Effects of a D-Cys^6/L-Cys^6 Interchange in Nonselective and Selective Vasopressin and Oxytocin Antagonists†

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We report the solid-phase synthesis of the D-Cys^6 analogues of arginine-vasopressin (AVP), peptide **1**, of the selective AVP vasopressor (V_{1a} receptor) antagonist [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin (d(CH_2)$_5$[Tyr(Me)^2]-AVP, (A)), peptide **2**, of the three nonselective antidiuretic/vasopressor (V_2/V_{1a} receptor) AVP antagonists d(CH_2)$_5$[Tyr(Et)^2]VAVP (B), d(CH_2)$_5$[D-Tyr(Et)^2]VAVP (C), and d(CH_2)$_5$[D-Phe^2]VAVP (D) (where V = Val^4), peptides **3**–**5**, of the nonselective oxytocin (OT) antagonists d(CH_2)$_5$-[Tyr(Me)^2]OVT (E) and d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH_2^{9}]OVT (F) (where OVT = ornithine-vasotocin), peptides **6** and **7**, and of the selective OT antagonists desGly-NH_2,d(CH_2)$_5$[Tyr(Me)^2,Thr^4]OVT (G) and d(CH_2)$_5$[D-Trp^2,Thr^4]OVT (H), peptides **8** and **9**. We also present the repeat syntheses of the previously reported d(CH_2)$_5$[D-Trp^2]AVT (peptide **10**) and its D-Cys^6 analogue (peptide **11**) (where AVT = arginine-vasotocin). Peptides **1**–**11** were assayed for agonistic and antagonistic activities in *in vivo* V_{1a}, V_2, and oxytocic assays and in *in vitro* oxytocic assays without and with 0.5 mM Mg^{2+}. With V_2 and V_{1a} agonistic potencies of 0.82 and 0.41 units/mg, [D-Cys^6]AVP has retained less than 0.3% of the V_2 and V_{1a} potencies of AVP. It exhibits no oxytocic activity and is an *in vitro* OT antagonist. pA_2 = 6.67 (no Mg^{2+}); pA_2 = 5.24 (0.5 mM Mg^{2+}). By contrast, with one or two exceptions, a D-Cys^6/L-Cys^6 interchange in antagonists **2**–**9**, although resulting in reductions of antagonistic potencies in all assays for virtually all peptides **2**–**9** relative to A–H, has been well tolerated. For peptides **2**–**5**, the anti-V_2 and anti-V_{1a} pA_2 values range from ~5.54 to 7.33 and from 7.19 to 8.06, respectively; the range of *in vitro* anti-OT pA_2 values (no Mg^{2+}) is 7.35–7.87; with 0.5 mM Mg^{2+}, the range is 7.24–8.21. Peptides **2** and **4** have *in vivo* anti-OT pA_2s = 6.60 and 7.16, respectively. For peptides **6**–**9**, the range of *in vitro* anti-OT pA_2 values (no Mg^{2+}) is 7.65–7.96; with 0.5 mM Mg^{2+}, the range is 7.41–7.65, and the *in vivo* anti-OT pA_2 values range from 6.85 to 7.33. With an *in vivo* anti-OT pA_2 = 7.33, peptide **6** is equipotent with its parent **E**. The *in vivo* anti-OT potencies of peptides **7**–**9** are significantly reduced relative to those of F–H. The *in vitro* anti-OT (0.5 mM Mg^{2+}) pA_2 values of **10** and **11** are 7.54 and 7.50, both significantly lower than those previously reported. Peptides **10** and **11** exhibit substantial V_{1a} antagonism. Their anti-V_{1a} pA_2 values are 7.56 and 7.53, respectively. The findings on peptides **2**–**9** show that a D-Cys^6/L-Cys^6 interchange in cyclic AVP and OT antagonists may be of limited value in enhancing antagonistic potency or selectivity. However, when combined with other appropriate molecular modifications, this interchange may be of merit for the design of orally active AVP and OT antagonists.

Antagonists of arginine-vasopressin (AVP) and oxytocin (OT) are widely used as powerful pharmacological tools in studies on the physiological, pathophysiological, and behavioral roles of these two peptides.[1] As tritiated and radioiodinated derivatives, AVP and OT agonists and antagonists are proving to be very useful radioligands in receptor localization and characterization studies.[2,3] AVP and OT agonists and antagonists have been utilized to help characterize the binding affinities and specifities of recently cloned AVP V_{1a}, V_{1b}, and V_2 receptors[4–9] from humans, rat, and pig and of the human oxytocin uterine receptor.[10a] They have continued to be valuable tools in the discovery of new receptor subtypes as shown by the recent demonstration of two receptor subtypes in the pregnant rat uterus.[10b,c] V_{1a} receptors present in the vasculature mediate the pressor action of AVP, and they are also present in brain, liver, and other tissues mediating the various biological

† Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1989**, *180*, A9–A11). All amino acids are in the L-configuration unless otherwise noted. Other abbreviations: Tyr(Et), *O*-ethyltyrosine; D-Tyr(Et), *O*-ethyl-D-tyrosine; Tyr(Me), *O*-methyltyrosine; AVP, arginine-vasopressin; OT, oxytocin; AVT, arginine-vasotocin; OVT, ornitnine-vasotocin; VAVP, [4-valine]arginine-vasopressin; desGly-NH_2, desglycinamide; d(CH_2)$_5$, 1-β-mercapto-β,β-pentamethylenepropionic acid; DCM, dichloromethane; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; AcOH, acetic acid; TFA, trifluoroacetic acid; HOBt, *N*-hydroxybenzotriazole; ONp, *p*-nitrophenyl ester; TEA, triethylamine; Z, benzyloxycarbonyl; SIADH, syndrome of inappropriate secretion of antidiuretic hormone.

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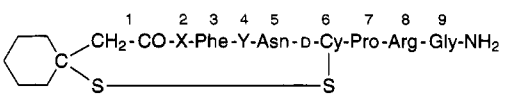
actions of AVP.[1,11-13] V_{1b} receptors, present in the anterior pituitary, mediate the ACTH-releasing effects of AVP.[13] V_2 receptors, present in the kidney, mediate the antidiuretic action of AVP.[13-15] OT receptors, present in the uterus, mediate the uterine-contracting (oxytocic) effect of oxytocin.[16] Besides their value as pharmacological tools and radioligands, AVP and OT antagonists are of potential clinical value.[1] AVP V_2 antagonists have potential therapeutic value for the treatment of hyponatremia caused by the syndrome of inappropriate secretion of the antidiuretic hormone (SIADH).[17] Antagonists of the vascular responses (V_{1a} receptor) to AVP may have clinical potential for the treatment of those patients with hypertension or congestive heart failure with concomitant elevated plasma AVP levels.[1,18] Antagonists of OT are of potential therapeutic value for the prevention of premature labor.[1] In this regard, an OT antagonist [1-deamino,D-Tyr(Et)^2,Thr^4]ornithine-vasotocin (tradename: ATOSIBAN) is currently undergoing clinical trial for this purpose.[19] Recently discovered orally active nonpeptide antagonists of OT and AVP[20,23] offer potentially attractive new therapeutic agents in this field. To date, no clinical trials with nonpeptide AVP or OT antagonists have been reported.

