$$\text{rate} = k_1k_3[\text{T}][\text{FAGLA}]/\left[(k_2 + k_3)\left(1 + \frac{[\beta\text{PPP}_\text{I}] + [\beta\text{PPP}_\text{II}] + [\beta\text{PPP}_\text{III}]}{K_\text{I}} + k_1[\text{FAGLA}]\right)\right] \quad (1)$$

literature:[27] $k_3 = 7.25 \times 10^2\ s^{-1}$ and $K_m = 2.5 \times 10^{-3}$ M. Since k_1 is probably on the order of $10^8\ M^{-1}\ s^{-1}$,[28] k_2 is approximately $2.5 \times 10^5\ s^{-1}$. Assuming $k_5 = 10^8\ M^{-1}\ s^{-1}$ and using $K_I = 1.6 \times 10^{-3}$ M[29] gives a value for k_4 of 1.6×10^5. These estimated rate constants can be converted into activation energy barriers using the equation $k = Ae^{E_a/RT}$, where A has the assumed value of 10^{13}. Figure 3 schematically relates the energies of the reactants and products based on these energy barriers. For this drawing, FAGLA and β-PPP_II were assumed to have the same energy. While the rates of interconversion of the conformers of β-phenylpropionyl-L-phenylalanine are not known, they are fast on the NMR time scale, and the barrier for interconversion was assumed to be approximately 4 kcal/mol.

Both the numerical values of the rate constants and this diagram clearly show that if the rate constant of equilibration of $\beta\text{PPP}_\text{A} \rightleftarrows \beta\text{PPP}_\text{B} \rightleftarrows \beta\text{PPP}_\text{C}$ is greater than 100 times the rate of conversion of T–FAGLA to product, then relative proportions of the conformers of the inhibitor will not influence its K_I. The K_I clearly depends only on the relative energies of the inhibitor–thermolysin and substrate–thermolysin complex. Only if the barrier to interconversion of the conformers of the inhibitor approaches that for the conversion of the enzyme–substrate–complex to product would the proportions of conformer be expected to influence the K_I. For many drugs this unusual condition implies restricted rotation and would clearly not apply.

Conclusion

These results show that the lowest energy solution conformer as determined by NMR spectroscopy is not always the conformer bound by an enzyme as determined by X-ray crystallography. In particular, thermolysin binds the lowest energy solution conformer of the inhibitor carbobenzoxy-L-phenylalanine but the highest energy solution conformer of β-phenylpropionyl-L-phenylalanine.

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Design of Potent and Selective Antagonists of the Vasopressor Responses to Arginine-vasopressin

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We have synthesized eight new 1-β,β-dialkyl-substituted analogues of [1-deamino]arginine-vasopressin (dAVP) to determine some of the structural features that account for antivasopressor potency and that improve selectivity by reducing antidiuretic agonistic activity. These analogues are as follows: **1**, [1-(β-mercapto-β,β-diethylpropionic acid)]arginine-vasopressin (dEt_2AVP); **2**, [1-(β-mercapto-β,β-diethylpropionic acid),4-valine,8-D-arginine]vasopressin (dEt_2VDAVP); **3**, [1-deaminopenicillamine,4-valine]arginine-vasopressin (dPVAVP); **4**, [1-(β-mercapto-β,β-diethylpropionic acid),4-valine]vasopressin (dEt_2VAVP); **5**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin [$d(CH_2)_5$VAVP]; **6**, [1-deaminopenicillamine,8-D-arginine]vasopressin (dPDAVP); **7**, [1-(β-mercapto-β,β-diethylpropionic acid),8-D-arginine]vasopressin (dEt_2DAVP); **8**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),8-D-arginine]vasopressin [$d(CH_2)_5$DAVP]. The protected precursors required for these analgoues were synthesized by a combination of solid phase and 8 + 1 couplings in solutions. These analogues were tested for agonistic and antagonistic activities in rat vasopressor, rat antidiuretic, and rat uterus assay systems. They exhibit no evident pressor activities. They are all highly effective antagonists of the vasopressor responses to AVP. They exhibit the following antivasopressor pA_2 values: **1**, 8.36 ± 0.07; **2**, 8.18 ± 0.06; **3**, 7.92 ± 0.07; **4**, 8.29 ± 0.08; **5**, 7.97 ± 0.06; **6**, 7.45 ± 0.08; **7**, 7.96 ± 0.08; **8**, 8.52 ± 0.03. These findings clearly indicate that in the four series of β,β-dialkyl-substituted analogues now completed, either the β,β-diethyl or the β,β-cyclopentamethylene substitution is far more effective than the β,β-dimethyl grouping in leading to enhanced antivasopressor potency. The antidiuretic activities of these analogues are also dramatically less than in analogues containing a β,β-dimethyl substituent. With antidiuretic activities of only 0.2, 0.06, and 0.3 unit/mg and relatively weak antioxytocic activities, analogues **5**, **7**, and **8** are among the most potent and selective vasopressor antagonists reported to date. These new analogues hold promise as additional tools for studies on the physiological roles of AVP.

We previously reported the synthesis and some pharmacological properties of a number of potent antagonists of vasopressor responses to arginine-vasopressin (AVP).[1-5] Their properties are summarized in Sawyer et al.[6] These antagonists are proving to be valuable tools in a variety of studies on the putative physiological roles of AVP. The

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findings from 24 such studies have been reported to date.[7-9]

Four of these antagonists, namely, [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin (dPVDAVP),[1] [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin [d$(CH_2)_5$VDAVP],[2] [1-deaminopenicillamine]arginine-vasopressin (dPAVP),[3] and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin [d$(CH_2)_5$AVP][4] were designed by incorporating β,β-dimethyl and β,β-cyclopentamethylene substituents on the β carbon at position 1 in two highly potent antidiuretic agonists, [1-deamino,4-valine,8-D-arginine]vasopressin (dVDAVP)[10] and [1-deamino]arginine-vasopressin (dAVP).[11] These dialkyl substituents, i.e., the β,β-dimethyl[12a] and the β,β-cyclopentamethylene,[12c] together with the intermediate β,β-diethyl[12b] substitution, were originally utilized in du Vigneaud's laboratory to produce promising antagonists of oxytocin[12] and of lysine-vasopressin.[13]

