the 2-amino group is replaced by a nonpolar substituent. Further studies aimed at elucidating the mechanism of action of these compounds and exploring their therapeutic potential are planned.

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A Carboxy-Terminus Truncated Analogue of Angiotensin II, [Sar^1]angiotensin II-(1-7)-Amide, Provides an Entry to a New Class of Angiotensin II Antagonists

Sir:

Potent antagonists to 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 Phe^8 by aliphatic amino acids and of Asp^1 by sarcosine.[2] The chemical and biological properties of saralasin, [Sar^1,Val^5,Ala^8]AII,[3] which has blood pressure lowering activity in humans, and related analogues, e.g., [Sar^1,Ile^8]AII and [Sar^1,Thr^8]AII, have been studied extensively.[4,5] A second class of antagonists differs structurally from AII by modification at the Tyr^4 residue. Sarmesin, [Sar^1,(Me)Tyr^4]AII, the prototype of this second class, is a less potent but fully competitive, reversible antagonist.[6]

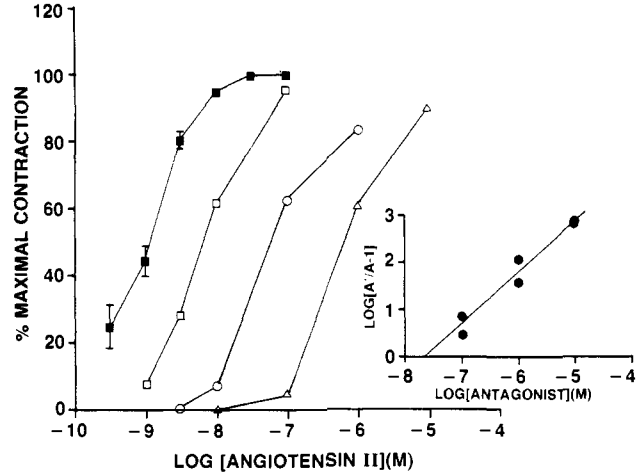

Figure 1. Concentration-response curves for angiotensin II in isolated rabbit aortic rings from a representative experiment in the absence (■) and presence (□) of 10^{-7} M, (○) 10^{-6} M, and (△) 10^{-5} M [Sar^1]AII-(1-7)NH_2. pD_2 for angiotensin II = 8.98 ± 0.07 (n = 6). Inset: Schild plot of all data with m = 1.08 ± 0.12 and pA_2 = 7.63 ± 0.20.

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.

One strategy to design peptidic hormone antagonists is based on the concept that a peptide hormone is composed of distinct binding and activating components.[7] Synthesizing the binding component alone should produce a fragment which binds to the receptor without activating second-messenger systems and initiating biological response. In the present study, we used this strategy to design [Sar^1]angiotensin II-(1-7)-amide ([Sar^1]AII-(1-7)-NH_2, 1) as the most rational probe based on the hypothesis that residues 1-7 define the specificity, intensity, and duration of action of the biological effect, while the nature of residue 8 invokes agonist or antagonist activity.[8,9] In addition Sar was introduced in position 1, a substitution known to increase the potency in other AII analogue series.

[Sar^1]AII-(1-7)NH_2 (1) and sarmesin were prepared by solid-phase synthesis[10] with the aid of an Applied Biosystems Inc. Model 430A peptide synthesizer. Purification was by preparative, reverse-phase, high-performance liquid chromatography on a C_{18} bonded silica gel column. The

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Table I. Effect of Saralasin, Sarmesin, and 1 on the Increase in Arterial Pressure Produced by Angiotensin II in the Anesthetized Rat

		variation in mean arterial pressure, % of control[b]						
				time after antagonist, min				
AII antagonist	dose, μg/kg per min	control	during AII X[a]	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 1 (n = 6)	60[e]	100	6/3	50/5	61/5	78/5	91/4	91/5

[a] AII X, angiotensin II antagonist. [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.

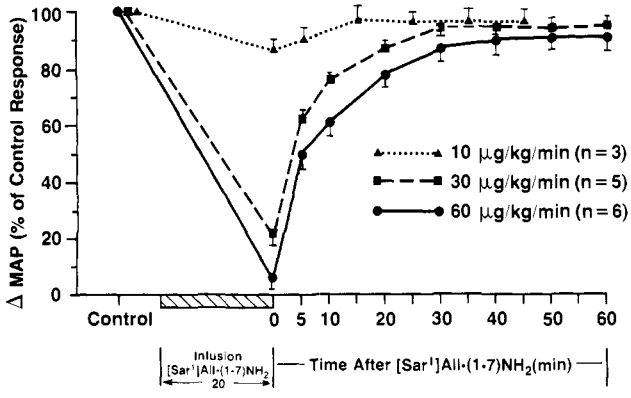

Figure 2. Increase in arterial pressure (expressed as % of control) produced by AII (30 ng/kg iv) before, during, and after iv infusion of [Sar^1]AII-(1–7)amide in ganglionic-blocked, anesthetized rats. Values are means ± SEM; the number of animals given each dose is shown in parentheses. [Sar^1]AII-(1–7)NH_2 exhibited dose-dependent inhibition of AII pressor activity which was completely reversible within 30 min after discontinuing the infusion of the antagonist.