Over the years, we and others have carried out extensive investigations aimed at improving the potency, receptor selectivity, and oral bioavailability of AVP V_{1a}, V_2, and OT uterine antagonists. For reviews, see refs 1 and 24-28. The present study on the effectiveness of a D-Cys⁶/L-Cys⁶ interchange in AVP and OT antagonist design is a continuation of a preliminary investigation carried out some years ago.[29] Our renewed interest in a D-Cys⁶/L-Cys⁶ interchange was sparked by a recent report[30] which is somewhat at variance with our earlier findings.[29] We had found that a D-Cys⁶/L-Cys⁶ interchange in the three nonselective AVP V_2/V_{1a} antagonists d(CH_2)$_5$[Tyr(Et)^2]VAVP,[32a] d(CH_2)$_5$[D-Tyr(Et)^2]VAVP,[32b] and d(CH_2)$_5$[D-Phe^2]VAVP[33] (peptides B-D; Table 2) to give peptides **3-5** (Table 2) led to a drastic loss of V_2 antagonism for peptide **3** and fairly substantial losses of V_2 antagonism for peptides **4** and **5**.[29] Although V_{1a} antagonism was fully retained in peptide **4**, substantial losses were sustained in peptides **3** and **5**. Furthermore, in unpublished findings, we found that *in vitro* oxytocic antagonism (in the absence of Mg^{2+}) was reduced 2-5-fold in peptides **3-5**. Oxytocic antagonism in the presence of 0.5 mM Mg^{2+} was also reduced to a lesser extent in all three peptides **3-5**. *In vivo* OT antagonism of peptide **4** was reduced by ~50% relative to that of its parent peptide C. These findings showed that while a D-Cys⁶/L-Cys⁶ interchange in AVP V_2/V_{1a} antagonists exerts variable and inconsistent effects on antagonistic potencies, in no instance was antagonistic potency enhanced.

We were thus most intrigued by a recent report[30] that a D-Cys⁶ substitution in the OT antagonist d(CH_2)$_5$[D-Trp^2]AVT[34] resulted in an apparent 3-fold enhancement in *in vitro* antioxytocic potency in the presence of 0.5 mM Mg^{2+} and which also suggested that a D-Cys⁶/L-Cys⁶ interchange might be a promising new lead in OT antagonist design.[30] These findings and conclusions were clearly different from our earlier published[29] and unpublished findings and with those more recently reported by others[31] on the effects of a D-Cys⁶/L-Cys⁶ interchange in a series of AVP V_2/V_{1a} antagonists.[31] However, since we and this more recent study[31] had utilized AVP-derived V_2/V_{1a} antagonists, which have a vasopressin-like ring with Phe at position 3, and this very recent study[30] had utilized an AVT-derived OT antagonist, which has an OT-like ring with Ile at position 3, there was the possibility that the contrasting effects on antagonistic potencies of a D-Cys⁶/L-Cys⁶ interchange observed in these studies[29-31] was due to the different ring structures of the parent AVP and OT antagonists. We thus decided to return to and expand our earlier investigation[29] to include an examination of the effects of D-Cys⁶/L-Cys⁶ interchange in four of our most potent and selective OT antagonists, d(CH_2)$_5$[Tyr(Me)^2]OVT,[35] d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH_2^9]OVT,[35] desGly-NH_2,d(CH_2)$_5$[Tyr(Me)^2,Thr^4]OVT,[35] and desGly-NH_2,d(CH_2)$_5$[D-Trp^2,Thr^4]OVT,[36] peptides E-H (Table 3), to give peptides **6-9** (Table 3). We also included an investigation of the effects of a D-Cys⁶/L-Cys⁶ interchange in the AVP V_{1a} antagonist d(CH_2)$_5$[Tyr(Me)^2]-AVP[37] (peptide A; Table 2) to give peptide **2** (Table 2). When our preliminary findings on peptides **2** and **6-9**[38] appeared to concur with our earlier findings[29] and those of Albrightson-Winslow and colleagues,[31] while being inconsistent with those of Flouret and colleagues for d(CH_2)$_5$[D-Trp^2,D-Cys⁶]AVT,[30] we decided to repeat the syntheses and pharmacological evaluation of this peptide[30] and its L-Cys⁶ parent d(CH_2)$_5$[D-Trp^2]AVT.[34] Our findings on our synthetic preparations of those two peptides (**10** and **11**) are also presented in this report. Finally, to compare and contrast the effects of a D-Cys⁶/L-Cys⁶ interchange in AVP and OT agonists and antagonists, we report here also the synthesis and some pharmacological properties of [D-Cys⁶]AVP (peptide **1**; Table 1). It will be recalled that [D-Cys⁶]OT (Table 1) was shown to be virtually devoid of agonistic activities.[39]

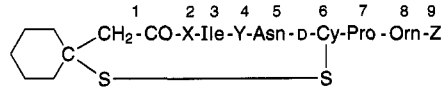
We thus report the synthesis and some pharmacological properties of the following 11 peptides designed according to the above rationale: **1**, [D-Cys⁶]AVP; **2**, d(CH_2)$_5$[Tyr(Me)^2,D-Cys⁶]AVP; **3**, d(CH_2)$_5$[Tyr(Et)^2,D-Cys⁶]VAVP; **4**, d(CH_2)$_5$[D-Tyr(Et)^2,D-Cys⁶]VAVP; **5**, d(CH_2)$_5$[D-Phe^2,D-Cys⁶]VAVP; **6**, d(CH_2)$_5$[Tyr(Me)^2,D-Cys⁶]OVT; **7**, d(CH_2)$_5$[Tyr(Me)^2,Thr^4,D-Cys⁶,Tyr-NH_2^9]-OVT; **8**, desGly-NH_2,d(CH_2)$_5$[Tyr(Me)^2,Thr^4,D-Cys⁶]OVT; **9**, desGly-NH_2,d(CH_2)$_5$[D-Trp^2,Thr^4,D-Cys⁶]OVT; **10**, d(CH_2)$_5$[D-Trp^2]AVT; and **11**, d(CH_2)$_5$[D-Trp^2,D-Cys⁶]-AVT. Peptide **1** is the D-Cys⁶ analogue of the V_2/V_{1a}/OT agonist AVP. Peptides **2-5** are D-Cys⁶ analogues of the AVP V_{1a}/OT antagonist A and the AVP V_2/V_{1a}/OT antagonists B-D. Peptides **6-9** are D-Cys⁶ analogues of nonselective (E, F) and selective (G, H) OT/V_{1a} antagonists. Peptides **10** and **11** are repeat syntheses of peptides originally synthesized in the Flouret laboratory.[30,34]

