Importance of the N-Terminal Domain of the Type II Angiotensin Antagonist Sarmesin for Receptor Blockade

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Analogues of the competitive angiotensin antagonist [Sar[1],Tyr(Me)[4]]angiotensin II (sarmesin) with modifications at the N-terminus have been prepared by the solid-phase method and purified by reversed-phase HPLC. Substitution of the Sar[1] residue of sarmesin with *N*,*N*-dimethyl-Gly, *N*-ethyl-Gly, aminoisobutyric, (methylamino)isobutyric, aminocaproic, and oxamic acids gave analogues that had the following respective antagonist activities (pA_2) in the rat isolated uterus assay: <6, 6.9, 5.5, 6.0, <6, and 5.3. The additional substitution of Ile for Phe at the C-terminus of the latter four peptides gave pA_2 values of 7.1, 5.1, <5, and 5. Substitution of the Arg[2] residue of sarmesin with Nle or Sar abolished antagonist activity. These data emphasize the stringent and discriminating structural requirements in the N-terminal domain of sarmesin that endow this analogue with its antagonist properties and suggest the presence of defined steric constraints in this region of the molecule during receptor blockade.

The octapeptide angiotensin II (ANG II, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) acts at receptors at peripheral and central sites to produce increased vascular resistance and extracellular fluid volume and has been implicated in blood pressure regulation in both normotensive and hypertensive states. Substitution of Sar at position 1 results in an increase in potency of both agonist and antagonist analogues of ANG II—an effect that may be attributed to an increased binding affinity as well as an increased biological half-life of the peptide. Two types of antagonist analogues have been identified from structure–activity studies. Type I antagonists are obtained by modifying the Phe[8] residue (e.g. Ala, Ile) and are characterized by slow receptor dissociation rates.[1,2] Type II antagonists are produced by methylating or omitting the Tyr hydroxyl group in [Sar[1]]ANG II and are reversible and competitive antagonists.[3,4] In previous studies on the type II antagonist [Sar[1],Tyr(Me)[4]]ANG II (sarmesin), it was found that modification or deletion of the Sar[1] residue resulted in a dramatic loss in biological activity.[4,5] Thus [Tyr(Me)[4]]-ANG II, [des[1],Tyr(Me)[4]]ANG II, [Ala[1],Tyr(Me)[4]]ANG II, and [Pro[1],Tyr(Me)[4]]ANG II were in each case biologically inactive. In the present paper, we have investigated further the role of the N-terminal domain of sarmesin in permitting this peptide to bind with high affinity to angiotensin receptors.

Results

Tables I and II give the chemical data and biological activities, respectively, of the various sarmesin analogues synthesized in the present study. As is evident from the data for the first five peptides listed in Table II, the requirement for a single alkyl substitution at the N-terminus of sarmesin is obligatory. Thus substitution of Sar by Me_2Gly diminishes antagonist activity ($pA_2 < 6$) whereas substitution with EtGly results in retention of significant antagonist activity ($pA_2 = 6.9$). Methylation of the α carbon atom of the N-terminal amino acid, as with Aib ($pA_2 = 5.5$), results in a marked reduction in antagonist activity, some of which is reinstated when the Aib residue is N-methylated ($pA_2 = 6.0$). The substitution of the long-chain Acp residue in position 1 of sarmesin abolishes antagonist activity ($pA_2 < 6$), as does the substitution of Oxm ($pA_2 = 5.3$).

The Arg residue in position 2 of sarmesin is also essential for receptor binding; replacement with the Nle isostere abolishes activity ($pA_2 < 5$). When the Sar residue of sarmesin is deleted, the resulting peptide is inactive,[4,5] and the activity is not reinstated by substituting Nle or Sar for the N-terminal Arg residue of these heptapeptide ANG III analogue (Table I).

