C-Terminal Deletions in Agonistic and Antagonistic Analogues of Vasopressin That Improve Their Specificities for Antidiuretic (V_2) and Vasopressor (V_1) Receptors†

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We report the solid-phase synthesis of 12 desGly and 12 desGly(NH_2) analogues of arginine-vasopressin (AVP), two highly selective antidiuretic (V_2) agonists, four vasopressor (V_1) antagonists, and five V_2/V_1 antagonists. The parent AVP agonists are (1) AVP, (2) 1-deamino[8-D-arginine]vasopressin (dDAVP), and (3) its 4-valine analogue, dVDAVP. The parent V_1 antagonists are (4) [1-(β-mercapto-β,β-pentamethylenepropionic acid)]arginine-vasopressin (d(CH_2)$_5$AVP), (5) d(CH_2)$_5$VDAVP, (6) [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin (dPVDAVP), and (7) d(CH_2)$_5$[Tyr(Me)]AVP. The parent V_2/V_1 antagonists are (8) d(CH_2)$_5$[D-Phe^2,Ile^4]AVP, (9) d(CH_2)$_5$[D-Phe^2]VAVP, (10) d(CH_2)$_5$[D-Tyr(Et)^2]VAVP, (11) d(CH_2)$_5$[Tyr(Et^2]VAVP, and (12) d(CH_2)$_5$[D-Ile^2,Ile^4]AVP. All 24 analogues were tested for agonistic and antagonistic activities in in vivo rat vasopressor and rat antidiuretic assays. The desGly and desGly(NH_2) analogues of **1–3** are either weak partial agonists or weak antagonists of the V_1 responses to AVP. Except for desGly(NH_2)AVP, which is a weak V_2 agonist, the remaining desGly and desGly(NH_2) analogues of **1–3** exhibit substantial V_2 agonism and are thus highly selective V_2 agonists. With antidiuretic activity of 321 units/mg, a resynthesized desGly(NH_2)dVDAVP is equipotent with AVP as a V_2 agonist. Thus our previously stated conclusion about the need for C-terminal $CONH_2$ for V_2 agonism is no longer valid. The four pairs of desGly/desGly(NH_2) analogues of the V_1 antagonists (**4–7**) all retained varying degrees of V_1 antagonism and some exhibited striking enhancements in anti-V_1/V_2 selectivity. Thus the desGly/desGly(NH_2) analogues of d(CH_2)$_5$Tyr(Me)AVP are highly potent V_1 antagonists/weak V_2 antagonists with anti-V_1/V_2 selectivities of 200 and 1200, respectively. The four pairs of desGly/desGly(NH_2) analogues of the V_2/V_1 antagonists (**8–11**) exhibited enhancements, full retention, or slight diminishment of both V_1 and V_2 antagonism, with the desGly analogue being usually the more potent of each pair. The desGly and desGly(NH_2) analogues of d(CH_2)$_5$[D-Ile^2,Ile^4]AVP (**12**) exhibited anti-V_2/V_1 selectivities of 46 and about 440, respectively. These are the most selective V_2 antagonists reported to date. Many of these analogues could serve as useful pharmacological tools in studies on the roles of AVP in normal and pathophysiological circumstances.

Deletion of the C-terminal glycinamide (Gly(NH_2)) from arginine-vasopressin (AVP) resulted in a complete loss of vasopressor (V_1) agonism and a drastic reduction of antidiuretic (V_2) agonism.[1,2] In striking contrast, the deletion of the C-terminal Gly(NH_2) from an AVP V_1 antagonist, [1-(β-mercapto-β,β-pentamethylenepripionic acid)]arginine-vasopressin (d(CH_2)$_5$AVP),[3] and from the AVP V_2/V_1 antagonist [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-phenylalanine,4-valine]arginine-vasopressin (d-(CH_2)$_5$[D-Phe^2],VAVP)[4] resulted in virtually full retention of V_1 and V_2/V_1 antagonism.[2] Deletion of just the C-terminal glycine of AVP (with retention of the C-terminal carboxamide) resulted in a drastic loss of V_1 agonism with a surprising retention of approximately 50% of the V_2 agonism of AVP.[2] Deletion of the C-teriminal Gly from d(CH_2)$_5$AVP and d(CH_2)$_5$[D-Phe^2]VAVP[4] and from three additional V_2/V_1 antagonists, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyltyrosine,4-valine]arginine-vasopressin (d(CH_2)$_5$[Tyr(Et)^2]VAVP),[5] [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyl-D-tyrosine,4-valine]arginine-vasopressin (d(CH_2)$_5$[D-Tyr-

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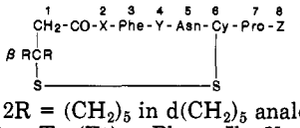
† Symbols and abbreviations are in accordance with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* 1984, *138*, 9). All amino acids are in the L configuration unless otherwise noted. Other abbreviations used are: Tyr(Me), *O*-methyltyrosine; Tyr(Et), *O*-ethyltyrosine; D-Tyr(Et), *O*-ethyl-D-tyrosine; AVP, arginine-vasopressin; dDAVP, 1-deamino[8-D-arginine]vasopressin; dVDAVP, 1-deamino[4-valine,8-D-arginine]vasopressin; desGly, desglycine [carboxy terminus is Arg(NH_2)]; desGly(NH_2), desglycinamide [carboxy terminus is Arg(OH)]; dP, 1-deaminopenicillamine or 1-β-mercapto-β,β-dimethylpropionic acid); d(CH_2)$_5$, 1-(β-mercapto-β,β-pentamethylenepropionic acid). The 1-(β-mercapto-β,β-dialkylpropionic acid) analogues of desGly AVP and desGly(NH_2)AVP have the following structure: where R = CH_3



in dP analogues; 2R = (CH_2)$_5$ in d(CH_2)$_5$ analogues; X = Tyr, Tyr(Me), Tyr(Et), D-Tyr(Et), D-Phe, D-Ile; Y = Gln, Val, Ile; Z = L- or D-Arg or L- or D-Arg(NH_2). DMF, dimethylformamide; DCCI, dicyclohexylcarbodiimide; BOC, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; AcOH, acetic acid; TFA, trifluoroacetic acid; HOBT, *N*-hydroxybenzotriazole; NPE, nitrophenyl ester; Et_3N, triethylamine; Z, benzyloxycarbonyl.