Analogues **2-5**, which have an AVP-like ring, have the following general structure:



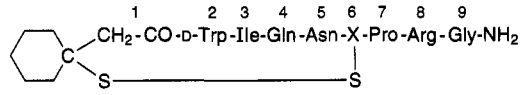
no.	X^2	Y^4
2	Tyr(Me)	Gln
3	Tyr(Et)	Val
4	D-Tyr(Et)	Val
5	D-Phe	Val

Analogues **6-9**, which have an OT-like ring, have the following general structure:



no.	X^2	Y^4	Z^9
6	Tyr(Me)	Gln	Gly-NH_2
7	Tyr(Me)	Thr	Tyr-NH_2
8	Tyr(Me)	Thr	
9	D-Trp	Thr	

Analogues **10** and **11**, which have an OT-like ring, have the following general structure:



no.	X^6
10	L-Cys
11	D-Cys

Peptide Synthesis

Starting from Boc-Gly-resin, Boc-Orn(Tos)-resin, or Boc-Tyr(Bzl)-resin, we synthesized the protected precursors **I**–**XI** of the free peptides **1**–**11** by solid-phase peptide synthesis according to previously published procedures.[31-33,40-42] HCl (1 M)/AcOH was used in all deprotection steps excluding those involving Boc-Gln.[42a] A 10% solution of TEA in DCM was used for neutralizations. Couplings were carried out by DCC preformed symmetrical anhydrides for peptides **I**–**V** and DCC/HOBt[43] for peptides **VI**–**XI**, except for Boc-Asn and Boc-Gln, which were incorporated as their *p*-nitrophenyl esters.[44] Also, in the case of peptides **II**–**V**, β-(benzylthio)-β,β-pentamethylenepropionic acid[45] was added as its *p*-nitrophenyl ester. Peptides were cleaved from the resin by ammonolysis as protected peptide amides (peptides **I**–**VII**, **X**, and **XI**)[42,46] or by HBr/TFA to give the protected peptide carboxylic acids (peptides **VIII** and **IX**).[40b,41,47,48] All protected peptides were purified by a series of precipitations. Peptides were deprotected by sodium in liquid ammonia[49] as previously described.[50-52] The disulfides were formed by oxidative cyclization with potassium ferricyanide using the normal procedure (peptides **1**–**5**, **10**, and **11**)[52] or a reverse procedure (peptides **6**–**9**).[53] The free peptides were desalted by gel filtration on a Sephadex G-15 column eluted with 50% AcOH and further purified on a G-15 column eluted with 0.2 M AcOH (peptides **1**–**5**) or on a LH-20 Sephadex column eluted with 2.0 M AcOH (peptides **6**–**11**) as previously described.[54] All free peptides were examined by TLC and HPLC for purity and by amino acid analysis and mass spectroscopy for chemical composition. Additionally, all free D-Cys^6 peptides were examined by HPLC for diastereoisomeric peptide contamination by coinjection with the corresponding L-Cys^6 peptide.

Bioassays

Peptides were assayed for agonistic and antagonistic activities in *in vitro* and *in vivo* rat oxytocic assays, rat vasopressor assays, and rat antidiuretic assays. For agonists, the 4-point assay design[55] was used, and for antagonists, the Schild's pA_2 method[56] was employed. The pA_2 is the negative logarithm of the molar concentration of the antagonist that will reduce the response to $2x$ units of the agonist to equal the response to x unit of the agonist in the absence of antagonist. In practice, this dose is estimated by finding doses above and below the pA_2 dose and interpolating on a logarithmic scale. In the rat *in vivo* assays, the pA_2 dose (effective dose, ED) is divided by an arbitrarily assumed volume of distribution of 67 mL/kg to estimate the molar concentration of the pA_2 dose. Thus, *in vivo* pA_2 values are estimates. USP posterior pituitary reference standards or synthetic oxytocin and arginine-vasopressin which had been standardized in oxytocic and vasopressor units against the USP posterior pituitary reference standard were used as working standards in all bioassays. *In vitro* oxytocic assays were performed on isolated uteri from diethylstilbestrol-primed rats in a Mg^{2+}-free van Dyke–Hasting solution.[57] *In vivo* anti-OT potencies were determined in urethane-anesthetized diethylstilbestrol-primed rats as previously described.[58] Vasopressor assays were performed on urethane-anesthetized and phenoxybenzamine-treated rats as described by Dekanski.[59] Antidiuretic assays were on water-loaded rats under ethanol anesthesia as described by Sawyer.[60] For all *in vivo* assays, doses of the peptide were injected intravenously. When standard errors are presented in the tables, the means reflect results from at least four independent assay groups.

Results and Discussion

Data on the effects of a D-Cys^6/L-Cys^6 interchange in AVP and OT[39] are given in Table 1. Data on the effects of a D-Cys^6/L-Cys^6 interchange in the AVP V_{1a} antagonist (A) and the three V_2/V_{1a} antagonists (B–D) are given in Table 2 (peptides **2**–**5**). The effects of a D-Cys^6/L-Cys^6 interchange in the two nonselective OT antagonists (E, F) and the two selective OT antagonists (G, H) are given in Table 3 (peptides **6**–**9**). Data from our resyntheses of $d(CH_2)_5$[D-Trp^2]AVT[34] and its D-Cys^6 analogue[30] (peptides **10** and **11**) together with the original data reported by Flouret and colleagues[34,30] are also presented in Table 3.

Comparison of Effects of D-Cys^6/L-Cys^6 Interchange in AVP and OT (Table 1). The replacement of L-Cys^6 by D-Cys^6 in oxytocin led to drastic losses of *in vitro* oxytocic activity and *in vivo* antidiuretic and vasopressor activities.[39] We now report similar losses of agonistic activities for [D-Cys^6]AVP (Table 1). Remarkably, [D-Cys^6]AVP is an antagonist in the *in vitro* oxytocic assays. Thus, these findings prove conclusively that a D-Cys^6/L-Cys^6 interchange in both oxytocin and vasopressin is not compatible with retention of agonistic activities. However, this does not rule out the possibility that a D-Cys^6/L-Cys^6 interchange might be better tolerated in other agonistic analogues of AVP and possibly also of OT.

