Eur J Med Chem (1990) 25, 589–594

Original article

Synthesis and biological activity of N-terminus modified [Ile8] angiotensin II analogues

PR Bovy*, JM O'Neal, E McMahon, M Palomo, GJ Smits, AJ Trapani, D McGraw

Cardiovascular Research, SRL, Monsanto Life Sciences Research Center, 700 Chesterfield Village Parkway, Chesterfield, MO 63198, USA

(Received 13 June 1989; accepted 23 January 1990)

Summary — Semi-peptidic analogues of the angiotensin II (AII) antagonist [Ile8]AII have been prepared by the solid phase method and, for some steps, by synthesis in solution. In the modified analogues **4–15**, the N-terminal di- or tripeptidic fragments (Asp-Arg or Asp-Arg-Val) in [Ile8]AII was replaced by non-peptidic moieties featuring amino or guanidino groups as substituent of simple alkyl spacers of various lengths. The compounds synthesized have been tested for their ability to produce an AII-like contraction and/or to inhibit AII-induced contractions in isolated rabbit aortae. All of them were found devoid of agonistic activity and most retained significant antagonistic activity in this assay. Analogue **5** antagonized AII-induced blood pressure effect in the anesthetized rat when infused at 30 μg/kg/min. The structure–activity relationships for these compounds are discussed.

Résumé — Synthèse et activité biologique d'analogues d'un antagoniste de l'angiotensine II, [Ile8] AII, à N-terminal modifié. *Une série d'analogues d'un antagoniste peptidique de l'angiotensine II (AII), [Ile8]AII, ont été préparés par une combinaison de techniques de synthèse en phase solide et en phase liquide. Les fragments di- ou tripeptidiques N-terminaux (Asp-Arg ou Asp-Arg-Val) du peptide antagoniste [Ile8]AII ont été remplacés dans les analogues **4–15** par un groupe ammonium ou guanidinium fixé sur des chaînes alkyles de différentes longueurs. La capacité de ces composés à antagoniser la réponse myotrope à l'angiotensine II a été testée sur tissus isolés (aorte de lapin). Tous les dérivés essayés se sont révélés, dans ce test, des antagonistes dépourvus d'activité agoniste. L'analogue **5**, infusé à 30 μg/kg/min, antagonise l'effet sur la pression sanguine de l'angiotensine II chez le rat anesthésié. Les relations de structure–activité sont discutées.*

angiotensin II / analogues / antagonists

Introduction

Potent peptidic angiotensin II (AII) antagonists have traditionally been obtained by a variety of alterations at positions 1 (aspartic acid), 4 (tyrosine) and 8 (phenylalanine) of the natural agonist sequence (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) [1–3]. Despite the extensive synthetic effort dedicated to the development of peptidic AII antagonists during the last 2 decades, comparatively little information has been published regarding their chain-length requirements, perhaps in relation to the structure of saralasin-like compounds for which the antagonist activity is directly linked to the presence of specific amino acid residues at both ends of the peptide (fig 1) [4, 18]. Some reports on the antagonistic activity of angiotensin III analogues have been reported previously [15–17].

EC Jorgensen *et al* had shown earlier that the N-terminal portion of AII could be simplified and yet retain significant pressor activity [12]. It was interesting to investigate the effect of N-terminal modifications on activity in a series of derivatives of the AII antagonist [Ile8]AII. Comparison between the effect of similar modifications in both the agonist and the antagonist series should provide further information on the structure–activity relationships of the peptide hormone. Also, such an approach could lead to antagonists of reduced molecular size by replacement of the N-terminal di- or tri-peptidic fragments by basic groups and alkyl spacers. Thus, we have designed several modified peptides in which amino or guanidino carboxylic acid, γ-Abu, δ-Ape, ε-Ahx, ω-Aoc, γ-Gbu, ε-Ghx were linked to the amino terminal function of the penta- or hexapeptides [Ile8]AII-(4–8) **1** and [Ile8]AII-(3–8) **2** derived from

*Correspondence and reprints

Abbreviations: these are according to the IUPAC-IUB Commission on Biochemical Nomenclature, Pure Appl Chemistry 1974, 40, 317. Other abbreviations include: iVal, 3-methyl-butanoic acid; DMF, dimethylformamide; TFA, trifluoro acetic acid; DCC, di-cyclohexylcarbodiimide; GDMP, 1-guanyl-3,5-dimethylpyrazole; HPLC, high pressure liquid chromatography; γ-Gbu, 4-guanidino-butanoic acid; ε-Ghx, 6-guanidino-hexanoic acid