Type I/II hybrid angiotensin antagonists are produced by the simultaneous substitution of Ile in position 8 and Tyr(Me) in position 4. Whereas [Sar[1],Tyr(Me)[4],Ile[8]]ANG II is a weak antagonist ($pA_2 = 6.6$),[4] [Aib[1],Tyr-(Me)[4],Ile[8]]ANG II has improved antagonist potency ($pA_2 = 7.1$). Somewhat surprisingly, methylation of the N-terminus of the latter, as in [MeAib[1],Tyr(Me)[4],Ile[8]]ANG II, results in a drastic reduction in the apparent binding affinity ($pA_2 = 5.1$). Neither Acp ($pA_2 < 5$) nor Oxm ($pA_2 < 5$) is tolerated in position 1 of these type I/II hybrid antagonist analogues (Table II).

To a first approximation, the relative sizes (sum of covalent radii) and relative lipophilicities (sum of methyl + methylene groups) of the amino acids occupying position 1 of these analogues can be compared. Relative sizes are given in parentheses with the amino acid structures shown in Figure 1. On this basis, it is apparent that the biological activity of each of these analogues (Table II) is not simply dependent on the molar volume or lipophilicity of residue 1, but is a more complex phenomenon involving considerations of shape, conformation, and receptor fit of the N-terminus.

Discussion

Previous structure–activity studies on sarmesin have shown that the Sar residue occupying position 1 is of great importance for the manifestation of the antagonist activity of this peptide.[4] The presence of the naturally occurring Asp residue at position 1 of ANG II, or substitution of Ala at the N-terminus of sarmesin, produces inactive analogues.[5] Even substitution of the secondary amino acid Pro at position 1 of sarmesin produces an inactive analogue,[5] illustrating that the nature of the secondary amino acid occupying position 1 is an important feature of the receptor-binding process for sarmesin. Conformational

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Table I. Chemical Data on Sarmes in Analogue[a]

analogue	TLC, R_f		amino acid analysis							
	BPAW	CMAW	Arg	His	Ile	Nle	Phe	Pro	Tyr	Val
[Sar^1,Tyr(Me)^4]ANG II (sarmesin)	0.26	0.23	1.00	1.00	0.94		1.22	1.06	0.67	1.00
[Me$_2$Gl^1,Tyr(Me)^4]ANG II	0.15	0.21	0.91	0.95	1.00		1.32	1.09	0.82	1.00
[EtGly^1,Tyr(Me)^4]ANG II	0.18	0.24	1.35	1.00	1.01		1.33	1.11	0.92	1.00
[Aib^1,Tyr(Me)^4]ANG II	0.25	0.23	1.17	0.86	0.93		1.14	1.03	0.55	1.00
[MeAib^1,Tyr(Me)^4]ANG II	0.25	0.22	1.22	0.83	0.94		1.09	1.00	0.55	1.00
[Acp^1,Tyr(Me)^4]ANG II	0.28	0.26	1.14	0.88	0.99		1.01	0.93	0.73	1.00
[Oxm^1,Tyr(Me)^4]ANG II	0.17	0.21	1.18	0.83	0.98		1.07	0.99	0.55	1.00
[Sar^1,Nle^2,Tyr(Me)^4]ANG II	0.27	0.28		0.96	0.89	1.08	1.31	1.00	1.00	1.00
[des^1,Nle^{2}Tyr(Me)^4]ANG II	0.19	0.22		0.96	0.88	1.08	1.31	1.08	1.00	1.00
[des^1,Sar^2,Tyr(Me)^4]ANG II	0.13	0.21		0.84	1.00		1.25	1.13	0.69	1.00
[Aib^1,Tyr(Me)^4,Ile^8]ANG II	0.26	0.22	1.17	0.86	1.03			0.94	0.71	1.00
[MeAib^1,Tyr(Me)^{4}Ile^8]ANG II	0.26	0.22	1.21	0.81	1.05			0.97	0.53	1.00
[Acp^1,Tyr(Me)^4,Ile^8]ANG II	0.19	0.22	1.11	0.85	1.03			0.89	0.73	1.00
[Oxm^1,Tyr(Me)^4,Ile^8]ANG II	0.17	0.21	1.10	0.88	1.03			0.88	0.70	1.00

[a] Conversion of the Tyr(Me) to Tyr during acid hydrolysis is not always quantitative. N-Methylated amino acids give very low color yields by amino acid analysis and could not be reliably estimated.

