deprotected peptide with diethyl ether and ethyl acetate. In all cases, purification was by preparative, reverse-phase, high-performance liquid chromatography on a C_{18}-bonded silica gel column. The homogeneous peptides conformed to theoretically expected amino acid composition and were further characterized by fast atom bombardment mass spectroscopy and in some cases by ^1H NMR (300 MHz, D_2O solutions, pH ≅ 2), except for compound 10, for which NMR data were collected at 500 MHz on a Varian VXR-500 spectrometer at 303 K. The phase-sensitive, double-quantum filtered COSY spectrum was obtained with 880 × 4096 hypercomplex data points using a 3-s recycle delay. The two-dimensional data were processed on a SUN Microsystems 4/110 workstation using VNMR software (Varian Associates, Palo Alto, CA). After zero filling to 4096 × 4096 data points, the data was processed by using phase-shifted sinebell apodization and base-line correction.

AII-(1–7) (1). Amino acid analysis (theoretical, found): Asp (1, 1.01), Arg (1, 1.00), Val (1, 0.88), Tyr (1, 0.99), Ile (1, 0.81), His (1, 0.85), Pro (1, 1.03). FAB mass spectrum $(M + H)^+$, MW: 899.5, 899.02.

[Sar^1]AII-(1–7) (2). Amino acid analysis (theoretical, found): Arg (1, 1.00), Val (1, 0.89), Tyr (1, 1.01), Ile (1, 0.81), His (1, 0.85), Pro (1, 1.03). FAB mass spectrum $(M + H)^+$, MW: 855.5, 855.01.

[Sar^1]AII-(1–7)-NH$_2$ (3). Amino acid analysis (theoretical, found): Arg (1, 1.01), Val (1, 0.95), Tyr (1, 0.99), Ile (1, 0.85), His (1, 0.88), Pro (1, 1.05). FAB mass spectrum $(M + H)^+$, MW: 854.4, 854.03.

[Sar^7]AII-(1–7)-NH$_2$ (4). Amino acid analysis (theoretical, found): Asp (1, 0.98), Arg (1, 1.05), Val (1, 0.94), Tyr (1, 1.02), Ile (1, 0.86), His (1, 1.02). FAB mass spectrum $(M + H)^+$, MW: 872.47, 872.02.

[Sar^1,Gly^7]AII-(1–7)-NH$_2$ (5). Amino acid analysis (theoretical, found): Arg (1, 1.05), Val (1, 0.90), Tyr (1, 1.07), Ile (1, 0.87), His (1, 1.00), Gly (1, 0.95). FAB mass spectrum $(M + H)^+$, MW: 814.5, 814.05.

[Sar^{1,7}]AII-(1–7)NH$_2$ (6). Amino acid analysis (theoretical, found): Arg (1, 1.03), Val (1, 0.91), Tyr (1, 1.03), Ile (1, 0.91), His (1, 1.00). FAB mass spectrum $(M + H)^+$, MW: 828.41, 827.99.

[Sar^1,*O*-MeTyr^4]AII-(1–7)-NH$_2$ (7). Amino acid analysis (theoretical, found): Arg (1, 0.98), Val (1, 0.98), Tyr (1, 0.85), Ile (1, 0.87), His (1, 0.94), Pro (1, 1.03). FAB mass spectrum $(M + H)^+$, MW: 868.6, 868.05.

AngII-(1–6)-NH$_2$ (8). Amino acid analysis (theoretical, found): Asp (1, 1.00); Arg (1, 1.08); Val (1, 0.96); Tyr (1, 1.03); Ile (1, 0.84); His (1, 0.94). FAB mass spectrum $(M + H)^+$, MW: 801.54, 800.97.

[Sar^1]AII-(1–6)-NH$_2$ (9). Amino acid analysis (theoretical, found): Arg (1, 1.06), Val (1, 0.98), Tyr (1, 0.95), Ile (1, 0.92), His (1, 1.06). FAB mass spectrum $(M + H)^+$, MW: 757.36, 756.91.