[Ile8]AII (fig 2). Such fragments are supposed to mimic the Arg2 guanidinium function and/or the amino terminus, both thought to be important in the interaction with the receptor [5, 12]. Since no hypothesis prevails on the conformational folding of the hormone and its analogues, we have chosen to look at methylene linkers of different lengths between the peptidic fragments **1** and **2** and the non-peptidic moieties anticipated to mimic the Arg2 guanidinium group or the amino terminus of the deleted N-terminal fragments.

Chemistry

The compounds listed in table I were synthesized using solid phase peptide synthesis (SPPS) or a combination of SPPS steps and synthesis in solution. In particular, the peptidic fragments containing natural or unnatural amino acids were assembled on a solid support from *t*-butyloxycarbonyl protected elements using dicyclohexylcarbodiimide as condensation agent. Acylations were performed on the protected peptide chain on the resin. Guanidino derivatives (**8**, **9**, **14** and **15**) were prepared by guanylation of the free deprotected peptides **5**, **7**, **10** and **12** (fig 3). The guanylation reaction proceeded smoothly in basic aqueous medium. As judged by HPLC observation of the crude reaction mixtures, the reaction was quite selective. Use of other solvents, such as DMF-water or dioxane-water mixtures, gave similar or poorer results. The homogeneous compounds obtained by preparative, reverse-phase, high performance chromatography were characterized by a combination of amino acid analysis, fast atom bombardment mass spectroscopy and, in some case, by ^{1}H NMR spectroscopy at 300 MHz in D_2O solution (pH ≈ 2.5).

Biology

The compounds were tested for agonist and antagonist activity in rabbit aortic ring and for *in vivo* antagonist properties according to the protocols described in the *Experimental protocols*.

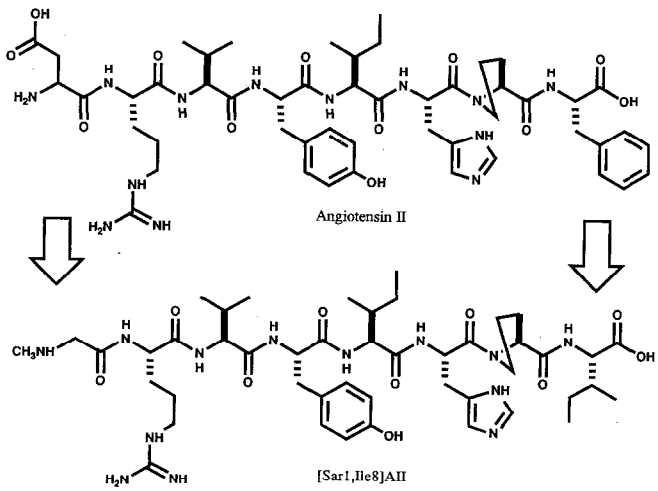

Fig 1. The structures of angiotensin II and [Sar1, Ile8]AII are compared in an arbitrary, fully extended, conformation. The arrows indicate where structural differences occur between the tissue hormone angiotensin II and its most documented class of synthetic peptidic antagonists, of which [Sar1, Ile8]AII is an example.