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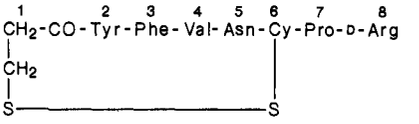
(Et)^2]VAVP),[6] and [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-phenylalanine,4-isoleucine]arginine-vasopressin (d(CH$_2$)$_5$[D-Phe^2,Ile^4]AVP)[7] led to almost full retention of V_1- and V_2-antagonistic potencies.[2,8] Subsequently, the desGly(NH$_2$) analogues of these latter three V_2/V_1 antagonists were also found to exhibit retention of their antagonistic potencies.[10] It was concluded from these observations, as well as the finding that AVP-acid, i.e., AVP that has the C-terminal CONH$_2$ replaced by a COOH group, is a very weak AVP V_2 agonist,[2] that, although a C-terminal carboxamide group is not a requirement for binding to AVP V_1 or V_2 receptors, it is essential for activation of both types of receptors. Preliminary data on the desGly(NH$_2$) analogue of the highly potent and selective AVP V_2 agonist 1-deamino[4-valine,8-D-arginine]-vasopressin (dVDAVP),[9] which was found to have drastically reduced V_2 agonism,[10,11] appeared to further substantiate these conclusions. However, our subsequent discovery, reported here, that the desGly(NH$_2$) analogue of 1-deamino[8-D-arginine]vasopressin (dDAVP)[12] retained substantial V_2 agonism prompted a reexamination of these conclusions. This entailed a resynthesis of desGly-(NH$_2$)dVDAVP with the discovery that the original synthesis was in error. We now report the synthesis and some pharmacological properties of the desGly and desGly(NH$_2$) analogues of AVP, dDAVP, and dVDAVP. Our findings on the desGly(NH$_2$) analogues will show that our original conclusions with regard to a critical requirement for a C-terminal CONH$_2$ group for activation of V_2 receptors are no longer valid. We also report the synthesis and more complete pharmacological properties of the aforementioned desGly and desGly(NH$_2$) analogues of V_1 and V_2/V_1 antagonists, together with the desGly and desGly(NH$_2$) analogues of three additional AVP V_1 antagonists, [1-(β-mercapto-β,β-pentamethylenepropionic acid),4-valine,8-D-arginine]vasopressin (d(CH$_2$)$_5$VDAVP),[13] [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin (dPVDAVP),[14] and [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin (d(CH$_2$)$_5$[Tyr(Me)^2]AVP),[3,15] and of the selective V_2/V_1 antagonist [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-isoleucine]arginine-vasopressin (d-(CH$_2$)$_5$[D-Ile^2,Ile^4]AVP.[15,16] We thus report the synthesis and some pharmacological properties of the following 24 desGly and desGly(NH$_2$) analogues. AVP agonists: **1a**, desGlyAVP; **1b**, desGly(NH$_2$)AVP; **2a**, desGlydDAVP; **2b**, desGly(NH$_2$)dDAVP; **3a** desGlydVDAVP; **3b**, desGly-(NH$_2$)dVDAVP. AVP V_1 antagonists: **4a**, desGlyd-(CH$_2$)$_5$AVP; **4b**, desGly(NH$_2$)d(CH$_2$)$_5$AVP; **5a**, desGlyd-(CH$_2$)$_5$VDAVP; **5b**, desGly(NH$_2$)d(CH$_2$)$_5$VDAVP; **6a**, desGlydPVDAVP; **6b**, desGly(NH$_2$)dPVDAVP; **7a**, desGlyd(CH$_2$)$_5$[Tyr(Me)^2]AVP; **7b**, desGly(NH$_2$)d(CH$_2$)$_5$-[Tyr(Me)^2]AVP. Nonselective AVP V_2/V_1 antagonists: **8a**, desGlyd(CH$_2$)$_5$[D-Phe^2,Ile^4]AVP; **8b**, desGly(NH$_2$)d-(CH$_2$)$_5$[D-Phe^2,Ile^4]AVP; **9a**, desGlyd(CH$_2$)$_5$[D-Phe^2]VAVP; **9b**, desGly(NH$_2$)d(CH$_2$)$_5$[D-Phe^2]VAVP; **10a**, desGlyd-(CH$_2$)$_5$[D-Tyr(Et)^2]VAVP; **10b**, desGly(NH$_2$)d(CH$_2$)$_5$[D-Tyr(Et)^2]VAVP; **11a**, desGlyd(CH$_2$)$_5$[Tyr(Et)^2]VAVP; **11b**, desGly(NH$_2$)d(CH$_2$)$_5$[Tyr(Et)^2]VAVP. Selective AVP V_2/V_1 antagonist: **12a**, desGlyd(CH$_2$)$_5$[D-Ile^2,Ile^4]AVP; **12b**, desGly(NH$_2$)d(CH$_2$)$_5$[D-Ile^2,Ile^4]AVP.

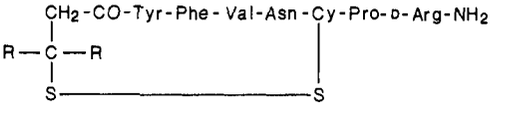
The following are representative structures in each category:

Agonist



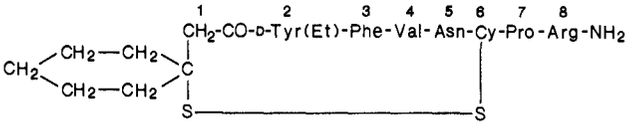
desGly(NH$_2$)dVDAVP

V_1 antagonists



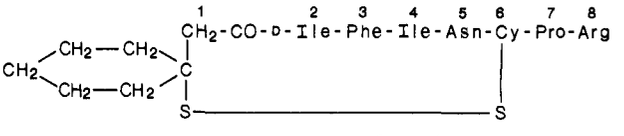
desGlydPVDAVP(R = CH_3) and desGlyd(CH$_2$)$_5$VDAVP(2R = $(CH_2)_5$)

Nonselective V_2/V_1 antagonist



desGlyd(CH$_2$)$_5$[D-Tyr(Et)^2]VAVP

Selective V_2/V_1 antagonist



desGly(NH$_2$)d(CH$_2$)$_5$[D-Ile^2,Ile^4]AVP

Peptide Synthesis. The protected precursors required for the synthesis of the desGly(NH$_2$) analogues of AVP, d(CH$_2$)$_5$AVP, and d(CH$_2$)$_5$[D-Phe^2]VAVP were originally synthesized in solution by stepwise couplings mediated by DCCI[17] (with HOBT[18] as an additive) with the appropriate BOC-amino acid derivatives, starting from BOC-Arg-(Tos)-*O*-Bzl in the case of the AVP and d(CH$_2$)$_5$AVP precursors and from a custom synthesized hexapeptide, BOC-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-*O*-Bzl (Alpha I Biochemicals), for the d(CH$_2$)$_5$[D-Phe^2]VAVP precursor.[19] Here we report the resyntheses, using the Merrifield solid-phase method,[20,21] and starting from either BOC-Arg-(Tos)-resin or BOC-D-Arg(Tos)-resin, of these three protected precursors and the syntheses of the remaining nine desGly(NH$_2$) analogues and of the 12 desGly analogue precursors. The procedures used were virtually identical

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Table I. Effects of desGly and desGly(NH_2) on Antidiuretic (V_2) and Vasopressor (V_1) Activities of Arginine-vasopressin (AVP), [1-Deamino,8-D-arginine]vasopressin (dDAVP), [1-Deamino,4-valine,8-D-arginine]vasopressin (dVDAVP)

no.	peptide[h]	antidiuretic activity (units/mg ± SE)	antivasopressor	
			effective dose,[a] nmol/kg	pA_2[b,c]
1	*AVP*[d]	330 ± 23	agonist (382 ± 5 units/mg)	
1a	desGlyAVP[e]	164 ± 4	partial agonist (<0.05 units/mg) partial antagonist ~60	~6.10
1b	desGly(NH_2)AVP[e]	5.6 ± 1.1	partial agonist (~0.02 units/mg)	
2	*dDAVP*[d]	1200 ± 126	agonist (0.39 ± 0.02 units/mg)	
2a	desGlydDAVP[f]	149 ± 16	26 ± 2	6.42 ± 0.04
2b	desGly(NH_2)dDAVP[f]	73 ± 8	298 ± 39	5.36 ± 0.06
3	*dVDAVP*[d]	1230	7.2 ± 1.4	7.03 ± 0.11
3a	desGlydVDAVP[f]	389 ± 60	20 ± 4	6.55 ± 0.08
3b	desGly(NH_2)dVDAVP[f]	321 ± 71	190 ± 24	5.55 ± 0.05
	desGly(NH_2)dVDAVP[g]	0.024	8.4 ± 0.8	6.90 ± 0.04

[a] The effective dose (ED) is defined as the dose (in nanomoles/kilogram) that reduces the response seen with $2x$ units of agonist to equal the response seen with $1x$ unit of agonist administered before antagonist. [b] Estimated in vivo pA_2 values represent the negative logarithms of the effective dose divided by the estimated volume of distribution (67 mL/kg). [c] Means ± SE. [d] Values reported in Manning et al.[12b] [e] From Manning et al.[2] [f] This publication. [g] Values for incorrect synthesis of **3b** as reported in ref 10 and 11. [h] DesGly, desglycine (Carboxy terminus is Arg(NH_2); desGly(NH_2), desglycinamide (carboxy terminus is Arg(OH).