Effects of a D-Cys^6/L-Cys^6 Interchange in AVP V_{1a} and V_2/V_{1a} Antagonists (Table 2). In a preliminary report,[29] we had previously shown that a D-Cys^6/L-Cys^6 interchange in the three nonselective V_2/V_{1a} AVP antagonists B–D (Table 2) to give peptides **3**–**5** (Table 2) resulted in significant, and in one case (peptide **3**) drastic, losses of both V_2 and V_{1a} antagonism. Thus, it is not surprising that the D-Cys^6 analogue of the selective V_{1a} antagonist A (Table 2), i.e., peptide **2** reported here, also exhibits a significant loss of V_{1a} antagonism relative to A. With an anti-V_{1a} $pA_2 = 7.87$, peptide **2** is only about $^1/_6$ as potent as its parent A. Interestingly, a D-Cys^6/L-Cys^6 interchange in $d(CH_2)_5$-[Tyr(Me)^2]AVP abolished V_2 agonism. The D-Cys^6 peptide **2** is a weak V_2 antagonist. We now report that all four

Table 1. Some Pharmacological Properties of D-Cys⁶ Analogues of Oxytocin (OT) and Arginine-vasopressin (AVP)

1 2 3* 4 5 6 7 8* 9
Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2

no.	peptide	oxytocic activity (U/mg) no Mg^{2+}	oxytocic activity (U/mg) 0.5 mM Mg^{2+}	antidiuretic act. (U/mg) (V_2 receptor)	vasopressor act. (U/mg) (V_{1a} receptor)
	oxytocin (OT)[a]	520 ± 12	486 ± 15	4 ± 0.8	4.3 ± 0.12
	[D-Cys⁶]OT[b]	0.62		<0.002	<0.002 (antagonist)
	Arg-vasopressin (AVP)[a]	13.9 ± 0.5	25.5 ± 0	323 ± 16	369 ± 6
1	[D-Cys⁶]AVP[c]	antagonist (pA_2 = 6.67 ± 0.06)	mixed[d] (pA_2 ~ 5.24)	0.82 ± 0.15	0.41 ± 0.03

* In oxytocin positions 3 and 8 are occupied by Ile and Leu, respectively. [a] Data from ref 16b. [b] Data from ref 39. [c] This publication. [d] Exhibits some agonist activity as well as antagonist activity.

Table 2. Pharmacological Properties of D-Cys⁶ Analogues of V_{1a} and V_2/V_{1a} Antagonists of Arginine-vasopressin

		antiantidiuretic (anti-V_2)		antivasopressor (anti-V_{1a})		antioxytocic *in vitro* pA_2[a]		antioxytocic *in vivo*	
no.	peptide	ED[b]	pA_2[c]	ED[b]	pA_2[c]	no Mg^{2+}	0.5 mM Mg^{2+}	ED[b]	pA_2[c]
A	$d(CH_2)_5$[Tyr(Me)²]AVP[f]	agonist (0.31 ± 0.07 U/mg)		0.16 ± 0.01[d]	8.62 ± 0.03	8.13 ± 0.12	7.24 ± 0.07	17 ± 3	6.62 ± 0.07
2	D-Cys⁶ analogue of A[e]	~194	~5.54	0.96 ± 0.24	7.87 ± 0.09	7.87 ± 0.06	7.60 ± 0.05	17 ± 1	6.60 ± 0.02
B	$d(CH_2)_5$[Tyr(Et)²]VAVP[e,g]	1.9 ± 0.2[g]	7.57 ± 0.6[g]	0.49 ± 0.11[g]	8.16 ± 0.09[g]	7.88 ± 0.10[e]	7.53 ± 0.07[e]	13 ± 2[e]	6.74 ± 0.0[e]
3	D-Cys⁶ analogue of B[e,j]	~72[j]	~6[j]	4.4 ± 0.5[j]	7.19 ± 0.05[j]	7.59 ± 0.09[e]	7.24 ± 0.10[e]		
C	$d(CH_2)_5$[D-Tyr(Et)²]VAVP[h,k]	1.1 ± 0.2	7.81 ± 0.7	0.45 ± 0.11	8.22 ± 0.12	8.32 ± 0.10[k]	8.38 ± 0.08[k]	2.6 ± 0.6[k]	7.47 ± 0.09[k]
4	D-Cys⁶ analogue of C[e,j]	3.3 ± 0.7[j]	7.33 ± 0.07[j]	0.60 ± 0.05[j]	8.06 ± 0.05[j]	7.43 ± 0.07[e]	8.21 ± 0.03[e]	4.8 ± 0.8[e]	7.16 ± 0.09[e]
D	$d(CH_2)_5$[D-Phe²]VAVP[i]	0.67 ± 0.13	8.07 ± 0.09	0.58 ± 0.04	8.06 ± 0.03	7.74 ± 0.06	8.29 ± 0.05	8.9 ± 1.9	6.92 ± 0.10
5	D-Cys⁶ analogue of D[e,j]	3.9 ± 0.5[j]	7.25 ± 0.06[j]	1.9 ± 0.4[j]	7.56 ± 0.07[j]	7.35 ± 0.05[e]	7.97 ± 0.06[e]		

[a] *In vitro* pA_2 values represent the negative logarithm to the base 10 of the average molar concentration (M) of antagonist which reduces the response to $2x$ units of agonist to the response with x unit of agonist. [b] The effective dose (ED) is defined as the dose (in nmols/kg) of antagonist that reduces the response to $2x$ units of agonist to the response with x unit of agonist administered in the absence of antagonist. [c] Estimated *in vivo* pA_2 values represent the negative logarithms of the "effective dose" in mol/kg divided by the estimated volume of distribution (67 mL/kg). [d] Means ± SE. [e] This publication. [f] Data from ref 37. [g] Data from ref 32a. [h] Data from ref 32b. [i] Data from ref 33. [j] Data from ref 29. [k] Data from ref 35.

