Banker for the preparation of this manuscript.

Registry No. 1, 7169-34-8; 2, 27404-31-5; 3, 97456-65-0; 4, 98678-87-6; 5, 98678-88-7; 6a, 98678-89-8; 6a·HCl, 98678-90-1; 6b, 98719-20-1; 6b·HCl, 98757-15-4; 6c, 98719-21-2; 6c·HCl, 98757-16-5; 3-(cyanomethyl)benzofuran, 52407-43-9; CH_2=C(OSiMe$_3$)CH=CH_2, 38053-91-7.

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Novel Vasopressin Analogues That Help Define a Minimum Effective Antagonist Pharmacophore

Sir:

In our attempt to ultimately design small molecules that act as vasopressin receptor antagonists, an initial goal has been to define the minimum active fragment of the class of peptide V_2-receptor antagonists first described by Manning et al. (exemplified by compound 1, Figure 1).[1] We have focused a portion of our effort on the tripeptide tail which is common to these antagonists as well as to vasopressin. This tail is postulated to contain key binding elements of the agonist pharmacophore.[2] It has recently been shown by Manning et al.[3] and independently by us[4,5] that potent octapeptide vasopressin antagonists can be obtained by deletion of the carboxyl-terminal glycine moiety, leaving a carboxyl-terminal argininamide, for example compounds **2** (SK&F 101926) and **3**. We have previously presented evidence that the pharmacophore requirements at the renal V_2-receptor differ for vasopressin agonists and antagonists.[6,7] For example, analogues of **1** (or its L-Tyr(Et)[2] congener) with either D- or L-arginine at position 8 are essentially equipotent, which is not the case with agonists.[1,6] This suggested that the terminal carboxamide group in **2** or **3** may not be essential in the antagonist pharmacophore since this situation would allow the D- and L-arginine analogues to be topochemically equivalent. This hypothesis was tested by the preparation of compound **4**, in which the lysine of **3** is replaced by an alkyldiamine.

The synthesis of **4** was dependent on proline acid **5** which was prepared by standard solid-phase synthesis techniques on Merrifield resin, cleaved from the resin with anhydrous HF, and cyclized under dilute conditions with aqueous potassium ferricyanide. Peptide **5** was then purified by flash chromatography on C-18 reverse phase using 50% aqueous acetonitrile containing 0.1% TFA[12] as eluant to afford partially purified acid. Condensation of this material with mono-Boc-cadaverine[8] using DCC/HOBT in DMF followed by deblocking with neat TFA afforded the crude peptide **4**. Purification was accomplished by ion exchange chromatography on BioRex 70 followed by preparative HPLC.[9]

The peptide was evaluated in vitro for vasopressin receptor binding and inhibition of LVP-sensitive adenylate cyclase in porcine renal medullary preparations, which have been shown to be similar to the human receptors.[5] It was also evaluated in vivo in a hydropenic rat assay.[10] The results are summarized in Table I. The aminoalkyl analogue **4** retains good activity when compared to **2**[11] or **3**, which supports our original hypothesis and suggests that

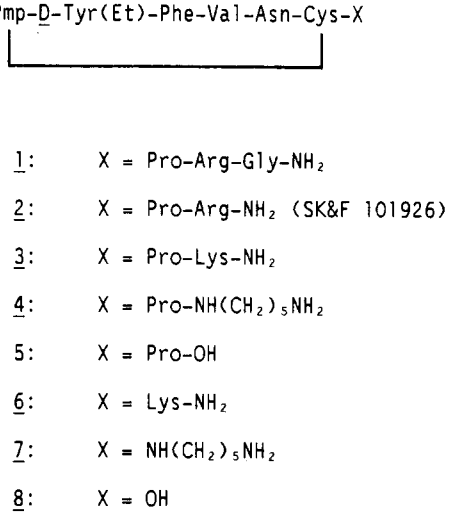

Figure 1.

Table I. Activity of Vasopressin Antagonists

compd	pig K_{bind},[a] nM	pig K_i,[b] nM	rat: ED_{300},[c] μg/kg
LVP	4.6	*b*	
1	12	6.4	11.2
2	12	3.9	9.2
3	37	9.4	22
4	30	7.2	27
6	26	5.8	59
7	19	6.8	94

[a] Inhibition constant for [^{3}H]LVP binding to medullary membranes of pig kidney (see ref 6). [b] Inhibition constant for vasopressin-stimulated adenylate cyclase of medullary membranes of pig kidney. K_{act} LVP was determined to be 1.2 nM (see ref 6). [c] Dose required to lower urine osmolality to 300 mOsm/kg (see ref 10).