Table II. Effects of DesGly/DesGly(NH_2) Modifications on Vasopressor (V_1) Antagonistic and Antidiuretic (V_2) Agonistic Potencies of Vasopressor Antagonists

no.	antagonist[j]	antidiuretic act (V_2), units/mg	antivasopressor act.	
			effective dose (ED),[a] nmol/kg	pA_2[b,c]
4	$d(CH_2)_5AVP$[d]	agonist 0.03	0.56 ± 0.11	8.16 ± 0.09
4a	desGlyd$(CH_2)_5$AVP[e]	antagonist [ED > 455 pA_2 < 5.2]	0.27 ± 0.04	8.40 ± 0.06
4b	desGly(NH_2)d$(CH_2)_5$AVP[e]	0.04	0.72 ± 0.07	7.88 ± 0.06
5	$d(CH_2)_5VDAVP$[f]	partial agonist 0.01	1.5 ± 0.2	7.68 ± 0.05
5a	desGlyd$(CH_2)_5$VDAVP[g]	0.075 ± 0.008	4.1 ± 0.8	7.25 ± 0.11
5b	desGly(NH_2)d$(CH_2)_5$VDAVP[g]	partial agonist 0.02	13.0 ± 2	6.73 ± 0.06
6	*dPVDAVP*[h]	123 ± 22	1.1 ± 0.3	7.82 ± 0.05
6a	desGlydPVDAVP[g]	5.8 ± 0.6	4.0 ± 0.8	7.25 ± 0.09
6b	desGly(NH_2)dPVDAVP[g]	4.6 ± 0.4	8.2 ± 1.0	6.92 ± 0.05
7	$d(CH_2)_5[Tyr(Me)^2]AVP$[d]	0.31 ± 0.07	0.16 ± 0.01	8.62 ± 0.03
7a	desGlyd$(CH_2)_5$[Tyr(Me)^2]AVP[g,i]	antagonist [ED 54 ± 5 pA_2 6.10 ± 0.04]	0.27 ± 0.06	8.44 ± 0.10
7b	desGly(NH_2)d$(CH_2)_5$[Tyr(Me)^2]AVP[g,i]	antagonist [ED 280 ± 28 pA_2 5.38 ± 0.04]	0.23 ± 0.02	8.46 ± 0.05

[a-c] See corresponding footnotes to Table I. [d] From Kruszynski et al.[3] [e] From Manning et al.[2] [f] From Lowbridge et al.[13] [g] This publication. [h] From Manning et al.[14] [i] From ref 15. [j] The abbreviations of the parent peptides and their full names: d$(CH_2)_5$AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid)] arginine-vasopressin; d$(CH_2)_5$VDAVP, [1-(β-mercapto-β,β-pentamethylenepropionic), 4-valine,8-D-arginine]vasopressin; dPVDAVP, [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin; d$(CH_2)_5$Tyr(Me)AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin; DesGly, DesGly(NH_2), see footnote *h*, Table I.

with those previously described for the synthesis of their Gly(NH_2)-containing parent molecules[3,7,9,12b,13,14,16,22] except that one less cycle of deprotection, neutralization, and coupling was required. HCl (1 N)/AcOH was used in all the deprotection steps except those involving BOC-Gln in which TFA was employed.[25] Neutralizations were carried out with 10% $Et_3N/MeCl_2$. Coupling reactions were mediated primarily by the preactivated DCCI/HOBT method[17,18] in $MeCl_2$ except for BOC-Asn and BOC-Gln, which were incorporated as their nitrophenyl active ester[23] derivatives in DMF. Ammonolytic cleavage[24,25] was used to generate the 12 desGly protected peptides. Acidolytic cleavage with HBr/TFA[21,26] was used to liberate the 12 desGly(NH_2) fully protected and, in some instances, partially protected peptides. All 24 protected peptides were purified by classical organic chemistry techniques[16] in the usual manner prior to deblocking with Na in NH_3,[27] as previously described.[16] Each of the deblocked disulfhydryl compounds was oxidized with dilute potassium ferricyanide[28] solution, and the resulting cyclized analogues were purifed by gel filtration on Sephadex G-15 as previously described.[29]

Bioassay Methods. The agonistic and antagonistic potencies of these analogues were measured by previously described methods.[30] These were antidiuretic assays in rats under ethanol anesthesia[31] and intravenous vasopressor assays in phenoxybenzamine-treated rats under urethane anesthesia.[32] The USP posterior pituitary reference standard was used in all assays for agonistic and antagonistic activities. Agonistic activities are expressed in units/milligram. Antagonistic potencies were determined and expressed as effective doses and pA_2 values were estimated. The effective dose is defined as the dose (in nanomoles/kilogram) that reduces the response seen from $2x$ units of agonist to the response seen with $1x$ unit of agonist.[33] Estimated in vivo pA_2 values represent the

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Table III. Effects of DesGly/DesGly(NH_2) Modifications on Antidiuretic (V_2) and Vasopressor (V_1) Antagonistic Potencies and Selectivities of AVP V_2/V_1 and a Selective V_2 Antagonist