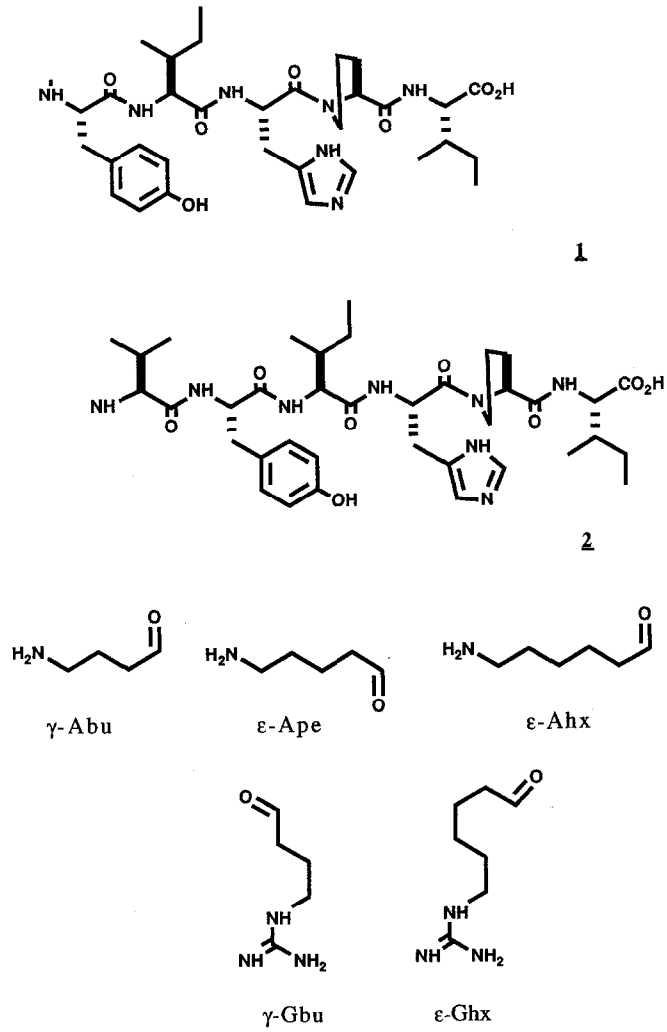

Fig 2. The structures (drawn with fully extended backbone conformations) of the peptidic (from [Ile8]AII) and non-peptidic fragments used to build the semi-peptidic analogues are displayed. The semi-peptidic analogues described have formally been obtained by acylation at the N-terminus of the fragments **1** and **2** by various amino- and guanidino-alkanoic acids.

Table I. Agonist and antagonist activities of compounds **1–15** in isolated rabbit aortae.

	Primary sequence [c]								in vitro *rabbit aorta*		
No	*1 Sar*	*2 Arg*	*3 Val*	*4 Tyr*	*5 Ile*	*6 His*	*7 Pro*	*8 Ile*	*No* [d] *of -(CH_2)*	*All like* [a] *%*	pA_2 [b]
1				Tyr	Ile	His	Pro	Ile		<0.1	<5
2			Val	Tyr	Ile	His	Pro	Ile		<0.1	<5
3			Ac-Val	Tyr	Ile	His	Pro	Ile		<0.1	6.6
4			iVal	Tyr	Ile	His	Pro	Ile		<0.1	5.6
5		γ-Abu	Val	Tyr	Ile	His	Pro	Ile	3	<0.1	7.2
6		δ-Ape	Val	Tyr	Ile	His	Pro	Ile	4	<0.1	7.4
7		ε-Ahx	Val	Tyr	Ile	His	Pro	Ile	5	<0.1	7.0
8		γ-Gbu	Val	Tyr	Ile	His	Pro	Ile	3	<0.1	7.5
9		ε-Ghx	Val	Tyr	Ile	His	Pro	Ile	5	<0.1	7.0
10			γ-Abu	Tyr	Ile	His	Pro	Ile	3	<0.1	5.2
11			δ-Ape	Tyr	Ile	His	Pro	Ile	4	<0.1	6.6
12			ε-Ahx	Tyr	Ile	His	Pro	Ile	5	<0.1	5.3
13			ω-Aoc	Tyr	Ile	His	Pro	Ile	7	<0.1	<5
14			γ-Gbu	Tyr	Ile	His	Pro	Ile	3	<0.1	6.15
15			ε-Ghx	Tyr	Ile	His	Pro	Ile	5	<0.1	<5

[a]Agonistic activity (% of AII-contractile response) tested by applying a dose of 10 μM of the peptide (or modified peptide) alone to the rabbit aorta preparation. [b]The pA_2 is the negative logarithm of the concentration of antagonist which halves the response to an ED_{50} dose of AII. The protocol used is essentially identical to that described by Scanlon *et al* [11]. For [Ile8]AII, pA_2 values of 7.9 and 9.2 have been reported [18]. [c]The compounds structure has been characterized by a combination of amino acid analysis, fast atom bombardment (FAB) mass spectroscopy and, in some cases, ^1H NMR at 300 MHz in D_2O solution (pH = ~ 2.5). [d]Refers to the number of methylene groups between the carbonyl group of the acylating fragment and its basic function.