Table II. Biological Activities of Sarmesin Analogues in the Rat Uterus Bioassay[a]

analogue	agonist act. (% of ANG II)	antagonist act. (pA_2)
[Sar^1,Tyr(Me)^4]ANG II (sarmesin)	<0.01	7.5 ± 0.1 (4)[5]
[Me$_2$Gly^1,Tyr(Me)^4]ANG II	0.09 ± 0.01 (5)	<6 (5)
[EtGly^1,Tyr(Me)^4]ANG II	<0.01	6.9 ± 0.1 (6)
[Aib^1,Tyr(Me)^4]ANG II	<0.01	5.5 ± 0.1 (5)
[MeAib^1,Tyr(Me)^4]ANG II	<0.01	6.0 ± 0.1 (5)
[Acp^1,Tyr(Me)^4]ANG II	<0.01	<5 (4)
[Oxm^1,Tyr(Me)^4]ANG II	<0.01	5.3 ± 0.1 (4)
[Sar^1,Nle^2,Tyr(Me)^4]ANG II	<0.01	<5 (4)
[des^1,Nle^2,Tyr(Me)^4]ANG II	<0.01	<5 (5)
[des^1,Sar^2,Tyr(Me)^4]ANG II	0.03 ± 0.006 (7)	<6 (7)
[Aib^1,Tyr(Me)^4,Ile^8]ANG II	<0.01	7.1 ± 0.2 (8)
[MeAib^1,Tyr(Me)^4,Ile^8]ANG II	<0.01	5.1 ± 0.2 (5)
[Acp^1,Tyr(Me)^4,Ile^8]ANG II	<0.01	<5 (4)
[Oxm^1,Tyr(Me)^4,Ile^8]ANG II	<0.01	<5 (4)
[Sar^1,Ile^8]ANG II	<0.01	8.1 ± 0.2 (3)[5]

[a] Values are given as mean ± SEM (number of experiments) with human ANG II as the standard. Abbreviations are standard except: Aib, 2-aminoisobutyric acid; MeAib, 2-(methylamino)isobutyric acid; Acp, 6-aminocaproic acid; Oxm, oxamic acid; des = amino acid omitted.

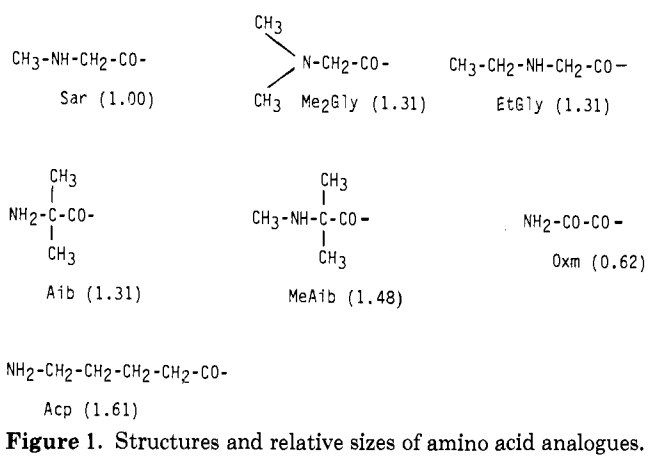

Figure 1. Structures and relative sizes of amino acid analogues.

studies on angiotensin carried out over more than two decades generally illustrate that the peptide takes up a folded structure in solution.[6] The presence of a β bend in the N-terminal region of the molecule results in overlap of the N-terminus with the peptide backbone.[7,8] Recent modeling of the angiotensin–receptor interaction has suggested a dual role for the N-terminus: (1) a charge interaction with an anionic site on the receptor, and (2) an intramolecular interaction within the peptide, possibly involving the Tyr ring.[8,9] When angiotensin is bound to its receptor, the combination of both influences could result in the N-terminus of the molecule being "sandwiched" between the receptor and the peptide backbone and, assuming this is a fairly tight interaction, would not permit a great deal of steric flexibility in the size and shape of substituents in this part of the molecule.[10] Consequently a residue such as Sar could well represent an optimum interaction between both the receptor and the peptide chain, such that very small changes in Sar result in poor accommodation of the substituents and loss of overall binding affinity of the peptide for the receptor.