		antiantidiuretic potency		antivasopressor potency		
no.	antagonist[m]	ED,[a] nmol/kg	pA_2[b,c]	ED,[a] nmol/kg	pA_2[b,c]	ED[d] ratio
8	*d*(CH_2)$_5$[D-*Phe*^{2},*Ile*^{4}]*AVP*[e]	0.46 ± 0.07	8.24 ± 0.01	0.99 ± 0.12	7.86 ± 0.05	2.0
8a	desGlyd(CH_2)$_5$[D-Phe^2,Ile^4]AVP[f]	0.66 ± 0.17	8.05 ± 0.09	0.45 ± 0.04	8.17 ± 0.04	0.7
8b	desGly(NH_2)d(CH_2)$_5$[D-Phe^2,Ile^4]AVP[g]	4.6 ± 1.2	7.20 ± 0.1	14 ± 2	6.74 ± 0.08	3.0
9	*d*(CH_2)$_5$[D-*Phe*^{2}]*VAVP*[h]	0.67 ± 0.13	8.06 ± 0.09	0.58 ± 0.04	8.06 ± 0.03	0.9
9a	desGlyd(CH_2)$_5$[D-Phe^2]VAVP[f]	0.58 ± 0.11	8.09 ± 0.08	0.47 ± 0.04	8.15 ± 0.03	0.8
9b	desGly(NH_2)d(CH_2)$_5$[D-Phe^2]VAVP[f]	1.3 ± 0.4	7.75 ± 0.11	0.80 ± 0.08	7.93 ± 0.05	0.6
10	*d*(CH_2)$_5$[D-*Tyr*(*Et*)^2]*VAVP*[i]	1.1 ± 0.2	7.81 ± 0.07	0.45 ± 0.11	8.22 ± 0.12	0.4
10a	desGlyd(CH_2)$_5$[D-Tyr(Et)^2]VAVP[f]	1.5 ± 0.3	7.69 ± 0.08	0.45 ± 0.04	8.17 ± 0.04	0.3
10b	desGly(NH_2)d(CH_2)$_5$[D-Tyr(Et)^2]VAVP[g]	1.1 ± 0.08	7.78 ± 0.03	1.20 ± 0.04	7.77 ± 0.02	1.1
11	*d*(CH_2)$_5$[*Tyr*(*Et*)^2]*VAVP*[j]	1.9 ± 0.2	7.57 ± 0.02	0.49 ± 0.11	8.16 ± 0.09	0.26
11a	desGlyd(CH_2)$_5$[Tyr(Et)^2]VAVP[f]	0.95 ± 0.2	7.89 ± 0.09	0.45 ± 0.02	8.18 ± 0.02	0.47
11b	desGly(NH_2)d(CH_2)$_5$[Tyr(Et)^2]VAVP[g]	2.0 ± 0.5	7.56 ± 0.1	1.1 ± 0.1	7.80 ± 0.03	0.55
12	*d*(CH_2)$_5$[D-*Ile*^{2},*Ile*^{4}]*AVP*[k]	0.67 ± 0.15	8.04 ± 0.10	26 ± 3	6.42 ± 0.06	39
12a	desGly[D-Ile^2,Ile^4]AVP[g,l]	0.37 ± 0.03	8.26 ± 0.04	~17 (mixed)	6.7	46
12b	desGly(NH_2)[D-Ile^2,Ile^4]AVP[g,l]	0.90 ± 0.1	7.88 ± 0.05	~400	5.2	440

[a-c] See corresponding footnotes to Table I. [d] ED ratio = antivasopressor ED/antiantidiuretic ED. [e] From ref 7. [f] From ref 2. [g] This publication. [h] From ref 4. [i] From ref 6. [j] From ref 5. [k] From ref 16. [l] From ref 15. [m] Abbreviations of the parent peptides and their full names: d(CH_2)$_5$[D-Phe^2,Ile^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-phenylalanine,4-isoleucine]arginine-vasopressin; d(CH_2)$_5$[D-Phe^2]VAVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-phenylalanine,4-valine]arginine-vasopressin; d(CH_2)$_5$[Tyr(Et)^2]VAVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyltyrosine,4-valine]arginine-vasopressin; d(CH_2)$_5$[D-Ile^2,Ile^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-isoleucine]arginine-vasopressin; DesGly/DesGly(NH_2), see footnote *h*, Table I.

negative logarithms of the effective doses divided by the estimated volume of distribution (67 mL/kg). Inhibition of antidiuretic responses was tested, in some cases, by injecting the standard 20 min after injecting the antagonist, to allow for recovery from the initial antidiuretic responses to some of the antagonists. For those analogues showing no agonistic activity, the standard could be injected 10 min after the antagonist. Each peptide was administered in two doses, a high dose, which reduced the response to 2*x* units of agonist to less than the response to 1*x* units of agonist, and a low dose, which did not fully reduce the response to that given by 1*x* unit of agonist. In each case, the effective dose was obtained by interpolation on a logarithmic scale between the two doses of antagonist.[33] The values for effective doses and pA_2's presented in the tables are means ± SE's of at least four independent estimates for each analogue.

Results and Discussion

The pharmacological properties of the desGly/desGly(NH_2) AVP agonists and antagonists and their respective parents are given in Tables I–III.

Effects of Deletions of Gly and Gly(NH_2) from AVP Agonists (Table I). (A) Effects on V_2 Agonism. The data in Table I clearly show that, for dDAVP and dVDAVP, the desGly(NH_2) modification does not have as drastic effects as was observed for AVP. Thus, desGly(NH_2)AVP retains only approximately 2% of the antidiuretic activity of AVP, whereas desGly(NH_2)dDAVP and desGly(NH_2)dVDAVP retain approximately 6% and 26% of the antidiuretic activities of their respective parents. The data for the resynthesized desGly(NH_2)dVDAVP is particularly noteworthy, for it represents a correction of preliminary data from an earlier synthesis that indicated that desGly(NH_2)dVDAVP had antidiuretic activity of only 0.024 units/mg.[10,11] Although the product from the incorrect synthesis of desGly(NH_2)dVDAVP has not been fully characterized, it now appears that the error in synthesis was due to a simple mix-up of the protected precursors of desGly(NH_2)dVDAVP and of its methyl ester, leading to the latter being reduced in the Na/liquid NH_3 step rather than the former. The mistake was not uncovered by amino acid analysis or by TLC examination and was not even suspected until we found that desGly(NH_2)dDAVP exhibited the unexpectedly high antidiuretic activity of 73 ± 8 units/mg reported here. With antidiuretic activity of 321 units/mg, resynthesized desGly(NH_2)dVDAVP is about as potent as AVP itself.

Deletion of Gly resulted in retention of appreciable antidiuretic activity by all three compounds. However, in the case of AVP, the desGlyAVP analogue retained the highest percentage, i.e., approximately 50% as compared to about 12% and 32% for desGlydDAVP and desGlydVDAVP, respectively.

(B) Effects on V_1 Agonism and V_1 Antagonism. The effects on vasopressor activities of the desGly/desGly(NH_2) modifications were highly consistent for all three analogues. For AVP, a potent V_1 agonist, these modifications resulted in drastic losses of vasopressor activity. desGlyAVP and desGly(NH_2)AVP are weak partial agonists. desGlyAVP is also a very weak V_1 antagonist. Deletion of Gly and Gly(NH_2) from dDAVP, which is a weak V_1 agonist, resulted in two very weak V_1 antagonists. Deletion of Gly/Gly(NH_2) from dVDAVP resulted in V_1 antagonists that have only approximately 5% and 0.5% of the V_1-antagonistic potency of dVDAVP itself.

These data further point to the hazards of basing structure–activity interpretations on one or two analogues. The drastic losses of V_2-agonistic activity exhibited by desGly(NH_2)AVP and by AVP-acid prompted the wrong conclusion about the necessity for the C-terminal $CONH_2$ group for V_2-receptor activation.[2] This appeared to be further substantiated by the extremely low V_2 agonism of the incorrectly synthesized desGly(NH_2)dVDAVP. From the data reported here, however, we can conclude that while the C-terminal Gly and Gly(NH_2) are important in preserving substantial V_1-agonistic activity, neither is as important in determining V_2-agonistic activity.

Effects of DesGly/DesGly(NH_2) Modifications on V_1 Antagonists (Table II). Deletion of the C-terminal Gly or Gly(NH_2) from the four V_1 antagonists in Table II resulted in molecules that retained V_1 antagonism but drastically reduced V_2-agonistic activity in those analogues of parent analogues that had appreciable V_2-agonistic potency. In all but one case, i.e., desGlyd(CH_2)$_5$AVP (**4a**), all of the desGly and desGly(NH_2) analogues were less

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potent as V_1 antagonists than their respective parents. Remarkably, desGlyd$(CH_2)_5$AVP is almost twice as potent as its parent. In three of the four analogues, the desGly-(NH_2) modification effected a significantly greater loss of antagonism than the desGly modification. However, for d$(CH_2)_5$[Tyr(Me)^2]AVP, the most potent V_1 antagonist of the series, the desGly/desGly(NH_2) modifications resulted in highly potent V_1 antagonists.[15] Remarkably also, the weak but definite antidiuretic activity of d$(CH_2)_5$[Tyr-(Me)^2]AVP[3] was completely abolished by both the desGly and desGly(NH_2) modifications. The two resulting analogues exhibited weak but definite antidiuretic antagonism. Both are thus highly selective V_1 antagonists with anti-V_1/V_2 selectivities of 200 and 1200, respectively. So, from this series of V_1 antagonists we can conclude that the desGly and desGly(NH_2) modifications result in (a) retention of V_1 antagonism (however, the degree of retention is very much structure-dependent) and (b) no change, reductions (and in some cases abolishment) in V_2 agonism, with inconsistent differences between the desGly and desGly(NH_2) modifications, and (c) some of the most selective V_1 antagonists known to date.