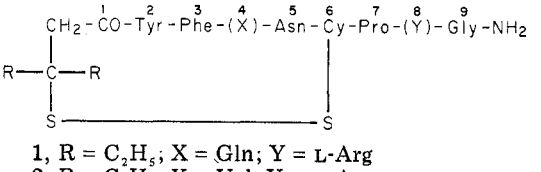
The antivasopressor potencies of these antagonists were measured by the method of Dyckes et al.[13a] and expressed as pA_2 values. pA_2 values as defined by Schild[14] represent the negative logarithms to the base 10 of the average molar concentrations of antagonist that will reduce the specific biological response to $2x$ units of agonist to equal the response to $1x$ units of agonist. The aformentioned four antagonists exhibited a curious pattern of antagonistic potencies.[4,6]. Thus, dPVDAVP and d$(CH_2)_5$VDAVP had pA_2 values of 7.82 and 7.68, respectively. Thus, in this pair it appeared that β,β-dimethyl and β,β-cyclopentamethylene substitutions were about equal in their influence on antagonistic potency. By contrast, the pA_2 values for dPAVP and d$(CH_2)_5$AVP were 7.45[3] and 8.35,[4] respectively—a dramatic difference from the earlier pattern. Thus, in this pair the β,β-cyclopentamethylene substitution produced an eightfold more potent antagonist than did the β,β-dimethyl substitution. Also comparison of the antagonistic potencies of the β,β-dimethyl and β,β-cyclopentamethylene analogue pairs points to another curious anomaly. Whereas in the β,β-dimethyl pair dPVDAVP is more potent than dPAVP, the opposite is true for the β,β-cyclopentamethylene pair; d$(CH_2)_5$AVP is much more potent than d$(CH_2)_5$VDAVP.

In seeking to clarify these apparent contradictions and in attempting to design more potent and/or more selective vasopressor antagonists, we have followed two interrelated approaches, which are the subject of this present report.

1. To determine the effects on antagonistic potencies and selectivities of the intermediate-sized β,β-diethyl[12b,13] substitution in dAVP and in dVDAVP relative to those of the aformentioned β,β-dimethyl and β,β-cyclopentamethylene substitutions.

2. To evaluate the individual contributions of the 4-valine and 8-D-arginine substitutions in the three β,β-dialkyl analogues of dVDAVP by synthesizing and pharmacologically evaluating two monosubstituted series of β,β-dimethyl, β,β-diethyl, and β,β-cyclopentamethylene dAVP analogues containing, respectively, valine and D-arginine substitutions at positions 4 and 8.

The eight peptides designed in this fashion are as follows: **1**, [1-(β-mercapto-β,β-diethylpropionic acid)]arginine-vasopressin (dEt$_2$AVP); **2**, [1-(β-mercapto-β,β-diethylpropionic acid),4-valine,8-D-arginine]vasopressin (dEt$_2$VDAVP); **3**, [1-deaminopenicillamine,4-valine]arginine-vasopressin (dPVAVP); **4**, [1-(β-mercapto-β,β-diethylpropionic acid),4-valine]arginine-vasopressin (dEt$_2$VAVP); **5**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin [d$(CH_2)_5$VAVP]; **6**, [1-deaminopenicillamine,8-D-arginine]-vasopressin (dPDAVP); **7**, [1-(β-mercapto-β,β-diethylpropionic acid),8-D-arginine]vasopressin (dEt$_2$DAVP); **8**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),8-D-arginine]vasopressin [d$(CH_2)_5$DAVP]. These analogues have the following general structures:



1, R = C_2H_5; X = Gln; Y = L-Arg
2, R = C_2H_5; X = Val; Y = D-Arg
3-5 R = CH_3, C_2H_5, $(CH_2)_5$; X = Val; Y = L-Arg
6-8, R = CH_3, C_2H_5, $(CH_2)_5$; X = Gln; Y = D-Arg

We now present the synthesis and some pharmacological properties of these eight analogues—all of which have been found to antagonize effectively pressor responses in rats to AVP.

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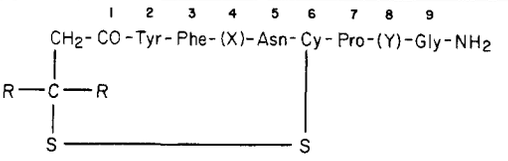
Table I. Antivasopressor and Antidiuretic Potencies of Eight New Antagonists of Vasopressor Responses to Arginine-vasopressin (AVP)



no.	antagonists[j]	R_2	X	Y	antivasopressor[c] effective dose,[a] nmol/kg	antivasopressor[c] pA_2[b]	antidiuretic act.,[c] units/mg
	dPAVP[e]	$(CH_3)_2$	Gln	L-Arg	3.2 ± 1.2 (8)	7.45 ± 0.11[d]	42 ± 3 (7)
1	dEt_2AVP	$(C_2H_5)_2$	Gln	L-Arg	0.32 ± 0.10 (10)	8.36 ± 0.07	0.38 ± 0.07 (3)
	$d(CH_2)_5AVP$[f]	$(CH_2)_5$	Gln	L-Arg	0.34 ± 0.07 (6)	8.35 ± 0.09	0.033 ± 0.005 (8)
	dPVDAVP[g]	$(CH_3)_2$	Val	D-Arg	1.1 ± 0.3 (9)	7.82 ± 0.05	123 ± 22 (7)
2	dEt_2VDAVP	$(C_2H_5)_2$	Val	D-Arg	0.35 ± 0.10 (7)	8.18 ± 0.06	0.71 ± 0.07 (4)
	$d(CH_2)_5VDAVP$[h]	$(CH_2)_5$	Val	D-Arg	1.5 ± 0.2 (11)	7.68 ± 0.05	0.10 ± 0.02 (5)
3	dPVAVP	$(CH_3)_2$	Val	L-Arg	0.84 ± 0.13 (4)	7.92 ± 0.07	312 ± 23 (4)
4	dEt_2VAVP	$(C_2H_5)_2$	Val	L-Arg	0.38 ± 0.09 (6)	8.29 ± 0.08	1.50 ± 0.01 (4)
5	$d(CH_2)_5VAVP$	$(CH_2)_5$	Val	L-Arg	0.76 ± 0.11 (8)	7.97 ± 0.06	0.32 ± 0.02 (4)
6	dPDAVP	$(CH_3)_2$	Gln	D-Arg	2.5 ± 0.5 (4)	7.45 ± 0.08	5.8 ± 0.7 (6)
7	dEt_2DAVP	$(C_2H_5)_2$	Gln	D-Arg	0.81 ± 0.15 (7)	7.96 ± 0.08	0.067 ± 0.004 (4)
8	$d(CH_2)_5DAVP$	$(CH_2)_5$	Gln	D-Arg	0.20 ± 0.02 (4)	8.52 ± 0.03	0.31 ± 0.01 (4)