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(6) Stassen, F.; Erickson, R.; Huffman, W.; Stefankiewicz, J.; Sulat, L.; Wiebelhaus, V. *J. Pharmacol. Exp. Ther.* **1982**, *223*, 50.
(7) Moore, M.; Huffman, W.; Roberts, G.; Rottschaefer, S.; Sulat, L.; Stefankiewicz, J.; Stassen, F. *Biochem. Biophys. Res. Commun.* **1984**, *121*, 878.
(8) Mono-Boc-cadaverine can be conveniently prepared by reacting an excess of diamine with di-*tert*-butyl dicarbonate in methylene chloride followed by an extractive workup.
(9) All new peptides were characterized by amino acid analysis and FAB mass spectrometry, and their purity was established by TLC and HPLC.
(10) Kinter, L.; Huffman, W.; Wiebelhaus, V.; Stassen, F. "Diuretics: Chemistry, Pharmacology and Clinical Applications"; Puschett, J., Ed.; Elsevier: New York, 1984; pp 72–81.
(11) Compound **2** (SK&F 101926) is one of the most potent V_2-receptor antagonists published to date.
(12) Abbreviations: Pmp, β,β-cyclopentamethylene-β-mercaptopropionic acid; AVP, arginine vasopressin; LVP, lysine vasopressin; DCC, *N*,*N*′-dicyclohexylcarbodiimide; HOBT, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; DMF, dimethylformamide; Boc, *tert*-butyloxycarbonyl.

0022-2623/85/1828-1759$01.50/0

the minimum effective antagonist pharmacophore might consist of a basic group attached to the cyclic hexapeptide ring. We therefore questioned the role of proline at position 7 in the antagonist. Proline is proposed to play a key role in the *agonist* pharmacophore both as an important binding element and in its ability to properly orient the tripeptide tail with respect to the cyclic hexapeptide ring.[2] In an attempt to evaluate the importance of proline at position 7, we prepared compounds **6** and **7** in which this group is deleted.

Compound **6** was prepared by solid-phase synthesis on benzhydrylamine resin, cleaved with anhydrous liquid HF, and cyclized with dilute aqueous potassium ferricyanide. This peptide was purified by countercurrent distribution followed by gel filtration. Compound **7** was prepared in an analogous way to compound **4** with the cyclic hexapeptide acid **8** as a key intermediate. Acid **8** was prepared by solid-phase synthesis on Merrifield resin, cleaved, and oxidized in the same manner as **5**.

The biological activities of **6** and **7** are given in Table I. Both compounds retain very good in vitro activity compared to that of **3**; however, their in vivo activities are somewhat reduced. This result may reflect metabolic as well as pharmacokinetic differences. It is clear from these results that both a terminal carboxamide and the proline residue are not essential for potent antagonist activity; a basic moiety attached directly to the cyclic hexapeptide ring presents an effective pharmacophore. These observations help to further refine our understanding of the molecular interactions of peptide antagonists with the vasopressin V_2-receptor.

Acknowledgment. We thank Gerald Roberts and Susan Rottschaefer for obtaining the FAB mass spectra. We are also indebted to Maurice Manning (Medical College of Ohio at Toledo) for providing a sample of antagonist **1**.

Registry No. **1**, 80148-24-9; **2**, 96827-97-3; **3**, 98612-53-4; **4**, 98612-54-5; **5**, 98612-55-6; **6**, 98612-56-7; **7**, 98612-57-8; **8**, 98612-58-9; mono-Boc-cadaverine, 51644-96-3; adenylate cyclase, 9012-42-4.

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Received June 7, 1985

Articles

Stereoselectivity of Muscarinic Receptors in Vivo and in Vitro for Oxotremorine Analogues. *N*-[4-(Tertiary amino)-2-butynyl]-5-methyl-2-pyrrolidones

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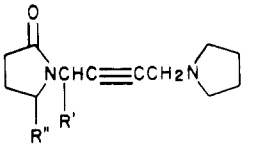
The enantiomers of three 5-methyl-2-pyrrolidone analogues of the muscarinic agent oxotremorine (**1**) were synthesized. The pyrrolidine derivative (*R*)-**13** was an antagonist to carbachol in the guinea pig ileum and also showed central and peripheral antimuscarinic activity in vivo. It was more potent and more selective than atropine in antagonizing the central effects of **1**. The dimethylamino analogue (*R*)-**14** and the trimethylammonium salt (*R*)-**15** were potent agonists in the guinea pig ileum. (*R*)-**14** showed both central muscarinic (hypothermia) and central antimuscarinic activity (antagonism of oxotremorine-induced tremor) in vivo. The *R* enantiomers of **13–15** were considerably more potent than the *S* enantiomers in vivo and in vitro irrespective of whether agonist or antagonist activity was measured. From a comparison of the contribution of the methyl group at the chiral center to the overall affinities, it is suggested that agonists and antagonists in this series bind in an essentially identical manner to the muscarinic receptor.

Methyl substitution in the potent muscarinic agent oxotremorine (**1**) has pronounced effects on its pharmacological activity.[1-4] Only one of the structural isomers resulting from the introduction of a single methyl group in **1** has oxotremorine-like properties in vivo.[4,5] The remaining isomers inhibit the effects of **1**.[1-4] The most potent among these are compounds **2** and **13**, which are more effective than atropine in antagonizing the central effects of **1**. In comparison to atropine, however, **2** and **13** show relatively weak peripheral parasympatholytic activity.[4-7] Practically all of the antimuscarinic activity of **2** resides in the *R* enantiomer.[8] For example, (*R*)-**2** was



1, R' = R" = H
2, R' = CH_3, R" = H
13, R' = H, R" = CH_3

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