Effects of DesGly/DesGly(NH_2) Modifications on AVP V_2/V_1 and AVP V_2 Antagonists (Table III). Preliminary reports on the properties of the desGly/des-Gly(NH_2) analogues in Table III have been presented elsewhere.[10,11,15] They all retain potent V_2 antagonism and, with the exception of the d$(CH_2)_5$[D-Ile^2,Ile^4]AVP analogues, potent V_1 antagonism.

(A) Relative Effects of DesGly/DesGly(NH_2) Modifications on V_2 Antagonism. The desGly modification resulted in a twofold enhancement in two cases and virtually full retention of V_2 antagonism in the remaining three cases. Thus, the desGly analogues of d$(CH_2)_5$[Tyr-(Et)^2]VAVP and d$(CH_2)_5$[D-Ile^2,Ile^4]AVP exhibited a twofold enhancement of V_2 antagonism relative to their respective parents, and the desGly analogues of d-$(CH_2)_5$[D-Phe^2]VAVP, d$(CH_2)_5$[D-Phe^2,Ile^4]AVP, and d-$(CH_2)_5$[D-Tyr(Et)^2]VAVP are virtually equipotent with their respective parent analogues. The desGly(NH_2) modifications resulted in virtually full retention of V_2 antagonism in three cases, a 50% reduction in one case and a 10-fold reduction in another. Thus the desGly(NH_2) analogues of d$(CH_2)_5$[D-Tyr(Et)^2]VAVP, d$(CH_2)_5$[Tyr-(Et)^2]VAVP, and d$(CH_2)_5$[D-Ile^2,Ile^4]AVP are virtually equipotent with their parents, whereas the desGly(NH_2) analogues of d$(CH_2)_5$[D-Phe^2]VAVP and d$(CH_2)_5$[D-Phe^2,Ile^4]VAVP are only $^1/_2$ and $^1/_{10}$ as potent as their parents, respectively. Thus the effects of the desGly(NH_2) modification appear to be much more structure-dependent than are those of the desGly modification.

(B) Relative Effects of desGly/desGly(NH_2) Modifications on V_1 Antagonism (Table III). The desGly modifications led to virtually full retention of V_1 antagonism in four out of the five analogues (**9a–12a**, Table **III**) and a twofold enhancement of V_1 antagonism in the remaining one, i.e., desGlyd$(CH_2)_5$[D-Phe^2,Ile^4]AVP (**8a**, Table III). By contrast, the desGly(NH_2) modification of all five analogues resulted in a decrease in V_1 antagonism (approximately 1.5–15-fold). Thus the desGly analgoue of d$(CH_2)_5$[D-Phe^2,Ile^4]AVP is twice as potent as its parent, whereas the desGly(NH_2) analogue is 14 times less potent than its parent. Thus, for this pair, the desGly analogue is 30 times more potent as a V_1 antagonist than its des-Gly(NH_2) counterpart. The desGly analogues of d-$(CH_2)_5$[D-Phe^2]VAVP, d$(CH_2)_5$[D-Tyr(Et)^2]VAVP, and d$(CH_2)_5$[Tyr(Et)^2]VAVP are virtually as potent V_1 antagonists as their respective parents and are each approximately twice as potent as the corresponding des-Gly(NH_2) analgoues. The desGly analogue of d$(CH_2)_5$[D-Ile^2,Ile^4]AVP appears to be somewhat more potent than its parent but is approximately 15 times more potent than the desGly(NH_2) analogue. from these data it is clear that for all but one of the four V_1 antagonists in Table II and for the V_2/V_1 antagonists in Table III the desGly modification results in greater retention of V_1 antagonism than the desGly(NH_2) modification.

Effects of DesGly/DesGly(NH_2) Modifications on Anti-V_2/V_1 Selectivity (Table III). For the V_2/V_1 antagonists in Table III no consistent pattern of increases or decreases in anti-V_2/V_1 selectively is evident. Some analogues exhibit very modest gains in selectivity, while for others slight losses were observed. However, for the highly selective V_2/V_1 antagonist d$(CH_2)_5$[D-Ile^2,Ile^4]AVP,[16] the desGly modification led to a modest increase in selectivity from 39 to 46 while the desGly(NH_2) modification resulted in a striking increase in selectivity. With an ED ratio of about 440, desGly(NH_2)d$(CH_2)_5$[D-Ile^2,Ile^4]AVP is clearly the most selective AVP V_2 antagonist reported to date.

Conclusions

Deletion of the C-terminal Gly and C-terminal Gly(NH_2) from AVP and the two highly selective V_2 agonists dDAVP and dVDAVP led to drastic reductions in V_1 agonism. Remarkably, the drastic loss of V_2-agonistic activities previously observed for desGly(NH_2)AVP[1,2] and for incorrectly synthesized desGly(NH_2)dVDAVP[10,11] was not found for desGly(NH_2)dDAVP or for a resynthesized version of desGly(NH_2)dVDAVP. The later is equipotent with AVP as a V_2 agonist. In the light of these findings, our previous conclusion, about the necessity of a C-terminal $CONH_2$ for activation of V_2 receptors, requires revision. This assertion is no longer valid. These findings, together with those for the series of four AVP V_1 antagonists and five AVP V_2/V_1 antagonists, indicate that a C-terminal GlyNH_2 or a C-terminal Gly is required neither for binding to nor for activation of AVP V_2 receptors. However, our prior conclusions that the C-terminal Gly-(NH_2) is not required for binding to V_1 receptors but is required for activation of V_1 receptors appear to remain tenable. These differences in the C-terminal structural requirements for binding to and activation of V_1 receptors are highly reminiscent of the contrasting effects of a proline deletion in AVP and in AVP antagonists[15,39,40] and would appear to further substantiate the view that AVP agonists and antagonists bind differently to AVP receptors.[39-41] These studies have also brought to light the highly selective