homogeneous peptides conformed to theoretically expected amino acid composition and were further characterized by fast atom bombardment mass spectroscopy and Edman sequence analysis.

[Sar^1]AII-(1–7)NH_2 was tested for agonist and antagonist activity in rabbit aortic rings. pD_2 values for AII were calculated from the AII concentration–response curves while pA_2s were determined from Schild plots.[11] Data for the Schild analysis was fitted by using the method of least squares. The in vivo antagonist activity of the peptides was evaluated in ganglionic-blocked, anesthetized rats. Figure 1 shows the concentration–response relationship for rabbit aorta produced by angiotensin II in the absence and in the presence of different concentrations of 1. In the presence of the latter, the dose–response curves are shifted to the right in a concentration-dependent manner, yielding parallel curves with the same maximal force of contraction. The pA_2 derived from the Schild plot of three concentrations of [Sar^1]AII-(1–7)NH_2 was 7.63 ± 0.20. In the same assay, saralasin ([Sar^1,Val^5,Ala^8]AII) does not produce a parallel dose–response curve and the maximum response to the tissue can not be achieved after extensive washing. Therefore, the pA_2 value reported for saralasin (8.61) has been determined by a modified method.[3,12] [Sar^1]AII-(1–7)NH_2 was devoid of agonistic activity in isolated rabbit aorta assay up to concentrations of 10^{-5} M. Figure 2 shows the in vivo effect of various doses of 1 on the pressor response produced by an iv bolus injection of 30 ng/kg AII. For comparison, the results obtained with two other peptidic antagonists, saralasin and sarmesin ([Sar^1,(Me)Tyr^4]AII), are also reported (Table I). [Sar^1]All-(1–7)NH_2 antagonized the increase in arterial pressure produced by AII in a dose-dependent manner (Figure 2), with essentially complete blockade at 60 μg/kg per min. Its potency was at least 6 times less than that of saralasin and marginally inferior to that of sarmesin (Table I). A transient agonist-like pressor response, typical of AII antagonists in vivo, was observed during the initial 5 min of analogue 1 infusion.

The present results indicate that C-truncation of the AII sequence can produce analogues that antagonize the natural hormone myotropic and pressor effects both in vitro and in vivo. This observation is unprecedented with respect to the traditional knowledge of AII agonists/antagonists structure–activity relationships which emphasize the crucial role of the residue at position 8. Indeed, convergent data from the literature suggest that the integrity of the carboxyl group at the C-terminus of the peptide is an absolute requirement for biological activity.[1-3,8,9,12] For example, removal of either Phe^8 or dipeptide Pro^7-Phe^8 completely destroys the pressor and myotropic activity of AII although Regoli reported a weak (pA_2 = 4) antagonistic effect of des[Phe^8]-AII.[12] More recently, Hsieh and Marshall reported that modification of the C-terminal carboxylic group results in analogues with negligible agonistic or antagonistic properties.[13] However, the report of a low binding affinity of [Val^5]-AII-(1–7) (des[Phe^8]-[Val^5]-AII) to AII adrenal and uterus 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[14] stimulated our interest in the design of AII C-terminus truncated analogues as potential antagonists.

The robust antagonistic effect that we observe with [Sar^1]AII-(1–7)NH_2 is taken as an indication that the approach[7] used to design this antagonist is a valid one. It also emphasizes the role of Phe^8 in eliciting biological response upon binding of AII to its receptor ([Sar^1]AII is a more potent agonist than the hormone itself[15]). Furthermore, analogue 1 behaves as a truly competitive and fully reversible inhibitor both in vivo and in vitro, properties that it shares only with sarmesin, the Tyr^4-modified AII antagonist. The success of producing antagonistic activity in our C-truncated analogue, in opposition with previous reports, resides in neutralization of the C-terminus combined with the introduction of Sar at position 1. These structural features in analogue 1 produced an AII antagonist about 2 orders of magnitude more potent than the parent AII-(1–7) fragment.[14] The very weak residual agonist activity of analogue 1 in vivo may suggest that some structural elements of the AII-(1–7) sequence participate in expression of biological activity. Residues 4 and 6 are the major candidates for that effect.[1,6,12]

The observations reported here indicate that C-terminus

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truncation provides a new route for the synthesis of AII antagonists. The effect of [Sar[1]]AII-(1-7)carboxamide on other angiotensin-mediated responses, e.g. secretion of catecholamines and aldosterone, renin release, and central nervous system effects, have yet to be determined. Further structure–activity relationship studies, including the effect of additional deletions at the C-terminus, are now under investigation.

