Potent V_2/V_{1a} Vasopressin Antagonists with C-Terminal Ethylenediamine-Linked Retro-Amino Acids†,‡

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Received March 17, 1992

We report the solid-phase synthesis and antagonistic potencies of 25 analogues (1–25) of [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyl-D-tyrosine,4-valine]arginine-vasopressin (d$(CH_2)_5$D-Tyr(Et)^2-VAVP) (A) and of the related Ile^4 (D) and [D-Phe^2,Ile^4] (E) analogues, potent antagonists of the antidiuretic (V_2-receptor) and of the vasopressor (V_{1a}-receptor) responses to arginine-vasopressin (AVP). Six of these peptides (**1**, **13**, **17**, **19**, **21**, and **23**) have the Pro-Arg-Gly-NH_2 tripeptide side chain fully or partially replaced or extended by ethylenediamine (Eda). The remaining 19 peptides have L- or D-amino acids retrolinked to these six C-terminal Eda peptides. Peptides **1**, **13**, **17**, and **19** all have the ring structure of (A). Their side-chain structures are as follows: **1**, Eda; **13**, Pro-Eda; **17**, Pro-Arg-Eda; **19**, Arg-Gly-Eda. Peptide **21** is the Pro-Arg-Eda analogue of D; peptide **23** is the Pro-Arg-Gly-Eda analogue of E. Peptide **2** is the retro-Arg analogue of **1**. Its side-chain structure is Eda←Arg. Peptides **3–6** are analogues of **2** which have the D-Tyr-(Et)^2 residue replaced by L-Tyr(Et)^2 (**3**), D-Phe^2 (**4**), D-Ile^2 (**5**), or D-Leu^2 (**6**), respectively. Peptides **7–12** are analogues of **2** which have the C-terminal retro-Arg replaced in retrofashion by D-Arg (**7**), Gly (**8**), Orn (**9**), D-Orn (**10**), D-Lys (**11**), or Arg-Arg (**12**). Peptides **14–16** have D-Orn (**14**), D-Lys (**15**), and D-Arg (**16**) retrosubstituted to peptide **13**. Peptides **18**, **20**, and **22** are the retro-Arg-substituted analogues of **17**, **19**, and **21**, respectively. Peptides **24** and **25** have Val and D-Val in retrolinkage with **23**, respectively. All 25 peptides were examined for agonistic and antagonistic potencies in AVP V_2/V_{1a} assays. With the exception of peptides **5** and **6**, all exhibit potent anti-V_{1a} antagonism, with anti-V_{1a} pA_2 values in the range 7.64–8.33. Peptides 1–25 exhibit the following anti-V_2 pA_2 values: **1**, 7.07 ± 0.05; **2**, 7.54 ± 0.10; **3**, 6.39 ± 0.04; **4**, 6.91 ± 0.06; **5**, ~5.8; **6**, <5.5; **7**, 7.95 ± 0.10; **8**, 6.59 ± 0.02; **9**, 7.55 ± 0.03; **10**, 7.24 ± 0.05; **11**, 7.76 ± 0.07; **12**, 7.61 ± 0.09; **13**, 7.64 ± 0.07; **14**, 7.92 ± 0.05; **15**, 7.78 ± 0.09; **16**, 7.92 ± 0.05; **17**, 7.79 ± 0.04; **18**, 7.68 ± 0.06; **19**, 8.03 ± 0.08; **20**, 7.87 ± 0.05; **21**, 8.00 ± 0.12; **22**, 8.10 ± 0.09; **23**, 8.10 ± 0.10; **24**, 8.10 ± 0.08; **25**, 8.09 ± 0.09. Comparison of the anti-V_2 potencies of peptides 1–6 clearly shows the superiority of the D-Tyr(Et)^2 substitution in leading to retention and enhancement of V_2 antagonism in this series. With only one exception (peptide **8**), the retromodified peptides exhibit either full retention and in a number of cases (**2**, **7**, **9–12**, **14**, and **16**) a 1.5–7.5-fold enhancement of V_2 antagonism compared to their respective parent C-terminal Eda peptides. The retro-Arg-substituted Ile^4 peptide **22** exhibits a 2-fold enhancement of anti-V_2 potency relative to its Val^4 counterpart **18**. The retromodified peptides **24** and **25**, which possess extensions at the C-terminal, also exhibit good retention of V_2 antagonism. Many of these retrosubstituted peptides are as potent as the most potent V_2 antagonists reported to date. Some of these may be orally active. These findings point to the usefulness of Eda retromodifications in the design of AVP antagonists. Furthermore they provide useful clues to the design of (a) more potent and selective AVP antagonists and (b) novel photoaffinity and radioiodinated ligands as probes of AVP receptors.

We reported the first effective antagonists of the antidiuretic (V_2-receptor) responses to arginine-vasopressin (AVP).[1,2] These early V_2 antagonists were also potent vasopressor (V_{1a}-receptor) antagonists.[2] Since then we and others have carried out extensive structural modifications of the early lead molecules in attempts (a) to enhance their antiantidiuretic potency, their selectivity, and their oral bioavailability and (b) to determine the

† This paper is dedicated to Professor Ralph Hirschmann on the occasion of his 70th birthday. It is a pleasure to participate in this tribute to his pioneering contributions and inspiring leadership in the peptide field.

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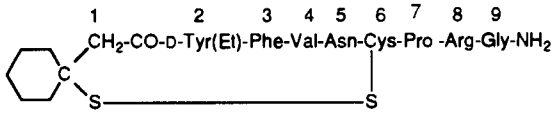
‡ Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* 1984, *138*, 9). All amino acids are in the L configuration unless otherwise noted. Other abbreviations used are as follows: d$(CH_2)_5$D-Tyr(Et)^{2}VAVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyl-D-tyrosine,4-valine]arginine-vasopressin; 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-Ile^2,Ile^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine,4-isoleucine]arginine-vasopressin; d$(CH_2)_5$[D-Tyr(Et)^2,Ile^4]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyl-D-tyrosine,4-isoleucine]arginine-vasopressin; AVP, arginine-vasopressin; Eda, ethylenediamine; DMF, dimethylformamide; DCC, dicyclohexylcarbodiimide; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; AcOH, acetic acid; TFA, trifluoroacetic acid; HOBt, *N*-hydroxybenzotriazole; ONp, *p*-nitrophenyl ester; Et_3N, triethylamine; Et_2O, diethyl ether, CH_2Cl_2, methylene chloride.