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Table IV. Physicochemical Properties of the Protected Des-Gly Precursors (Ia–XIIa)[a]

no.	protected desGly peptide	formula	yield,[b,c] %	mp, °C	$[\alpha]^{25}_D$ (c 1, DMF)	R_f B	R_f C	R_f D
Ia	AVP	$C_{80}H_{94}N_{14}O_{15}S_3 \cdot 2H_2O$	29.0	219–220	−22.0[d]	0.43	0.60	0.87
IIa	dDAVP	$C_{72}H_{87}N_{13}O_{13}S_3 \cdot 2H_2O$	63.0	193–196	−21.3	0.75	0.79	0.72
IIIa	dVDAVP	$C_{72}H_{89}N_{12}O_{12}S_3 \cdot 2H_2O$	61.0	220–224	−26.0	0.85	0.92	0.79
IVa	$d(CH_2)_5$AVP	$C_{77}H_{95}N_{13}O_{13}S_3 \cdot 2H_2O$	42.0	198–201	−37.7	0.52	0.80	0.82
Va	$d(CH_2)_5$VDAVP	$C_{77}H_{96}N_{12}O_{12}S_3 \cdot 2H_2O$	70.0	80–183	−35.0	0.85	0.87	0.88
VIa	dPVDAVP	$C_{74}H_{91}N_{12}O_{12}S_3 \cdot 2H_2O$	70.3	184–187	−35.5	0.77	0.96	0.96
VIIa	$d(CH_2)_5$[Tyr(Me)^2]AVP	$C_{71}H_{97}N_{12}O_{12}S_3 \cdot H_2O$	26.6	177–180	−36.5	0.78	0.80	0.70
VIIIa	$d(CH_2)_5$[D-Phe^{2}Ile^4]AVP	$C_{71}H_{92}N_{12}O_{11}S_3 \cdot 2H_2O$	47.8	217–219	−20.1	0.70	0.81	0.81
IXa	$d(CH_2)_5$[D-Phe^2]VAVP	$C_{70}H_{90}N_{12}O_{11}S_3$	58.6	222–225	−21.0	0.57	0.88	0.75
Xa	$d(CH_2)_5$[D-Tyr(Et)^2]VAVP	$C_{72}H_{94}N_{12}O_{12}S_3 \cdot 2H_2O$	48.6	224–226	−11.5	0.73	0.79	0.71
XIa	$d(CH_2)_5$[Tyr(Et)^2]VAVP	$C_{72}H_{94}N_{12}O_{12}S_3 \cdot 2H_2O$	39.5	218–219	−42.3	0.57	0.84	0.93
XIIa	$d(CH_2)_5$[D-Ile^2,Ile^4]AVP	$C_{68}H_{94}N_{12}O_{11}S_3$	52.9	209–210	−27.1	0.67	0.73	0.84

[a] Structures of the linear protected sequences are given in the Experimental Section. [b] Yields were calculated on the basis of the arginine content of the starting resin. [c] All the protected peptides gave the expected amino acid ratios after hydrolysis ± 3%. [d] c = 0.5, DMF.

Table V. Physicochemical Properties of the Protected DesGly(NH_2) Precursors (Ib–XIIb)[a]

no.	protected desGly(NH_2) peptides	formula	yield,[b,c] %	mp, °C	$[\alpha]^{25}_D$ (c 1, DMF)	R_f B	R_f C	R_f D
Ib	AVP·HBr	$C_{72}H_{87}N_{13}O_{14}S_3 \cdot HBr$	35.5	122–125	−5.5	0.49	0.68	0.39
IIb	dDAVP	$C_{72}H_{86}N_{12}O_{14}S_3 \cdot 2H_2O$	52.6	172–174	−37.5	0.62	0.83	0.10
IIIb	dVDAVP	$C_{72}H_{88}N_{11}O_{13}S_3 \cdot 2H_2O$	89.6	180–182.5	−35.5	0.80	0.84	0.29
IVb	$d(CH_2)_5$AVP	$C_{77}H_{92}N_{12}O_{14}S_3 \cdot 2H_2O$	19.7	188–190	−34.4	0.70	0.75	0.41
Vb	$d(CH_2)_5$VDAVP	$C_{77}H_{95}N_{11}O_{13}S_3 \cdot 2H_2O$	87.5	138–140	−34.5	0.74	0.79	0.30
VIb	dPVDAVP	$C_{74}H_{90}N_{11}O_{13}S_3 \cdot 2H_2O$	86.5	144–145	−43.2	0.79	0.84	0.69
VIIb	$d(CH_2)_5$[Tyr(Me)^2]AVP	$C_{71}H_{94}N_{12}O_{16}S_3 \cdot 2H_2O$	60.3	182–186	−21.5[d]	0.67	0.82	0.08
VIIIb	$d(CH_2)_5$[D-Phe^2,Ile^4]AVP	$C_{71}H_{91}N_{11}O_{12}S_3 \cdot 2H_2O$	60.4	179–182	−19.5	0.78	0.85	0.34
IXb	$d(CH_2)_5$[D-Phe^2]VAVP	$C_{70}H_{89}N_{11}O_{12}S_3 \cdot 2H_2O$	27.0	180–183	−24.9	0.77	0.82	0.30
Xb	$d(CH_2)_5$[D-Tyr(Et)^2]VAVP	$C_{72}H_{93}N_{11}O_{13}S_3 \cdot 2H_2O$	56.5	192–194	−25.7	0.76	0.86	0.23
XIb	$d(CH_2)_5$[Tyr(Et)^2]VAVP	$C_{72}H_{93}N_{11}O_{13}S_3 \cdot 2H_2O$	41.0	204–206	−38.1	0.72	0.78	0.28
XIIb	$d(CH_2)_5$[D-Ile^2,Ile^4]AVP	$C_{68}H_{93}N_{11}O_{12}S_3 \cdot 2H_2O$	40.3	221–223	−26.0	0.62	0.71	0.32

[a] Structures of the linear protected sequences are given in the Experimental Section. [b] Yields were calculated on the basis of the arginine content of the starting resin. [c] All the protected peptides gave the expected amino acid ratios after hydrolysis ± 3%. [d] c = 0.5, DMF.

Table VI. Physicochemical Properties of the DesGly Peptides (**1a–12a**)

no.	desGly peptide	yield,[a,b] %	$[\alpha]^{25}_D$ (c = 0.3, 1 N AcOH)	R_f B	R_f A	R_f C
1a	AVP	45.4	−9.3	0.23	0.03	0.24
2a	dPAVP	60.0	−65.0	0.20	0.06	0.31
3a	dVDAVP	23.7	−83.3	0.24	0.39	0.49
4a	$d(CH_2)_5$AVP	37.1	−42.7	0.33	0.24	0.43
5a	$d(CH_2)_5$VDAVP	42.6	−31.7	0.29	0.28	0.64
6a	dPVDAVP	25.0	−37.6	0.30	0.04	0.40
7a	$d(CH_2)_5$[Tyr(Me)^2]AVP	34.6	−63.3	0.33	0.21	0.51
8a	$d(CH_2)_5$[D-Phe^2,Ile^4]AVP	28.6	−115.4	0.40	0.41	0.63
9a	$d(CH_2)_5$[D-Phe^2]VAVP	56.8	−111.2	0.39	0.39	0.59
10a	$d(CH_2)_5$[D-Tyr(Et)^2]VAVP	31.6	−95.2	0.38	0.37	0.60
11a	$d(CH_2)_5$[Tyr(Et)^2]VAVP	37.3	−38.0	0.41	0.32	0.65
12a	$d(CH_2)_5$[D-Ile^2,Ile^4]yAVP	15.0	−6.5	0.35	0.34	0.61

[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%.

AVP V_1/V_2 antagonists, desGly- and desGly(NH_2)d-$(CH_2)_5$[Tyr(Me)^2]AVP[15], some highly potent AVP V_2/V_1 antagonists, and the most selective AVP V_2 antagonist reported to date, desGly(NH_2)d$(CH_2)_5$[D-Ile^2,Ile^4]AVP.[15] Many of these peptides are valuable new pharmacological tools for studies on the physiological and pathophysiological roles of AVP.