[Sar^1]AII-(1–6)-N(CH$_3$)$_2$ (10). The dry resin from SPPS (1 g; 0.5 mequiv/g) is suspended in dimethylamine in a pressure tube for 5 days at 25 °C. After evaporation of dimethylamine, the residue is extracted with methanol. After removal of the methanol, an oil is obtained which is extracted with chloroform. By ^1H NMR, this chloroform extract contains the carbamate derived from the reaction of dimethylamine on the benzyloxycarbonyl group from the Tyr(Br-Z). After HF deprotection the remaining peptide is purified by HPLC as usual. Amino acid analysis (theoretical, found): Arg (1, 0.99), Val (1, 1.0), Tyr (1, 1.01), Ile (1, 0.91), His (1, 0.82). FAB mass spectrum $(M + H)^+$, MW: 785.5, 784.97. ^1H NMR (δ, ppm, D_2O/H_2O, pH ≅ 2,5): 8.37 (b s, 1 H, C^2H His), 7.2 (s, 1 H, C^4H His), 7.08 (d, 2 H, $C^{2,6}H$ Tyr), 6.76 (d, 2 H, $C^{3,5}H$ Tyr), 5.01 (t, 1 H, C^α His), 4.6 (t, 1 H, $C^\alpha H$ Tyr), 4.3, (t, 1 H, $C^\alpha H$ Arg), 4.15 (d, 1 H, $C^\alpha H$ Val), 4.02 (d, 1 H, $C^\alpha H$ Ile), 3.9 (s, 2 H, $C^\alpha H$ Sar), 3.1 (m, 2 H, $C^\delta H$ Arg), 3.07 (m, 2 H, $C^\beta H$ His), 3.05 (s, 3 H, NCH_3), 2.9 (m, 2 H, $C^\beta H$ Tyr), 2.85 (s, 3 H, NCH_3), 2.75 (s, 3 H, NCH_3), 1.91 (m, 1 H, $C^\beta H$ Val), 1.71 (m + m, 1 H + 2 H, $C^\beta H$ Ile + $C^\beta H$ Arg), 1.5 (m, 2 H, $C^\gamma H$ Arg); 1.37 (m, 1 H, $C^{\gamma 1}H_2$ Ile), 1.1 (m, 1 H, $C^{\gamma 1}H_2$ Ile), 0.95 (d, 3 H, $C^{\gamma 1}H$ Val), 0.86 (d, 3 H, $C^{\gamma 2}H$ Val); 0.8 (t, 3 H, $C^{\gamma 2}H_3$ Ile), 0.75 (d, 3 H $C^\delta H$ Ile).

Biology. Peptides 1–10 were tested for agonist and antagonist activity in rabbit aortic rings. Male New Zealand white rabbits (2–2.5 kg) were sacrificed by an overdose of pentobarbital and exsanguinated via the carotid arteries. The thoracic aorta was removed, cleaned of adherent fat and connective tissue, and then cut into 3-mm ring segments. The endothelium was removed from

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the rings by gently sliding a rolled up piece of filter paper into the vessel lumen. The rings were then mounted in a water-jacketed tissue bath, maintained at 37 °C, between a moveable and a fixed stainless steel wire, with the moveable end attached to an FT03 Grass transducer coupled to a Model 7D Grass Polygraph for recording isometric force responses. The bath was filled with 20 mL of oxygenated (95% oxygen/5% carbon dioxide) Krebs solution of the following composition (nM):(130) NaCl, (15) $NaHCO_3$, (15) KCl, (1.2) NaH_2PO_4, (1.2) $MgSO_4$, (2.5) $CaCl_2$, and (11.4) glucose. The preparations were equilibrated for 1 h before approximately 1 g of passive tension was placed on the rings. For the agonist assay, the rings were exposed to increasing concentrations of the test compound, at 30-min intervals, during which time the tissue was washed three times with 20 mL of fresh Krebs solution. For the measurement of antagonistic activity, paired rings from the same rabbits were used; one was exposed to increasing concentrations of AII (at 30-min intervals) and a second ring was exposed to increasing concentrations of AII in the presence of the test compound, which was added 5 min prior to the addition of AII. The concentration–response curves for AII in the presence of the antagonist were evaluated in terms of the percent of the maximal contraction of the control ring exposed only to AII. pD_2 values for AII were calculated from the AII concentration response curves while pA_2s were determined according to the method of Schild.[23]