0022-2623/92/1835-3895$03.00/0

minimum pharmacophore required for V_2/V_{1a} antagonism[3-25] (for reviews see refs 26–34). One of the early promising V_2/V_{1a} antagonists to emerge from these studies was [1-β-mercapto-β,β-pentamethylenepropionic acid),2-*O*-ethyl-D-tyrosine,4-valine]arginine-vasopressin (d$(CH_2)_5$D-Tyr(Et)^{2}VAVP) (A, Table II).[3] d$(CH_2)_5$D-Tyr(Et)^{2}VAVP has the following structure:



Modifications at position 2 with D-Phe, D-Ile, and D-Leu coupled with Abu or Ile substitutions at position 4 brought about enhancements of anti-V_2 potency and/or anti-V_2/anti-V_{1a} selectivity.[4-6] We and others later found that the C-terminal glycine or glycine amide in d$(CH_2)_5$D-Tyr(Et)^{2}VAVP and/or in related analogues could be deleted or replaced by a wide variety of amino acids and by ethylenediamine (Eda,–NH$(CH_2)_2$NH$_2$) with full retention of V_2 and V_{1a} antagonistic potencies.[7-11,15,16,18] It was subsequently found that the entire Pro-Arg-Gly-NH$_2$ side chain could be deleted and replaced by a series of dialkyl amines and (aminoalkyl)guanidines with good retention of V_2/V_{1a} antagonistic potencies.[21] In a preliminary communication, we reported that the molecule obtained by the replacement of the entire side chain of d$(CH_2)_5$D-Tyr(Et)^{2}VAVP by Eda still retained ~10% of the V_2 antagonism and almost 80% of the V_{1a} antagonistic potency of the parent, d$(CH_2)_5$D-Tyr(Et)^{2}VAVP.[27] Thus it was clear from these studies that the side chain in AVP

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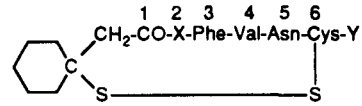
Table I. Antiantidiuretic and Antivasopressor Potencies of Position 2 Substituted Arginine-Vasopressin (AVP) Antagonists Having Arginine Attached to the Ring in Retrolinkage via Ethylenediamine (Eda)



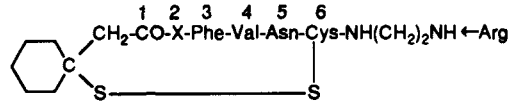
			antiantidiuretic (anti-V_2)		antivasopressor (anti-V_{1a})	
no.	X	Y	ED[a] (nmol/kg)	pA_2[b]	ED[a] (nmol/kg)	pA_2[b]
1	D-Tyr(Et)	$NH(CH_2)_2NH_2$[d]	5.7 ± 0.6[c]	7.07 ± 0.05	0.47 ± 0.03	8.15 ± 0.03
2	D-Tyr(Et)	$NH(CH_2)_2NH$←Arg	2.1 ± 0.4	7.54 ± 0.10	0.75 ± 0.02	7.95 ± 0.02
3	L-Tyr(Et)	$NH(CH_2)_2NH$←Arg	27 ± 6	6.39 ± 0.04	0.76 ± 0.14	7.97 ± 0.08
4	D-Phe	$NH(CH_2)_2NH$←Arg	8.4 ± 1.2	6.91 ± 0.06	1.7 ± 0.4	7.64 ± 0.09
5	D-Ile	$NH(CH_2)_2NH$←Arg	~100[e]	~5.8[e]	47 ± 6	6.16 ± 0.06
6	D-Leu	$NH(CH_2)_2NH$←Arg	>234[e]	<5.5[e]	58 ± 9	6.08 ± 0.08

[a] The effective dose is defined as the dose (in nanomoles/kilogram) that reduces the response seen with $2x$ units of agonist to equal the response seen with x units of agonist administered in the absence of the antagonist. [b] Estimated in vivo pA_2 values represent the negative logarithms of the effective doses divided by the estimated volume of distribution (67 mL/kg). [c] Mean ± SE. [d] Preliminary data reported in ref 27. [e] These peptides showed weak agonism as well as antagonism on these assays. Only rough estimates of their EDs and pA_2s are shown.

V_2/V_{1a} antagonists could tolerate a broad degree of structural deletion and modification without serious consequences to receptor binding.

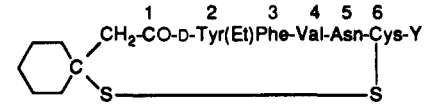
Our findings and those of others that the Eda modification is well tolerated at position 7 as a replacement for the entire Pro-Arg-Gly-NH_2 side chain, at position 8 as a replacement for the Arg-Gly-NH_2 moiety, and at position 9 as a replacement for Gly-NH_2 in d$(CH_2)_5$D-Tyr(Et)^{2}VAVP and in related V_2/V_{1a} antagonists offered the possibility of exploring the effects of amino acid substitutions coupled to the ring of d$(CH_2)_5$D-Tyr(Et)^{2}VAVP and of related V_2/V_{1a} antagonists in retrofashion via Eda, Pro-Eda, Pro-Arg-Eda, Arg-Gly-Eda, and Pro-Arg-Gly-Eda linkers. We were particularly intrigued by the possibility that should such modifications be well tolerated, they might in some instances confer greater resistance to enzymatic degradation and might thus enhance oral bioavailability and would also offer a novel approach to AVP antagonist design.

On the basis of earlier findings on the need for a basic residue in the side chain,[15,27] we selected arginine for the first retrocoupling to the [ring]Eda[27] derivative 1, where the [ring] is that of d$(CH_2)_5$D-Tyr(Et)^{2}VAVP(A)[3] to give the [ring]Eda←Arg analogue 2. When preliminary findings with 2 were promising, we wished to obtain the optimal ring before progressing to further Eda and retro-Eda modifications. We thus synthesized the L-Tyr(Et)^2, D-Phe^2, D-Ile^2, and D-Leu^2 analogues of 2. The structures of 2 and of the resulting four [ring]Eda←Arg analogues (3–6) are as follows:



X = D-Tyr(Et (**2**), L-Tyr(Et) (**3**), D-Phe (**4**), D-Ile (**5**), D-Leu (**6**)