Experimental Section

Amino acid derivatives were purchased from Bachem Inc. or from Chemalog Inc. BOC-D-Tyr(Et),[34] β-(benzylthio)propionic acid,[28] β-(benzylthio)-β,β-dimethylpropionic acid,[35] and β-(benzylthio)-β,β-pentamethylenepropionic acid[36] were synthesized by previously published procedures. The protected precursors required for the synthesis of the 12 desGly analogues and the 12 desGly(NH_2) peptides were synthesized by the manual solid-phase method[20,21] as described below, using previously described modifications employed for the synthesis of their respective Gly-(NH_2)-containing parent analogues.[3,7,9,12b,13,14,16,22] The physicochemical properties of the purified 12 desGly and the 12 desGly(NH_2) protected peptides are given in Tables IV and V, respectively. All 24 protected precursors were converted to the required free cyclized peptides by deblocking with Na/liquid NH_3,[27] oxidation with dilute ferricyanide solution,[28] desalting, and purification in a two-step procedure using gel filtration on Sephadex G-15 as previously described.[29] The physicochemical properties of the free desGly and desGly(NH_2) peptides are given in Tables VI and VII, respectively. The conversion of the protected precursor for desGly(NH_2)d$(CH_2)_5$[Tyr(Me)]AVP (VIIb, Table V) to its free cyclized derivative (**7b**, Table VII) is described below. This description is representative of the procedure followed for the synthesis of all of the remaining 23 peptides in Tables VI and VII from their respective precursors in Tables IV and V. Dimethylformamide (DMF) was distilled under reduced pressure. Other solvents and reagents were analytical grade. Thin-layer chromatography (TLC) was 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–

Table VII. Physicochemical Properties of the DesGly(NH_2) Peptides (**1b–12b**)

no.	desGly(NH_2) peptide	yield,[a,b] %	$[\alpha]^{25}_D$ (*c* 0.3, 1 N AcOH)	R_f B	R_f A	R_f C
1b	AVP	48.3	−13.3	0.23	0.07	0.18
2b	dDAVP	31.5	−76.7	0.27	0.02	0.23
3b	dVDAVP	54.7	−85.0	0.22	0.24	0.51
4b	$d(CH_2)_5$AVP	44.0	−70.0	0.33	0.27	0.32
5b	$d(CH_2)_5$VDAVP	29.2	−31.6	0.42	0.40	0.48
6b	dPVDAVP	57.8	−23.7	0.38	0.41	0.49
7b	$d(CH_2)_5$[Tyr(Me)^2]AVP	30.3	−50.7	0.25	0.16	0.30
8b	$d(CH_2)_5$[D-Phe^2,Ile^4]AVP	24.2	−125.0	0.38	0.45	0.45
9b	$d(CH_2)_5$[D-Phe^2]VAVP	22.5	−144.0	0.39	0.40	0.46
10b	$d(CH_2)_5$[D-Tyr(Et)^2]VAVP	16.2	−104.3	0.38	0.30	0.50
11b	$d(CH_2)_5$[Tyr(Et)^2]VAVP	18.4	−49.8	0.36	0.42	0.57
12b	$d(CH_2)_5$[D-Ile^2,Ile^4]AVP	31.4	−82.0	0.30	0.32	0.44

[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%.

water–pyridine (15:3:3:10, v/v/v/v), (D) chloroform–methanol (7:3, v/v). Loads of 10–50 μg were appplied and chromatograms were a minimum length of 10 cm. Iodine vapor was used for detection.[9] 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,[37] 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 121M Beckman automatic amino acid analyzer. Molar ratios were referred to Arg or Phe = 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%.

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-Arg(Tos) or BOC-D-Arg(Tos) to an incorporation of approximately 0.25 mmol/g by the cesium salt method.[38] Seven separate cycles of deprotection, neutralization, and coupling were carried out for the synthesis of the 12 peptidyl resins required for the preparation of the 12 pairs of desGly and desGly(NH_2) protected precursors. The following amino acid derivatives were utilized in successive but separate coupling steps, starting from either BOC-Arg(Tos)-resin or BOC-D-Arg(Tos)-resin. First coupling BOC-Pro; second coupling BOC-Cys(Bzl); third coupling BOC-Asn NPE; fourth coupling BOC-Gln NPE, BOC-Val, BOC-Ile; fifth coupling BOC-Phe; sixth coupling BOC-Tyr(Bzl), BOC-Tyr(Me), BOC-D-Tyr(Et), BOC-D-Phe, BOC-D-Ile. DCCI[17]/HOBT[18] was employed to mediate all couplings other than those involving Asn and Gln, which were incorporated as their respective nitrophenyl esters.[23] For the final separate coupling steps the following derivatives were used: Z-Cys(Bzl), β-(benzylthio)propionic acid,[28] β-(benzylthio)-β,β-dimethylpropionic acid,[35] and β-(benzylthio-β,β-pentamethylenepropionic acid.[36] HCl (1 N)/AcOH was used in all deprotection steps except those involving BOC-Gln (TFA only, without acetic acid washes, was used). Each protected acylheptapeptide was divided into two portions. For the 12 desGly protected peptides, ammonolysis[24,25] in MeOH was used as the cleaving agent from the resin. For the 12 desGlyNH_2 protected peptides, HBr/TFA[26] in $MeCl_2$ cleavages were performed. All of the protected precursors were purifed 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. The following syntheses of the protected precursors of desGlyd$(CH_2)_5$[D-Ile^2,Ile^4]AVP and of desGly(NH_2)d$(CH_2)_5$-[D-Ile^2,Ile^4]AVP from a common protected peptide resin are representative of methods used to synthesize all of the protected peptides described in Tables IV and V.

[β-(Benzylthio)-β,β-pentamethylenepropionyl]-D-Ile-Phe-Ile-Asn-Cys(Bzl)Pro-Arg(Tos)NH_2 (XIIa, Table IV. (i) **By Ammonolytic Cleavage.**[25] BOC-Arg(Tos)-resin (5.85 g, 2.4 mmol) was placed in a glass synthesis vessel. Manual methods of solid phase synthesis methodology,[20,21] i.e., seven cycles of deprotection with 1 M HCl/AcOH, neutralization with 10% Et_3N in $MeCL_2$, and coupling (mediated by DCCI/HOBT) or active esters, were used to incorporate successively BOC-Pro, BOC-Cys(Bzl), BOC-Asn NPE, BOC-Ile, BOC-Phe, BOC-D-Ile, and β-(benzylthio)-β,β-pentamethylenepropionic acid to give the protected peptidyl resin: [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Ile-Phe-Ile-Asn-Cys(Bzl)-Pro-Arg(Tos)-resin (3.85 g) (A). This peptidyl resin was divided into two portions for ammonolytic cleavage as described here and for acidolytic cleavage as described below.

The protected acyloctapeptide-resin (A) (1.92 g, 0.6 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 in over a period of approximately 30 min. The tightly stoppered flask was kept at room temperature in an explosion-proof environment in the hood for 5 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. 50 mL) and the product precipitated by the addition of H_2O (ca. 700 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 ether (200 mL), collected, and dried in vacuo over P_2O_5 to give the required desGly acylheptapeptide amide (XIIa, Table IV). (Pertinent physiochemical data for all desGly protected peptides are given in Table IV.) The remaining 11 desGly protected precursors were prepared in essentially the same manner by ammonolysis from the appropriate peptidyl resin. The structures of the protected desGly peptides Ia–XIa are as follows: Ia, Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)Pro-Arg(Tos)NH_2; IIa, [β-(benzylthio)-propionyl)]-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg(Tos)NH_2; IIIa, [β-(benzylthio)propionyl]-Tyr(Bzl)-Phe-Val-Asn-Cys-(Bzl)-Pro-D-Arg(Tos)-NH_2; IVa, [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg-(Tos)NH_2; Va, [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)NH_2; VIa, [β-(benzylthio)-β,β-dimethylpropionyl]-Tyr(Bzl)-Phe-Val-Asn-Cys-(Bzl)-Pro-D-Arg-(Tos)NH_2; VIIa, [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg-(Tos)NH_2; VIIIa, [β-(benzylthio)-β,β-pentamethylene-propionyl]-D-Phe-Phe-Ile-Asn-Cys(Bzl)-Pro-Arg(Tos)NH_2; IXa, [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Phe-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)NH_2; Xa, [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg-(Tos)NH_2; XIa, [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)NH_2.

