Solid-Phase Synthesis of 16 Potent (Selective and Nonselective) in Vivo Antagonists of Oxytocin†

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We describe the synthesis and some pharmacological properties of 16 new in vivo antagonists of oxytocin. These are based on modifications of three peptides: A, B, and C. A is our previously reported potent and selective antagonist of the vasopresor (V_1 receptor) responses to arginine-vasopressin (AVP)/weak oxytocin antagonist, [1-(β-mercapto-β,β-pentamethylenepropionic acid), 2-*O*-methyltyrosine]arginine-vasopressin (d(CH_2)$_5$[Tyr(Me)^2]AVP. B reported here, the Ile^3 analogue of A, is d(CH_2)$_5$[Tyr(Me)^2]AVT (**5** below) and C is our previously reported potent nonselective oxytocin antagonist/AVP V_1 antagonist, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine,8-ornithine]vasotocin (d(CH_2)$_5$[Tyr(Me)^2]OVT). The following substitutions and deletions, alone or in combination, were employed in A, B, and C: 1-deaminopenicillamine (dP); D-Tyr(Alk)^2 (where Alk = Me or Et), D-Phe^2; Val^4, Thr^4; Δ^3-Pro^7; Lys^8, Cit^8, Orn^8; desGly^9, desGly-NH_2^{9}, Ala-NH_2^{9}; Leu-NH_2^{9}; Arg-NH_2^{9}. The 16 new analogues are (1) d(CH_2)$_5$[D-Tyr(Me)^2]AVP, (2) d(CH_2)$_5$[D-Tyr(Me)^2,Val^4,Δ^3-Pro^7]AVP, (3) d(CH_2)$_5$[D-Tyr(Et)^2,Val^4,Lys^8]VP, (4) d(CH_2)$_5$[D-Tyr(Et)^2,Val^4,Cit^8]VP, (5) d(CH_2)$_5$[Tyr(Me)^2]AVT, (6) d(CH_2)$_5$[Tyr(Me)^2,Lys^8]VT, (7) dP[Tyr(Me)^2]AVT, (8) dP[Tyr(Me)^2,Val^4]AVT, (9) d(CH_2)$_5$[D-Tyr(Me)^2,Val^4]AVT, (10) d(CH_2)$_5$[D-Phe^2,Val^4]AVT, (11) d(CH_2)$_5$[Tyr(Me)^2,Thr^4]OVT, (12) d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Ala-NH_2^{9}]OVT, (13) d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Leu-NH_2^{9}]OVT, (14) d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Arg-NH_2^{9}]OVT, (15) desGly-NH_2^{9},d(CH_2)$_5$[Tyr(Me)^2,Thr^4]OVT, (16) desGly^9,d(CH_2)$_5$[Tyr(Me)^2,Thr^4]OVT. **1-4** are analogues of A, **5-10** are analogues of B, and **11-16** are analogues of C. Their protected precursors were synthesized either entirely by the solid-phase method or by a combination of solid-phase and solution methods (1 + 8 or 8 + 1 couplings). All analogues were tested in rats for agonistic and antagonistic activities in oxytocic (in vitro, without and with Mg^{2+}, and in vivo) assays as well as by antidiuretic and vasopressor assays. All analogues exhibit potent oxytocic antagonism in vitro and in vivo. With an in vitro pA_2 (in the absence of Mg^{2+}) = 9.12 ± 0.09, dP[Tyr(Me)^2]AVT (**7**) is one of the most potent in vitro oxytocin antagonists reported to date. Fifteen of these analogues (all but **6**) appear as potent or more potent in vivo oxytocin antagonists than C (pA_2 = 7.37 ± 0.17). Analogues **1-9** and **14** are potent AVP V_1 antagonists. Their anti-V_1 pA_2 values range from 7.92 to 8.45. They are thus nonselective oxytocin antagonists. The remaining six analogues (**10-13**, **15**, **16**) exhibit substantially reduced anti-V_1 potencies relative to their antioxytocic potencies in vivo and are thus far more selective as oxytocin antagonists than C. d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Leu-NH_2^{9}]OVT (**13**) and desGly-NH_2^{9},d(CH_2)$_5$[Tyr(Me)^2,Thr^4]OVT (**15**) are among the most selective in vivo oxytocin antagonists reported to date. These findings provide useful clues for the design of even more potent and selective in vivo oxytocin antagonists. Some of the analogues reported here may be of value for the treatment of premature labor and/or as pharmacological tools for studies on the physiological and pathophysiological roles of oxytocin and of AVP.

Delaying premature delivery remains a challenging goal in obstetrical practice.[1] Suppression of oxytocin-induced uterine contractions, through the use of clinically effective in vivo antagonists of oxytocin, could be a major step toward this goal. Over the years we[2-4] and others[5-7] have reported many promising in vivo antagonists of oxytocin. Structural modifications first reported by du Vigneaud's and Rudinger's laboratories that produced in vitro oxytocin antagonists have been valuable in the design of later antagonists that were fully effective in vivo. For reviews, see ref 12-20. These modifications are (1) Tyr(Me) substitution at position two[8] and (2) β,β-dialkyl substitutions at

† Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* 1984, *138*, 9]. All amino acids are in the L configuration unless otherwise noted. Other abbreviations used are Tyr(Me), *O*-methyltyrosine; Tyr(Et), *O*-ethyltyrosine; D-Tyr(Et), *O*-ethyl-D-tyrosine; Cit, citrulline; Δ^3-Pro, 3,4-dehydroproline; AVP, arginine-vasopressin; LVP, lysine-vasopressin; AVT, arginine-vasotocin; OVT, ornithine-vasotocin; desGly^9, des-9-glycine [carboxy, terminus is Orn-NH_2^{8}]; desGly-NH_2^{9}, des-9-glycinamide [carboxy terminus is Orn-OH^8]; dP, 1-deaminopenicillamine or 1-β-mercapto-β,β-dimethylpropionic acid; d(CH_2)$_5$, 1-β-mercapto-β,β-pentamethylenepropionic acid; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; Boc, *tert*-butoxycarbonyl; Bzl, benzyl; Tos, tosyl; AcOH, acetic acid; TFA trifluoroacetic acid; HOBt, *N*-hydroxybenzotriazole; ONp, *p*-nitrophenyl ester; Et_3N, triethylamine; Z, benzyloxycarbonyl.

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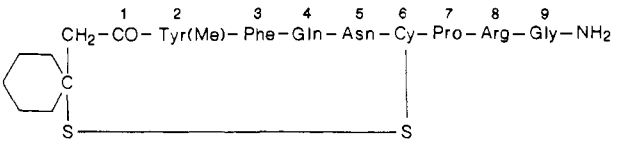
position one.[9] In the latter the two hydrogens on the β-carbon atom at position 1 are replaced with (a) dimethyl $(CH_3)_2$[9] (b) diethyl $(C_2H_5)_2$[10] and (c) pentamethylene $(CH_2)_5$[11] substituents. The combination of the Tyr(Me)^2 and the β,β-dimethyl modifications in [1-deamino]oxytocin (dOT)[34], to give [1-deaminopenicillamine,2-*O*-methyltyrosine]oxytocin,[2b] produced one of the first truly effective in vivo oxytocin antagonists.[2b] Modifications of oxytocin antagonists that were subsequently found to produce enhancements of in vivo oxytocic antagonism are (3) Thr^4[2] and (4) Orn^8[3,4] substitutions. One of our most potent in vivo oxytocin antagonists was designed by combining three of these four modifications, namely, $(CH_2)_5$, Tyr(Me)^2, and Orn^8 in dOT to give [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine,8-ornithine]vasotocin (d$(CH_2)_5$[Tyr(Me)^2]OVT).[4] While exploring the properties of our antagonists of the vasopressor (V_1 receptor) and of the antidiuretic (V_2 receptor) responses to arginine-vasopressin (AVP),[13-17,19,22] we uncovered two additional modifications that resulted in significant enhancements of in vivo antioxytocic potencies. These are (5) D-Tyr-(Alk)[2,17,19,22c] (where Alk = Me, Et) and (6) D-Arg^8 substitutions.[17,19,22b] Also, an oxytocin antagonist from the Ferring group, [1-deamino,2-*O*-ethyl-D-tyrosine,4-threonine,8-ornithine]vasotocin, is currently undergoing clinical trial.[21]

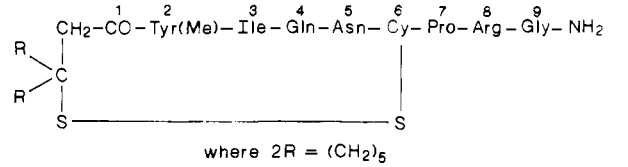
One of the most serious drawbacks of virtually all of our previously reported in vivo oxytocin antagonists is their lack of specificity with regard to V_1 receptors. In fact, most of our oxytocin antagonists are more potent as V_1 antagonists than as in vivo oxytocin antagonists.[4,13-17,19] Thus d$(CH_2)_5$[Tyr(Me)^2]OVT is 5 times more potent as a V_1 antagonist than as an oxytocin antagonist.[4] In searching for clues to the design of more selective in vivo antagonists of oxytocin, we have followed three parallel but interrelated pathways based on modifications of three different antagonists: (1) d$(CH_2)_5$[Tyr(Me)^2]AVP,[22a] (2) d$(CH_2)_5$-[Tyr(Me)^2]AVT, and (3) d$(CH_2)_5$[Tyr(Me)^2]OVT.[4] We have utilized five of the six modifications listed above plus some additional modifications uncovered in our search for selective AVP V_1 and V_2 antagonists.[13-17,22] These additional modifications utilized alone or in combination are Val^4, Δ^3-Pro^7, Lys^8, citrulline^8 (Cit^8) substitutions, multiple substitutions at position 9, and C-terminal Gly/Gly-NH_2 deletions.[23,28]

1. Analogues of d$(CH_2)_5$[Tyr(Me)^2]AVP.[22a] d-$(CH_2)_5$[Tyr(Me)^2]AVP is one of our most potent V_1 antagonists.[22a] Although also a highly potent in vitro antagonist of oxytocin, it is 100 times less potent as an in vivo oxytocin antagonist than as a V_1 antagonist.[22] We report here four new analogues (1–4) of d$(CH_2)_5$[Tyr(Me)^2]AVP, which have the following structure:



Analogues 1–4 possess the following modifications at positions 2, 4, 7, and 8: (1) D-Tyr(Me)^2; (2) D-Tyr(Me)^2, Val^4, Δ^3-Pro^7; (3) D-Tyr(Et)^2, Val^4, Lys^8; (4) D-Tyr(Et)^2, Val^4, Cit^8.