[a] The effective dose is defined as the dose (in nanomoles per kilogram) that reduces the response to $2x$ units of agonist to equal the response to $1x$ units of agonist. [b] Estimated in vivo pA_2 values represent the negative logarithms of the "effective dose" dose divided by the estimated volume of distribution (67 mL/kg). [c] Means ± SE; number of assay groups in parentheses. [d] Mixed agonist/antagonist. [e] From Bankowski et al.[3] [f] From Kruszynski et al.[4] [g] From Manning et al.[1] [h] From Lowbridge et al.[2] The abbreviations and their full names are as follows: dPAVP, [1-deaminopenicillamine]-arginine-vasopressin; dEt_2AVP, [1-(β-mercapto-β,β-diethylpropionic acid)]arginine-vasopressin; $d(CH_2)_5AVP$, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin; dPVDAVP, [1-deaminopenicillamine,4-valine,8-D-arginine]-vasopressin; dEt_2VDAVP, [1-(β-mercapto-β,β-diethylpropionic acid),4-valine,8-D-arginine]vasopressin; $d(CH_2)_5VDAVP$, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin; dPVAVP, [1-deaminopenicillamine,4-valine]arginine-vasopressin; dEt_2VAVP, [1-(β-mercapto-β,β-diethylpropionic acid),4-valine]arginine-vasopressin; $d(CH_2)_5VAVP$, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin; dPDAVP, [1-deaminopenicillamine,8-D-arginine]vasopressin; dEt_2DAVP, [1-(β-mercapto-β,β-diethylpropionic acid),8-D-arginine]vasopressin; $d(CH_2)_5DAVP$, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),8-D-arginine]vasopressin.

Peptide Synthesis. The protected peptide precursors required for the synthesis of all eight peptide antagonists were prepared by a combination of solid-phase peptide synthesis[15,16] and by 8 + 1 active ester[17] and dicyclohexylcarbodiimide (DCCI)[18a] couplings in solution, both facilitated by *N*-hydroxybenzotriazole (HOBT).[18b] Four protected octapeptides were synthesized on the resin. Three of these were deprotected with HCl/acetic acid to give, following ammonolysis,[19a] I, Tyr(Bzl)Phe-Gln-Asn-Cys(Bzl)Pro-Arg(Tos)-Gly-NH_2;[19c] II, Tyr(Bzl)Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2; III, Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2.[19c] The fourth protected octapeptide resin on ammonlysis yielded IV, Boc-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2,[19c] which was deprotected with trifluoroacetic acid (F_3AcOH) immediately prior to coupling. Aliquots of the partially protected octapeptides I–III and that obtained from IV were coupled, respectively, with β-(*S*-benzylmercapto)-β,β-diethylpropionic acid[12b] by the DCCI–HOBT preactivation method[18b] to give the immediate protected precursors of dEt_2AVP, dEt_2VAVP, dEt_2DAVP, and dEt_2VDAVP. Separate aliquots of the partially protected peptides II and III were coupled with *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-dimethylpropionate[12a] and *p*-nitrophenyl β-(*S*-benzylmercapto)-β,β-cyclopentamethylenepropionate[12c] to give, respectively, the immediate protected precursors of dPVAVP, dPDAVP, $d(CH_2)_5VAVP$, and $d(CH_2)_5DAVP$. Each protected precursor was deblocked with Na in NH_3[20] as previously described,[10a,19] and the resulting disulfhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$.[21] The analogues were desalted and purified by gel filtration[22] on Sephadex G-15.[23]

Bioassay Methods. The agonistic and antagonistic properties of these analogues were measured by previously described methods.[1-5,24] These included intravenous antidiuretic assays in rats under ethanol anesthesia, vasopressor assays in phenoxybenzamine-treated rats under urethane anesthesia, and assays on the rat uterus in vitro in a medium containing (a) no Mg^{2+} and (b) 0.5 mM Mg^{2+}. The USP posterior pituitary reference standard was used in all assays for agonistic and antagonistic activities.

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Table II. Antioxytocic Potencies of Analogues 1-8

no.	antagonist	antioxytocic (in vitro) pA_2 [a] no Mg^{2+}	antioxytocic (in vitro) pA_2 [a] 0.5 mM Mg^{2+}
	dPAVP [b]	6.93 ± 0.10 (14)	5.93 ± 0.13 [f] (16)
1	dEt_2AVP	7.30 ± 0.07 (4)	7.16 ± 0.09 (8)
	$d(CH_2)_5AVP$ [c]	7.32 ± 0.09 (5)	7.27 ± 0.10 (12)
	dPVDAVP [d]	7.23 ± 0.04 (13)	7.12 ± 0.15 (9)
2	dEt_2VDAVP	7.29 ± 0.11 (6)	7.21 ± 0.04 (9)
	$d(CH_2)_5VDAVP$ [e]	6.63 ± 0.05 (8)	6.23 ± 0.11 (9)
3	dPVAVP	8.09 ± 0.08 (4)	7.72 ± 0.10 (6)
4	dEt_2VAVP	7.32 ± 0.11 (6)	7.66 ± 0.11 (10)
5	$d(CH_2)_5VAVP$	7.34 ± 0.07 (4)	7.31 ± 0.03 (10)
6	dPDAVP	7.87 ± 0.15 (6)	6.71 ± 0.18 (17)
7	dEt_2DAVP	6.95 ± 0.05 (4)	6.70 ± 0.05 (8)
8	$d(CH_2)_5DAVP$	6.97 ± 0.10 (4)	6.59 ± 0.10 (9)

[a] In vitro pA_2 values were calculated as described in Bankowski et al.[24b] Means ± SE; number of assay groups in parentheses. [b] From Bankowski et al.[3] [c] From Kruszynski et al.[4] [d] From Manning et al.[1] [e] From Lowbridge et al.[2] [f] Mixed agonist/antagonist.

Agonistic activities are expressed in units per milligram. In vivo antagonistic activities were determined and expressed as "effective doses" and estimated pA_2 values. The "effective dose" is defined as the dose in nanomoles per kilogram that reduces the response to $2x$ units of agonist to equal the response to $1x$ units of agonist. Estimated in vivo "pA_2" values represent the negative logarithms of the effective doses divided by the estimated volume of distribution (67 mL/kg).

Results and Discussion

The antivasopressor and antidiuretic potencies of the eight new analogues, together with those of related compounds, are presented in Table I. Their antioxytocic (in vitro) potencies are given in Table II. None of the eight analogues exhibit any pressor activity. All are effective antagonists of the vasopressor responses to AVP. They possess antidiuretic activities of widely differing potencies.