In the present study, we have found that whereas Me$_2$Gly is not tolerated in position 1 of sarmesin (pA_2 < 6), EtGly is acceptable although with some loss of apparent binding affinity (pA_2 = 6.9). The difference in antagonist activities of the [Me$_2$Gly^1]- and [EtGly^1]sarmesin analogues could be due to functional or steric influences. From a functional viewpoint, Me$_2$Gly represents a tertiary amine whereas EtGly represents a secondary amine. However, at physiological pH both of these groups would be protonated and both should be able to participate as proton donors in hydrogen-bonding interactions, and therefore it is difficult to disseminate these two analogues on this basis. From a steric viewpoint, Me$_2$Gly is more bulky than EtGly—in the lateral sense rather than the longitudinal sense—and if the N-terminal residue of sarmesin exists within a restricted space allocation during interaction of the peptide with the receptor, steric considerations could explain the difference in biological activities. Proton NMR studies on these peptides have illustrated that the Sar residue of sarmesin and other [Sar^1]-containing analogues is subject to a strong shielding influence,[9,10] suggesting proximity to neighboring groups in solution. This effect may be exacerbated at the receptor, and could explain the difference in antagonist activity between [Me$_2$Gly^1]sarmesin and [EtGly^1]sarmesin.

The very low activity of [Aib^1]sarmesin (pA_2 = 5.5) is in accord with the low activity associated with [Ala^1]sar-

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mesin ($pA_2 < 6$).[5] Thus transposition of the *N*-methyl group of sarmesin to the neighboring α carbon of the N-terminal amino acid virtually abolishes biological activity. This illustrates that general steric hindrance of a relatively nondiscriminating nature at position 1 is not sufficient to create a favorable binding interaction between the receptor and sarmesin. In other words, the important steric influence of the methyl group at the N-terminus of sarmesin derives from its very precise location. In agreement with this, methylation of the Aib residue, as in [MeAib^1]sarmesin, improves biological activity ($pA_2 = 6.0$). However, the antagonist activity of [MeAib^1]sarmesin falls well below the activity of the parent peptide sarmesin and illustrates an inability of the receptor to accommodate branching at the α carbon atom. This finding, together with the absence of biological activity for [Pro^1]sarmesin,[5] demonstrates that for the secondary amino acid occupying position 1 of sarmesin there is a preference not only for a single *N*-methyl group but also the absence of a substantial side chain at the α carbon. The size and shape of the N-terminal residue of sarmesin appears to be subject to unusually severe restrictions.

The biological activity of [Acp^1]sarmesin ($pA_2 < 6$) shows, as might be expected in the light of previous findings, that a carbon chain extending beyond the normal positioning of the *N*-methyl group of Sar cannot be accommodated in the receptor-binding interaction with sarmesin. The finding that [Oxm^1]sarmesin ($pA_2 = 5.3$) has very low activity also demonstrates that a Gly isostere, in which the α methylene group is replaced by a carbonyl group, is not acceptable at position 1. It is not clear if this is due to functional or steric changes, the latter resulting from the rigidity introduced by two planar carbonyl groups in tandem. It would be of interest to determine if *N*-methyloxamic acid could assume the role of Sar in sarmesin, although one suspects that the *N*-methyl group would be inappropriately positioned in MeOxm.

In the present study the importance of the Arg residue occupying position 2 of sarmesin was examined. Thus the Arg residue was replaced with the charged isostere Nle to give [Nle^2]sarmesin, which was biologically inactive ($pA_2 < 5$). This demonstrates that the positive charge located on the guanidino group of the Arg residue of sarmesin is mandatory for biological activity. This is of no great surprise since it is well known that the positive charge at position 2 of ANG II and its analogues is an important feature of receptor binding. Similarly the heptapeptide [des^1,Nle^2]ANG II is biologically inactive ($pA_2 < 5$). It was of interest to investigate the biological activity of the heptapeptide [des^1,Sar^2]sarmesin, particularly since the equivalent type I angiotensin antagonist [des^1,Sar^2,Ile^8]-ANG II is a potent antagonist that may be specific for ANG III receptors.[11] However, [des^1,Sar^2]sarmesin was essentially biologically inactive ($pA_2 < 6$), exemplifying another readily discernible difference between type I and type II antagonists, which extends to heptapeptide species in these two classes.