2. Analogues of d$(CH_2)_5$[Tyr(Me)^2]AVT. In preliminary studies reported here, we found that substitution of isoleucine for phenylanine at position 3 in d$(CH_2)_5$[Tyr-(Me)^2]AVP to give the corresponding vasotocin analogue, d$(CH_2)_5$[Tyr(Me)^2]AVT, resulted in a dramatic enhancement in in vivo oxytocic antagonism. d$(CH_2)_5$[Tyr-(Me)^2]AVT has the following structure:



In addition to d$(CH_2)_5$[Tyr(Me)^2]AVT, we report five analogues (6–10) of d$(CH_2)_5$[Tyr(Me)^2]AVT, modified at positions 1, 2, 4, and 8. These are (6) 2R = $(CH_2)_5$; Lys^8; (7) 2R = $(CH_3)_2$; (8) 2R = $(CH_3)_2$, Val^4; (9) 2R = $(CH_2)_5$; D-Tyr(Me)^2, Val^4; (10) 2R = $(CH_2)_5$; D-Phe^2, Val^4.

3. Analogues of d$(CH_2)_5$[Tyr(Me)^2]OVT^4. We report six new analogues (11–16) all having Thr^4 in combination

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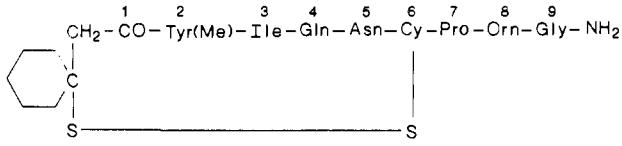
with either desGly, desGly-NH_2, or a variety of substituents at position 9.[23] d$(CH_2)_5$[Tyr(Me)^2]OVT has the following structure:



Analogues 11–16 have the following modifications: (11) Thr^4; (12) Thr^4, Ala-NH_2^9; (13) Thr^4, Leu-NH_2^9; (14) Thr^4, Arg-NH_2^9; (15) desGly-NH_2^9, Thr^4; (16) desGly^9, Thr^4.

It should be noted that a related analogue, d$(CH_2)_5$-[Tyr(Me)^2,Thr^4,Tyr-NH_2^9]OVT, a highly potent and selective in vivo oxytocin antagonist, has recently been shown to be a very valuable precursor for conversion to a radioiodinated ligand for use in the localization and identification of central and peripheral oxytocin receptors.[24a] Also, in another in vitro binding study, the desGly-NH_2^9,d-$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT reported here has been utilized as a highly selective oxytocin receptor ligand.[24b]

The full names and their abbreviations for these 16 new analogues are as follows: (1) [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyl-D-tyrosine]arginine-vasopressin (d$(CH_2)_5$[D-Tyr(Me)^2]AVP); (2) [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyl-D-tyrosine,4-valine, 7-(3,4-dehydro)proline]arginine-vasopressin (d$(CH_2)_5$[D-Tyr(Me)^2,Val^4,Δ^3-Pro^7]AVP); (3) [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyl-D-tyrosine,4-valine]lysine-vasopressin (d$(CH_2)_5$-[D-Tyr(Et)^2,Val^4]LVP); (4) [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyl-D-tyrosine,4-valine,8-citrulline]vasopressin (d$(CH_2)_5$[D-Tyr(Et)^2,Val^4,Cit^8]VP); (5) [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasotocin (d$(CH_2)_5$[Tyr-(Me)^2]AVT); (6) [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine,8-lysine]vasotocin (d-$(CH_2)_5$[Tyr(Me)^2,Lys^8]VT); (7) [1-deaminopenicillamine-,2-*O*-methyltyrosine]arginine-vasotocin (dP[Tyr(Me)^2]-AVT); (8) [1-deaminopenicillamine,2-*O*-methyltyrosine,4-valine]arginine-vasotocin (dP[Tyr(Me)^2,Val^4]AVT); (9) [1-(β-mercapto-β,β-pentamethylenepropionic acid,2-*O*-methyl-D-tyrosine,4-valine]arginine-vasotocin (d$(CH_2)_5$[D-Tyr(Me)^2,Val^4]AVT); (10) [1-(β-mercapto-β,β-pentamethylenepropionic acid,2-D-phenylalanine,4-valine]arginine-vasotocin (d$(CH_2)_5$[D-Phe^2,Val^4]AVT); (11) [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine,4-threonine]ornithine-vasotocin (d-$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT); (12) [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine,4-threonine,9-alaninamide]ornithine-vasotocin (d$(CH_2)_5$-[Tyr(Me)^2,Thr^4,Ala-NH_2^9]OVT); (13) [1-(β-mercapto-β,β-pentamethylenepropionic acid,2-*O*-methyltyrosine,4-threonine,9-leucinamide]ornithine-vasotocin (d$(CH_2)_5$-[Tyr(Me)^2,Thr^4,Leu-NH_2^9]OVT); (14) [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine,4-threonine,9-argininamide]ornithine-vasotocin (d$(CH_2)_5$-[Tyr(Me)^2,Thr^4,Arg-NH_2^9]OVT); (15) des-9-glycinamide-[1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine,4-threonine]ornithine-vasotocin (desGly-NH_2^9,d$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT); (16) des-9-glycine-[1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine,4-threonine]ornithine-vasotocin (des-Gly^9,d$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT).

While these studies were in progress others have reported potent antagonists of oxytocin[6,7] related to our earlier in vivo antagonists[2-4] and to some of those reported here. Hruby and co-workers have employed the 1-penicillamine grouping, D-amino acids at position 2, Thr^4 and Orn^8 substitutions extensively. Thus their analogues differ from many of our analogues primarily by having an α-amino group at position 1.[7] The Ferring group has developed some highly potent oxytocin antagonists also using D-Tyr(Et)^2, Thr^4, Val^4, Orn^8, Cit^8, and L- and D-Arg^8 substitutions but lacking any alkyl substituents at position 1, i.e., theirs are 1-deamino analogues.[6]

Peptide Synthesis. Starting from either Boc-Gly-resin or Boc-Orn(Tos)-resin, we synthesized the protected precursors (I–XVI) of the free peptides 1–16 either entirely by the solid-phase method[25-27] (peptides I–IV, VI, IX, X, XV, and XVI) or by a combination of solid-phase and solution methods of peptide synthesis (peptides V, VII, and VIII by 1 + 8 couplings;[2-4,22a] peptides XI–XIV by 8 + 1 couplings) using previously described procedure.[24a,8,42] HCl (1 M)/AcOH was used in all the deprotection steps except those involving Boc-Gln in which TFA was employed.[27a] Neutralizations were carried out with 10% $Et_3N/MeCl_2$. Coupling reactions were mediated primarily by the DCCI method in $MeCl_2$ except for Boc-Asn and Boc-Gln, which were incorporated as their *p*-nitrophenyl esters[31a] in DMF. β-(S-benzylthio)-β,β-pentamethylenepropionic acid[11] was coupled either as its activated ester (for the 1 + 8 coupling in solution) or by using DCCI/HOBt.[30] Likewise, *p*-nitrophenyl β-(S-benzylthio)-β,β-dimethylpropionate[9] was used for the 1 + 8 couplings in solution. Cleavage from the resin was by either ammonolysis[27,31c] in methanol to give the protected peptide amides or by HBr/TFA[25b,26,28,32] to give the protected des-Gly-NH_2 precursor (XV) and the precursors required for the 8 + 1 couplings in solution. The later were carried out as recently described.[24a] Na in NH_3[33] was used to deblock each protected precursor as previously described,[2-4,22,24] and the resulting disulfhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$.[34] The free peptides were desalted and purified by gel filtration on Sephadex G-15 in a two-step procedure using 50% and 0.2 M AcOH as eluents, respectively, as previously described.[35]

Bioassay Methods. Antagonistic activities of analogues are expressed as pA_2's.[40] The pA_2 is the negative logarithm of the "effective" molar concentration of an antagonist that reduces the response to a dose of agonist to the level of the response to one-half that dose of agonist given in the absence of antagonist. Antioxytocic potencies in vitro were assayed on isolated uteri from estrogen-pretreated rats suspended with a resting tension of 1 g in a Mg^{2+}-free van Dyke–Hastings solution[37] or in the same

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medium enriched with 0.5 mM Mg^{2+}. The media were maintained at 32 °C and gassed with 5% CO_2 and 95% O_2. Concentrations of antagonists above and below the effective concentration were administered and the effective concentration was estimated by interpolation on a semilogarithmic scale.[40]

Antagonistic activities in vivo were calculated from "effective doses" of antagonists given iv. These doses were estimated as were the effective concentrations in in vitro assays. The effective concentrations in in vivo assays were estimated by assuming an arbitrary volume of distribution for the antagonists (67 mL/kg).[47] In vivo antioxytocic activity was assayed by measuring changes in integrated intrauterine pressure in estrogen-pretreated rats under urethane anesthesia.[3] Antagonism of milk-ejection responses was assayed by recording changes in mammary pressure in lactating rats under pentobarbital anesthesia.[3] Antivasopressor activity was assayed on phenoxybenzamine-pretreated rats under urethane anesthesia[39,47] and antiantidiuretic activity on hydrated rats under ethanol anesthesia.[38] The USP Posterior Pituitary Reference Standard was used as agonist in all assays except those for antioxytocic activity in vivo. Synthetic oxytocin (Syntocinon, Sandoz) was used in these assays. When standard errors of means are indicated in the tables, these refer to the means of at least four independent estimates of effective doses or pA_2's.