Relative Effects of the β,β-Dialkyl Substitutions on Vasopressor Antagonism. No clearly consistent pattern of increasing or decreasing potencies with increasing size of the β,β-dialkyl substituents has emerged. Thus, the trend in each of the four series, i.e., in the dAVP, dVDAVP, dVAVP, and dDAVP series, is different. (a) In the dAVP series, the β,β-diethyl and the β,β-cyclopentamethylene substitutions resulted in equipotent analogues which are eight to nine times more potent than the β,β-dimethyl-containing analogue. (b) In the dVDAVP series, the β,β-diethyl substitution resulted in the most potent analogue; thus, dEt_2VDAVP exhibited a twofold enhancement in antivasopressor potency over that exhibited by dPVDAP. In this series, the order of increasing effectiveness is β,β-cyclopentamethylene, β,β-dimethyl, β,β-diethyl. (c) In the dVAVP series, the β,β-diethyl substitution again resulted in the most potent analogue; thus, dEt_2VAVP is about twice as potent as the other two β,β-dialkyl compounds. (d) In the dDAVP series, there appears to be a clear correlation between increasing size of the β,β-dialkyl group and antivasopressor potency. Thus, with an antivasopressor pA_2 of 8.52, $d(CH_2)_5DAVP$ is clearly the most potent member of this series. It is also the most potent of all the eight new antagonists and is, in fact, one of the most potent vasopressor antagonists reported to date.

Effects of the Individual Val[4] and D-Arg[8] Substitutions on Vasopressor Antagonism. These substitutions also had inconsistent effects on antivasopressor potencies. Thus, the substitution of valine for glutamine in dPAVP affected a threefold enhancement in antivasopressor potency. In dEt_2AVP, this substitution caused virtually no change in potency, whereas in $d(CH_2)_5AVP$ it led to a 50% reduction in potency. The substitution of D-arginine for L-arginine in dPAVP and in dEt_2AVP affected, respectively, no change and a 50% reduction. In $d(CH_2)_5AVP$, this substitution brought about a modest increase in antivasopressor potency.

Effects of the Combined Val[4] and D-Arg[8] Substitutions on Vasopressor Antagonism. In comparing the potencies of the three β,β-dialkyl analogues in the dVDAVP series with those of the corresponding members of the dVAVP or dDAVP series, it is clear that combining the Val[4] and D-Arg[8] modifications in a single molecule has had inconsistent effects on antivasopressor potentices. In the case of the β,β-cyclopentamethylene-containing analogues, the combination of the Val[4] and the D-Arg[8] substitutions has led to marked reduction in antivasopressor potencies relative to $d(CH_2)_5DAVP$. In the β,β-diethyl series, this combination had little influence on potency.

Relative Effects of Arg[8] vs. Lys[8] on Vasopressor Antagonism. A comparison of the antivasopressor potency of [1-(β-mercapto-β,β-diethylpropionic acid)lysine-vasopressin (dEt_2LVP, pA_2 = 7.15)[13a] with that of dEt_2AVP (pA_2 = 8.36) reported here clearly shows that in this β,β-diethyl-containing analogue pair, Arg[8] is far superior to Lys[8] in leading to enhanced antivasopressor potency. It remains to be seen whether this obtains also for the β,β-dimethyl and/or the β,β-cyclopentamethylene containing analogues reported here.

Relative Effects of β,β-Dialkyl Substitutions on Antivasopressor/Antidiuretic Selectivity. Comparisons of the antidiuretic activities of the analogues in each of the four series of β,β-dialkyl-substituted analogues in Table I reveals a very consistent pattern of drastically reduced antidiuretic activities in analogues with larger β,β-dialkyl substituents. In three of four series, the least potent analogue is that containing a β,β-cyclopentamethylene grouping. Thus, $d(CH_2)_5VAVP$ and $d(CH_2)_5DAVP$ join $d(CH_2)_5AVP$[4] and $d(CH_2)_5VDAVP$[2] in being among the most selective vasopressor antagonists reported to date. The β,β-dimethyl-containing analogues exhibit an interesting spectrum of antidiuretic potencies, ranging from a high of 312 units/mg for dPVAVP to a low of 5.8 units/mg for dPDAVP.

All of the new analogues effectively antagonize oxytocic responses by rat uteri in vitro (Table II). In previously reported oxytocin analogues, increasing the size of the β,β-dialkyl substituent from dimethyl to diethyl to cyclopentamethylene results in a progressive enhancement of antagonistic potency.[6] In these vasopressin analogues, however, no such relationship is apparent. In fact, in all but the dPAVP series, the β,β-cyclopentamethylene analogue is the least potent in assays done in the presence of Mg^{2+}.

Conclusion

The eight new analogues reported here are all highly potent antagonists of vasopressor responses to AVP. Comparisons of their antivasopressor potencies clearly indicates that these do not follow a consistent pattern based on the relative sizes of the β,β-dimethyl, β,β-diethyl, and β,β-cyclopentamethylene substitutions. Early studies from du Vigneaud's laboratory had shown that the antivasopressor potencies of a series of β,β-dialkyl-substituted oxytocin analogues decreasing with increasing size of the β,β-dialkyl groups.[12c] Our previous findings were inconclusive on this point.[1-4] One partial series [dPVDAVP,[1] $d(CH_2)_5VDAVP$[2]] followed this pattern, the other [dPAVP,[3] $d(CH_2)_5AVP$[4]] clearly did not. Our present

findings show clearly that the relative effects of these substituents is entirely dependent on the nature of the other substituents in the dAVP molecule. Nonetheless, it is clear from the four completed series of β,β-dialkyl antagonists reported here, i.e., dAVP, dVDAVP, dVAVP, and dDAVP, that in any given series either the β,β-diethyl or the β,β-cyclopentamethylene grouping is more effective than the β,β-dimethyl grouping in leading to enhanced antivasopressor potency.

A number of the new analogues reported here, namely, dEt_2AVP (pA_2 = 8.36), dEt_2VAVP (pA_2 = 8.29), and d-$(CH_2)_5DAVP$ (pA_2 = 8.52) are among the most potent vasopressor antagonists reported to date. In addition, their low antidiuretic activities give them attractive antivasopressor/antidiuretic selectivity. With these properties, these peptides promise to be valuable tools for future studies on the physiological roles of AVP.