[β-Benzyl-β,β-pentamethylenepropionyl]-D-Ile-Phe-Ile-Asn-Cys(Bzl)Pro-Arg(Tos) (XIIb, Table V). (ii) **By Acidolytic Cleavage.**[26] Hydrogen bromide was bubbled through a suspension of the above protected acylheptapeptide-resin (A) (1.92 g, 0.6 mmol) in TFA (35 mL) and anisole (6 mL) in a glass funnel fitted with a fritted disk as described in Stewart and Young.[21] After 35 min the filtrate was collected. The resin was resuspended in $MeCl_2$ (13 mL), TFA (13 mL), and anisole (6 mL). HBr bubbling was resumed for a further 35 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 evaporation. 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; 581 mg. This material was dissolved in warm DMF (ca. 10 mL), reprecipitated with water, collected, and dried in vacuo over P_2O_5 to give the desired protected acylheptapeptide (XIIb, Table V). The remaining desGly(NH_2) protected precursors were obtained in essentially the same manner by acidolytic cleavage from the appropriate peptidyl resin. Their structures are as follows: Ib, HBr-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos); IIb, [β-(benzylthio)propionyl]-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-D-Arg(Tos); IIIb, [β-(benzylthio)propionyl]-Tyr-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos); IVb, [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg-(Tos); Vb, [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos); VIb, [β-(benzylthio)-β,β-dimethylpropionyl]-Tyr-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos); VIIb, [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos); VIIIb, [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Phe-Ile-Asn-Cys(Bzl)-Pro-Arg(tos); IXb, [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Phe-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos); Xb, [β-(benzylthio)-β,β-pentamethylenepropionyl]-D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg-(Tos); XIb, [β-(benzylthio)-β,β-pentamethylenepropionyl]-Tyr-(Et)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos). The physiochemical properties of all 12 desGlyNH_2 protected peptide (Ib–XIIb) are given in Table V.

Desglycinamide[1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-methyltyrosine]arginine-vasopressin [desGly(NH_2)d(CH_2)$_5$Tyr(Me)AVP] (7b, Table VII). The protected acylheptapeptide amide (VIIb, Tables V) 120 mg) was dissolved in dry ammonia (500 mL) redistilled from sodium. The solution was treated at the boiling point and with stirring with sodium from a stick of sodium contained in a small-bore glass tube until a light-blue color persisted in the solution for ca. 30 s. Dry acetic acid (0.4 mL) 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%, 50 mL) and quickly poured into ice-cold water (ca. 1000 mL). The pH was adjusted to ca. 7.0 with concentrated ammonium hydroxide. Following the neutralization, an excess of a solution of potassium ferricyanide (0.01 M, 15 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 water (200 mL), and the combined filtrate and washings were lyophilized. The resulting powder (2.51 g) 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 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 (43 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 4 mL/h. The peptide was eluted in a single peak (absorbance 254 nm). Lyophilization of the pertinent fractions gave the vasopressin analogue (**7b**, Table VII). Its physiochemical properties are given in Table VII. With minor modifications this procedure was utilized to give all the free peptides in Tables VI and VII.

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Registry No. **1a**, 47914-57-8; **1b**, 37552-33-3; **2a**, 110551-37-6; **2b**, 84236-22-6; **3a**, 110551-38-7; **3b**, 102136-54-9; **4a**, 90332-78-8; **4b**, 90332-79-9; **5a**, 110551-39-8; **5b**, 110612-13-0; **6a**, 110551-40-1; **6b**, 110551-43-4; **7a**, 110551-41-2; **7b**, 110551-44-5; **8a**, 90332-83-5; **8b**, 105107-73-1; **9a**, 90352-21-9; **9b**, 90332-80-2; **10a**, 90332-82-4; **10b**, 105107-72-0; **11a**, 90332-81-3; **11b**, 110567-67-4; **12a**, 110551-42-3; **12b**, 110551-45-6; Ia, 110551-14-9; Ib, 110551-26-3; IIa, 110551-15-0; IIb, 110567-66-3; IIIa, 110551-16-1; IIIb, 110551-27-4; IVa, 110551-17-2; IVb, 110551-28-5; Va, 110551-18-3; Vb, 110551-29-6; VIa, 110551-19-4; VIb, 110551-30-9; VIIa, 110551-20-7; VIIb, 110551-31-0; VIIIa, 110551-21-8; VIIIb, 110551-32-1; IXa, 110551-22-9; IXb, 110551-33-2; Xa, 110551-23-0; Xb, 110551-34-3; Xia, 110551-24-1; XIb, 110551-35-4; XIIa, 110551-25-2; XIIb, 110551-36-5; BOC-Arg(Tos), 13836-37-8; BOC-D-Arg(Tos), 61315-61-5; BOC-Pro, 15761-39-4; BOC-Cys-(Bzl), 5068-28-0; BOC-Asn NPE, 4587-33-1; BOC-Gln NPE, 15387-45-8; BOC-Val, 13734-41-3; BOC-Ile, 13139-16-7; BOC-Phe, 13734-34-4; BOC-Tyr(Bzl), 2130-96-3; BOC-Tyr(Me), 53267-93-9; BOC-D-Tyr(Et), 76757-92-1; BOC-D-Phe, 18942-49-9; BOC-D-Ile, 55721-65-8; Z-Cys(Bzl), 3257-18-9; β-(benzylthio)propionic acid, 2899-66-3; β-(benzylthio)-β,β-dimethylpropionic acid, 7536-39-2; β-(benzylthio)-β,β-pentamethylenepropionic acid, 55154-80-8.

Synthesis and 5-Hydroxytryptamine Antagonist Activity of 2-[[2-(Dimethylamino)ethyl]thio]-3-phenylquinoline and Its Analogues

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A series of 2-[(2-aminoethyl)thio]quinolines substituted at the 3-position with alkyl, aryl, or heteroaryl groups has been prepared in the search for novel and selective 5-HT_2 antagonists. The affinity of the compounds for 5-HT_1 receptor sites was measured by their ability to displace [^{3}H]-5-HT from rat brain synaptosomes whereas the affinity for 5-HT_2 receptor sites was measured by their ability to displace [^{3}H]spiperone from synaptosomes prepared from rat brain cortex. The 5-HT_2 antagonist properties of the compounds were measured in vivo by their antagonism of 5-hydroxytryptophan-induced head twitches in the mouse and by their antagonism of hyperthermia induced by fenfluramine (*N*-ethyl-α-methyl-*m*-(trifluoromethyl)phenethylamine hydrochloride) in the rat. The structure–activity relationships in this series are discussed and the properties of 2-[[2-(dimethylamino)ethyl]thio]-3-phenylquinoline hydrochloride (**70**) are highlighted.

During the past few years there has been a marked increase in research on 5-hydroxytryptamine (5-HT) as summarized in recent reviews.[1,2] Attempts have been made to explain the physiology of 5-HT and the pharmacology of various 5-HT agonists and antagonists by the

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