Examination of the anti-V_2 potencies of these five peptides (Table I) clearly showed that only the D-Tyr-(Et)^2, containing peptide 2, led to a retromodified antagonist possessing enhanced V_2 antagonism greater than that of the [ring]Eda compound 1.[27] Thus the ring of d$(CH_2)_5$D-Tyr(Et)^{2}VAVP[3] (A) was selected for further exploration of the effects of Eda and retrosubstituted Eda modifications on V_2 and V_{1a} antagonism. Initially we decided to explore the effects of other retro-amino acid replacements for retro-Arg in the [ring]Eda←Arg peptide 2. The retro-amino acids selected were D-Arg, Gly, Orn, D-Orn, and D-Lys to give analogues 7–11 (Table II). Analogue 12 (Table II) was designed to determine the effect of the dipeptide Arg-Arg linked in retrofashion to the [ring]Eda compound 1. Replacement of the C-terminal Arg-Gly-NH_2 in d$(CH_2)_5$D-Tyr(Et)^{2}VAVP by Eda gave compound 13 ([ring]Pro-Eda, Table II) this has been reported independently to be a potent V_2 antagonist.[21] This was modified in retrofashion with D-Orn, D-Lys, and D-Arg to give peptides 14–16 (Table II). Replacement of the C-terminal Gly-NH_2 in d$(CH_2)_5$D-Tyr(Et)^{2}VAVP by Eda gave the [ring]Pro-Arg-Eda compound 17 (Table II). This was modified in retrofashion with Arg to give peptide 18 (Table II). Extension of the C-terminal Gly in desProd-$(CH_2)_5$D-Tyr(Et)^{2}VAVP[10,16] with an Eda residue gave the [ring]Arg-Gly-Eda peptide 19 (Table II). This was modified in retrofashion with Arg to give peptide 20 (Table II). Peptides 7–20 have the following [ring]Y general structure, where the [ring] is that of d$(CH_2)_5$D-Tyr(Et)^2-VAVP (A, Table II) and Y denotes various modifications of its side-chain Pro-Arg-Gly-NH_2 as noted:

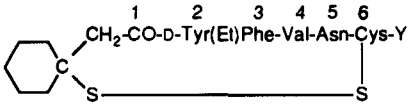


[ring]Y

Y = Eda←D-Arg (**7**), Eda←Gly (**8**), Eda←Orn (**9**), Eda←D-Orn (**10**), Eda←D-Lys (**11**), Eda←Arg←Arg (**12**), Pro-Eda (**13**), Pro-Eda←D-Orn (**14**), Pro-Eda←D-Lys (**15**), Pro-Eda←D-Arg (**16**), Pro-Arg-Eda (**17**), Pro-Arg-Eda←Arg (**18**), Arg-Gly-Eda (**19**), Arg-Gly-Eda←Arg (**20**)

All of the remaining five peptides are analogues of d$(CH_2)_5$D-Tyr(Et)^{2}VAVP which have an Ile residue in place of Val at position 4 (Table III). Three of them also have a D-Phe/D-Tyr(Et) interchange at position 2. Peptide 21 (Table III) which terminates in Eda is simply the Ile^4 analogue of 17 (Table II). This was modified in retrofashion with Arg to give peptide 22 which is the Ile^4 analogue of peptide 18 (Table II). Peptide 23 has the C-terminal Gly in the highly potent V_2/V_{1a} antagonist d$(CH_2)_5$[D-Phe^2,Ile^4]AVP[5] extended with an Eda residue. Retromodification of this peptide with Val and D-Val gave, respectively, the side-chain extended retromodified pep-

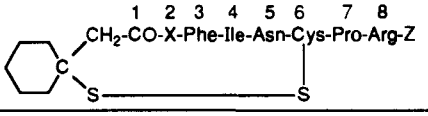
Table II. Antiantidiuretic and Antivasopressor Potencies of C-Terminal Retromodified AVP Antagonists Together with Those of Their Parent C-Terminal Ethylenediamine-Substituted Parent Peptides and of Related AVP Antagonists



		antiantidiuretic (anti-V_2)		antivasopressor (anti-V_{1a})	
no.	Y	ED[a] (nmol/kg)	pA_2[b]	ED[a] (nmol/kg)	pA_2[b]
2	$NH(CH_2)_2NH$←Arg[d]	2.1 ± 0.4[c]	7.54 ± 0.10	0.75 ± 0.02	7.95 ± 0.02
7	$NH(CH_2)_2NH$←D-Arg	0.83 ± 0.17	7.95 ± 0.10	0.47 ± 0.04	8.15 ± 0.03
8	$NH(CH_2)_2NH$←Gly	18 ± 1	6.59 ± 0.02	1.66 ± 0.10	7.61 ± 0.02
9	$NH(CH_2)_2NH$←Orn	1.9 ± 0.2	7.55 ± 0.03	1.06 ± 0.07	7.80 ± 0.03
10	$NH(CH_2)_2NH$←D-Orn	4.0 ± 0.5	7.24 ± 0.05	0.59 ± 0.08	8.06 ± 0.06
11	$NH(CH_2)_2NH$←D-Lys	1.2 ± 0.2	7.76 ± 0.07	0.82 ± 0.08	7.92 ± 0.04
12	$NH(CH_2)_2NH$←Arg←Arg	1.8 ± 0.4	7.61 ± 0.09	0.86 ± 0.18	7.93 ± 0.09
13	Pro-$NH(CH_2)_2NH_2$[e,f]	1.6 ± 0.2	7.64 ± 0.07	0.75 ± 0.05	7.95 ± 0.03
14	Pro-$NH(CH_2)_2NH$←D-Orn	0.82 ± 0.10	7.92 ± 0.05	0.95 ± 0.11	7.86 ± 0.05
15	Pro-$NH(CH_2)_2NH$←D-Lys	1.2 ± 0.3	7.78 ± 0.09	0.57 ± 0.07	8.08 ± 0.05
16	Pro-$NH(CH_2)_2NH$←D-Arg	0.81 ± 0.10	7.92 ± 0.05	0.48 ± 0.05	8.15 ± 0.04
(A)	Pro-Arg-Gly-NH_2[g]	1.1 ± 0.2	7.81 ± 0.07	0.45 ± 0.11	8.22 ± 0.12
17	Pro-Arg-$NH(CH_2)_2NH_2$	1.1 ± 0.1	7.79 ± 0.04	0.69 ± 0.06	8.00 ± 0.04
18	Pro-Arg-$NH(CH_2)_2NH$←Arg	1.4 ± 0.2	7.68 ± 0.06	0.51 ± 0.10	8.15 ± 0.09
(B)	Arg-Gly-NH_2[h,i]	1.1 ± 0.1	7.82 ± 0.07	0.56 ± 0.09	8.09 ± 0.07
(C)	Pro-Arg-Gly-$NH(CH_2)_2NH_2$[k]	1.2 ± 0.2	7.82 ± 0.11	0.32 ± 0.04	8.33 ± 0.05
19	Arg-Gly-$NH(CH_2)_2NH_2$	0.65 ± 0.12	8.03 ± 0.08	0.59 ± 0.05	8.05 ± 0.04
20	Arg-Gly-$NH(CH_2)_2NH$←Arg	0.91 ± 0.12	7.87 ± 0.05	0.73 ± 0.06	7.96 ± 0.04

[a-c] See corresponding footnotes to Table I. [d] Data from Table I. [e] Independent synthesis reported in ref 21. [f] The data shown here from assays on peptide 13 were erroneously reported in an earlier publication[11] to be from assays on an analogue with the structure here designated as peptide 17. The data given here for peptide 17 are from assays on a subsequent synthesis of this analogue. [g] V_2/V_{1a} antagonist, $d(CH_2)_5$D-Tyr(Et)^{2}VAVP, data from Manning et al.[3] [h] desProd$(CH_2)_5$D-Tyr(Et)^{2}VAVP, independent synthesis reported in ref 16. [i] Preliminary data reported in ref 10. [k] Data from Manning et al., ref 8.