Experimental Section

The procedure of "solid phase" synthesis followed that previously published.[15,16,19] Chloromethylated resin (Bio-Rad Bio-Beads SX-1) was esterified[25] with Boc-Gly to an incorporation of 0.56 mmol/g. Amino acid derivatives were supplied by Bachem Inc. β-(S-Benzylmercapto)-β,β-diethylpropionic acid[12b] was generously supplied by Dr. Martha Ferger, Cornell University. p-Nitrophenyl β-(S-benzylmercapto)-β,β-dimethylpropionate[12a] and p-nitrophenyl β-(S-benzylmercapto)-β,β-cyclopentamethylene propionate[12c] were synthesized. Dimethylformamide (DMF) was distilled under reduced pressure immediately prior to its use. Other solvents and reagents were analytical grade. Thin-layer chromatography (TLC) was on silica gel (0.25 mm, Brinkman Silplate). The following solvent systems were used: A, 1-butanol–acetic acid–water (4:1:5, v/v, upper phase); B, chloroform–methanol (7:3, v/v); C, 1-butanol–acetic acid–water–pyridine (15:3:3:10, v/v); D, chloroform–methanol–acetic acid (9:1:1, v:v). Loads of 10–50 μg were applied, and chromatograms were minimum length 10 cm. Iodine vapor was used for detection. For amino acid analysis,[26] peptides (~0.8 mg) were hydrolyzed with constant-boiling hydrochloric acid (500 μL) containing phenol (10 μL) in evacuated and sealed ampules for 18 h at 110 °C. The analyses were performed using a Beckman automatic amino acid analyzer Model 121. Molar ratios were referred to Gly = 100. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. The analytical results for elements indicated by their symbols are within ±0.4% of theoretical values. The yields of the free peptides are based on the weights of the lyophilized powders and are uncorrected for water and acetic acid content. Optical rotations were measured with a Rudolph polarimeter Model 80.

Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (I). Boc-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin[10b] (4.47 g, 1.6 mmol) was treated with HCl/AcOH (c 1 M), washed with AcOH, EtOH, and MeOH, and dried to give the octapeptide resin hydrochloride (4.34 g, 1.6 mmol). This was ammonolyzed,[19a] and the cleaved product was extracted with warm DMF. The crude product was precipitated by addition of water and reprecipitated from DMF–methanol–ether to give the purified product: yield 1.75 g (84.9%); 211–215 °C; $[\alpha]^{23}_D$ −30.3° (c 1.5, DMF); TLC R_f (A) 0.42; R_f (B) 0.64. Anal. ($C_{64}H_{81}N_{13}O_{12}S_2 \cdot 2H_2O$) C, H, N. Amino acid analysis:[26] Tyr, 0.98; Phe, 1.02; Val, 1.02; Asp, 1.01; Cys(Bzl), 0.97; Pro, 1.03; Arg, 0.98; Gly, 1.00; NH_3, 1.96.

Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (II). The *tert*-butyloxycarbonyl octapeptide resin[19c] (1.40 g, 0.5 mmol) was deprotected in the manner detailed above for I to give the octapeptide resin hydrochloride (1.37 g, 0.5 mmol), which was ammonolyzed and purified as for I to give the octapeptide amide II: yield 430 mg (65.4%); mp 173–172 °C; $[\alpha]^{23}_D$ −34.3° (c 1, DMF); TLC R_f (A) 0.3, R_f (B) 0.62. Anal. ($C_{64}H_{80}N_{14}O_{13}S_2 \cdot 2H_2O$) C, H, N. Amino acid analysis: Tyr, 1.02; Phe, 0.99; Glu, 1.02; Asp, 1.01; Cys(Bzl), 0.98; Pro, 1.03; Arg, 1.00; Gly, 1.00; NH_3, 3.11.

Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (III). The *tert*-butyloxycarbonyl octapeptide resin[19c] (4.20 g, 1.5 mmol) was deprotected as above to give the octapeptide resin hydrochloride (4.11 g, 1.5 mmol), which was ammonolyzed; the product was then worked up as for I to give III: yield 1.72 g (87.3%); mp 161–162 °C; $[\alpha]^{22}_D$ −21.0° (c 1, DMF); TLC R_f (A) 0.32; R_f (B) 0.56. Anal. ($C_{64}H_{80}O_{13}S_2 \cdot 2H_2O$) C, H, N. Amino acid analysis: Tyr, 0.99; Phe, 0.99; Glu, 1.03, Asp, 1.02; Cys(Bzl), 0.97; Pro, 1.02; Arg, 0.98; Gly, 1.00; NH_3, 2.91.

Boc-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (IV). The *tert*-butyloxycarbonyl octapeptide resin[10a] (1.52 g, 0.5 mmol) was ammonolyzed, and the product was worked up as for I to give IV: yield 465 mg (67.0%); mp 210–211 °C; $[\alpha]^{23}_D$ −16.8° (c 1, DMF); TLC R_f (A) 0.62, R_f (C) 0.70. Anal. ($C_{69}H_{89}N_{13}O_{14}S_2 \cdot 2H_2O$) C, H, N. Amino acid analysis: Tyr, 0.98; Phe, 1.02; Val, 1.03; Asp, 1.01; Cys(Bzl), 0.98; Pro, 1.02; Arg, 1.00; Gly, 1.00; NH_3, 1.94.

β-(S-Benzylmercapto)-β,β-diethylpropionyl-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (Pr-dEt_2VAVP, V). A solution of β-(S-benzylmercapto)-β,β-diethylpropionic acid[12b] (201.7 mg, 0.8 mmol) and N-hydroxybenzotriazole monohydrate (HOBT)[18b] (183.7 mg, 1.2 mmol) in DMF (0.7 mL) was cooled in ice and treated with DCCI[18a] (172 mg, 0.83 mmol). This mixture was stirred for 15 min in ice and for 90 min at room temperature. The precipitated dicyclohexylurea was filtered off, and the filtrate was added to the solution of Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (I; 322.2 mg, 0.25 mmol) in DMF (2.5 mL). N-Methylmorphine (NMM; 75 μL) was added to give a solution of pH ~7.5 to moist pH paper.[17b] The reaction mixture was stirred for 2 days. The crude product was precipitated by the addition of ethyl acetate–ethanol (1:1) and then reprecipitated from DMF–methanol–ethyl ether to give V: yield 288 mg (75.6%); mp 218–220 °C; $[\alpha]^{23}_D$ −41.3° (c 1, DMF); TLC R_f (A) 0.72, R_f (D) 0.91. Anal. ($C_{78}H_{99}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 0.98; Phe, 1.01; Val, 1.02; Asp, 1.01; Cys(Bzl), 0.97; Pro, 1.02; Arg, 0.98; Gly, 1.00; NH_3, 2.04.