Results

The antioxytocic (in vitro and in vivo), antivasopressor, and antidiuretic (agonistic or antagonistic) properties of the three series of analogues together with those of some related peptides are presented in Table I–III. It should be noted at the outset that none of these new peptides exhibited either oxytocic or vasopressor agonism.

A. Analogues of $d(CH_2)_5$[Tyr(Me)^2]AVP (Table I). These four new peptides all exhibit substantial in vitro oxytocic antagonism in the presence and in the absence of Mg^{2+}. pA_2 values in the absence of Mg^{2+} range from 7.87 for $d(CH_2)_5$[D-Tyr(Me)^2]AVP (1) and $d(CH_2)_5$[D-Tyr(Et)^2,Val^4,Lys^8]VP (3) to 8.61 for $d(CH_2)_5$[D-Tyr(Et)^2,Val^4,Cit^8]VP (4). pA_2 values in the presence of Mg^{2+} range from 6.96 for $d(CH_2)_5$[D-Tyr(Me)^2,Val^4,Δ^3-Pro^7]AVP (2) to 8.32 for $d(CH_2)_5$[D-Tyr(Me)^2]AVP (1). All four new peptides (1–4) are also potent antioxytocics in vivo. With pA_2 values of 7.47 to 7.75 they are all at least as potent as $d(CH_2)_5$[Tyr(Me)^{2}Orn^8]VT[4] [pA_2 = 7.37 (Table III)]. However, each of these new peptides (1–4) is even more potent as an antivasopressor than as an antioxytocic. Their antivasopressor pA_2 values range from 7.93 to 8.32. It will be recalled that $d(CH_2)_5$[Tyr(Me)^2]AVP is a weak antidiuretic agonist. Not unexpectedly these four new D-Tyr(Alk)-containing analogues of $d(CH_2)_5$[Tyr(Me)^2]AVP all exhibit substantive antiantidiuretic potency (anti-V_2

Table I. Some Pharmacological Properties of Nonselective Antagonists of Oxytocin (1–4) Based on Modifications of $d(CH_2)_5$[Tyr(Me)^2]AVP at Positions 2, 4, 7, and 8

		antioxytocic							
		in vitro pA_2[c,d]		in vivo		antivasopressor		antiantidiuretic	
no.	peptide	no Mg^{2+}	0.5 mM Mg^{2+}	ED[b,d]	pA_2[c,d]	ED[b,d]	pA_2[c,d]	ED[b,d]	pA_2[c,d]
	$d(CH_2)_5$[Tyr(Me)^2]AVP[e]	8.13 ± 0.12	7.24 ± 0.07	17 ± 3	6.62 ± 0.08	0.16 ± 0.01	8.62 ± 0.03	agonist 0.31 unit/mg	
1	$d(CH_2)_5$[D-Tyr(Me)^2]AVP[f]	7.87 ± 0.11	8.32 ± 0.11	1.3 ± 0.3	7.47 ± 0.11	0.36 ± 0.02	8.27 ± 0.02	7.3 ± 1.3	6.98 ± 0.08
1a	$d(CH_2)_5$[Tyr(Me)^2,Val^4]AVP[f,i,j]	8.36 ± 0.13	7.87 ± 0.11	8.0 ± 1.9	6.94 ± 0.08	0.29 ± 0.06	8.32 ± 0.08	3.1 ± 0.4	7.35 ± 0.06
1b	$d(CH_2)_5$[D-Tyr(Me)^2,Val^4]AVP[f,h,j]	7.96 ± 0.09	7.83 ± 0.12	1.9 ± 0.3	7.57 ± 0.09	0.23 ± 0.04	8.48 ± 0.08	1.2 ± 0.13	7.77 ± 0.06
2	$d(CH_2)_5$[D-Tyr(Me)^2,Val^4,Δ^3-Pro^7]AVP[f]	7.88 ± 0.08	6.96 ± 0.06	1.17 ± 0.04	7.75 ± 0.01	0.55 ± 0.04	8.08 ± 0.03	1.7 ± 0.2	7.61 ± 0.07
2b	$d(CH_2)_5$[D-Tyr(Et)^2,Val^4]AVP[f,h,j]	8.32 ± 0.10	8.38 ± 0.08	2.6 ± 0.6	7.47 ± 0.09 [6.74][k]	0.45 ± 0.11	8.22 ± 0.12	1.1 ± 0.2	7.81 ± 0.07
3	$d(CH_2)_5$[D-Tyr(Et)^2,Val^4,Lys^8]VP[f,i]	7.87 ± 0.04	7.98 ± 0.04	1.9 ± 0.03	7.57 ± 0.09	0.48 ± 0.08	8.16 ± 0.07	1.5 ± 0.4	7.72 ± 0.10
4	$d(CH_2)_5$[D-Tyr(Et)^2,Val^4,Cit^8]VP[f,i]	8.61 ± 0.12	7.83 ± 0.10	1.8 ± 0.3	7.59 ± 0.07	0.81 ± 0.11	7.93 ± 0.07	23 ± 6	6.51 ± 0.12

[a] In vitro pA_2 values were calculated as described in Bankowski et al.[4] [b] The effective dose (ED) is defined as the dose (in nanomoles per kilogram) that reduces the response to 2× units of agonist to equal the response to 1× unit. [c] Estimated in vivo pA_2 values represent the negative logarithms of the effective doses divided by the estimated volume of distribution (67 mL/kg). [d] Means ±S.E. [e] From Kruszynski et al.[22a] / This publication. [g] From Manning et al.[22b] [h] Anti-V_1 and anti-V_2 properties from Manning et al.[22c] [i] Preliminary data on the anti-V_1 and anti-V_2 properties of these molecules are given in Manning et al.[45] / Preliminary in vivo pA_2 values reported in Manning and Sawyer[17] and Manning et al.[19] [k] Value for $d(CH_2)_5$[Tyr(Et^2),Val^4]AVP[22b] reported in Manning and Sawyer[17] and Manning et al.[19]

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Table II. Some Pharmacological Properties of Nonselective Antagonists of Oxytocin (5–10) Based on Modifications of $d(CH_2)_5$[Tyr(Me)^2]AVT at Positions 1, 2, 4, and 8

		antioxytocic potency				antivasopressor		
		in vitro pA_2[a,d]		in vivo		ED[b,d]		antidiuretic act.,
no.	peptide	no Mg^{2+}	0.5 mM Mg^{2+}	ED[b,d]	pA_2[c,d]	(nmol/kg)	pA_2[c,d]	unit/mg
	$d(CH_2)_5$[Tyr(Me)^2]AVP[e]	8.13 ± 0.12	7.24 ± 0.07	17 ± 3	6.62 ± 0.08	0.16 ± 0.01	8.62 ± 0.03	0.31
5	$d(CH_2)_5$[Tyr(Me)^2]AVT[f]	8.72 ± 0.16	8.49 ± 0.03	1.1 ± 0.3	7.80 ± 0.09	0.64 ± 0.13	8.08 ± 0.10	~0.03
6	$d(CH_2)_5$[Tyr(Me)^2,Lys^8]VT[f]	7.63 ± 0.08	7.81 ± 0.07	4.6 ± 1.3	7.23 ± 0.14	1.5 ± 0.3	7.68 ± 0.09	~0.003
7	dP[Tyr(Me)^2]AVT[f]	9.12 ± 0.09	8.71 ± 0.09	2.2 ± 0.5	7.61 ± 0.14	0.25 ± 0.02	8.45 ± 0.05	~1.6
8	dP[Tyr(Me)^2,Val^4]AVT[f]	7.75 ± 0.07	8.11 ± 0.11	1.9 ± 0.4	7.57 ± 0.10	0.23 ± 0.02	8.46 ± 0.04	1.6 ± 0.2
9	$d(CH_2)_5$[D-Tyr(Me)^2,Val^4]AVT[f]	7.65 ± 0.06	8.25 ± 0.11	2.4 ± 0.6	7.48 ± 0.10	1.1 ± 0.2	7.82 ± 0.08	antagonist ED = 9.4 ± 1.7 pA_2 = 6.88 ± 0.08
10	$d(CH_2)_5$[D-Phe^2,Val^4]AVT	7.89 ± 0.09	8.25 ± 0.10	2.5 ± 0.6	7.46 ± 0.11	17 ± 4	6.64 ± 0.11	antagonist ED = 45 ± 14 pA_2 = 6.21 ± 0.13

[a–e] See corresponding footnotes to Table I. [f] This publication.