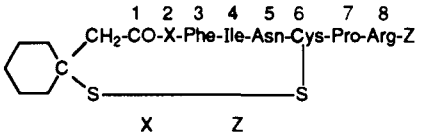
Table III. Antiantidiuretic and Antivasopressor Potencies of C-Terminal Retromodified Analogues of the Potent AVP Antagonists $d(CH_2)_5$[D-Tyr(Et)^2,Ile^4]AVP and $d(CH_2)_5$[D-Phe^2,Ile^4]AVP and Those of Their Parent C-Terminal Eda Peptides



			antiantidiuretic (anti-V_2)		antivasopressor (anti-V_{1a})	
no.	X	Z	ED[a] (nmol/kg)	pA_2[b]	ED[a] (nmol/kg)	pA_2[b]
(D)	D-Tyr(Et)	Gly-NH_2[d]	1.2 ± 0.3[c]	7.75 ± 0.05	1.2 ± 0.03	7.78 ± 0.09
21	D-Tyr(Et)	$NH(CH_2)_2NH_2$[e]	0.77 ± 0.22	8.00 ± 0.12	0.32 ± 0.04	8.33 ± 0.06
22	D-Tyr(Et)	$NH(CH_2)_2NH$←Arg	0.57 ± 0.10	8.10 ± 0.09	0.36 ± 0.03	8.27 ± 0.03
(E)	D-Phe	Gly-NH_2[f]	0.46 ± 0.07	8.24 ± 0.08	0.99 ± 0.12	7.86 ± 0.05
(F)	D-Phe	$NH(CH_2)_2NH_2$[g]	0.55 ± 0.08	8.11 ± 0.06	0.88 ± 0.10	7.89 ± 0.04
23	D-Phe	Gly-$NH(CH_2)_2NH_2$	0.57 ± 0.13	8.10 ± 0.10	1.8 ± 0.03	7.58 ± 0.07
24	D-Phe	Gly-$NH(CH_2)_2NH$←Val	0.53 ± 0.08	8.10 ± 0.08	3.3 ± 0.1	7.30 ± 0.02
25	D-Phe	Gly-$NH(CH_2)_2NH$←D-Val	0.60 ± 0.15	8.09 ± 0.09	1.2 ± 0.2	7.77 ± 0.09

[a-c] See corresponding footnotes to Table I. [d] $d(CH_2)_5$[D-Tyr(Et)^2,Ile^4]AVP, data from Sawyer et al., ref 11. [e] Preliminary data reported in ref 8 and 11. [f] $d(CH_2)_5$[D-Phe^2,Ile^4]AVP, data from Manning et al., ref 5. [g] Preliminary data reported in ref 8.

tides 24 and 25 (Table III). Peptides 21–25 have the following general structures:



	X	Z
21	D-Tyr(Et)	Eda
22	D-Tyr(Et)	Eda←Arg
23	D-Phe	Gly-Eda
24	D-Phe	Gly-Eda←Val
25	D-Phe	Gly-Eda←D-Val

We present here the synthesis and antagonistic potencies of the peptides 1–25 designed according to the above rationale. To our knowledge, this use of ethylenediamine as a linker for the retroincorporation of amino acids is unprecedented in the design of AVP antagonists and in peptide design in general.

Peptide Synthesis

The Merrifield sold-phase method[35-37] was used for the synthesis of the C-terminal ethylenediamine (Eda) protected peptides I, IIIA, IVA, VA, VIA, XIII, XVII, XIX, XXI, and XXIII (Table IV) as previously described.[2-9,38,39] For the syntheses on the resin the appropriate number of cycles of deprotection (with HCl (1 M)/AcOH), neutralization (with 10% Et_3N/CH_2Cl_2), and coupling (by DCC[40]/

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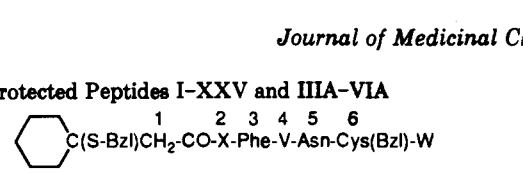
Table IV. Physicochemical Properties of Protected Peptides I-XXV and IIIA-VIA