β-(S-Benzylmercapto)-β,β-diethylpropionyl-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (Pr-dEt_2AVP, VI). Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (II; 218 mg, 0.165 mmol) was coupled with β-(S-benzylmercapto)-β,β-diethylpropionic acid in the manner described for V to give the acyl octapeptide amide VI: yield 124 mg (48.4%); mp 201–203 °C; $[\alpha]^{23}_D$ −41.1° (c 1, DMF); TLC R_f (A) 0.50; R_f (B) 0.42. Anal. ($C_{78}H_{98}N_{14}O_{14}S_3$) C, H, N. Amino acid analysis: Tyr, 0.98; Phe, 1.02; Glu, 1.01; Asp, 1.02; Cys(Bzl), 0.98; Pro, 1.01; Arg, 0.99; Gly, 1.00; NH_3, 2.94.

β-(S-Benzylmercapto)-β,β-diethylpropionyl-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (Pr-dEt_2DAVP, VII). The octapeptide amide III (210 mg, 0.16 mmol) was coupled with β-(S-benzylmercapto)-β,β-diethylpropionic acid in the manner described for V to give the acyl octapeptide amide VII: yield 133 mg (53.6%); mp 194–196 °C; $[\alpha]^{23}_D$ −29.6° (c 1, DMF); TLC R_f (A) 0.54; R_f (B) 0.42. Anal. ($C_{78}H_{98}N_{14}O_{14}S_3$) C, H, N. Amino acid analysis: Tyr, 0.99; Phe, 1.01; Glu, 1.03; Asp, 1.01; Cys(Bzl), 0.97; Pro, 1.02; Arg, 0.98; Gly, 1.00; NH_3, 2.94.

β-(S-Benzylmercapto)-β,β-diethylpropionyl-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (Pr-dEt_2VDAVP, VIII).[27] Boc-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (IV; 347 mg, 0.25 mmol) was dissolved in F_3AcOH (3 mL) and stirred at room temperature for 40 min. Cold ether (30 mL) was added, and the precipitated material was filtered and washed with ether. The product was dried in vacuo over sodium hydroxide pellets. The product (317 mg, 90.4%) was dissolved in DMF (1 mL) and NMM was added to give a solution of pH ~7.5 to moist pH paper.[17b] This solution of the free

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(27) The syntheses of the protected precursor (VIII) and free peptide (XX) were first accomplished in this laboratory by Dr. John Lowbridge. The free peptide was not, however, pharmacologically evaluated. The relatively low yield of the free peptide, which resulted from difficulty in its purification, necessitated the complete resyntheses of VIII and XX reported here. The authors thank Dr. Lowbridge for these early contributions to this study.

octapeptide amide was coupled with β-(S-benzylmercapto)-β,β-diethylpropionic acid in the manner described for V to give the acyl octapeptide amide VIII:[27] yield 302 mg (79.3%); mp 204–206 °C; $[\alpha]^{22}_D$ −28.8° (c 1, DMF); TLC R_f (A) 0.66; R_f (D) 0.91. Anal. ($C_{78}H_{99}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 0.98; Phe, 1.01; Val, 1.02; Asp, 1.01; Cys(Bzl), 0.98; Pro, 1.02; Arg, 0.98; Gly, 1.00; NH_3, 2.06.

β-(S-Benzylmercapto)-β,β-dimethylpropionyl-Tyr-(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (PrdPVAVP, IX). The octapeptide amide I (387 mg, 0.3 mmol) was dissolved in DMF (1 mL). A solution of p-nitrophenyl β-(S-benzylmercapto)-β,β-dimethylpropionate[12a] (333 mg, 1.2 mmol) and N-hydroxybenzotriazole monohydrate (184 mg, 1.2 mmol) in DMF (1.5 mL) was added. NMM was added to give a solution of pH 7–8 to moist pH paper. The reaction mixture was stirred at room temperature overnight, and TLC (in system A) showed that the reaction was complete. Methanol (80 mL) and ether were added with vigorous stirring. The precipitated material was filtered, washed with a mixture of methanol–ether (8:3), and dried in vacuo. The crude product was reprecipitated from DMF–methanol–ethyl ether to give the acyl octapeptide amide IX: yield 311 mg (69.35%); mp 220–222 °C; $[\alpha]^{23}_D$ −50.7° (c 1, DMF); TLC R_f (A) 0.52; R_f (B) 0.72. Anal. ($C_{76}H_{95}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 1.01; Phe, 0.97; Val, 1.02; Asp, 0.98; Cys(Bzl), 0.96; Pro, 1.02; Arg, 0.99; Gly, 1.00; NH_3, 2.06.

β-(S-Benzylmercapto)-β,β-dimethylpropionyl-Tyr-(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2 (PrdPDAVP, X). The octapeptide amide III (395 mg, 0.3 mmol) was treated with p-nitrophenyl β-(S-benzylmercapto)-β,β-dimethylpropionate[12a] (333 mg, 1.2 mmol) and HOBT (184 mg, 1.2 mmol), and the product was isolated as described above for IX to give the acyloctapeptide amide X: yield 290 mg (63.4%): mp 197–199 °C; $[\alpha]^{23}_D$ −31.9° (c 1, DMF); TLC R_f (A) 0.51, R_f (B) 0.70. Anal. ($C_{76}H_{94}N_{14}O_{14}S_3$) C, H, N. Amino acid analysis: Tyr, 1.01; Phe, 1.02; Gln, 1.02; Asp, 1.01; Cys(Bzl), 0.98; Pro, 1.02; Arg, 0.99; Gly, 1.00; NH_3, 2.92.

β-(S-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 [Pr-d(CH_2)$_5$VAVP, XI]. The octapeptide amide I (387 mg, 0.3 mmol) was treated with p-nitrophenyl β-(S-benzylmercapto)-β,β-cyclopentamethylenepropionate[12c] (232 mg 0.6 mmol) and HOBT (92 mg, 0.6 mmol), and the product was isolated as described above for IX to give XI: yield 313 mg (70.0%); mp 216–218 °C; $[\alpha]^{23}_D$ −44.3° (c 1, DMF); TLC R_f (A) 0.55; R_f (B) 0.74. Anal. ($C_{79}H_{99}N_{13}O_{13}S_3$) C, H, N. Amino acid analysis: Tyr, 1.02; Phe, 0.98; Val, 1.02; Asp, 1.00; Cys(Bzl), 0.98; Pro, 1.01; Arg, 0.98; Gly, 1.00; NH_3, 1.98.