The final part of this investigation involved studies on type I/II hybrid angiotensin antagonists. Previous studies[4,5] with analogues of the type [Sar^1,Tyr(Me)^4,Ile^8]ANG II have shown that the type I/II hybrid of this type has lower antagonist activity than either of the parent analogues, i.e. [Sar^1,Ile^8]ANG II (type I) or the type II parent analogue [Sar^1,Tyr(Me)^4]ANG II (sarmesin). Furthermore the characteristically slow rate of receptor dissociation observed for [Sar^1,Ile^8]ANG II is eliminated when its Tyr hydroxyl group is methylated.[2,4] Remarkably, the nonmethylated analogue [Aib^1,Tyr(Me)^4,Ile^8]ANG II turned out to be a potent antagonist at angiotensin receptors ($pA_2 = 7.1$), whereas [MeAib^1,Tyr(Me)^4,Ile^8]ANG II had paradoxically reduced antagonist activity ($pA_2 = 5.1$) by comparison. Apparently a modification, i.e. N-terminal methylation, which usually enhances the antagonist activity of either a type I or a type II antagonist (e.g. [Aib^1]sarmesin, $pA_2 = 5.5$ and [MeAib^1]sarmesin, $pA_2 = 6.0$), virtually abolishes the activity of this type I/II hybrid antagonist. This is an interesting observation that deserves further study since it may illustrate the possibility of obtaining hybrid analogues with superior antagonist properties by eliminating the *N*-methyl group. Perhaps more importantly, apparently paradoxical findings of this type can provide new insights into the conformational properties of these analogues as they relate to intramolecular interactions within the peptide backbone and intermolecular interactions between the peptide and the receptor. There is a suggestion here of a more constrained N-terminal domain for the type I/II hybrid analogue during receptor binding than for either of its parent type I or type II antagonists. There may also be a great deal of indirect interaction/communication between the N- and C-terminal of the molecule: the increased binding affinity of [Sar^1,Tyr(Me)^4]ANG II ($pA_2 = 7.5$) compared to [Sar^1,Tyr(Me)^4,Ile^8]ANG II ($pA_2 = 6.6$[5]) contrasts with the decreased affinity of [Aib^1,Tyr(Me)^4]Ang II ($pA_2 = 5.5$) compared to [Aib^1,Tyr(Me)^4,Ile^8]ANG II ($pA_2 = 7.1$) and illustrates a marked interdependence between position 1 and position 8 in the determination of receptor-binding affinity.

[Acp^1,Tyr(Me)^4,Ile^8]ANG II was found to be essentially biologically inactive, presumably because overextension of the N-terminus beyond an optimum length obviates any favorable receptor interaction. This finding might be anticipated since MeAib is apparently already too lengthy to be accommodated in hybrid analogues (Table II). [Oxm^1,Tyr(Me)^4,Ile^8]ANG II represents an interesting analogue since the Oxm group provides the same peptide backbone length as the Aib group. However, [Oxm^{1}Tyr-(Me)^4,Ile^8]ANG II is essentially biologically inactive ($pA_2 < 5$), illustrating that the isopropyl group of Aib cannot be replaced by a carbonyl group in hybrid analogues. Whether this is due to the different steric influences associated with Aib and Oxm, or due to functional changes brought by the presence of a carbonyl group in place of a hydrocarbon, has yet to be determined.