pA_2 values range from 6.51 to 7.72). Thus although these peptides exhibit substantial gains in antioxytocic potencies, there are no gains in antioxytocic/anti-V_1 selectivity except in the case of **4**. In this instance the substitution of Cit^8 for Arg^8 (**2b**) or Lys^8 (**3**) resulted in a 15- to 20-fold reduction in anti-V_2 activity and a proportionate gain in OT/V_2 specificity. The usefulness of the L-Tyr(Alk)/D-Tyr(Alk)[22c] [where Alk = Me or Et] interchange for enhancing antioxytocic potency is clearly illustrated by comparisons of the ED and pA_2 values for the $d(CH_2)_5$[Tyr(Me)^2]AVP/$d(CH_2)_5$[D-Tyr(Me)^2]AVP and $d(CH_2)_5$[Tyr(Me)^2,Val^4]AVP/$d(CH_2)_5$[D-Tyr(Me)^2,Val^4]AVP pairs.[17,19,22b,c] The D-Tyr(Me) substitution effected 10-fold and 4-fold enhancements, respectively, in antioxytocic potencies. Likewise, the substitutions of D-Tyr(Et) for L-Tyr(Et) in $d(CH_2)_5$[Tyr(Et)^2,Val^4]AVP[22b] (pA_2 = 6.74)[17,19] effected about an 8-fold enhancement in antioxytocic potency in the resulting peptide, $d(CH_2)_5$[D-Tyr(Et)^2,Val^4]-AVP[22c] (pA_2 = 7.47 in vivo).[17,19] It should be noted that similar effects of D-amino acid substitutions on oxytocic antagonism have been found independently by others.[6b,7b] Val^4 substitutions had little effect on antioxytocic potency when substituted in $d(CH_2)_5$[D-Tyr(Me)^2]AVP (**1**) (pA_2 = 7.47) to give $d(CH_2)_5$[D-Tyr(Me)^2,Val^4]AVP[22c] (pA_2 = 7.57).[17,19] Similarly, the substitution of Pro^7 by Δ^3-Pro^7 in $d(CH_2)_5$[D-Tyr(Me)^2,Val^4]AVP (pA_2 = 7.57)[17,19] to give $d(CH_2)_5$[D-Tyr(Me)^2,Val^4,Δ^3-Pro^7]AVP (**2**) (pA_2 = 7.75) resulted in an insignificant gain in antioxytocic potency. Remarkably, the Arg^8 residue in $d(CH_2)_5$[D-Tyr(Et)^2]-VAVP[22] (pA_2 = 7.47)[17,19] can be replaced by either lysine or citrulline with retention of potent oxytocic antagonism. Thus $d(CH_2)_5$[D-Tyr(Et)^2,Val^4,Lys^8]VP (**3**) (pA_2 = 7.57) and $d(CH_2)_5$[D-Tyr(Et)^2,Val^4,Cit^8]VP (**4**) (pA_2 7.59) are among the most potent antioxytocics reported to date.

We have previously noted the dramatic effects of a D-Arg^8/L-Arg^8 interchange on in vivo antioxytocic antagonism, as illustrated by two of our original AVP, V_2 antagonists, $d(CH_2)_5$[Tyr(Et)^2]VAVP and $d(CH_2)_5$[Tyr-(Et)^2]VDAVP.[22b] Their antioxytocic in vivo pA_2 values are 6.74[17,19] and 8.19,[17,19] respectively. Thus the latter is one of the most potent in vivo oxytocin antagonists reported to date. The investigation of a similar D-Arg^8/L-Arg^8 interchange in the analogues in Table I is now well warranted.

B. Analogues of $d(CH_2)_5$[Tyr(Me)^2]AVT (5–10, Table II). These six peptides all exhibit potent oxytocic antagonism in vitro. Their in vitro pA_2 values in the absence of Mg^{2+} range from 7.63 for $d(CH_2)_5$[Tyr-(Me)^2,Lys^8]VT (**6**) to 9.12 for dP[Tyr(Me)^2]AVT (**7**). This latter compound is one of the most potent in vitro antagonists of oxytocin reported to date. Their in vitro pA_2 values in the presence of 0.5 mM Mg^{2+} range from 7.81 to 8.71. With the possible exception of the Lys^8 analogue (**6**), these are all potent in vivo antagonists of oxytocin, with pA_2 values ranging from 7.46 for the D-Phe^2 analogue (**10**) to 7.80 for $d(CH_2)_5$[Tyr(Me)^2]AVT. This latter peptide is thus one of the most potent in vivo oxytocic antagonists reported to date.

The findings in this series point to curious anomalies in the effects of similar substitutions here and in the first series (1–4, Table I). It is particularly noteworthy that $d(CH_2)_5$[Tyr(Me)^2]AVT is almost 17 times more potent than $d(CH_2)_5$[Tyr(Me)^2]AVP as an in vivo oxytocin antagonist. In this pair, the Ile/Phe interchange at position 3 has had a very dramatic effect on oxytocic antagonism in vivo. On the basis of this finding, one might have predicted that a similar Ile/Phe interchange at position 3 in $d(CH_2)_5$[D-Tyr(Me)^2]AVP (Table I) (anti-OT pA_2 = 7.57) would result in a similar dramatic increase in in vivo antioxytocic potency. Examination of the properties of $d(CH_2)_5$[D-Tyr(Me)^2,Val^4]AVT (**9**, Table II) (pA_2 = 7.48) indicates that this did not occur. These peptides are virtually equipotent. Likewise, the effects of replacing of Arg^8 in the two series were strikingly dissimilar. Thus the substitution of Lys^8 for Arg^8 in $d(CH_2)_5$[Tyr(Me)^2]AVT resulted in a 4-fold reduction in in vivo oxytocic antagonism. The resulting $d(CH_2)_5$[Tyr(Me)^2,Lys^8]VT (**6**) has an in vivo pA_2 = 7.23 compared to 7.80 for its Arg^8 counterpart, i.e., $d(CH_2)_5$[Tyr(Me)^2]AVT (**5**). This is in striking contrast to the aforementioned effects of a similar Lys^8/Arg^8 switch in the $d(CH_2)_5$[Tyr(Me)^2]AVP series (Table I). These inconsistant comparisons serve to further illustrate the uncertainties inherent in predicting the effects of a given structural change based on its effects in a single molecule. Apart from $d(CH_2)_5$[D-Phe^2,Val^4]AVT, which has an anti-V_1 pA_2 = 6.64 and an anti-OT/anti-V_1 selectivity = 7, these AVT-like peptides in Table II exhibit potent antivasopressor potencies with pA_2 values in the range of 7.68 for the Lys^8 analogue (**6**) to 8.46 for dP-[Tyr(Me)^2,Val^4]AVT (**8**). Thus they are all nonselective oxytocin antagonists with respect to vascular receptors. Peptides **5** and **6** are very weak antidiuretic agonists, exhibiting approximately 0.03 and approximately 0.003 unit/mg, respectively. The dP analogues (**7** and **8**) exhibit 1.6 unit/mg of antidiuretic activity. Two of these peptides (**9** and **10**) are weak V_2 antagonists, with anti-V_2 pA_2 values of 6.88 and 6.21, respectively. Thus these AVT-type peptides are highly potent and selective oxytocin antagonists with respect to AVP V_2 renal receptors. Replacement of the Arg^8 residue by D-Arg^8 should also be worthwhile with this series of analogues.

C. Analogues of $d(CH_2)_5$[Tyr(Me)^2]OVT (11–16, Table III). Except for $d(CH_2)_5$[Tyr(Me)^2]OVT, all of the analogues in Table III exhibit significant increases in an-

tioxytocic potency in the presence of Mg^{2+} compared to their potencies in the absence of Mg^{2+}. Thus in the absence of Mg^{2+}, the pA_2 values range from 7.62 for the Ala-NH_2[9] analogue (**12**) to a high of 8.17 for the desGly analogue (**16**), whereas in the presence of Mg^{2+} the pA_2 values range from 8.20 (**12**) to 8.56 (**16**). All of these peptides exhibit at least as much in vivo oxytocic antagonism as d(CH_2)$_5$[Tyr(Me)[2]]OVT[4] (pA_2 = 7.37). Furthermore, with the exception of the Arg-NH_2[9] analogue (**14**), all exhibit substantially reduced antivasopressor antagonism relative to d(CH_2)$_5$[Tyr(Me)[2]]OVT (anti-V_1 pA_2 = 7.96).[4] In fact, all are much weaker as vasopressor antagonists than as in vivo oxytocin antagonists. They thus exhibit highly significant gains in antioxytocic/anti-V_1 selectivity relative to d(CH_2)$_5$[Tyr(Me)[2]]OVT (ED ratio = 0.2).[4] With effective dose ratios of 17 and 15, respectively, desGly-NH_2[9],d(CH_2)$_5$[Tyr(Me)[2],Thr[4]]OVT (**15**) and d(CH_2)$_5$[Tyr(Me)[2],Thr[4],Leu-NH_2[9]]OVT (**13**) are among the most selective oxytocin antagonists reported to date. As noted earlier the monoiodinated derivative of d(CH_2)$_5$-[Tyr(Me)[2],Thr[4],Tyr-NH_2[9]]OVT has recently been shown to exhibit very high selectivity for oxytocin receptors in vitro.[24a] The Leu-NH_2[9] and Tyr-NH_2[9] analogues (**13** and **14b**) exhibit very weak V_2 agonism. The desGly-NH_2[9] analogue (**15**) is a weak V_2 antagonist (pA_2 = 5.3). The remaining peptides (**11**, **12**, **14**, and **16**) exhibit weak partial V_2 agonism/weak V_2 antagonism. Thus these are all highly selective for oxytocin receptors relative to renal tubular receptors. In comparing the properties of d(CH_2)$_5$[Tyr-(Me)[2]]OVT with those of its Thr[4] derivative (peptide **11**), it is clear that the Thr[4] substitution has brought about a 2-fold increase in antioxytocic potency in vivo while at the same time leading to a 12-fold reduction in anti-V_1 potency. This further illustrates the usefulness of the Thr[4] substitution for the enhancement of antioxytocic potency and selectivity.[2]

We have previously shown that the C-terminal Gly-NH_2[9] in a variety of vasopressin V_2 and V_2/V_1 antagonists can be either deleted or substituted with a variety of other amino acid amides and non-amino acids, with retention of good antagonistic potencies.[23,28] Also, in a preliminary report, Lebl and colleagues have shown that the desGly-NH_2[9] analogue of 1-deamino[D-Phe(Me)[2], carba[6]]oxytocin exhibits potent oxytocic antagonism in vitro in the absence of Mg^{2+}.[46] It was thus not surprising that these modifications are also well tolerated in oxytocic antagonists. However, the enhanced in vivo oxytocic antagonism exhibited by these peptides is surprising and highly gratifying. Thus one or more of these peptides may be useful for the treatment of premature labor. All of these are potentially useful as pharmacological tools for studies on oxytocin receptors and on the physiological roles of oxytocin.