D-Cys⁶ analogues, **2**–**5** (Table 2), exhibit reductions in antioxytocic potencies *in vitro* (0 Mg^{2+}). Three of the four peptides, i.e., **3**–**5** (Table 2), also exhibit reductions in antioxytocic potencies *in vitro* (0.5 mM Mg^{2+}). Only one analogue (**2**) exhibited an enhancement of anti-OT potency *in vitro* (0.5 mM Mg^{2+}). *In vivo* anti-OT potency was retained in one case (peptide **2**) and reduced in the other (peptide **4**) of the two D-Cys⁶ analogues tested on this assay to date. Thus, in AVP V_2/V_{1a} antagonists, while a D-Cys⁶/L-Cys⁶ interchange appears to preserve the ability to bind to V_2/V_{1a} and OT receptors and to thereby retain antagonistic potencies, it has not enhanced antagonistic potencies. Furthermore, the results of a D-Cys⁶/L-Cys⁶ interchange appear to be very structure dependent with regard to losses of V_2 antagonism. Thus, the D-Cys⁶ analogues **3**–**5** retain respectively only ~3%, ~33%, and 17% of the V_2 antagonism of their respective parent peptides B–D (Table 2). Whether a D-Cys⁶ substitution in an AVP V_2/V_{1a} antagonist will result in a drastic or a fairly substantial loss of V_2 antagonistic potency cannot be predicted. As noted above, the effects of a D-Cys⁶ substitution on the V_{1a} antagonism of V_2/V_{1a} antagonists appear not to be as drastic as the losses of V_2 antagonism. Although losses of V_{1a} antagonism were sustained for peptides **3** and **5**, these were much less in each case than the loss in each case of V_2 antagonism. Peptide **4** is virtually equipotent with its parent peptide C as a V_{1a} antagonist.

We can conclude from the data on peptides **2**–**5**, all of which contain a vasopressin ring, that a D-Cys⁶/L-Cys⁶ interchange in a V_{1a} antagonist and in three V_2/V_{1a} antagonists, although leading to losses of V_2, V_{1a}, and OT antagonism, does not abolish the ability to bind to V_2, V_{1a}, or OT receptors. These findings suggest the possibility that D-Cys⁶ substitutions could, in combination with other appropriate structural modifications, be of value in the design of orally active AVP V_2/V_{1a} and OT antagonists. The findings on the effects of a D-Cys⁶/L-Cys⁶ interchange in cyclic AVP V_2/V_{1a} antagonists are remarkably similar to those we have recently reported for a D-Abu⁶/L-Abu⁶ interchange in a series of linear AVP V_2/V_{1a} antagonists.[61]

Effects of a D-Cys⁶/L-Cys⁶ Interchange in Oxytocin Antagonists, Peptides 6–9 and 11 (Table 3). In peptides **6**, **8**, and **9**, a D-Cys⁶/L-Cys⁶ interchange reduced anti-OT potencies *in vitro*, in the absence of Mg^{2+}. The anti-OT potency of peptide **7** remained unchanged in this assay. In the presence of Mg^{2+}, the anti-OT potencies of peptides **6**, **7**, and **8** were diminished but that of peptide **9** was unchanged. These findings are in striking contrast to those reported by others[30] for the D-Cys⁶/L-Cys⁶ analogue pair **10** and **11** (Table 3). In our assays, the newly synthesized peptides **10** and **11** exhibit reduced anti-OT potency (*in vitro*, 0.5 mM Mg^{2+}) compared to those reported originally.[30,34] Furthermore, it may be noted that although *in vitro* OT antagonism is fully retained in the D-Cys⁶ analogue (**11**), we have not observed the 3-fold enhancement in OT antagonism observed in the original report on the peptide.[30] We have no explanations for these discrepancies except to point out that assays were carried out on different preparations of these two peptides in different laboratories. Our findings on the effects of a D-Cys⁶/L-Cys⁶ interchange in all the OT antagonists studied to date appear to be consistent with those we had previously observed for this interchange in AVP V_2/V_{1a} antagonists.[29] This interchange appears not to enhance antagonistic potency as suggested by the Flouret et al. report.[30] On the contrary, with only one or two exceptions, it results in losses of antagonistic potencies. When assayed *in vivo*, the three D-Cys⁶ OT antagonists, **7**–**9**, exhibited significant losses of anti-

Table 3. Some Pharmacological Properties of D-Cys^6 Analogues of Selective and Nonselective Oxytocin Antagonists

		antioxytocic							
		in vitro pA_2[a]		*in vivo*		antivasopressor		antidiuretic activity	
no.	peptide	no Mg^{2+}	0.5 mM Mg^{2+}	ED[b]	pA_2[c]	ED[b]	pA_2[c]	U/mg	ED[j] ratio
E	$d(CH_2)_5$[Tyr(Me)^2]OVT[e]	8.52 ± 0.10[d]	7.88 ± 0.10	4.2 ± 1.6	7.37 ± 0.17	0.80 ± 0.16	7.96 ± 0.10	~0.01	0.2
6	D-Cys^6 analogue of E[f]	7.65 ± 0.07	7.44 ± 0.05	3.11 ± 0.51	7.33 ± 0.07	3.73 ± 0.15	7.25 ± 0.02	antagonist (pA_2 ~ 5.9)	1.0
F	$d(CH_2)_5$[Tyr(Me)^2,Thr^4, Tyr-NH_2^9]OVT[e]	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	6.6
7	D-Cys^6 analogue of F[f]	7.96 ± 0.05	7.41 ± 0.08	5.63 ± 0.93	7.08 ± 0.08	10 ± 2	6.83 ± 0.09	antagonist (pA_2 ~ 5.5)	1.8
G	desGly-NH_2,$d(CH_2)_5$ [Tyr(Me)^2,Thr^4]OVT[e]	7.89 ± 0.04	8.24 ± 0.09	1.3 ± 0.2	7.69 ± 0.07	23 ● 4	6.48 ± 0.08	antagonist (pA_2 ~ 5.5)	17.7
8	D-Cys^6 analogue of G[f]	7.73 ± 0.07	7.60 ± 0.06	9.24 ± 1.85	6.86 ± 0.10	17.3 ± 2.0	6.56 ± 0.05	antagonist (pA_2 ~ 5.4)	
H	desGly-NH_2,$d(CH_2)_5$ [D-Trp^2,Thr^4]OVT[g]	8.02 ± 0.03	7.64 ± 0.05[f]	3.08 ± 0.59	7.34 ± 0.09	248 ± 23	5.43 ± 0.04	~0.01	80
9	D-Cys^6 analogue of H[f]	7.83 ± 0.04	7.65 ± 0.08	9.42 ± 1.82	6.85 ± 0.08	20 ± 2	6.52 ± 0.03	pA_2 ~ 5.5	2.1
10	$d(CH_2)_5$[D-Trp^2]AVT[f,h]		7.77 ± 0.03[h]					antagonist (pA_2 < 5.9)[h]	
			7.54 ± 0.07[f]			2.0 ± 0.3[f]	7.56 ± 0.06[f]		
11	D-Cys^6 analogue of **10**[f,i]		8.29 ± 0.23[i]					antagonist (pA_2 < 6.3)	
			7.50 ± 0.06[f]			2.0 ± 0.3[f]	7.53 ± 0.05[f]	pA_2 < 5.6	

[a,b,c,d] See corresponding footnotes Table 2. [e] Data from reference 35. [f] This publication. [g] Data from reference 36. [h] Data from reference 34. [i] Data from reference 30. [j] ED ratio = antivasopressor ED/antioxytocic ED.