β-(S-Benzylmercapto)-β,β-cyclopentamethylenepropionyl-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg-(Tos)-Gly-NH_2 [Pr-d(CH_2)$_5$DAVP, XII]. The octapeptide amide III (264 mg, 0.2 mmol) was treated with p-nitrophenyl β-(S-benzylmercapto)-β,β-cyclopentamethylenepropionate[12c] (155 mg, 0.4 mmol) and HOBT (62 mg, 0.4 mmol), and the product was isolated as described above for IX to give XII: yield 174 mg (55.6%); mp 194–196 °C; $[\alpha]^{23}_D$ −30.1° (c 1, DMF); TLC R_f (A) 0.47; R_f (B) 0.64. Anal. ($C_{79}H_{98}N_{14}O_{14}S_3 \cdot H_2O$) C, H, N. Amino acid analysis: Tyr, 1.01; Phe, 0.99; Glu, 1.02; Asp, 1.00; Cys(Bzl), 0.98; Pro, 1.02; Arg, 0.98 Gly, 1.00; NH_3, 3.04.

[1-(β-Mercapto-β,β-dimethylpropionic acid),4-valine]arginine-vasopressin (dPVAVP, XIII). A solution of the protected acyl octapeptide amide IX (145 mg, 0.09 mmol) in sodium-dried and redistilled ammonia (400 mL) was treated at the boiling point and with stirring with sodium[20] 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 (0.4 mL) was added to discharge the color. The solution was evaporated, the residue was dissolved in aq. acetic acid (0.2%, 800 mL), and this solution was treated with 2 M ammonium hydroxide solution to give a solution of pH 6.5. An excess of a solution of potassium ferricyanide[21] (0.01 M, 18 mL) was added gradually with stirring. The yellow solution was stirred for an additional 10 min and then for 10 min with anion-exchange resin (Bio-Rad Ag-3, Cl^- form, 10 g damp weight). The suspension was slowly filtered through a bed of resin (50 g damp weight). The bed was washed with aqueous acetic acid (0.2%, 200 mL),[10a] and the combined filtrate and washings were lyophilized. The resulting powder (1.53 g) was desalted on a Sephadex G-15[22] column (110 × 2.7 cm) eluting with aqueous acetic acid (50%)[23] with a flow rate of 5 mL/h. The eluate was fractionated and monitored for absorbance of 280 nm. The fractions comprising the major peak were pooled and lyophilized, and the residue (90 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) with a flow rate of 3 mL/h.[23] The peptide was eluted in a single peak (absorbance 280 nm). Lyophilization of the pertinent fractions gave the vasopressin analogue: yield 80 mg (77.2%); TLC R_f (A) 0.27, R_f (C) 0.42; $[\alpha]^{23}_D$ −61.6° (c 0.5, 1 M AcOH). Amino acid analysis:[26] Tyr, 0.98; Phe, 0.99; Val, 1.01; Asp, 1.00; Pro, 1.04; Arg, 1.02; Gly, 1.00; NH_3, 2.1.

Analysis following performic acid oxidation prior to hydrolysis[28] gave a Cys(O_3H)-Gly ratio of 1.05:1.00.

[1-(β-Mercapto-β,β-diethylpropionic acid),4-valine]arginine-vasopressin (dEt$_2$VAVP, XIV). The peptide intermediate V (168 mg, 0.11 mmol) was reduced by sodium in liquid ammonia, reoxidized, deionized, and purified as for XIII: yield 64 mg (53.1%); TLC R_f (A) 0.28; R_f (C) 0.42; $[\alpha]^{21}_D$ −60.2° (c 0.4, 1 M AcOH). Amino acid analysis: Tyr, 0.98; Phe, 1.00; Val, 1.02; Asp, 0.99; Pro, 1.01; Arg, 1.01; Gly, 1.00; NH_3, 2.08.

Analysis following performic acid oxidation prior to hydrolysis gave a Cys(O_3H)-Gly ratio of 1.02:1.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin [d(CH_2)$_5$VAVP, XV]. The analogue XV was prepared from the intermediate XI (150 mg, 0.098 mmol) in the manner detailed above for XIII: yield 57 mg (52.5%); TLC R_f (A) 0.25; R_f (C) 0.44; $[\alpha]^{21}_D$ −70.8° (c 0.5, 1 M AcOH). Amino acid analysis: Tyr, 0.98; Phe, 0.99; Val, 1.02; Asp, 1.01; Pro, 1.03; Arg, 1.01; Gly, 1.00; NH_3, 2.08.

Analysis following performic acid oxidation prior to hydrolysis gave a Cys(O_3H)-Gly ratio of 1.02:1.00.

[1-(β-Mercapto-β,β-dimethylpropionic acid),8-D-arginine]vasopressin (dPDAVP, XVI). Treatment of the protected acyl octapeptide amide X (167 mg, 0.11 mmol) as detailed for XIII gave the analogue XVI: yield 91 mg (75.8%); TLC R_f (A) 0.21, R_f (C) 0.23; $[\alpha]^{21}_D$ −47.8° (c 0.5, 1 M AcOH). Amino acid analysis: Tyr, 0.97; Phe, 0.99; Glu, 0.97; Asp, 0.98; Pro, 1.02; Arg, 1.01; Gly, 1.00; NH_3, 2.96.

Analysis following performic acid oxidation prior to hydrolysis gave a Cys(O_3H)-Gly ratio of 1.02:1.00.

[1-(β-Mercapto-β,β-diethylpropionic acid),8-D-arginine]-vasopressin [d(CH_2)$_5$DAVP, XVII]. The peptide intermediate VII (130 mg, 0.084 mmol) was reduced by sodium in liquid ammonia, reoxidized, deionized, and purified as for XIII: yield 56 mg (59.3%); TLC R_f (A) 0.20; R_f (C) 0.28; $[\alpha]^{21}_D$ −53.8° (c 0.4, 1 M AcOH). Amino acid analysis: Tyr, 0.98; Phe, 1.00; Glu, 1.00; Asp, 1.01; Pro, 1.03; Arg, 0.99; Gly, 1.00; NH_3, 3.08.

Analysis following performic acid oxidation prior to hydrolysis gave a Cys(O_3H)-Gly ratio of 1.04:1.00.

[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid],8-D-arginine]vasopressin [d(CH_2)$_5$DAVP, XVIII]. The analogue XVIII was prepared from the intermediate XII (161 mg, 0.103 mmol) in the manner detailed above for XIII: yield 68 mg (59.6%); TLC R_f (A) 0.23; R_f (C) 0.28; $[\alpha]^{23}_D$ −40.8° (c 0.5, 1 M AcOH). Amino acid analysis: Tyr, 0.98; Phe, 0.99; Glu, 0.99; Asp, 1.01; Pro, 1.02; Arg, 1.00; Gly, 1.00; NH_3, 3.03.