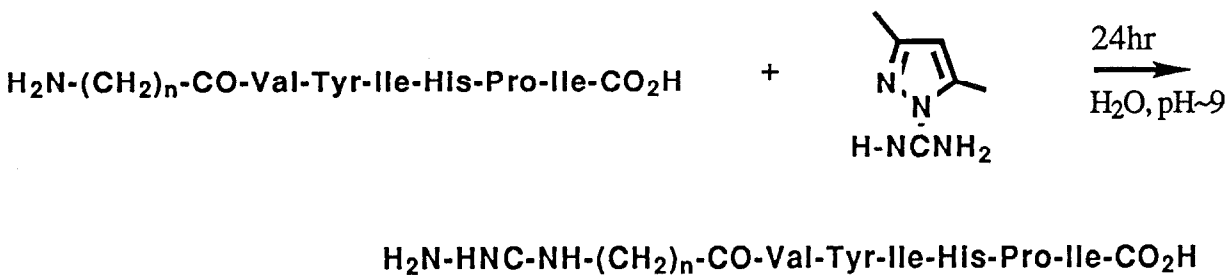

Fig 3. Schematic description of the guanylation reaction performed on the free peptides in aqueous solution using 3,5-dimethylpyrazole-1-carboxamidine.

Experimental protocols

Synthesis

The peptidic fragments were prepared by solid phase synthesis [6] with the aid of an Applied Biosystems Inc Model 430A peptide synthesizer. The synthesis was performed with Boc-Ile esterified to a 1% DVB resin serving as an insoluble support. Most amino acids were purchased from Peninsula Laboratories, Peptide International Inc, Serva or Chemalog. The *t*-butyloxycarbonyl group (Boc) was used for amino protection during the coupling procedure. Reactive side chain protections were: Tyr, O-2-bromo-benzyl-oxycarbonyl; His, π-*N*-benzyloxymethyl. When the protected amino acid was not commercially available, the Boc protecting group was introduced using 1,2,2,2-tetrachloroethyl *tert*-butyl carbonate and triethylaminc [7].

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. Non-alpha amino acids were coupled either using a 'glycine' cycle or by hand [8]. Acylation of the terminal amino group by isobutyric acid in **4** was performed by hand using a 4-fold excess of the acid and DCC in a solvent mixture of 30% DMF in dichloromethane. After 2 h of reaction at 25°C, the coupling was incomplete (Kaiser test) and was repeated for 4 h. Acetylation was performed with acetic anhydride in the usual manner [8]. Removal of the peptide from the resin and simultaneous deprotection of the side chain functions were achieved by treatment with anhydrous hydrogen fluoride (0°C, 60–90 min). The guanidinium groups were introduced by using 1-guanyl-3,5-dimethylpyrazole [9] (GDMP, commercially available from Aldrich) on a purified peptide according to the procedure described for compound **8** from table I. Purification was by preparative, reverse-phase, high performance liquid chromatography on a C_{18} bonded silica gel column. The homogeneous peptide conformed to theoretically expected amino acid composition and were further characterized by fast atom bombardment mass spectroscopy and, in some cases, by ^1H NMR spectroscopy at 300 MHz in D_2O solutions (pH ≈ 2.5).

[Ile8]AII-(4–8) ***1***
Amino acid analysis (theoretical, found): Pro (1, 1.01); Ile (2, 1.78); Tyr (1, 0.84); His (1, 0.67). FAB mass spectrum (calc $C_{32}H_{47}N_7O_7$; base peak of isotope cluster, $[M + H]^+$): 641.77; 642.1.

[Ile8]AII-(3–8) ***2***
Amino acid analysis (theoretical, found): Pro (1, 1.06); Ile (2, 1.87); Tyr (1, 0.94); His (1, 0.77); Val (1, 0.97). FAB mass spectrum (calc $C_{37}H_{56}N_8O_8$; base peak of isotope cluster, $[M + H]^+$): 740.90; 741.5.

[N-AcVal3,Ile8]AII-(3–8) ***3***
Amino acid analysis (theoretical, found): Pro (1, 0.96); Ile (2, 1.83); Tyr (1, 0.87); His (1, 0.79); Val (1, 0.95). FAB mass spectrum (calc $C_{39}H_{58}N_8O_9$; base peak of isotope cluster, $[M + H]^+$): 782.94; 783.5.

[iVal3,Ile8]AII-(3–8) ***4***
Amino acid analysis (theoretical, found): Pro (1, 1.01); Ile (2, 1.15); Tyr (1, 0.70); His (1, 0.58). FAB mass spectrum (calc $C_{39}H_{58}N_8O_9$; base peak of isotope cluster, $[M + H]^+$): 782.94; 783.5.