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Articles

Synthesis of Gastrin Antagonists, Analogues of the C-Terminal Tetrapeptide of Gastrin, by Introduction of a β-Homo Residue

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A series of analogues of Boc-Trp-Leu-Asp-Phe-NH_2, a potent gastrin agonist, were synthesized by introducting a β-homo residue in the sequence. These compounds were tested in vivo on acid secretion, in the anesthetized rat, and for their ability to inhibit binding of labeled gastrin to its receptors on gastric mucosal cells. These analogues behaved as gastrin antagonists. The most potent compounds in this series were Boc-Trp-Leu-β-homo-Asp-$NHCH_2C_6H_5$ (**10**) (IC_{50} = 1 μM, ED_{50} = 0.2 mg/kg), Boc-Trp-Leu-β-homo-Asp-$NHCH_2CH_2C_6H_5$ (**11**) (IC_{50} = 0.75 μM, ED_{50} = 0.5 mg/kg), Boc-Trp-Leu-β-homo-Asp-Phe-NH_2 (**12**) (IC_{50} = 1.5 μM, ED_{50} = 0.1 mg/kg), and Boc-Trp-Leu-β-homo-Asp-D-Phe-NH_2 (**13**) (IC_{50} = 2 μM, ED_{50} = 0.1 mg/kg). We could demonstrate the importance of the region of the peptide bond between leucine and aspartic acid and of the structure of the C-terminal dipeptide Asp-Phe-NH_2, for exhibiting biological activity on acid secretion.

Gastrin is a peptide hormone which was isolated from hog antral mucosa by Gregory and Tracy.[1] It exists in an unsulfated (gastrin I) and in a sulfated form (gastrin II). Among the numerous biological actions of gastrin, we have been particularly interested in its property of stimulating gastric acid secretion.[2] It was demonstrated early on that the C-terminal tetrapeptide of gastrin was able to display the whole range of the biological activities of gastrin.[3] Extensive structure–activity relationships[4] have shown that replacement of tryptophan and aspartic acid lead to weakly active or inactive compounds, whereas methionine can be substituted by a leucine or norleucine without significant loss of activity. We have recently demonstrated the importance of the peptide bonds of the C-terminal tetrapeptide of gastrin for biological activity, particularly the bond between methionine and aspartic acid. The pseudopeptide Boc-Trp-Leu-ψ(CH_2NH)-Asp-Phe-NH_2, in which the peptide bond between leucine and aspartic acid had been replaced by a CH_2NH bond, was found to bind to the gastrin receptor with the same apparent affinity as the C-terminal tetrapeptide analogue of gastrin, Boc-Trp-Leu-Asp-Phe-NH_2, but was unable to stimulate gastric acid secretion. In fact, this compound was an inhibitor of gastrin-induced acid secretion, with an ED_{50} value of 0.3 mg/kg.[5] To the contrary, the pseudopeptide analogue bearing a "reduced bond" between tryptophan and leucine, i.e. Boc-Trp-ψ(CH_2NH)-Leu-Asp-Phe-NH_2, behaved as a full agonist of gastrin, with the same potency and efficacy as Boc-Trp-Leu-Asp-Phe-NH_2. We thus postulated that the bond between methionine (or leucine) and aspartic acid should be a peptide bond, in order to exhibit agonist activity on gastric acid secretion. Moreover, we observed an enzymatic cleavage between Met (or Leu) and Asp when gastrin analogues were incubated with a gastric mucosal cell membrane preparation[6] and even with gastric mucosal cells.[7] Whether or not this enzymatic cleavage is related to biological activity remains to be demonstrated. In previous works, we also investigated several modifications of this C-terminal dipeptide, i.e. substitution of the phenylalanine amide by a 2-phenylethylamine[8] or a 2-phenylethanol,[9] or retro-inverso modifications,[10] which all led to potent gastrin antagonists.

We thus speculated that modifications involving either the bond between methionine (or leucine) and aspartic acid (that would make it stable to enzymatic degradation) or specific modifications affecting the dipeptide Asp-Phe-NH_2

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