Analogues **11**–**16** were also assayed for their ability to antagonize milk-ejection responses to oxytocin in lactating rats.[3] The antagonistic actions of these analogues often lasted for an hour or more. This made it impractical to attempt to estimate precise effective doses. Nonetheless, each analogue was tested on at least four rats and they were all effective antagonists with roughly estimated pA_2's in the range of 7 to 8. The long duration of antagonistic action of these analogues on both uterine and milk-ejection assays in vivo resembles the long durations of antagonistic activities of related analogues previously reported by Sawyer et al.[3] and Chan et al.[7]

Discussion

We have reported the synthesis of 16 new antagonists of oxytocin. Ten of these analogues were designed by

Table III. Some Pharmacological Properties of Selective Oxytocin Antagonists (**11**–**16**) Based on Modifications of d(CH_2)$_5$[Tyr(Me)[2]]OVT at Positions 4 and 9

		antioxytocic				antivasopressor		antidiuretic act.,	
		in vitro pA_2[a,d]		in vivo					
no.	peptide	no Mg^{2+}	0.5 mM Mg^{2+}	ED[b,d]	pA_2[c,d]	ED[b,d]	pA_2[c,d]	unit/mg	ED ratio[e]
	d(CH_2)$_5$[Tyr(Me)[2]]OVT[f]	8.52 ± 0.10	7.88 ± 0.10	4.2 ± 1.6	7.37 ± 0.17	0.80 ± 0.16	7.96 ± 0.10	0.01	0.2
11	d(CH_2)$_5$[Tyr(Me)[2],Thr[4]]OVT[g]	8.02 ± 0.09	8.41 ± 0.07	2.0 ± 0.3	7.55 ± 0.08	9.9 ± 1.6	6.85 ± 0.07	weak partial agonist/antagonist ED = ~330 pA_2 = ~5.3	5.0
12	d(CH_2)$_5$[Tyr(Me)[2],Thr[4],Ala-NH_2[9]]OVT[g]	7.62 ± 0.11	8.20 ± 0.10	2.4 ± 0.3	7.46 ± 0.05	8.1 ± 1.9	6.95 ± 0.09	weak partial agonist/antagonist	3.4
13	d(CH_2)$_5$[Tyr(Me)[2],Thr[4],Leu-NH_2[9]]OVT[g]	7.78 ± 0.10	8.46 ± 0.02	1.8 ± 0.2	7.57 ± 0.04	27 ± 2	6.39 ± 0.03	0.029 ± 0.009	15
14	d(CH_2)$_5$[Tyr(Me)[2],Thr[4],Arg-NH_2[9]]OVT[g]	7.69 ± 0.11	8.26 ± 0.08	2.3 ± 0.6	7.54 ± 0.12	0.76 ± 0.14	7.96 ± 0.08	weak partial agonist/antagonist	0.3
14b	d(CH_2)$_5$[Tyr(Me)[2],Thr[4],Tyr-NH_2[9]]OVT[h]	7.63 ± 0.07	8.28 ± 0.10	1.0 ± 0.1	7.83 ± 0.04	6.6 ± 0.9	7.02 ± 0.07	0.015 ± 0.006	6.6
15	desGly-NH_2,d(CH_2)$_5$[Tyr(Me)[2],Thr[4]]OVT[g,i]	7.89 ± 0.04	8.24 ± 0.09	1.3 ± 0.2	7.69 ± 0.07	23 ± 4	6.48 ± 0.08	weak antagonist ED = ~300 pA_2 = ~5.3	17
16	desGly,d(CH_2)$_5$[Tyr(Me)[2],Thr[4]]OVT[g]	8.17 ± 0.08	8.56 ± 0.09	1.7 ± 0.3	7.61 ± 0.08	7.3 ± 1.3	6.98 ± 0.07	weak partial agonist/antagonist ED = ~100 pA_2 = ~5.8	4.3

[a–d] See corresponding footnotes to Table I. [e] ED ratio = antivasopressor ED/antioxytocic ED. [f] From Bankowski et al.[4] [g] This publication. [h] From Elands et al.[24a] [i] Preliminary unpublished properties were given by Elands et al.[24b]

Table IV. Physicochemical Properties of the Protected Peptides (I–XVI)[a]

no.	protected peptide	(elemental analysis) formula	yield, %[b,c]	mp, °C	$[\alpha]^{25}_D$, deg (c = 1, DMF)	TLC, R_f a	b	c	d
I	d(CH$_2$)$_5$[D-Tyr(Me)^2]AVP	$C_{73}H_{94}N_{14}O_{14}S_3 \cdot H_2O$	75.8	188–189	−22.6	0.60		0.83	0.59
II	d(CH$_2$)$_5$[D-Tyr(Me)^2,Val^4,Δ^3-Pro^7]AVP	$C_{73}H_{93}N_{13}O_{13}S_3$	91.8	212–214	−51.5	0.73		0.76	0.75
III	d(CH$_2$)$_5$[D-Tyr(Et)^2,Val^4,Lys^8]VP	$C_{74}H_{97}N_{11}O_{13}S_3 \cdot 3H_2O$	73.1	205–209	−20.5	0.70		0.90	
IV	d(CH$_2$)$_5$[D-Tyr(Et)^2,Val^4,Cit^8]VP	$C_{67}H_{90}N_{12}O_{12}S_2$	65.0	237–240	−23.0	0.56		0.84	0.43
V	d(CH$_2$)$_5$[Tyr(Me)^2]AVT	$C_{70}H_{96}N_{14}O_{14}S_3$	85.0	240–242	−35.3		0.61		0.70
VI	d(CH$_2$)$_5$[Tyr(Me)^2,Lys^8]VT	$C_{70}H_{96}N_{12}O_{14}S_3 \cdot H_2O$	82.0	208–212	−36.1		0.50	0.79	
VII	dP[Tyr(Me)^2]AVT	$C_{67}H_{92}N_{14}O_{14}S_3$	85.2	243–246	−36.6		0.51		0.74
VIII	dP[Tyr(Me)^2,Val^4]AVT	$C_{67}H_{93}N_{13}O_{13}S_3$	85.1	229–231	−37.4		0.48		0.55
IX	d(CH$_2$)$_5$[D-Tyr(Me)^2,Val^4]AVT	$C_{70}H_{97}N_{13}O_{13}S_3 \cdot H_2O$	68.2	220–224	−14.8	0.68		0.83	0.73
X	d(CH$_2$)$_5$[D-Phe^2,Val^4]AVT	$C_{69}H_{95}N_{13}O_{12}S_3$	75.2	223–227	−19.5	0.72		0.90	0.76
XI	d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4]OVT	$C_{68}H_{93}N_{11}O_{14}S_3$	75.6	197–201	−28.8		0.71	0.84	0.73
XII	d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Ala-NH$_2^9$]OVT	$C_{69}H_{95}N_{11}O_{14}S_3 \cdot H_2O$	81.6	219–222	−20.7	0.63		0.82	0.30
XIII	d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Leu-NH$_2^9$]OVT	$C_{72}H_{101}N_{11}O_{14}S_3$	85.1	206–209	−22.7		0.80	0.82	0.91
XIV	d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Arg-NH$_2^9$]OVT	$C_{79}H_{108}N_{14}O_{16}S_4 \cdot H_2O$	59.9	197–199	−23.3	0.62		0.71	0.27
XV	desGlyNH$_2$,d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4]OVT	$C_{66}H_{89}N_9O_{14}S_3$	74.3	186–188[d]	−29.5[d]	0.68	0.77	0.72	
XVI	desGly,d(CH$_2$)$_5$[Gyr(Me)^2,Thr^4]OVT	$C_{73}H_{96}N_{10}O_{13}S_3$	48.6	227–231	−22.6	0.81	0.80	0.91	

[a] Structures of the protected peptides are given in the Experimental Section. [b] Yields were calculated on the basis of the glycine or ornithine content of the starting resin. [c] All the protected peptides gave the expected amino acid ratios after hydrolysis ±3%. The acidic hydrolysis of compound IV caused a conversion of citrulline residue to ornithine in a range of about 50%. [d] (Lit.[24a] mp 184–185 °C, lit.[24a] $[\alpha]^{20}_D$ −29.5° (c = 1.0, DMF).

modifying the AVP V_1 antagonist d(CH$_2$)$_5$[Tyr(Me)^2]-AVP[22a] in two separate ways to give, on the one hand, AVP-like molecules (with phenylalanine at position 3) and, on the other, six vasotocin-like molecules (with isoleucine at position 3). The remaining six analogues were designed by substituting threonine for glutamine at position 4, alone or in combination with a variety of substitutions or deletions at position 9, in the potent but nonselective oxytocin antagonist d(CH$_2$)$_5$[Tyr(Me)^2]OVT.[4] All 16 new peptides appear to be at least as potent in vivo oxytocin antagonists as d(CH$_2$)$_5$[Tyr(Me)^2]OVT (pA_2 = 7.37). However, only the analogues of d(CH$_2$)$_5$[Tyr(Me)^2]OVT (**11–13, 14b–16**) (Table III) also exhibited substantial gains in antioxytocic/antivasopressor selectivities.