no.[a,b]	X	V	W	formula	yield (%)[c]	MP (°C)	$[\alpha]^{25}$ (C = 1, DMF) (deg)	TLC[d] A	B	C	D
I	D-Tyr(Et)	Val	$NH(CH_2)_2NH_2$	$C_{56}H_{74}O_8N_8S_2$	72.0	218–222	−19.3	0.69	0.62	0.86	0.11
II	D-Tyr(Et)	Val	$NH(CH_2)_2NH$←Z-Arg(Tos)	$C_{77}H_{98}O_{13}N_{12}S_3$	78.0	226–228	−14.2	0.85	0.78	0.86	0.90
IIIA	L-Tyr(Et)	Val	$NH(CH_2)_2NH_2$	$C_{56}H_{74}O_8N_8S_2$	88.3	219–221	−30.0	0.53	0.62	0.88	0.05
III	L-Tyr(Et)	Val	$NH(CH_2)_2NH$←Z-Arg(Tos)	$C_{77}H_{98}O_{13}N_{12}S_3$	87.2	204–209	−24.6	0.87	0.86	0.81	0.96
IVA	D-Phe	Val	$NH(CH_2)_2NH_2$	$C_{54}H_{70}O_7N_8S_2$	91.6	205–209	−13.0	0.52	0.59	0.81	0.07
IV	D-Phe	Val	$NH(CH_2)_2NH$←Z-Arg(Tos)	$C_{75}H_{94}O_{12}N_{12}S_3$	86.2	205–208	−5.3	0.90	0.93	0.89	0.98
VA	D-Ile	Val	$NH(CH_2)_2NH_2$	$C_{61}H_{72}O_7N_8S_2$	86.0	223–225	−9.4	0.52	0.60	0.88	0.07
V	D-Ile	Val	$NH(CH_2)_2NH$←Z-Arg(Tos)	$C_{72}H_{98}O_{12}N_{12}S_3$	81.2	207–211	+7.1	0.86	0.90	0.86	0.96
VIA	D-Leu	Val	$NH(CH_2)_2NH_2$	$C_{51}H_{72}O_7N_8S_2$	80.8	208–211	−19.5	0.52	0.60	0.77	0.11
VI	D-Leu	Val	$NH(CH_2)_2NH$←Z-Arg(Tos)	$C_{72}H_{98}O_{12}N_{12}S_3$	70.6	194–198	+28.2	0.91	0.95	0.87	0.96
VII	D-Tyr(Et)	Val	$NH(CH_2)_2NH$←Boc-D-Arg(Tos)	$C_{74}H_{96}O_{13}N_{12}S_3$	79.9	210–212	−12.2		0.99	0.98	0.95
VIII	D-Tyr(Et)	Val	$NH(CH_2)_2NH$←Z-Gly	$C_{66}H_{83}O_{11}N_9S_2$	64.1	238–239	−16.7	0.93		0.93	0.92
IX	D-Tyr(Et)	Val	$NH(CH_2)_2NH$←Z-Orn(Z)	$C_{77}H_{96}O_{13}N_{10}S_2$	63.4	235–237	−13.8	0.00		0.00	0.95
X	D-Tyr(Et)	Val	$NH(CH_2)_2NH$←Boc-D-Orn(Tos)	$C_{73}H_{96}O_{13}N_{10}S_3$	81.0	220–223	−12.0		0.99	0.92	0.94
XI	D-Tyr(Et)	Val	$NH(CH_2)_2NH$←Boc-D-Lys(Z)	$C_{75}H_{100}O_{13}N_{10}S_2$	44.0	214–216	−12.4	0.92		0.95	0.93
XII	D-Tyr(Et)	Val	$NH(CH_2)_2NH$←Z-Arg(Tos)←Arg(Tos)	$C_{90}H_{116}O_{18}N_{18}S_4$	61.0	172–176	−14.6	0.81	0.86	0.90	0.99
XIII	D-Tyr(Et)	Val	Pro-$NH(CH_2)_2NH_2$	$C_{61}H_{81}O_9N_9S_2$	89.3	189–191	−18.5	0.44	0.50	0.72	0.05
XIV	D-Tyr(Et)	Val	Pro-$NH(CH_2)_2NH$←Boc-D-Orn(Z)	$C_{79}H_{95}O_{14}N_{11}S_2$	71.6	191–195	−15.4	0.81	0.90	0.98	0.99
XV	D-Tyr(Et)	Val	Pro-$NH(CH_2)_2NH$←Boc-D-Lys(Z)	$C_{80}H_{97}O_{14}N_{11}S_2$	71.3	198–201	−14.1	0.82	0.91	0.97	0.99
XVI	D-Tyr(Et)	Val	Pro-$NH(CH_2)_2NH$←Boc-D-Arg(Tos)	$C_{79}H_{107}O_{14}N_{13}S_3$	56.7	183–185	−15.3	0.76	0.88	0.96	0.98
XVII	D-Tyr(Et)	Val	Pro-Arg(Tos)$NH(CH_2)NH_2$	$C_{74}H_{99}O_{12}N_{13}S_3$	50.0	188–192	−17.2	0.43	0.54	0.82	0.12
XVIII	D-Tyr(Et)	Val	Pro-Arg(Tos)$NH(CH_2)NH$←Z-Arg(Tos)	$C_{95}H_{123}O_{17}N_{17}S_4$	96.5	168–173	−11.4	0.80	0.84	0.89	0.99
XIX	D-Tyr(Et)	Val	Arg(Tos)-Gly-$NH(CH_2)_2NH_2$	$C_{71}H_{95}O_{12}N_{13}S_3$	91.9	188–190	−3.9	0.59	0.51	0.77	0.09
XX	D-Tyr(Et)	Val	Arg(Tos)-Gly-$NH(CH_2)_2NH$←Arg(Tos)	$C_{92}H_{119}O_{17}N_{17}S_4$	82.2	173–177	−8.5	0.85	0.84	0.92	0.88
XXI	D-Tyr(Et)	Ile	Pro-Arg(Tos)-$NH(CH_2)_2NH_2$	$C_{75}H_{101}O_{13}N_{13}S_3$	22.2	189–191	−17.2	0.45		0.75	0.14
XXII	D-Tyr(Et)	Ile	Pro-Arg(Tos)-$NH(CH_2)_2NH$←Z-Arg(Tos)	$C_{96}H_{125}O_{17}N_{17}S_4$	91.7	182–185	−13.1	0.83	0.81	0.92	0.92
XXIII	D-Phe	Ile	Pro-Arg(Tos)-Gly-$NH(CH_2)_2NH_2$	$C_{75}H_{100}O_{12}N_{14}S_3$	74.8	186–188	−18.4	0.53	0.44	0.79	
XXIV	D-Phe	Ile	Pro-Arg(Tos)-Gly-$NH(CH_2)_2NH$←Z-Val	$C_{88}H_{115}O_{15}N_{15}S_3$	79.3	214–216	−13.9	0.74	0.86	0.94	0.98
XXV	D-Phe	Ile	Pro-Arg(Tos)-Gly-$NH(CH_2)NH$←Boc-D-Val	$C_{85}H_{117}O_{15}N_{15}S_3$	73.4	198–201	−16.4	0.76	0.87	0.95	0.97

[a] The protected peptides I-XXV are the immediate protected precursors for the free peptides 1–25 given in Tables I–III and V. [b] The protected peptides IIIA–VIA were not converted to their respective free peptides. They were synthesized solely to serve as precursors for their respective protected retro-arginine derivatives III–VI. [c] Yields were calculated on the basis of the amino acid content on the resin in the case of the C-terminal Eda-protected peptides and on the coupling in solution for the protected Eda retrosubstituted peptides. [d] The solvent systems are described in the Experimental Section.