[γ-Abu2,Ile8]AII-(2–8) ***5***
Amino acid analysis (theoretical, found): Pro (1, 1.09); Ile (2, 1.77); Tyr (1, 0.98); His (1, 0.86); Val (1, 0.95). FAB mass spectrum (calc $C_{41}H_{63}N_9O_9$; base peak of isotope cluster, $[M + H]^+$): 826.01; 826.5.

[δ-Ape2,Ile8]AII-(2–8) ***6***
Amino acid analysis (theoretical, found): Pro (1, 1.11); Ile (2, 1.67); Tyr (1, 0.88); His (1, 0.82); Val (1, 0.90). FAB mass spectrum (calc $C_{42}H_{65}N_9O_9$; base peak of isotope cluster, $[M + H]^+$): 840.04; 840.5.

[ε-Ahx2,Ile8]AII-(2–8) ***7***
Amino acid analysis (theoretical, found): Pro (1, 0.99); Ile (2, 1.62); Tyr (1, 0.99); His (1, 0.80); Val (1, 1.02). FAB mass spectrum (calc $C_{43}H_{67}N_9O_9$; base peak of isotope cluster, $[M + H]^+$): 854.07; 854.5.

[γ-Gbu2,Ile8]AII-(3–8) ***8***
An aliquot (50 mg, ≈ 0.05 mmol) of [γ-Abu2,Ile8]AII-(2–8) **5** was dissolved in 5 ml H_2O and GDMP (60 mg, 0.3 mmol as 180 µl of a stock solution of 2 g GDMP in 6 ml H_2O at pH = 9.5) was added through a syringe. The pH of the resulting reaction mixture was adjusted to ≈ 9 with 2.5 N NaOH. After 24 h stirring at 25°C the conversion of the amino group to the guanidino group was almost complete as shown by analytical HPLC. The guanidino derivative had a higher retention time on C18 bonded silica gel (Vydac 218TP54) eluted with a linear gradient of 15–35% of acetonitrile in water containing 0.05% TFA over 25 min. The desired compound, [γ-Gbu2,Ile8]AII-(2–8) was purified by reverse phase HPLC (yield 45%) and characterized by amino acid analysis and FAB mass spectroscopy. Amino acid analysis (theoretical, found): Pro (1, 1.05); Ile (2, 1.67); Tyr (1, 0.98); His (1, 0.76); Val (1, 1.05). FAB mass spectrum (calc $C_{42}H_{65}N_{11}O_9$; base peak of isotope cluster, $[M + H]^+$): 868.05; 868.5.

[ε-Ghx2,Ile8]AII-(3–8) ***9***
Amino acid analysis (theoretical, found): Pro (1, 0.99); Ile (2, 1.71); Tyr (1, 0.88); His (1, 0.87); Val (1, 15). FAB mass spectrum (calc $C_{44}H_{69}N_{11}O_9$; base peak of isotope cluster, $[M + H]^+$): 896.11; 897.

[γ-Abu3,Ile8]AII-(3–8) ***10***
Amino acid analysis (theoretical, found): Pro (1, 1.11); Ile (2, 1.75); Tyr (1, 0.98); His (1, 0.80). FAB mass spectrum (calc $C_{36}H_{54}N_8O_8$; base peak of isotope cluster, $[M + H]^+$): 726.88; 727.4.

[δ-Ape3,Ile8]AII-(3–8) ***11***
Amino acid analysis (theoretical, found): Pro (1, 1.01); Ile (2, 1.85); Tyr (1, 0.95); His (1, 0.74). FAB mass spectrum (calc $C_{37}H_{56}N_8O_8$; base peak of isotope cluster, $[M + H]^+$): 740.91; 741.4.

[ε-Ahx3,Ile8]AII-(3–8) ***12***
Amino acid analysis (theoretical, found): Pro (1, 1.08); Ile (2, 1.79); Tyr (1, 0.99); His (1, 0.69). FAB mass spectrum (calc $C_{38}H_{58}N_8O_8$; base peak of isotope cluster, $[M + H]^+$): 754.93; 727.5.

[ω-Aoc3,Ile8]AII-(3–8) ***13***
Amino acid analysis (theoretical, found): Pro (1, 1.02); Ile (2, 1.68); Tyr (1, 0.96); His (1, 0.74). FAB mass spectrum (calc $C_{40}H_{62}N_8O_8$; base peak of isotope cluster, $[M + H]^+$): 782.99; 783.5.