Analysis of these findings reveals clear differences in the effects of a given substitution depending on the ring structure (pressin-like/tocin-like) of the peptide in which it is employed. Thus the D-Tyr(Alk) [where Alk = Me, Et] substitutions at position 2 in analogues of d(CH$_2$)$_5$[Tyr-(Me)^2]AVP (Table I) brought about striking increases in in vivo antioxytocic potency relative to their L-Tyr(Alk) counterparts. However in analogues of d(CH$_2$)$_5$[Tyr-(Me)^2]AVT (Table II), a D-Tyr(Me)^2 substitution appears to have had little effect on antioxytocic potency. Position 8 in the analogues of d(CH$_2$)$_5$[Tyr(Me)^2]AVP is very tolerant of change. Thus, arginine, lysine, and citrulline are interchangeable at position 8 in d(CH$_2$)$_5$[D-Tyr-(Et)^2,Val^4]AVP with all three peptides exhibiting virtually identical in vivo antioxytocic potencies (**2b**, **3**, **4**, Table I), whereas d(CH$_2$)$_5$[Tyr(Me)^2,Lys^8]VT (**6**, Table II) is 3 times less potent than its Arg^8 parent. Similarly, although the Ile/Phe switch at position 3 in d(CH$_2$)$_5$[Tyr(Me)^2]AVP to give d(CH$_2$)$_5$[Tyr(Me)^2]AVT resulted in a 17-fold enhancement in in vivo antioxytocic potency, this same modification in d(CH$_2$)$_5$[D-Tyr(Me)^2,Val^4]AVP (**2b**, Table I) to give d(CH$_2$)$_5$[D-Tyr(Me)^2,Val^4]AVT (**9**, Table II) gave rise to virtually no change in antioxytocic potency. These findings urge the use of extreme caution in predicting the usefulness of a given modification for oxytocin antagonist design. In this regard it is both significant and high gratifying that the Thr/Gln interchange in d(CH$_2$)$_5$[Tyr-(Me)^2]OVT resulted in a 2-fold enhancement in in vivo antioxytocic potency exhibited by the resulting analogue, d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4]OVT (**11**, Table III). This is consistent with what had been previously observed for a similar Thr/Gln interchange in a series of early oxytocin antagonists.[2-4] It is also noteworthy that in d(CH$_2$)$_5$-[Tyr(Me)^2,Thr^4]OVT the deletion of the C-terminal Gly or Gly-NH$_2$ and replacement of the latter by a series of amino acid amides have had effects similar to those previously observed for these modifications in AVP V_1 and V_2 antagonists,[23,28] i.e., retention of antagonism. Indeed, both the desGly[9] and the desGly-NH$_2$[9] analogues of d-(CH$_2$)$_5$[Tyr(Me)^2,Thr^4]OVT (**15**, **16**, Table III) are as potent in vivo antioxytocics as is d(CH$_2$)$_5$[Tyr(Me)^2]OVT itself. Since both analogues are much less potent as V_1 antagonists, they possess greatly increased antioxytocic/antivasopressor selectivities with respect to d(CH$_2$)$_5$-[Tyr(Me)^2]OVT. This is particularly true for the des-Gly-NH$_2$ analogue. Likewise the replacement of the C-terminal Gly-NH$_2$ in d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4]OVT by either Ala-NH$_2$, Leu-NH$_2$, Arg-NH$_2$, or, as recently reported, Tyr-NH$_2$,[24a] resulted in good retention or slight enhancement of antioxytocic potency by all of the resulting analogues (**12–14**, Table III).

Conclusion

With in vivo antioxytocic pA_2 values = 7.80 and 7.83, d(CH$_2$)$_5$[Tyr(Me)^2]AVT (**5**, Table II) and d(CH$_2$)$_5$[Tyr-(Me)^2,Thr^4,Tyr-NH$_2^9$]OVT (**14b**, Table III) are among the most potent antioxytocics reported to date. With antioxytocic/anti-V_1 selectivity ratios of 15 and 17 respectively d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Leu-NH$_2^9$]OVT (**13**, Table III) and desGly-NH$_2^9$,d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4]OVT (**15**, Table III) are among the most selective oxytocin antagonists reported to date. The findings from these three series together with those recently reported by others[6,7,21] provide useful clues for the design of more potent and selective oxytocin antagonists. Furthermore, some of these antagonists could be useful pharmacological tools for studies on the physiological roles of oxytocin and hold promise for development as clinically useful agents for the prevention of premature births in humans.

Experimental Section

Amino acid derivatives were purchased from Bachem Inc. or from Chemalog Inc. Boc-D-Tyr(Et),[41] Boc-Tyr(Me),[41] Boc-D-Tyr(Me),[41] β-(benzylthio)-β,β-pentamethylenepropionic acid,[11,42] β-(benzylthio)-β,β-dimethylpropionic acid,[9] p-nitrophenyl β-(benzylthio)-β,β-pentamethylenepropionate,[11] and p-nitrophenyl β-(benzylthio)-β,β-dimethylpropionate[9] were synthesized by previously published procedures. The protected precursors required for the synthesis of nine of the analogues reported here (compounds I–IV, VI, IX, X, XV, XVI, Table IV) and two protected octapeptides, Boc-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH$_2$ (D) and Boc-Tyr(Me)-Ile-Val-Asn-Cys-(Bzl)-Pro-Arg(Tos)-Gly-NH$_2$ (E), were synthesized by the manual

Table V. Physicochemical Properties of the Free Peptides (1–16)

no.	peptide	yield, %[a,b]	$[\alpha]^{25}_D$, deg (c = 0.3, 1 M AcOH)	TLC, R_f a	b	c
1	d$(CH_2)_5$[D-Tyr(Me)^2]AVP	57.2	−109.3	0.14		0.19
2	d$(CH_2)_5$[D-Tyr(Me)^2,Val^4,Δ^3-Pro^7]AVP	26.0	−114.8	0.11		0.25
3	d$(CH_2)_5$[D-Tyr(Et)^2,Val^4,Lys^8]VP	40.2	−98.0	0.16		0.65
4	d$(CH_2)_5$[D-Tyr(Et)^2,Val^4,Cit^8]VP	14.0	−54.5	0.23		0.62
5	d$(CH_2)_5$[Tyr(Me)^2]AVT	55.3	−44.8		0.31	0.61
6	d$(CH_2)_5$[Tyr(Me)^2,Lys^8]VT	46.6	−56.8	0.11		0.37
7	dP[Tyr(Me)^2]AVT	69.6	−31.2		0.30	0.61
8	dP[Tyr(Me)^2,Val^4]AVT	81.6	−32.8		0.22	0.46
9	d$(CH_2)_5$[D-Tyr(Me)^2,Val^4]AVT	57.6	−77.5	0.26	0.08	0.52
10	d$(CH_2)_5$[D-Phe^2,Val^4]AVT	55.1	−92.3	0.25	0.31	0.61
11	d$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT	57.5	−32.6	0.46	0.31	0.55
12	d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Ala-NH_2^9]OVT	74.0	−34.0	0.30		0.50
13	d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Leu-NH_2^9]OVT	53.1	−58.9	0.24	0.33	0.39
14	d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Arg-NH_2^9]OVT	40.5	−26.0	0.05	0.16	0.35
15	desGly-NH_2,d$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT	71.6	−52.5	0.24	0.24	0.39
16	desGly,d$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT	63.6	−43.5	0.25	0.30	0.49

[a] Yields are based on the amount of protected peptide used in the reduction–reoxidation step in each case. [b] All the free peptides gave the expected amino acid analysis ratios after hydrolysis ±3%. The acidic hydrolysis of compound 4 caused a conversion of citrulline residue to ornithine in a range of about 50%.

solid-phase method[25,26] as described below using previously described modifications.[24a,27] The protected intermediates XI–XIV (Table IV) were obtained by an 8 + 1 approach, using [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Thr-Asn-Cys(Bzl)-Pro-Orn(Tos)-OH (XV)[24a] and Gly-NH_2 (XI), Ala-NH_2 (XII), Leu-NH_2 (XIII), and Arg(Tos)-NH_2 (XIV) by methods already described.[24a,28] Removal of the N-terminal protecting group from the two octapeptide amides (D, E) and coupling with either *p*-nitrophenyl β-(benzylthio)-β,β-pentamethylenepropionate[11] or *p*-nitrophenyl β-(benzylthio)-β,β-dimethylpropionate[9] by described methods[2–4,22a] yielded the immediate precursors V, VII, and VIII (Table IV). The physicochemical properties of the 16 purified protected peptides (I–XVI) are given in Table IV. All 16 protected precursors were converted to the required free cyclized peptides by deblocking with Na/liquid NH_3,[33] oxidation with dilute ferricyanide solution,[34] desalting, and purification in a two-step procedure using gel filtration on Sephadex G-15 as previously described.[35] The physicochemical properties of the free peptides are given in Table V. The conversion of the protected precursor for d$(CH_2)_5$[D-Tyr(Et)^2,Val^4,Cit^8]VP (IV, Table 4) to its free cyclized derivative (4, Table V) is described below. This description is representative of the procedure followed for the synthesis of all of the remaining 15 peptides in Table V from their respective precursors in Table IV. Dimethylformamide (DMF) was distilled under reduced pressure. Other solvents and reagents were analytical grade. Thin-layer chromatography (TLC) was performed on silica gel (0.25 mm, Brinkmann Silpate). The following solvent systems were used: (a) butan-1-ol–acetic acid–water (4:1:1, v/v/v), (b) butan-1-ol–acetic acid–water (4:1:5, v/v/v, upper phase), (c) butan-1-ol–acetic acid–water–pyridine (15:3:3:10, v/v/v/v), (d) chloroform–methanol (7:3, v/v). Loads of 10–50 μg were applied and chromatograms were a minimum length of 10 cm. Iodine vapor was used for detection. Elemental analyses were performed by Integral Microanalytical Laboratories, Inc., Raleigh, NC. The analytical results for elements indicated by their symbols were within 0.4% of theoretical values. Optical rotations were measured with a Rudolph Model 80 polarimeter or a Rudolph Autopol III. For amino acid analysis,[43] peptides (approximately 0.7 mg) were hydrolyzed with constant boiling hydrochloric acid (500 μL) containing phenol (10 μL) in evacuated and sealed ampules for 18 h at 118 °C. The analyses were performed on a Model 121 M Beckman automatic amino acid analyzer or Beckman System 6300 amino acid analyzer. Molar ratios were referred to Gly or Orn = 1.00. The cysteine content of the free peptides was estimated as $^1/_2$ cystine. All peptides (protected and free) gave the expected amino acid ratios ± 3%. Melting points of the protected peptides are uncorrected.