HOBt[41]) were carried out with Boc-Cys(Bzl)-resin, Boc-Pro-resin, Boc-Arg(Tos)-resin or Boc-Gly-resin, as previously described,[2-9,39] to give the respective protected acyl resins. Boc-Asn was incorporated as its nitrophenyl ester:[42] β-(*S*-benzylthio)-β,β-pentamethylenepropionic acid[43] was used in the final DCC/HOBt-mediated coupling step in all cases. Cleavage from the resin was by aminolysis with ethylenediamine in methanol as previously described[38,39] to give the appropriate C-terminal Eda-protected peptides I, IIIA–VIA, XIII, XVII, XIX, XXI, and XXIII (Table IV). The protected retromodified peptides II–XII, XIV–XVI, XVIII, XX, XXII, XXIV, and XXV (Table IV) were obtained by acylating the appropriate protected Eda peptide I, IIIA–VIA, XIII, XVII, XIX, XXI, and XXIII with the desired Z- or Boc-protected amino acid derivative in a DCC/HOBt[40,41]-mediated reaction or as a nitrophenyl ester[42] in solution. Na in liquid NH_3[44] was used to deblock each protected precursor (I–XXV, Table IV) as previously described,[2-9,39] and the resulting disulfhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$.[45] Purification by gel filtration on Sephadex G-15 in a two-step procedure using 50% and 0.2 M AcOH as eluents, respectively, as previously described[46] gave the free peptides 1–25 (Tables I–III and V).

Bioassay Methods

Assays for antidiuretic V_2 agonism or antagonism were done by measuring changes in urine flow in water-loaded rats under ethanol anesthesia.[47] Assays for vasopressin V_{1a}-antagonistic activities were performed by following blood pressure responses in rats anesthetized with urethane and pretreated with phenoxybenzamine.[48] The USP posterior pituitary reference standard was used as a standard in agonistic assays and as an agonist in assays

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Table V. Physicochemical Properties of the Free Peptides 1–25 Given in Tables I–III

no.	yield (%)[a,b]	$[\alpha]^{25}_D$ (1M AcOH) (deg)	TLC[c] A	TLC[c] B	TLC[c] C
1	11.0	−141.6, c = 0.2	0.41	0.52	0.59
2	40.3	−124.3, c = 0.3	0.30	0.42	0.56
3	24.4	−11.5, c = 0.1	0.28	0.32	0.62
4	39.6	−117.6, c = 0.1	0.26	0.29	0.58
5	44.3	−113.0, c = 0.1	0.26	0.29	0.57
6	36.2	−122.0, c = 0.1	0.30	0.29	0.60
7	20.0	−87.0, c = 0.3	0.29	0.29	0.59
8	10.2	−121.6, c = 0.1	0.37	0.47	0.57
9	50.0	−71.6, c = 0.3	0.25	0.25	0.33
10	13.5	−86.0, c = 0.3	0.27	0.17	0.33
11	37.0	−118.6, c = 0.3	0.33	0.29	0.38
12	19.6	−90.4, c = 0.1	0.18	0.07	0.27
13	10.8	−116.8, c = 0.28	0.36	0.39	0.50
14	33.2	−107.8, c = 0.46	0.28	0.16	0.26
15	20.8	−101.9, c = 0.48	0.26	0.10	0.25
16	14.2	−90.5, c = 0.38	0.29	0.20	0.36
17	40.0	−99.0, c = 0.1	0.25	0.14	0.34
18	27.3	−72.5, c = 0.1	0.18	0.05	0.28
19	40.6	−83.4, c = 0.2	0.25	0.18	0.33
20	28.5	−63.8, c = 0.1	0.17	0.06	0.25
21	68.2	−72.0, c = 0.3	0.26	0.16	0.36
22	31.3	−79.5, c = 0.1	0.17	0.05	0.25
23	27.6	−39.8, c = 0.3	0.21	0.20	0.32
24	9.9	−24.0, c = 0.26	0.29	0.20	0.49
25	37.9	−33.8, c = 0.32	0.28	0.21	0.49

[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 ±3%. [c] The solvent systems and conditions are given in the Experimental Section.

for antagonism. All injections of agonists and antagonists were given intravenously. In assays for antagonistic activities an "effective dose" (ED) of the antagonist was estimated. The ED is the dose of an antagonist that reduces the response to a subsequent dose of agonist to equal the response to one-half that dose of agonist given in the absence of antagonist. In practice, this is estimated by finding doses of antagonist above and below the ED and interpolating on a log scale.[49] An in vivo pA_2 can be estimated from the ED by dividing the ED by an arbitrarily assumed volume of distribution of 67 mL/kg.[50] When standard errors are presented in Tables I–III, the means reflect results from at least four independent assay groups. None of these peptides exhibited any evident vasopressor agonism. Some peptides showed both agonistic and antagonistic activities on antidiuretic assays. In these instances the presence of agonism precluded reliable measurement of antagonistic potencies. EDs and pA_2 could only be roughly estimated, and means and standard errors are not indicated for these estimates in Table I.

Results and Discussion

The antiantidiuretic (anti-V_2) and antivasopressor (anti-V_{1a}) potencies of peptides 1–25 together with those of some related peptides are given in Tables I–III. These 25 new peptides include the 6 parent C-terminal Eda peptides 1, 13, 17, 19, 21, and 23 and their retromodified derivatives. As expected, all of the C-terminal Eda peptides are V_2/V_{1a} antagonists with some being quite potent. Most of the retromodified peptides, with the exception of 3–6 and 8, are potent V_2 antagonists with anti-V_2 pA_2 values in the range 7.24 (10) to 8.10 (24 and 25). Virtually all of the retromodified peptides, with the exceptions of 5 and 6, are also potent V_{1a} antagonists, with anti-V_{1a} pA_2 values in the range 7.30 (24) to 8.27 (22). In a number of instances the retromodified peptides exhibited significant enhancement in anti-V_2 potency relative to the parent Eda peptide. Thus the retromodified peptides 2, 7, and 9–12 are clearly more potent than the parent Eda peptide 1. Likewise the retromodified peptides 14 and 16 are more potent than the parent Eda peptide 13. In other instances the retromodified peptides are virtually equipotent with the parent Eda peptides. Thus peptide 22 and 21 exhibit virtually identical anti-V_2 potencies. This is also the case for the C-terminal retropeptides 24 and 25 which, with anti-V_2 pA_2 valus of 8.10 and 8.09 are equipotent with the parent Eda peptide 23 (anti-V_2 pA_2 = 8.10). Only in one instance is anti-V_2 potency clearly decreased in the retromodified peptide 8 which has a pA_2 of 6.59; significantly lower than that of the parent Eda peptide 1. Anti-V_2 potency is not significantly decreased in any peptide containing a basic retro-amino acid at the C-terminus. It is clear that the retromodifications reported here are in general very well tolerated, leading to excellent retention and, in many cases, significant enhancement of anti-V_2 potencies.