Analysis following performic acid oxidation prior to hydrolysis gave a Cys(O_3H)-Gly ratio of 1.01:1.00.

[1-(β-Mercapto-β,β-diethylpropionic acid)]arginine-vasopressin (dEt$_2$AVP, XIX). Treatment of the protected acyl octapeptide amide VI (120 mg, 0.077 mmol) as detailed for XIII gave the analogue XIX: yield 42 mg (47.1%); TLC R_f (A) 0.30, R_f (C) 0.31; $[\alpha]^{23}_D$ −53.8° (c 0.4, 1 M AcOH). Amino acid analysis: Tyr, 0.98; Phe, 1.01; Glu, 1.00; Asp, 1.01; Pro, 1.03; Arg, 1.02; Gly, 1.00; NH_3, 3.01.

Analysis following performic acid oxidation prior to hydrolysis gave a Cys(O_3H)-Gly ratio of 1.05:1.00.

[1-(β-Mercapto-β,β-diethylpropionic acid),4-valine,8-D-arginine]vasopressin (dEt$_2$VDAVP, XX).[27] Treatment of the protected acyl octapeptide amide VIII (161 mg, 0.112 mmol) as detailed for XIII gave the analogue XX: yield 69 mg (55.9%); TLC R_f (A) 0.29, R_f (C) 0.44; $[\alpha]^{23}_D$ −62.7° (c 0.5, 1 M AcOH).

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Amino acid analysis: Tyr, 0.98; Phe, 0.98; Val, 1.03; Asp, 1.01; Pro, 1.01; Arg, 1.01; Gly, 1.00; NH_3, 2.11.

Analysis following performic acid oxidation prior to hydrolysis gave a Cys(O_3H)-Gly ratio of 1.05:1.00.

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Design of More Potent and Selective Antagonists of the Antidiuretic Responses to Arginine-vasopressin Devoid of Antidiuretic Agonism

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Substitution of D-tyrosine at position 2 of [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin, d(CH_2)$_5$VAVP, turned this weak antidiuretic agonist and antagonist of vasopressor responses into an effective antagonist of the antidiuretic response. d(CH_2)$_5$-D-TyrVAVP, however, like other reported antagonists of the antidiuretic response, retains some antidiuretic agonistic activity. It is also a relatively strong antagonist of vasopressor and oxytocic responses. In attempting (a) to increase the specificity of antagonists of the antidiuretic response, (b) to eliminate residual agonistic activity and enhance antagonistic potency, and (c) to help delineate structural features at position 2 required for antidiuretic antagonism, we have synthesized eight new analogues substituted at position 2 by the solid-phase method: **1**, d(CH_2)$_5$-D-PheVAVP; **2**, d(CH_2)$_5$-D-PheVDAVP; **3**, d-(CH_2)$_5$-D-IleVAVP; **4**, d(CH_2)$_5$-D-LeuVAVP; **5**, d(CH_2)$_5$-D-ValVAVP; **6**, d(CH_2)$_5$-D-AlaVAVP; **7**, d(CH_2)$_5$GlyVAVP; **8**, d(CH_2)$_5$-D-ArgVAVP. These analogues were tested for agonistic and antagonistic activities by antidiuretic, vasopressor, and oxytocic assays in rats. Analogues **1**, **3**, **4**, and **5** exhibit no agonistic activities in these assays. These four analogues, as well as analogue **2**, effectively antagonize antidiuretic responses to AVP. Their antiantidiuretic pA_2 values are as follows: **1**, 8.07 ± 0.09; **2**, 7.07 ± 0.1; **3**, 7.98 ± 0.05; **4**, 7.79 ± 0.12; **5**, 7.48 ± 0.06. Analogues **6–8** are weak antidiuretic agonists and exhibit no detectable antiantidiuretic activity. Analogues **3–8** show greatly reduced potencies as antagonists of vasopressor and oxytocic responses. Thus, analogues **3–5** show much greater specificity as antagonists of the antidiuretic response than any previously reported. Analogues **1** and **3** are also the most potent antagonists of the antidiuretic response yet reported. The combination of increased antiantidiuretic potency and specificity should make these analogues useful tools for studies on the role of AVP in causing water retention in experimental animals and in man. They may also serve as prototypes for the design of even more potent and selective antidiuretic antagonists. Potent and specific antagonists of the antidiuretic action of ADH could be valuable for treating water retention in a variety of clinical situations.

We recently reported the first known effective antagonists of in vivo antidiuretic responses to both exogenous and endogenous arginine-vasopressin (AVP).[1,2] These antagonists were designed by incorporating *O*-alkyltyrosine residues (where alkyl = methyl, ethyl, isopropyl, or *n*-propyl) at position 2 in two vasopressor antagonists, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin [d(CH_2)$_5$VDAVP][3] and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin [d(CH_2)$_5$VAVP].[4] We subsequently found that the substitution of an *O*-alkyl-D-tyrosine residue in each of these eight antidiuretic antagonists brought about substantial increases in their respective antiantidiuretic potencies.[5,6] This led to the discovery that the incorporation of an unalkylated D-tyrosine residue for L-tyrosine at position 2 in d(CH_2)$_5$VAVP and in d(CH_2)$_5$VDAVP converted these weak antidiuretic agonists into potent antagonists of the antidiuretic responses to AVP.[5,6] All of these antidiuretic antagonists exhibit transient antidiuretic agonistic activity and are also potent antagonists of the vasopressor responses to AVP.[2,5,6] The finding that the substitution of an unalkylated D-tyrosine residue in d(CH_2)$_5$VAVP and in d(CH_2)$_5$VDAVP led to peptides possessing antidiuretic antagonism raised the possibility that the incorporation of other D-amino acids at position 2 in d(CH_2)$_5$VAVP and in d-(CH_2)$_5$VDAVP might lead to more potent and/or more selective antidiuretic antagonists lacking any residual antidiuretic agonistic activity. Initially, we substituted D-phenylalanine at position 2 in d(CH_2)$_5$VAVP and in d-(CH_2)$_5$VDAVP. Preliminary data on the resulting peptides were highly encouraging; both peptides were effective antidiuretic antagonists. The L-arginine-containing peptide was, however, much more potent than the D-arginine-containing one—a result highly consistent with all of our earlier data.[1,2,5,6] Thus, we decided to restrict further explorations of position 2 with D-amino acid substitutions to d(CH_2)$_5$VAVP. Therefore, following these findings with

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