Experimental Section

tert-Butyloxycarbonyl-blocked amino acids were synthesized in our laboratories or were purchased from Peninsula Laboratories, Bachem Inc., or Chemical Dynamics Corp. Protected-amino acid-resins, ANG II, and [Sar^1,Ile^8]ANG II, were purchased from Peninsula Laboratories. Solid-phase peptide synthesis was carried out with Beckman 990 or 990B peptide synthesizers essentially by procedures described previously.[5] Coupling steps with 2.5 equiv of Boc-protected amino acid and coupling reagent were employed for each amino acid; the first coupling was mediated by dicyclohexylcarbodiimide (DCC) in the presence of 1-hydroxybenzotriazole (HBT) for 3 h and the second, when proved necessary by the ninhydrin test, was mediated by *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) for 8 h. Deprotection steps (2 and 25 min) utilized 25% CF_3CO_2H in $CHCl_3$ containing indole (0.1%). Neutralization steps (2 and 25 min) utilized 10% triethylamine in $CHCl_3$. Peptides were synthesized on a 0.5-mmol scale up to the heptapeptide stage, and thereafter the protected resin was divided in half and the next Boc-amino acid was added to half. Yields of fully protected peptide-resins were in excess of 90%. The completed peptide was removed from

(11) Moore, G. J.; Ganter, R. C.; Goghari, M. H.; Franklin, K. J., submitted for publication.

the resin and simultaneously deprotected by reaction with anhydrous HF (10 mL) in the presence of *p*-cresol (1 g) for 30 min at 0 °C. The peptide was dissolved in CF_3CO_2H (100 mL), and the resin was removed by filtration. The solvent was removed by rotary evaporation, and the crude product was isolated by trituration with ether and filtration. Yield 200–300 mg. The crude product obtained after the reaction with HF was dissolved in 7% acetic acid (10 mL), clarified by centrifugation, and purified by HPLC.

Preparative Reversed-Phase HPLC. Purification of peptides was accomplished with use of a Varian HPLC system equipped with a Vista 401 microprocessor controller. Separations were achieved on a Bio-Rad Hi-Pore 318 reversed-phase preparative column (25.0 × 2.15 cm) at 25 °C with a stepped linear gradient of acetonitrile in 0.1% CF_3CO_2H at a flow rate of 7.5 mL/min. Automated repetitive injections of peptides (5 × 5 mg) were made from a nitrogen pressurized Rheodyne injector with a 2.0-mL sample loop. One-fifth of the total sample was injected during each run by lowering the flow rate to 4.0 mL/min for a 0.1-min "inject" period. One cycle consisted of the following events: 0 → 10 min, 7.5 mL/min, 90% H_2O/10% of 1% aqueous CF_3CO_2H; 10 → 11 min, → 4.0 mL/min; 11 → 11.1 min, "inject"; 11.1 → 13 min, → 7.5 mL/min, → 70% H_2O/20% CH_3CN/10%; of 1% CF_3CO_2H; 13 → 30 min, → 45% H_2O/45% CH_3CN/10% of 1% CF_3CO_2H; 30 → 42 min, → 90% CH_3CN/10% of 1% CF_3CO_2H; 42 → 50 min, → 100% H_2O.

Fractions were collected at 0.1-min intervals with a Gilson Model 201 fraction collector programmed to collect for a 5-min period centered around the elution time (27–29 min) of the major product. The fraction collector was restarted by the Vista 401 at the beginning of each HPLC run so that the material eluting with the same retention time was repeatedly deposited in the same tubes. Elution of the peptide was detected simultaneously from the absorbances at 254 nm (Varian UV-1) and 230 nm (Kratos SF769Z). Fractions containing the required peptide were pooled; after removal of CH_3CN on a rotary evaporator at 40 °C, the fractions were lyophilized and stored at −20 °C. Yield 5–20%.

Amino acid analyses (Beckman 121 M) were carried out after acid hydrolysis in 6 N HCl (containing 1% cresol to prevent loss tyrosine) at 100 °C for 18 h in vacuo. The purity of products was established by analytical HPLC reruns and by thin-layer chromatography (TLC). TLC was carried out with precoated silica gel on glass (Merck Kieselgel 60 F254) TLC plates. The two solvent systems used were 1-butanol–pyridine–acetic acid–water (15:10:3:6) (BPAW) and chloroform–methanol–acetic acid–water (15:10:2:3:) (CMAW). Peptides were detected by fluorescence quenching under UV light followed by development with ninhydrin (0.3%) in 1-butanol/acetic acid (100:3, v/v).