Solid-Phase Synthesis. Chloromethylated resin (Chemalog, 1% cross-linked S-DVB, 200–400 mesh, 0.75–1.00 mmol/g) was esterified with either Boc-Gly or Boc-Orn(Tos) to an incorporation of approximately 0.5 mmol/g by the cesium salt method.[44] Eight (or seven) separate cycles of deprotection, neutralization, and coupling were carried out for the synthesis of the 16 peptidyl resins. Ammonolysis[27a,31c] in MeOH or acidolytic cleavage (HBr/TFA)[25b,26,28,32] in $MeCl_2$ was used to split the protected peptides from the resin. All of the protected precursors were purified by the same general method: extraction with hot DMF followed by reprecipitations with H_2O and with MeOH/Et_2O until adjudged pure by TLC.

[β-(Benzylthio)-β,β-pentamethylenepropionyl]-D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-Cit-Gly-NH_2 (IV, Table IV). Boc-Gly-resin (1.14 g, 0.5 mmol) was placed in a glass synthesis vessel. Manual methods of solid-phase synthesis methodology,[25a,26] i.e., eight cycles of deprotection with 1 M HCl/AcOH, neutralization with 10% Et_3N in $MeCl_2$, and coupling (mediated by DCC[29] or active esters[31a]) were used to incorporate successively Boc-Cit-ONp, Boc-Pro, Boc-Cys(Bzl), Boc-Asn-ONp, Boc-Val, Boc-Phe, Boc-D-Tyr(Et), and *p*-nitrophenyl β-(benzylthio)-β,β-pentamethylenepropionate (the last coupling was facilitated by the addition of *N*-hydroxybenzotriazole (HOBt).[30] The resulting protected peptidyl resin, [β-(benzylthio)-β,β-pentamethylenepropionyl]]-D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-Cit-Gly-resin (1.7 g, 0.5 mmol), was suspended in dry methanol (ca. 60 mL) at −10 °C in a 250-mL round-bottomed flask and NH_3 (ca. 20 mL) was bubbled through the suspension for a period of approximately 30 min. The tightly stoppered flask was kept at room temperature in an explosion-proof environment in the hood for 3 days. The suspension was recooled to −15 °C for approximately 15 min before removal of the stopper and the NH_3 was allowed to evaporate at room temperature. Following removal of the MeOH, the protected peptide was extracted with hot (ca. 70 °C) DMF (ca. 32 mL) and the product precipitated by the addition of hot (ca. 70 °C) water (ca. 500 mL). Following overnight storage at 4 °C, the product was collected, dried in vacuo over P_2O_5, reprecipitated from hot DMF (5 mL) with ethyl ether (300 mL), collected, and dried in vacuo over P_2O_5 to give the required acyloctapeptide amide (IV, Table IV). Pertinent physicochemical data for all protected acyloctapeptide amides are given in Table IV.

Boc-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (D). The protected octapeptide resin, Boc-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (3.0 g, 1.0 mmol), was prepared from Boc-Gly-resin (2.28 g, 1.0 mmol) by solid-phase peptide synthesis methodology,[25a,26] i.e., seven cycles of deprotection, neutralization, and coupling with Boc-Arg(Tos), Boc-Pro, Boc-Cys(Bzl), Boc-Asn-ONp, Boc-Gln-ONp, Boc-Ile, and Boc-Tyr(Me), respectively. The desired protected octapeptide amide (D) was prepared in essentially the same manner as compound IV, by ammonolysis from the peptidyl resin: yield 1.07 g (81.8%); mp 225–227 °C dec; $[\alpha]^{25}_D$ = −30.9° (c = 1.0, DMF); TLC 0.52 (b), 0.48 (d). Anal. $C_{60}H_{86}N_{14}O_{15}S_2 \cdot H_2O$ (C, H, N).

Boc-Tyr(Me)-Ile-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (E). Boc-Gly-resin (2.28 g, 1.0 mmol of Gly) was subjected to seven cycles of deprotection, neutralization, and coupling to yield the

protected octapeptidyl resin (3.51 g, weight gain 1.23 g, 99.6% theory). Ammonolysis of the protected peptidyl resin and product isolation as for compound IV yielded the protected octapeptide amide (E): yield 1.01 g (78.8%); mp 212–213 °C dec; $[\alpha]^{25}_D$ = −25.8° (c = 1.0, DMF); TLC 0.64 (b), 0.82 (d). Anal. $C_{60}H_{87}N_{13}O_{14}S_2 \cdot 2H_2O$ (C, H, N).

[β-(Benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Thr-Asn-Cys(Bzl)-Pro-Orn(Tos)-OH (XV, Table IV). Boc-Orn(Tos)-resin (5.5 g, 3.0 mmol of Orn) was subjected to seven cycles of deprotection, neutralization, and coupling to yield the protected octapeptidyl resin (8.6 g, weight gain 3.1 g, 89.9% theory). The protected heptapeptide (XV)[24a] was liberated from the resin by acidolytic cleavage.[25b,26,28,32] Hydrogen bromide was bubbled through a suspension of the above-protected acylheptapeptide-resin (8.6 g, 3.0 mmol) in TFA (50 mL) and anisole (5 mL) in a glass funnel fitted with a fritted disk as described in Stewart and Young.[26] After 30 min the filtrate was collected. The resin was resuspended in $MeCl_2$ (25 mL), TFA (25 mL), and anisole (5 mL). HBr bubbling was resumed for a further 30 min, whereupon the filtrate was collected and the resin was washed with $MeCl_2$–TFA (1:1, 40 mL × 3). The filtrates and washings were combined and evaporated to dryness on a rotary evaporator. Addition of ether (ca. 200 mL) to the residual anisole solution gave a precipitate, which following 3 h at 4 °C was collected, washed with ether, and dried over P_2O_5; 2.7 g. This material was dissolved in warm DMF (ca. 25 mL) reprecipitated with water, collected, and dried in vacuo over P_2O_5 to give the protected acylheptapeptide (XV) (2.173 g). The physicochemical data for this product are given in Table IV.

[β-(Benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (V, Table IV) (1 + 8 Coupling). The Boc-octapeptide amide (D) (981.0 mg, 0.75 mmol) was dissolved in cold TFA (4 mL) and left to stand at room temperature for 25 min. Cold ether (ca. 100 mL) was added, and the precipitated material was collected, washed with ether, and dried in vacuo over sodium hydroxide pellets (yielding 940.0 mg of the TFA salt); TLC 0.40 (b). This material TFA·x-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (330 mg, 0.25 mmol) and *N*-hydroxybenzotriazole (HOBt, 77 mg, 0.5 mmol) were dissolved in DMF (3 mL) and *N*-methylmorpholine (NMM) was added gradually to give a solution of pH 7–8 to moist pH paper.[31b] A solution of *p*-nitrophenyl β-(benzylthio)-β,β-pentamethylenepropionate[11] (194 mg, 0.5 mmol, in 0.4 mL of DMF) was added. The reaction mixture was stirred at room temperature overnight, then diluted with an ethyl acetate–methanol mixture (1:1, v/v, ca. 10 mL), and precipitated by addition of dry ether (ca. 200 mL). The precipitate was filtered, washed with ether, and dried in vacuo over P_2O_5. The crude product (330 mg) was reprecipitated from DMF–methanol to give the acylpeptide amide (V) (309 mg).

The remaining two protected peptides obtained by utilization of (1 + 8) coupling methodology[22a] (VII and VIII, Table IV) were prepared in essentially the same manner, using *p*-nitrophenyl β-(benzylthio)-β,β-dimethylpropionate and Boc-octapeptide amides (D and E). Pertinent physicochemical properties of above three protected peptides V, VII, and VIII are given in Table IV.

[β-(Benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Thr-Asn-Cys(Bzl)-Pro-Orn(Tos)-Leu-NH_2 (XIII, Table IV) (8 + 1 Coupling). The protected octapeptide XV (709.4 mg, 0.5 mmol) and HOBt (202.7 mg, 1.5 mmol) were dissolved in DMF (5 mL) and cooled to 0 °C. DCC (103.2 mg, 0.5 mmol) was added and the mixture was stirred at 0 °C for 30 min. HCl·xLeu-NH_2 (333.3 mg, 2 mmol) was added, followed by a few drops of triethylamine until pH 7 was reached. After overnight stirring at room temperature the dicyclohexylurea was filtered off and the product was precipitated from the filtrate by addition of water (ca. 400 mL). The solid was collected, washed with water, and dried in vacuo over P_2O_5. Reprecipitation from DMF-ethanol and drying in vacuo gave the desired protected precursor XIII (650 mg). The remaining protected precursors XI, XII, and XIV were prepared in the same manner. The physicochemical data of compounds XI–XIV are given in Table IV.