OT potencies with respect to their parent peptides F–H. By contrast, the D-Cys^6 OT antagonist **6**, although losing *in vitro* anti-OT potency, retained full *in vivo* anti-OT potency relative to its parent peptide E. All four D-Cys^6 OT antagonists, **6**–**9**, exhibited weak anti-V_2 activity. Remarkably, a D-Cys^6/L-Cys^6 interchange appears to have had inconsistent effects on the anti-V_{1a} potencies of the four new D-Cys^6/L-Cys^6 pairs and in the resynthesized pair (peptides **10** and **11**). One of the peptides (peptide **6**) exhibits a substantial reduction in anti-V_{1a} potency with respect to its parent peptide E. Another peptide (**9**) exhibits a significant gain in anti-V_{1a} potency with respect to its parent peptide H. The anti-V_{1a} potencies of the remaining three peptides, **7**, **8**, and **11**, are not significantly different than those of their respective parents F, G, and **10**. Thus, none of the D-Cys^6 OT antagonists (**6**–**8**) exhibit significant gains in anti-OT/anti-V_{1a} selectivity *in vivo*. While a D-Cys^6/L-Cys^6 substitution in these OT antagonists has not resulted in enhanced OT antagonism or selectivity, the retention of full *in vivo* OT antagonism by peptide **6**, and the partial but significant retention of *in vivo* OT antagonism by peptides **7**–**9**, lends support to the view that D-Cys^6 substitutions may be of value in OT antagonist design.[30] As noted above for AVP V_2/V_{1a} and V_{1a} antagonists, D-Cys^6 substitutions, in combination with other structural modifications, may have potential in the design of orally active OT antagonists.

Conclusions

Our findings on the effects of a D-Cys^6/L-Cys^6 interchange in AVP are consistent with those previously reported for this interchange in OT.[39] Thus, a D-Cys^6/L-Cys^6 interchange in AVP also leads to drastic losses of agonistic activities. By contrast, as we and others have previously noted, a D-Cys^6/L-Cys^6 interchange is much better tolerated in AVP and OT antagonists.[29,30] Although we could not confirm the dramatic increase in *in vitro* (0.5 mM Mg^{2+}) OT antagonism previously reported for $d(CH_2)_5$[D-Trp^2,D-Cys^6]AVT,[30] our findings show that the effects of a D-Cys^6/L-Cys^6 interchange in OT antagonists reported here are consistent with those we have reported earlier on the effects of this interchange in AVP V_2/V_{1a} antagonists.[29] With only one or two exceptions, a D-Cys^6/L-Cys^6 interchange does not enhance antagonistic potencies. In only a few instances is full antagonism retained. In the majority of cases, a D-Cys^6 substitution brought about substantial losses of antagonistic potencies in one or more assays. Nevertheless, all the D-Cys^6 antagonists retain the ability to bind to their receptors. Although a D-Cys^6/L-Cys^6 interchange appears to have limited value for increasing either the potency or the selectivity of the AVP or OT antagonists reported in this paper, nonetheless, the retention of full *in vivo* antagonism by the D-Cys^6 peptide **6** is encouraging. Thus, the potential of a D-Cys^6/L-Cys^6 interchange for the design of other AVP V_{1a} and V_2 antagonists and OT antagonists and also for orally active peptides merits further exploration.

Experimental Section

Amino acid derivatives were purchased from Bachem Inc. or Chemalog Inc. Boc-Tyr(Me),[62] Boc-Tyr(Et),[62] Boc-D-Tyr-(Et),[62] β-(benzylthio)-β,β-pentamethylenepropionic acid,[45] and *p*-nitrophenyl β-(benzylthio)-β,β-pentamethylenepropionate[45] were synthesized by previously published procedures. Thin layer chromatography (TLC) was run on precoated silica gel plates (60F-254, E. Merck) with the following systems: (a) 1-butanol:AcOH:H_2O (4:1:5, upper phase), (b) 1-butanol:AcOH:H_2O (4:1:1), (c) 1-butanol:AcOH:H_2O:pyridine (15:3:3:10), and (d) chloroform:methanol (7:3). Loads of 10–15 μg were applied, and chromatograms were developed at minimal length of 10 cm. The chlorine gas procedure for the KI-starch reagent and I_2 were used for detection.[41] Optical rotations were measured with a Rudolph Autopol III polarimeter. Amino acid analyses were done by Commonwealth Biotechnologies Inc. or the University of Louisville Core Facility. Molar ratios were referred to Phe or Gly = 1. Tyr(Et) and Tyr(Me) were detected as Tyr without substantial losses in 28 h hydrolyses. All peptides gave the expected amino acid ratios ±5%. Analytical high-performance liquid chromatography (HPLC) was performed on a Waters 810 instrument equipped with a Rainin Microsorb C_{18} column using a linear gradient (1%/min) of 10–70% acetonitrile containing 0.05% of TFA in 0.05% aqueous trifluoroacetic acid at a flow rate of 1 mL/min with UV detection at 210 nm. All peptides were at least 95% pure. Furthermore, coinjection of each D-Cys^6 peptide with its L-Cys^6 diastereoisomer, with the exception of the D-Cys^6/L-Cys^6 AVP