Rat Uterus Bioassay. Defatted uterine horns from diethylstilbestrol-primed virgin Sprague–Dawley rats (150–250 g) were cut in half, and each tissue was suspended under 1 g of tension in a 3-mL tissue bath containing 150 mM NaCl, 5.6 mM KCl, 0.18 mM $NaHCO_3$, and 1.4 mM glucose at pH 7.0 gassed with oxygen. Contractions were monitored with Gould Metripak 763341-4202 isotonic transducers coupled to Gould 13-4615-50 transducer amplifiers housed in a Gould 2600S recorder. Agonist activities of peptides were determined by matching the response with an equivalent response to ANG II (human). Antagonist activities (pA_2) were determined as the negative logarithm of the concentration of antagonist required to reduce the response to an EC_{50} dose of ANG II to the response to half the EC_{50} dose.

Registry No. Sarmesin, 88874-29-7; [Me_2Gly^1,Tyr(Me)^4]ANG II, 114396-29-1; [EtGly^1,Tyr(Me)^4]ANG II, 114396-30-4; [Aib^{1}Tyr(Me)^4]ANG II, 114396-31-5; [MeAib^{1}Tyr(Me)^4]ANG II, 114396-32-6; [Acp^1, Tyr(Me)^4]ANG II, 114396-33-7; [Oxm^1, Tyr (Me)^4]ANG II, 114396-34-8; [Sar^1, Nle^8, Tyr(Me)^4]ANG II, 114396-35-9; [des^1, Nle^2, Tyr(Me)^4]ANG II, 114396-36-0; [des^1, Sar^8, Tyr(Me)^4]ANG II, 114396-37-1; [Aib^1, Tyr(Me)^4, Ile^8]ANG II, 114396-38-2; [MeAib^1, Tyr(Me)^4, Ile^8]ANG II, 114422-57-0; [Acp^1, Tyr(Me)^4, Ile^8]ANG II, 114396-39-3; [Oxm^1, Tyr(Me)^4, Ile^8]ANG II, 114396-40-6; [Sar^1, Ile^8]ANG II, 37827-06-8.

Synthesis and Structure–Activity Relationships of 2-Sulfonamido-1,3,4,6,7,11bα-hexahydro-2*H*-benzo[*a*]quinolizines as α_2-Adrenoceptor Antagonists

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A series of 2-sulfonamido-1,3,4,6,7,11bα-hexahydro-2*H*-benzo[*a*]quinolizines were synthesized and examined for α_2- and α_1-adrenoceptor antagonist activity on the rat vas deferens and anococcygeus muscle, respectively. A number of compounds in this series were shown to be potent and selective α_2-adrenoceptor antagonists. Studies on the resolved enantiomers of compounds **6**, **10**, and **16** showed that α_2-adrenoceptor antagonist activity resided primarily in the 2*R*,11b*S* isomers, related to the absolute configuration of the α_2-antagonist yohimbine, such that the benzene ring and sulfonamide groups in this series were superimposable on the pyrrole and ester groups of yohimbine.

α-Adrenoceptors have been classified into α_1 or α_2 subtypes depending on the relative affinities of various agonists and antagonists.[1,2] Initially the two receptors were also defined anatomically, α_1-adrenoceptors being located postsynaptically and α_2-adrenoceptors at presynaptic sites on sympathetic nerve terminals. Subsequently α_2-adrenoceptors were identified at postsynaptic sites in several organs and in platelets.[3] Presynaptic α_2-adrenoceptors mediate a negative-feedback mechanism which modulates release of norepinephrine from nerve endings.[1] It follows that blockade of central presynaptic α_2-adrenoceptors should lead to enhanced neuronal release of norepinephrine, an effect which is predicted from the catecholamine theory[4] of depressive illness to be of potential value for the treatment of depression. Furthermore chronic treatment of rats with desipramine produces a

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