The in vivo antagonist activity of the peptides was evaluated in ganglion-blocked, anesthetized rats. Male Sprague–Dawley rats (240–400 g) were anesthetized with Inactin (100 mg/kg ip) and instrumented with two catheters (PE-50), one in a single femoral vein and one in a single femoral artery to administer drugs and determine arterial pressure (MAP), respectively. Autonomic neurotransmission was interrupted by treatment with mecamylamine (3 mg/kg iv) and atropine (400 mg/kg ip). When MAP stabilized, AII (30 ng/kg iv bolus) was administered four times at 10-min intervals to achieve a reproducible pressor response. The test peptide was then infused iv for 20 min before rechallenging with AII. Infusion of the antagonist was discontinued, and AII was again administered at 5–10-min intervals during the hour which followed.

The determination of angiotensin II stimulated aldosterone release was performed on isolated glomerulosa cells from Sprague–Dawley rats prepared as reported previously.[24] Incubation tubes contained 1-mL cell suspension ($0.5–2 \times 10^5$ cells) and various concentrations of peptides. All incubations were done in triplicate. The tubes were gassed with 95% O_2/5% CO_2, capped, gently mixed, and placed in a shaking water bath at 37 °C. After incubating for 2 h, the tubes were centrifuged at 100 × g for 15 min at 4 °C. The cell-free media were decanted and stored at −80 °C for aldosterone determination by direct radioimmunoassay (Diagnostic Products Corp.). The aldosterone concentration was determined from a standard curve. Intra- and interassay coefficients of variation were 3.2% and 5.3%, respectively.

The ability of the peptides to alter the drinking response to intracerebroventricular (icv) injection of angiotensin II was studied in conscious Sprague–Dawley rats. Each rat had a guide cannula implanted in the lateral cerebral ventricle under pentobarbital sodium or chloral hydrate anesthesia. Rats were allowed 3–4 days of recovery. All compounds were given icv. The test peptides (500 or 2500 pmol) were given immediately prior to AII (100 pmol). Angiotensin II induced drinking was verified in each rat the day before testing of the experimental compounds. Each experimental compound was tested in a separate group of rats.

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N-(3-[^{18}F]Fluoropropyl)-*N*-nordiprenorphine: Synthesis and Characterization of a New Agent for Imaging Opioid Receptors with Positron Emission Tomography

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A series of *N*-fluoroalkyl (**1–5**) and *N*-alkyl (**6–8**) analogues of the high-affinity opioid receptor antagonist diprenorphine (**9**) has been synthesized and evaluated with in vitro binding assays. Three of the *N*-fluoroalkyl compounds were prepared with the positron-emitting radionuclide ^{18}F (**1a**, **2a**, **5a**), and their biodistribution was determined in rats. Compounds **2a** and **5a** were made by using a two-step labeling procedure, [^{18}F]fluoride displacement of an iodoalkyl triflate followed by *N*-alkylation, that required 2 h and proceeded in 4–6% overall radiochemical yield at the end of synthesis. The effective specific activity of compounds **2a** and **5a**, determined by competitive receptor binding assay, was 840–1820 Ci/mmol. Compound **1a** was made by the same two-step procedure, with the bromoalkyl triflate, in 0.3–0.6% radiochemical yield at an effective specific activity of 106–264 Ci/mmol. Specificity of binding in vivo was measured as the percent injected dose/gram of striatal tissue divided by the percent injected dose/gram of cerebellar tissue. The best striatum to cerebellum ratio (3.32 ± 0.74 at 30 min) was achieved with *N*-(3-[^{18}F]-fluoropropyl)-*N*-nordiprenorphine (**2a**, [^{18}F]FPND). The high specific binding demonstrated by this compound indicates that it may be useful for in vivo imaging of opioid receptors with positron emission tomography.

In recent years, positron emission tomography (PET) has emerged as an important technique for studying receptors in living animals and humans.[1] In particular, our current research has focused on the opioid receptor system.[2] Several positron-emitting receptor ligands have been developed and used for imaging opioid receptors in living humans. These include the μ type specific ($\mu \gg \delta > \kappa$) ligand [^{11}C]carfentanil,[3] the universal non type specific

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