Table 4. Physicochemical Properties of Protected Peptides **I–XI**

no.[a]	structure	yield[b] (%)	mp (°C)	$[\alpha]^{25}_D$ (deg) (*c* 1, DMF)	TLC, R_f[c] a	b	c	d
I	Cbz-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-D-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	30	167–9	−25.6	0.71	0.63	0.86	0.87
II	d$(CH_2)_5$(Bzl)-Tyr(Me)-Phe-Gln-Asn-D-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	64	148–50	−22.4	0.68	0.61	0.86	0.84
III	d$(CH_2)_5$(Bzl)-Tyr(Et)-Phe-Val-Asn-D-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	58.4	158–60	−28.9	0.75	0.77	0.82	0.90
IV	d$(CH_2)_5$(Bzl)-D-Tyr(Et)Phe-Val-Asn-D-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	29.0	167–9	−7.6		0.64	0.88	0.92
V	d$(CH_2)_5$(Bzl)-D-Phe-Phe-Val-Asn-D-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	83.5	134–6	−9.3		0.60	0.84	0.90
VI	d$(CH_2)_5$(Bzl)-Tyr(Me)-Ile-Gln-Asn-D-Cys(Bzl)-Pro-Orn(Tos)-Gly-NH_2	85.0	124–6	−25.2	0.67	0.74	0.74	
VII	d$(CH_2)_5$(Bzl)-Tyr(Me)-Ile-Thr(Bzl)-Asn-D-Cys(Bzl)-Pro-Orn(Tos)-Tyr(Bzl)-NH_2	71.3	198–200	−2.3	0.87	0.84	0.82	
VIII	d$(CH_2)_5$(Bzl)-Tyr(Me)-Ile-Thr(Bzl)-Asn-D-Cys(Bzl)-Pro-Orn(Tos)-OH	50.2	121–3	−10.0	0.71	0.78	0.72	
IX	d$(CH_2)_5$(Bzl)-D-Trp-Ile-Thr(Bzl)-Asn-D-Cys(Bzl)-Pro-Orn(Tos)-OH	52.6	136–9	+3.6	0.76	0.74	0.71	
X	d$(CH_2)_5$(Bzl)-D-Trp-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	80.0	179–82	−12.0		0.47	0.69	0.61
XI	d$(CH_2)_5$(Bzl)-D-Trp-Ile-Gln-Asn-D-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	70.6	139–43	−15.0		0.62	0.78	0.89

[a] The protected peptides **I–XI** are the immediate protected precursors for the free peptides **1–11** given in Tables 1–5. [b] Yields were calculated on the basis of the amino acid content of the resin. [c] Solvent systems are described in the Experimental Section.

Table 5. Physicochemical Properties of the Free Peptides **1–11**

no.	peptide	yield (%)[a,b]	$[\alpha]^{25}_D$ (deg) (*c* 0.1, 50% AcOH)	TLC, R_f[c] a	b	d	HPLC[d] t_R (min)	HPLC[e] L^6	D^6	formula	MW	$[M+H]^+$[f]
1	[D-Cys⁶]AVP	60.6	−99.0		0.06	0.09	25.2	24.6	24.6	$C_{46}H_{65}N_{16}O_{12}S_2$	1084.2	1085
2	d$(CH_2)_5$[Tyr(Me)²,D-Cys⁶]AVP	21.3	+10.0		0.19	0.44	39.3	35.3	39.3	$C_{52}H_{74}N_{15}O_{12}S_2$	1151.4	1153
3	d$(CH_2)_5$[Tyr(Et)²,D-Cys⁶]VAVP	34.5	+21.0		0.29	0.68	45.3	43.2	45.7	$C_{53}H_{77}N_{14}O_{11}S_2$	1136.4	1137.5
4	d$(CH_2)_5$[D-Tyr(Et)²,D-Cys⁶]VAVP	19.0	−75.2		0.15	0.64	46.1	42.1	46.3	$C_{53}H_{77}N_{14}O_{11}S_2$	1136.4	1137.5
5	d$(CH_2)_5$[D-Phe²,D-Cys⁶]VAVP	41.6	−84.0		0.20	0.65	43.7	39.7	43.9	$C_{51}H_{73}N_{14}O_{10}S_2$	1092.3	1094
6	d$(CH_2)_5$[Tyr(Me)²,D-Cys⁶]OVT	51.7	−7.0	0.17	0.10	0.20	36.6	32.9	37.0	$C_{48}H_{74}N_{12}O_{12}S_2$	1075.32	1077
7	d$(CH_2)_5$[Tyr(Me)²,Thr⁴,D-Cys⁶,Tyr-$NH_2$⁹]OVT	50.6	−23.0	0.31	0.17	0.50	40.0	36.1	40.3	$C_{54}H_{79}N_{11}O_{13}S_2$	1155.42	1156
8	desGly-NH_2,d$(CH_2)_5$[Tyr(Me)²,Thr⁴,D-Cys⁶]OVT	55.9	−25.0	0.19	0.12	0.17	39.1	36.6	39.8	$C_{45}H_{69}N_9O_{12}S_2$	992.23	993.5
9	desGly-NH_2,d$(CH_2)_5$[D-Trp²,Thr⁴,D-Cys⁶]OVT	19.1	−67.0	0.20	0.13	0.19	43.7	40.1	43.9	$C_{46}H_{67}N_{10}O_{11}S_2$	1000.3	1002
10	d$(CH_2)_5$[D-Trp²]AVT	58.8	−118.0	0.11	0.17	0.25	35.2	35.2	39.7	$C_{50}H_{73}N_{15}O_{11}S_2$	1126.37	1127
11	d$(CH_2)_5$[D-Trp²,D-Cys⁶]AVT	26.7	−24.0	0.16	0.20	0.30	39.5	35.2	39.7	$C_{50}H_{73}N_{15}O_{11}S_2$	1126.37	1127

[a] Yields are based on the amount of protected peptide used in the reduction–reoxidation step in each case and are uncorrected for acetic acid and water content. [b] All peptides gave the expected amino acid analysis ratios after hydrolysis ±5%. [c] Solvent systems and conditions are given in the Experimental Section. [d] All peptides were at least 95% pure and, on the basis of this HPLC study and that shown under footnote e, were not contaminated by detectable amounts of the corresponding L-Cys⁶ diastereoisomer. For elution conditions, see the Experimental Section. [e] Retention times were obtained following coinjection of each D-Cys⁶ peptide with its corresponding L-Cys⁶ diastereoisomer. For elution conditions, see the Experimental Section. [f] Data obtained by ES-MS.

pair (peptide **1** and AVP), gave an excellent resolution of the D-Cys⁶/L-Cys⁶ diastereoisomers in each pair as reflected by the clear differences in retention times of the D-Cys⁶ and L-Cys⁶ diastereoisomers in all pairs (Table 5). We can conclude from these HPLC single-injection and coinjection studies that none of the D-Cys⁶-containing AVP and OT antagonists is contaminated by detectable amounts of its L-Cys⁶ diastereoisomer. Electron spray mass spectrometry (ES-MS) spectra, obtained at the University of Michigan Protein and Carbohydrate Structura Facility, were in agreement with the composition of each peptide.