Critical Requirement for D-Tyr(Et)^2 in Tail-Shortened Retromodified V_2 Antagonists (Table I). The parent [ring]Eda peptide (1, Table I) where the ring is that of the potent V_2/V_{1a} antagonist, d(CH$_2$)$_5$D-Tyr(Et)^2-VAVP[3] has an anti-V_2 pA_2 = 7.07 and an anti-V_{1a} pA_2 = 8.15. The attachment of an Arg residue in retrofashion to the amino group of the C-terminal Eda residue gave the [ring]Eda←Arg analogue (2, Table II). This peptide has an anti-V_2 pA_2 = 7.54 and an anti-V_{1a} pA_2 = 7.95. Thus, in this instance the retro-Arg modification led to a 3-fold enhancement in anti-V_2 potency and a slight decrease in anti-V_{1a} potency relative to the parent [ring]Eda peptide 1 (Table I). Compound 2 was further modified at position 2 by replacing D-Tyr(Et) with L-Tyr(Et), D-Phe, D-Ile, and D-Leu, respectively, in an effort to determine the most potent V_2 [ring]-substituted Eda←Arg antagonist to use for further studies of the side chain. The D-Phe^2 and D-Ile^2 substituents were selected because we had previously shown that these modifications led to highly potent V_2 antagonists[4-6] and, in the case of the D-Ile^2 analogues, highly selective as well.[4,6] Examination of the properties of peptides 3–6 (Table I) shows clearly that these are all much less potent V_2 antagonists than peptide 2. The respective anti-V_2 pA_2 values of the L-Tyr(Et)^2, D-Phe^2, D-Ile^2, and D-Leu^2 analogues are 6.39, 6.91, ~5.8, and <5.5. Thus with an anti-V_2 pA_2 = 7.54, the D-Tyr(Et)^2 analogue 2 is respectively about 4 and >100 times more potent than the most and least potent of these four peptides. These findings are in striking contrast to the relative anti-V_2 potencies of the parent d(CH$_2$)$_5$X^{2}VAVP (where X = D-Tyr(Et), L-Tyr(Et), D-Phe, D-Ile, and D-Leu peptides.[3-6] Here the D-Phe^2, D-Ile^2, and D-Tyr(Et)^2 analogues are virtually equipotent and only about twice as potent as the L-Tyr(Et)^2 and D-Leu^2 analogues. The very low anti-V_2 potency of the D-Ile^2 analogue is clearly reiminiscent of the drastic reduction in anti-V_2 potency observed upon the deletion of proline from d(CH$_2$)$_5$[D-Ile^2,Ile^4]AVP,[10] in

(49) Schild, H. O. pA, a new scale of the measurement of drug antagonism. *Br. J. Pharmacol. Chemother.* **1947**, *2*, 189–206.

(50) Dyckes, D. F.; Nestor, J. J., Jr.; Ferger, M. F.; du Vigneaud, V. [1-β-mercapto-β,β-diethylpropionic acid]-8-lysine-vasopressin, a potent inhibitor of 8-lysine-vasopressin and of oxytocin. *J. Med. Chem.* **1974**, *17*, 250–252.

striking contrast to the effects of proline deletion from d(CH$_2$)$_5$D-Tyr(Et)^{2}VAVP[10,16] which did not reduce its anti-V_2 potency. Thus the findings in Table I clearly illustrate the profound influence of the position 2 substituent on the anti-V_2 potencies of side chain deleted analogues of AVP V_2 antagonists. The data in Table I also show the superiority of the D-Tyr(Et)^2 substituent compared to the other position 2 substituents evaluated here. Hence, we elected to use compound **2** as the model for the design of the peptides shown in Table II.

Effects of L- and D-Amino Acids Retrolinked to [Ring]Eda (1) (Peptides 7–12, Table II). All of the peptides in Table II have the ring structure of the cyclic V_2/V_{1a} antagonist d(CH$_2$)$_5$D-Tyr(Et)^{2}VAVP[3] and differ only in the nature of the side chain (Y) attached to the ring. Thus, as noted above, compound 1 (Table I) is [ring]-Eda and compound 2 is [ring]Eda←Arg. Compounds 7–12 (Table II) are analogues of [ring]Eda←Arg in which the Arg is replaced, respectively, by D-Arg, Gly, Orn, D-Orn, D-Lys, and Arg-Arg. Apart from the retro-Gly peptide 8 all of these retrosubstituted peptides are more potent V_2 antagonists than the parent [ring]Eda peptide (1, Table I). All of the peptides 7–12 are also potent V_{1a} antagonists. While the retro-D-Arg analogue 7 (anti-V_2 pA_2 = 7.95) appears to be about twice as potent as its retro-L-Arg counterpart 2 (anti-V_2 pA_2 = 7.54) the opposite is true for the retro-L-Orn/D-Orn pair 9 and 10. The relatively low anti-V_2 potency of the retro-Gly peptide 8 (anti-V_2 pA_2 = 6.59) relative to all of the other [ring]Eda retromodified peptides (2, 7, and 9–12, Table II) is in line with earlier observations about the need for a cationic substituent in the side chain as a prerequisite for V_2 antagonism.[15,27] With anti-V_2 pA_2 values of 7.95 and 7.76 the [ring]Eda←D-Arg (7) and [ring]Eda←D-Lys (11) side chain deleted analogues are 5–7 times more potent than the [ring]Eda peptide 1 (anti-V_2 pA_2 – 7.07) and are virtually equipotent with the parent antagonist (A) (anti-V_2 pA_2 = 7.81) which possesses the entire Pro-Arg-Gly(NH$_2$) side chain, thus illustrating the usefulness of this approach in AVP V_2 antagonist design.

Basic D-Amino Acids Retrolinked to [Ring]Pro-Eda (13) Enhance V_2 Antagonism (14–16, Table II). The parent [ring]Pro-Eda peptide (13, Table II) exhibits a significant enhancement in anti-V_2 potency (pA_2 = 7.64) compared to its desPro parent [ring]Eda peptide 1 (Table I), anti-V_2 pA_2 = 7.07. This finding confirms an independent observation by others.[21] Peptides 14–16 have D-Orn, D-Lys, and D-Arg linked in retrofashion to [ring]-Pro-Eda. In all three cases, the retromodification is very well tolerated with the retro-D-Orn and retro-D-Arg peptides 14 and 16 being almost twice as potent V_2 antagonists as the parent [ring]Pro-Eda peptide 13. However, comparison of the properties of peptides 14–16 with their desPro[ring]Eda←D-Orn, ←D-Lys, and ←D-Arg counterpart retropeptides 10, 11, and 7, respectively, shows that in the case of the D-Lys and D-Arg peptides, the addition of a proline residue did not enhance anti-V_2 potency. In fact, both of the D-Lys and D-Arg pairs exhibit virtually identical, anti-V_2 pA_2 values. However, in the case of the D-Orn pair, peptides 10 and 14, the anti-V_2 potency difference is quite striking. With an anti-V_2 pA_2 = 7.92 peptide 14 is almost 5 times more potent than peptide 10 (anti-V_2 pA_2 = 7.24). The high anti-V_2 potencies of these D-Orn, D-Lys, and D-Arg peptides (14–16) bodes well for (a) their potential bioavailability, (b) their use as precursors for photoaffinity probes for receptor characterization, and (c) the use of retrosubstituted D-amino acids in the design of more potent AVP V_2 antagonists.