[γ-Gbu3,Ile8]AII-(3–8) ***14***
Amino acid analysis (theoretical, found): Pro (1, 1.09); Ile (2, 1.65); Tyr (1, 0.95); His (1, 0.76). FAB mass spectrum (calc $C_{37}H_{56}N_{10}O_8$; base peak of isotope cluster, $[M + H]^+$): 769.92; 769.6.

[ε-Ghx3,Ile8]AII-(3–8) ***15***
Amino acid analysis (theoretical, found): Pro (1, 1.02); Ile (2, 1.56); Tyr (1, 0.98); His (1, 0.76). FAB mass spectrum (calc $C_{39}H_{60}N_{10}O_8$; base peak of isotope cluster, $[M + H]^+$): 796.97; 797.5.

Biology

The compounds **1–15** were tested for agonist and antagonist activity in rabbit aortic rings. Male New Zealand white rabbits (2–2.5 kg) were sacrificed using 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 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 $NaHCO_3$, 5 KCl, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, and 11.4 glucose. For the agonist assay, the rings were exposed to increasing concentrations of the compounds, at 30 min intervals, during which time the tissue was washed 3 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 compound acting as an antagonist. The compound tested for antagonist activity 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 Schild [10].

The *in vivo* antagonist activity of the peptides was evaluated in ganglionic-blocked, anesthetized rats. Male Sprague–Dawley rats (240–400 g) were anesthetized with inactin (100 mg/kg ip) and instrumented with 2 catheters (tapered 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 neuro-transmission was interrupted by treatment with mecamylamine (3 mg/kg iv) and atropine (400 mg/kg ip). When MAP stabilized, AII (30 mg/kg iv bolus) was administered 4 times at 10 min intervals, to achieve a reproducible pressor response. Saralasin of **5** 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.

Results and Discussion

The modified peptides obtained have been tested in the isolated rabbit aorta assay for agonistic and antagonistic effects. None of the compounds tested had any agonist activity in this assay. The reference hexa- and pentapeptides [Ile8]AII-(3–8) **2** and [Ile8]AII-(4–8) **1** did not produce any agonistic or antagonistic effect. However, acylation at the amino group of Tyr4 in the pentapeptide [Ile8]AII-(4–8) with isovaleric acid (iVal, 3-methyl-butyric acid to mimic the Val side chain) brought forth a weak antagonistic effect (compound **4**, pA_2 = 5.6). Similarly, simple acylation at the amino terminus of the hexapeptide [Ile8]AII-(3–8) **2** produced significant antagonistic activity (compound **3**, pA_2 of 6.6). The difference between biological properties of **2**, **3** and **4** may be indicative of the importance of a non-charged group, capable of hydrogen bonding, between the positions 2 and 3 of the peptide.

The potencies of antagonistic effect observed *in vitro* for the modified peptides **5–15** are reported in table I. From these data, several conclusions can be drawn. First, acylation of the inactive peptidic fragments **1** and **2** with non-peptidic moieties usually results in a compound with significant antagonistic effect. Interestingly, all the active compounds reported behaved as competitive, fully reversible antagonists, an observation which contrasts with the behavior of salarasin and related antagonists with aliphatic residues at position 8 [13, 19]. Second, the Val3 residue does contribute to increase affinity upon formation of the complex with the receptor (compare the penta- and hexapeptide series). Such an observation is coherent with data collected before in the agonistic series [12]. In the derivatives from the hexapeptide series, the influence of the polymethylene chain lengths is minimal and the nature of the positively charged group (guanidinium *vs* ammonium) does not produce strikingly different results. In the modified pentapeptide series, a positively charged group exerts the most beneficial influence on antagonistic activity when the chain length of the alkyl spacer is 3 to 4 methylenes. In addition, for the pentapeptide **1**, introduction of a terminal guanidinium group (**14**) resulted in a stronger antagonistic effect than a terminal ammonium group (**10**) when the spacer chain is 3 methylenes long. However, a longer spacer resulted in a total loss of detectable antagonistic activity upon replacement of an ammonium function by a guanidinium group (as in **15** compared to **12**). Also, in this series, introduction of ω-amino-octanoic acid, a known mimic for tripeptidic fragments [12], did not produce a biologically active compound (analogue **13**).