Solid-Phase Synthesis Procedure. Protected peptides were synthesized by manual solid-phase peptide synthesis (SPPS) using a Boc protocol similar to those published before.[32,40–42,47,50–51] Chloromethylated resin (Chemalog or Bachem Inc.; 1% cross-linked S-DVB, 200–400 mesh, 0.7–1.0 mmol/g) was esterified with Boc-Gly, Boc-Orn(Tos), or Boc-Tyr(Bzl) to an incorporation of approximately 0.5 mmol/g by the cesium salt method.[63] Peptidyl resins **VI–XI** were obtained from the corresponding Boc amino acid resins in eight or seven cycles of SPPS. Peptidyl resins **I–V** were obtained by the split synthesis method. Initially, a pentapeptidyl precursor resin was synthesized in four cycles of SPPS and then split, and each sequence was completed in four additional cycles. The protected peptides were cleaved from the resin by ammonolysis in methanol[41,46] (peptides **I–VII, X,** and **XI**) or by HBr/TFA treatment[40b,47,48] (peptides **VIII** and **IX**). Protected peptide amides were extracted by hot DMF, precipitated by hot water, and further purified by reprecipitations with DMF/MeOH/Et_2O, until considered pure by TLC. The protected peptides cleaved by HBr were purified by reprecipitations with DMF/MeOH/Et_2O and DMF/water. The protected peptides (**I–XI**; see Table 4) were deblocked with sodium in liquid ammonia.[49] The resulting disulfhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$ using the normal[52] or a modified reverse procedure.[53] The free cyclic peptides were purified by a two-step gel filtration procedure[54] on Sephadex G-15 and Sephadex LH-20 columns. The physicochemical properties of the free cyclic peptides (**1–11**) are given in Table 5.

[β-(Benzylthio)-β,β-pentamethylenepropionyl]-D-Tyr-(Et)-Phe-Val-Asn-D-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (IV; Table 4), Ammonolytic Cleavage. The corresponding C-terminal glycine-containing protected octapeptidyl resin (1.85 g, 0.5 mmol) obtained from eight cycles of solid-phase synthesis as described above was treated with ammonia in the presence of methanol for 72 h in a sealed flask. Following evaporation of the methanolic ammonia, the protected peptide was extracted by hot DMF (32 mL) and precipitated by hot water. The product was collected, dried, and dissolved in DMF (5 mL) and EtOH (20 mL). Ethyl ether was added until the solution became turbid. Solid material was filtered. The solution was evaporated to dryness. The residue was dissolved in hot EtOH, and the product was reprecipitated with ethyl ether (200 mL). Collection and drying in vacuo over P_2O_5 gave the required acyloctapeptide amide **IV** (Table 4). A similar procedure was used for the cleavage and purification of the protected nonapeptide amide **I** and protected acyloctapeptide amides **II, III, V–VII, X,** and **XI** (Table 4).

[β-(Benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Ile-Thr(Bzl)-Asn-D-Cys(Bzl)-Pro-Orn(Tos)-OH (VIII; Table 4), Acidolytic Cleavage. The corresponding C-terminal tosylornithine-containing protected heptapeptidyl resin (1.43 g, 0.6 mmol) obtained from seven cycles of solid-phase synthesis as described above was suspended in TFA (25 mL) containing anisole (5 mL), and hydrogen bromide was bubbled through the suspension for 45 min. The filtrate was collected, and the resin was resuspended in DCM (12 mL), TFA (12 mL), and anisole (5 mL). HBr bubbling was resumed for a further 40 min. The filtrate was collected, and the resin was washed with DCM/TFA (1:1, v/v, 2 × 20 mL). The filtrates and washings were combined and evaporated on a rotary evaporator. The residue was treated with Et_2O (ca. 250 mL) and placed in the refrigerator for several hours. The resulting

precipitate was collected, washed with ether, and dried in vacuo over P_2O_5. The product was dissolved in DMF (5 mL) and MeOH (15 mL) and precipitated with ether (200 mL). The collected material was dissolved in DMF (10 mL), reprecipitated with water, collected, and dried in vacuo over P_2O_5 to give the desired protected acylheptapeptide **VIII** (Table 4). The same procedure was used for the cleavage and purification of the protected carboxyl-terminating acylheptapeptide **IX** (Table 4).

[1-(β-Mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyl-tyrosine,4-valine,6-D-cysteine]arginine-vasopressin (4; Tables 2 and 5). A stirred solution of protected acyloctapeptide amide **IV** (120 mg) in sodium-dried ammonia (ca. 400 mL) was treated at boiling point with a stick of sodium metal contained in a small-bore glass tube until a light-blue color persisted in the solution for ca. 30 s. NH_4Cl was added to discharge the color. The ammonia was evaporated, and the flask was flushed with nitrogen. The residue was dissolved in 50 mL of degassed 50% AcOH and poured into ice-cold water (1000 mL). The pH was adjusted to approximately 7.0 with concentrated ammonium hydroxide. A solution of potassium ferricyanide (0.01 M, 12.0 mL) was added gradually with stirring while the pH was maintained at 7 by the addition of concentrated ammonium hydroxide until a yellow color persisted in the solution. The yellow solution was stirred for an additional 20 min and, after acidification with AcOH to pH 5, then for 10 min with anion-exchange resin (Bio-Rad AG-3, Cl^- form, 10 g damp weight). The suspension was filtered, and the bed was washed with 0.2 N AcOH; the combined filtrate and washings were lyophilized. The resulting powder was desalted on a Sephadex G-15 column (110 × 2.7 cm) eluting with aqueous acetic acid (50%) with a flow rate of 5 mL/h. The eluent was fractionated and monitored for absorbance at 254 nm. The fractions comprising the major peak were checked by TLC, pooled, and lyophilized. The residue 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 4 mL/h. The peptide was eluted in a single peak (absorbance at 254 nm). Lyophilization of pertinent fractions gave the desired peptide **4** (Tables 2 and 5). The same procedure was used to obtain peptides **1–3**, **5**, **10**, and **11** (Tables 1–3 and 5).

Des-9-glycinamide[1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine,4-threonine,6-D-cysteine]ornithine-vasotocin (8; Tables 3 and 5). The protected acylheptapeptide **VIII** (120 mg) was deprotected by sodium in liquid ammonia as above. Reoxidation of the deblocked disulfhydryl peptide was carried out by a modified reverse procedure[53] as follows. The residue was dissolved in 25 mL of 50% AcOH, and the solution was diluted with 75 mL of H_2O. The peptide solution was added dropwise with stirring over a period of 15–30 min to an 800 mL aqueous solution which contained 20 mL of a 0.01 M solution of potassium ferricyanide. Meanwhile, the pH was adjusted to approximately 7.0 with concentrated ammonium hydroxide. The yellow solution was stirred for an additional 20 min. The peptide was isolated and purified as above. However, for the second gel filtration, a Sephadex LH-20 column eluted with 2 M AcOH was used. The same procedure was used for reoxidation of the free cyclic peptides **6**, **7**, and **9** (Tables 3 and 5).

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