The structures of all 16 protected precursors (Table IV) are as follows: (I) [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2; (II) [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Tyr(Me)-Phe-Val-Asn-Cys(Bzl)-Δ³-Pro-Arg(Tos)-Gly-NH_2; (III) [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2; (IV) [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-Cit-Gly-NH_2; (V) [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2; (VI) [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2; (VII) [β-(benzylthio)-β,β-dimethylpropionyl]-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2; (VIII) [β-(benzylthio)-β,β-dimethylpropionyl]-Tyr(Me)-Ile-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2; (IX) [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Tyr(Me)-Ile-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2; (X) [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Phe-Ile-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2; (XI) [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Thr-Asn-Cys(Bzl)-Pro-Orn(Tos)-Gly-NH_2; (XII) [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Thr-Asn-Cys(Bzl)-Pro-Orn(Tos)-Ala-NH_2; (XIII) [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Thr-Asn-Cys(Bzl)-Pro-Orn(Tos)-Leu-NH_2; (XIV) [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Thr-Asn-Cys(Bzl)-Pro-Orn(Tos)-Arg(Tos)-NH_2; (XV) [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Thr-Asn-Cys(Bzl)-Pro-Orn(Tos)-OH; (XVI) [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Pro-Orn(Tos)-NH_2.

[1-(β-Mercapto-β,β-pentamethylenepropionic acid),2-O-ethyl-D-tyrosine,4-valine,8-citrulline]vasopressin (d-$(CH_2)_5$[D-Tyr(Et)^2,Val^4,Cit^8]VP) (4, Table V). A solution of the protected acyloctapeptide amide (IV, Table IV), (170 mg, 0.13 mmol) in sodium-dried and redistilled ammonia (ca. 500 mL) was treated at the boiling point and with stirring with sodium[33] from a stick of the metal contained in a small-bore glass tube until a light-blue color persisted in the solution for 30 s. Dry acetic acid was added to discharge the color. The ammonia was evaporated, and nitrogen was passed through the flask. After 5 min, the residue was dissolved in degassed aqueous acetic acid (50%, 80 mL) and quickly poured into ice-cold water (ca. 1000 mL). The pH was adjusted to approximately 7 with concentrated ammonium hydroxide. Following neutralization, an excess of a solution of potassium ferricyanide (0.01 M, 12.5 mL) was added gradually with stirring. The yellow solution was stirred for an additional 20 min and for 10 min with anion-exchange resin (Bio-Rad AG-3, Cl^- form, 30 g damp weight). The suspension was slowly filtered through a bed of resin (30 g damp weight). The bed was washed with 0.2 M AcOH (3 × 100 mL) and the combined filtrate and washings were lyophilized. The resulting powder (2.3 g) was desalted on a Sephadex G-15 column (110 × 2.7 cm), eluting with aqueous acetic acid (50%)[35] with a flow rate of 5 mL/h. The eluate was fractioned and monitored for absorbance at 254 nm. The fractions comprising the major peak were checked by TLC (a), pooled, and lyophilized, and the residue (51 mg) was further subjected to gel filtration on a Sephadex G-15 column (100 × 1.5 cm), eluting with aqueous acetic acid (0.2 M)[35] with a flow rate of 4 mL/h. The peptide was eluted in a single peak (absorbance at 254 nm). Lyophilization of the pertinent fractions gave the vasopressin analogue 4. With minor modifications this procedure was utilized to give all the remaining free peptides. The physicochemical properties of all 16 free peptides are given in Table V.

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Registry No. 1, 98375-38-3; 2, 117710-38-0; 3, 92954-82-0; 4, 92954-83-1; 5, 117710-39-1; 6, 117710-40-4; 7, 117710-41-5; 8, 117734-02-8; 9, 117734-03-9; 10, 117734-04-0; 11, 117710-42-6; 12, 117710-43-7; 13, 117710-44-8; 14, 117710-45-9; 15, 115499-13-3; 16, 117734-05-1; I, 117710-46-0; II, 117710-47-1; III, 117710-48-2; IV, 117710-49-3; V, 117710-50-6; VI, 117710-51-7; VII, 117710-52-8; VIII, 117710-53-9; IX, 117710-54-0; X, 117710-55-1; XI, 117710-56-2; XII, 117710-57-3; XIII, 117710-58-4; XIV, 117710-59-5; XV,

114025-21-7; XVI, 117710-60-8; BOC-Cit-ONp, 56612-88-5; BOC-Pro-OH, 15761-39-4; BOC-Cys(Bzl)-OH, 5068-28-0; BOC-Asn-ONp, 4587-33-1; BOC-Val-OH, 13734-41-3; BOC-Phe-OH, 13734-34-4; BOC-D-Tyr(Et)-OH, 76757-92-1; 1-($CH_2COOC_6H_4$-p-NO_2)-1-(SCH_2Ph)-c-C_6H_{10}, 55154-81-9; BOC-Arg(Tos)-OH, 13836-37-8; BOC-Gln-ONp, 15387-45-8; BOC-Ile-OH, 13139-16-7; BOC-Tyr(Me)-OH, 53267-93-9; BOC-Tyr(Me)-Ile-Gln-Asn-Cys-(Bzl)-Pro-Arg(Tos)-Gly-NH_2, 117710-62-0; H-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2·TFA, 117710-65-3; H-Leu-NH_2·HCl, 10466-61-2; BOC-Tyr(Me)-Ile-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, 117710-66-4; $PhCH_2SC(CH_3)_2CH_2COOC_6H_4$-p-$NO_2$, 7536-40-5; oxytocin, 50-56-6.

Structure-Activity Relationships of Novel Vasopressin Antagonists Containing C-Terminal Diaminoalkanes and (Aminoalkyl)guanidines[1]

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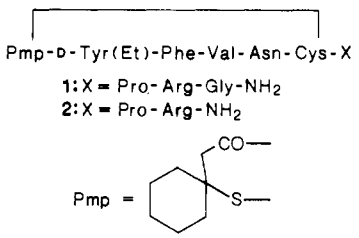
We report the synthesis and biological activity of a series of analogues of the vasopressin antagonists [Pmp[1],D-Tyr(Et)[2],Val[4]]arginine-vasopressin (1) and [Pmp[1],D-Tyr(Et)[2],Val[4],desGly[9]]arginine-vasopressin (2), where part or all of the tripeptide tail has been replaced by a simple alkyldiamine [$NH(CH_2)_nNH_2$] or (aminoalkyl)guanidine [$NH(CH_2)_nNHC(=NH)NH_2$] in order to examine the effects that variation of the length and orientation of the tripeptide tail have on renal vasopressin (V_2) receptor antagonist activity. The results show that the entire tripeptide tail (Pro-Arg-Gly-NH_2) can be replaced by an alkyldiamine or an (aminoalkyl)guanidine, compounds **15** and **16**, respectively, indicating that there is no orientational requirement for the basic functional group coming off the cyclic hexapeptide ring. Also, there seems to be an "optimal" distance between the basic functional group and the hexapeptide ring since receptor affinity of the antagonists begins to fall off when the basic functional group is too close (compound **13**) or extends too far (compounds **8-10**) from the hexapeptide ring. These results suggest all that is necessary for retention of antagonist affinity and potency is a basic functional group, amine or guanidine, extended an optimal distance from the hexapeptide ring.

In a preliminary communication,[1] we outlined the effects of removing parts of the tripeptide tail of the potent vasopressin antagonists [Pmp[1],D-Tyr(Et)[2],Val[4]]arginine-vasopressin (**1**)[2,3] and [Pmp[1],D-Tyr(Et)[2],Val[4],desGly[9]]arginine-vasopressin (**2**)[4,5] and replacing them with simple diaminoalkanes. In this paper we examine in greater



detail the effects that variation of the length and orientation of the tripeptide tail have on renal vasopressin (V_2) receptor antagonist activity.

When this study was initiated, little was known concerning the contribution of the tripeptide tail (Pro-Arg-Gly-NH_2) to the renal vasopressin (V_2) antagonist pharmacophore. The model proposed by Walter et al.[6] for the V_2 agonist pharmacophore emphasized the important role of the complete tripeptide tail in the binding of AVP to the renal V_2 receptor. The full tripeptide tail was also a common feature in many of the early V_2 antagonists.[3] However, more recent studies have shown that the tripeptide tail of V_2 antagonists can be significantly modified without affecting receptor affinity or antagonist potency.[4,5,7,8] Indeed, it has been shown that the C-terminal glycine can be deleted[4,5] and the proline at position 7 can be freely substituted for[7] or even deleted[8] all with retention

Scheme I. Synthesis of Mono-Boc-diamines[a]

path A

$H_2N(CH_2)_nNH_2 \xrightarrow{(a)}$ Boc-$NH(CH_2)_nNH_2$

path B

$H_2NCH_2CH_2OH \xrightarrow{b}$ Boc-$NHCH_2CH_2OH \xrightarrow{c}$

Boc-$NHCH_2CH_2N_3 \xrightarrow{d}$ Boc-$NHCH_2CH_2NH_2$

[a] (a) $(Boc)_2O$, CH_2Cl_2; (b) $(Boc)_2O$, Et_3N, CH_2Cl_2; (c) Ph_3P, DEAD, HN_3, THF/toluene; (d) Ra-Ni, $H_2NNH_2·H_2O$, EtOH.

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(1) For a preliminary communication of this work, see: Huffman, W. F.; Ali, F. A.; Bryan, W. M.; Callahan, J. F.; Moore, M. L.; Silvestri, J. S.; Yim, N. C. F.; Kinter, L. B.; McDonald, J. E.; Ashton-Shue, D.; Stassen, F. L.; Heckman, G. D.; Schmidt, D. B.; Sulat, L. *J. Med. Chem.* **1985**, *28*, 1759.

(2) Abbreviations of amino acids follow the recommendations of the IUPAC-IUB Commision on Biochemical Nomenclature: *Eur. J. Biochem.* **1984**, *158*, 9. All optically active amino acids are assumed to be L unless otherwise specified. The following additional abbreviations are used: Pmp, β-thio-β,β-pentamethylenepropionic acid; AVP, [Arg[8]]vasopressin; LVP, [Lys[8]]vasopressin.

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