The non-peptidic moieties that we have tested are successful in mimicking, at least, part of the di- or tripeptide fragment removed from the model peptide, [Ile8]AII. For example, a single basic group separated by 3–5 methylenes is sufficient to significantly increase antagonistic property of the hexa- or penta-

Table II. Effect of saralasin and analogue **5** on the increase in arterial pressure produced by angiotensin II in anesthetized rat.

AII antagonist	*Dose* μg/kg/min	*Control*	*Variation in mean arterial pressure (% of control)* [b]					
			During AII X [a]	*Time after antagonist (min)* 5	10	20	40	60
Saralasin ($n = 4$)	10	100	0	29 ± 5	50 ± 5	78 ± 5	96 ± 4	94 ± 6
Analogue **5** ($n = 2$)	30	100	4 ± 1	41 ± 1	66 ± 6	82 ± 4	94 ± 4	99 ± 2

[a]AII X, angiotensin II antagonist; [b]Values are mean ± SEM

peptide. We have also found that the presence of the Val3 side chain influences favorably affinity to the receptor, as judged from the pA_2s for compounds **5–9**. Previous studies in the agonistic series had shown similar importance of the Val3 residue [12]. The importance of this residue both for agonistic and antagonistic activity indicates its influence on the formation of the peptide receptor complex rather than in eliciting biological response. One of the analogues (**5**) has been tested *in vivo* (ganglion blocked, anesthetized rats) and shown to produce complete blockade of the AII response when infused at 30 μg/kg/min (table II). However, no increase in duration of action (as compared to that of saralasin) was observed in these conditions and a weak agonistic effect which subsided after a few minutes following the beginning of infusion was recorded.

Acknowledgments

We acknowledge JF Zobel for amino acid analysis and P Toren and EW Kolodziej for the assistance of mass spectroscopy. We also wish to thank EH Blaine and RE Manning for support during this work.

References

1 Bumpus FM, Khosla MC (1977) *Hypertension, Physiopathology and Treatment* (Genest J, Koiw E, Kuchel O, eds) Mc Graw Hill, NY, 183–201
2 Marshall GR, Wine W, Needleman P (1970) *Proc Natl Acad Sci USA* 67, 1624
3 Goghari MH, Franklin KJ, Moore GJ (1986) *J Med Chem* 29, 1121–1124
4 Khosla MC, Smeby RR, Bumpus FM (1973) *In: Handbook of Experimental Pharmacology* (Page IH, Bumpus FM, eds) Springer–Verlag, Heidelberg, 37, 126–161
5 Pals DT, Masucci FD, Sipos F, Denning Jr DS, Fessler DC (1971) *Circ Res* 29, 673
6 Merrifield RB (1963) *J Am Chem Soc* 85, 2149
7 Barcelo G, Sennet JP, Sennyey G (1986) *J Org Chem* 30, 3951–3953
8 Stewart JM, Young JD (1984) *In: Solid Phase Peptide Synthesis*. Pierce Chemical Company, Rockford, IL 73
9 Habbeb AFSA (1959) *Biochim Biophys Acta* 34, 294–304
10 Schild HO (1947) *Br J Pharmacol Chemother* 2, 189–206
11 Matsoukas JM, Goghary MH, Scanlon MN, Franklin KJ, Moore GJ (1985) *J Med Chem* 28, 780–783
12 Jorgensen EC, Windridge GC, Lee TC (1970) *J Med Chem* 13, 744–745
Jorgensen EC, Kirlay-Olah IC, Lee TC, Windridge GC (1974) *J Med Chem* 17, 323–326
13 Scanlon MN, Matsoukas JM, Franklin KJ, Moore GJ (1984) *Life Sci* 34, 317–321
14 Samanen J, Brandeis E, Narindray D, Adams W, Cash T, Yellin T, Regoli D (1988) *J Med Chem* 31, 737–741
15 Paiva TB, Goissis G, Juliano L, Miyamoto ME, Paiva ACM (1974) *J Med Chem* 17, 238–241
16 Schiller PW (1979) *Can J Biochem* 57, 402–407
17 Moore GJ (1981) *Proc 7th Am Pept Symp* 245–248
18 Regoli D, Park WK, Rioux F, Chan CS (1971) *Rev Can Biol* 30, 319–329
19 Regoli D, Rioux F, Park WK (1972) *Rev Can Biol* 31, 73–77