Retro-Arg Analogue of [Ring]Pro-Arg-Eda (17) (18, Table II). The replacement of the C-terminal glycineamide in d(CH$_2$)$_5$D-Tyr(Et)^{2}VAVP[3] ([ring]Pro-Arg-GlyNH$_2$ (A), Table II) by Eda gave the [ring]Pro-Arg-Eda compound 17 (Table II). With an anti-V_2 pA_2 = 7.79 this peptide is equipotent with its Gly-NH$_2$ containing parent (A) (anti-V_2 pA_2 = 7.81). The retroaddition of Arg to this peptide to give the [ring]Pro-Arg-Eda←Arg peptide (18, Table II) (anti-V_2 pA_2 = 7.68) did not reduce anti-V_2 potency significantly. The investigation of a retro-D-Arg substitution would now appear to be well warranted. The properties of peptide 18 clearly shows that the retroaddition of Arg to the side chain of the [ring]Pro-Arg-Eda peptide 17 is well tolerated with excellent retention of both V_2 and V_{1a} antagonism.

[Ring]-Arg-Gly-Eda←Arg (20, Table II) Is Not More Potent than Parent Eda Peptide (19, Table II). The addition of an Eda residue to the known [ring]Arg-Gly-NH$_2$ peptide (B)[10,16] (Table II) to give the [ring]Arg-Gly-Eda peptide 19 (Table II) did not reduce anti-V_2 potency. The former peptide (B) has an anti-V_2 pA_2 = 7.82,[10] while the Eda-containing peptide (19) exhibits an impressive anti-V_2 pA_2 = 8.03. The retroaddition of Arg to peptide 19 to give the [ring]Arg-Gly-Eda←Arg peptide 20 (Table II) (anti-V_2 pA_2 = 7.87) did not reduce anti-V_2 potency significantly. With the exception of the retro-Gly analogue 8 the retro-D-Lys analogue 15 and the retro-L-Arg analogues 18 and 20, all of the remaining nine retro-amino acid Eda-substituted peptides in Table II are significantly more potent than their parent C-terminal Eda peptides. These findings clearly illustrate the usefulness of this approach for the design of V_2/V_{1a} antagonists and offer very promising clues to the design of even more potent and more selective V_2 antagonists.

Fully Extended Side-Chain Eda and Retrosubstituted Eda-Ile^4 Analogues Exhibit Potent V_2/V_{1a} Antagonism (Peptides 21–25, Tables III). Table III contains analogues of the potent V_2/V_{1a} antagonists d(CH$_2$)$_5$[D-Tyr(Et)^2,Ile^4]AVP[11] (D) and d(CH$_2$)$_5$[D-Phe^2,-Ile^4]AVP[5] (E) in which the C-terminal Gly-NH$_2$ has been replaced by Eda to give peptide 21 and F, respectively, or extended by Eda to give peptide 23. The C-terminal Eda peptide 21 was further modified by retrolinkage with arginine to give peptide 22. Retroadditions of L-Val and D-Val to peptide 23 resulted in peptides 24 and 25. It will be noted that peptides 21 and 22 differ from peptide 17 and 18 (Table II) only by an Ile/Val interchange at position 4. All of these five peptides, 21–25, are potent V_2 antagonists, possessing anti-V_2 pA_2 values of 8.00–8.10. Their anti-V_{1a} pA_2 values range from a low of 7.30 for 24 to a high of 8.33 for 21. With an anti-V_2 pA_2 value = 8.00 the C-terminal Eda peptide 21 is clearly as potent as its C-terminal Gly-NH$_2$ parent (D) (anti-V_2 pA_2 = 7.75).[11] Furthermore, the retro-Arg modification of 21 was very well tolerated, as evidenced by the high anti-V_2 potency (pA_2 = 8.10) of the resulting retro-Arg analogue 22. Comparison of the anti-V_2 potencies of the retro-Arg,Val^4 analogue (18, Table II) and its retro-Arg,Ile^4 counterpart (22, Table III) points to the superiority of the Ile^4 substitution relative to the Val^4 substitution in leading to enhanced anti-V_2 potency in the analogue pair 18 and 22. With an anti-V_2 pA_2 = 8.10, peptide 22 is over twice as

potent as analogue 18. This would not have been predicted on the basis of the anti-V_2 potencies of their C-terminal Gly-NH_2 parents A[3] (Table II) and D[11] (Table III) which exhibit virtually identical anti-V_2 potencies. Thus, this finding regarding the relative effectiveness of the Ile[4]/Val[4] substitutions may represent a promising clue for the design of retro-type V_2 antagonists. d(CH_2)$_5$[D-Phe[2],Ile[4]]-AVP[5] (E, Table III) with an anti-V_2 pA_2 = 8.24, is one of the most potent V_2 antagonists reported to date. Remarkably, as shown here replacement or extension of the C-terminal Gly-NH_2 of E by Eda to give peptides F and 23, respectively, resulted in almost full retention of anti-V_2 potency in each case. The retrocoupling of either L-Val or D-Val to peptide 23 was very well tolerated. The resulting retro-Val and retro-D-Val peptides 24 and 25 exhibit anti-V_2 pA_2 values of 8.10 and 8.09, respectively. We have previously shown that peptides D and 21 exhibit oral activity.[8] Investigation of the oral activity of analogues 22, 24, and 25 would now appear to be well warranted. These findings also raise many questions with regard to (a) how far the side chain of E or related AVP V_2/V_{1a} antagonists could be extended and modified with Eda and other dialkylamines and with retro-Eda and other dialkylamine substitutions without loss of V_2 antagonism and (b) what other acyl substituents could be coupled to the side chain via Eda or other dialkylamines with retention of ability to bind to V_2 and V_{1a} receptors. These findings also offer promising clues to the design of a variety of Eda and other diamine linked retroanalogues of potent, selective, and orally active V_2 and V_{1a} antagonists including radioiodinated and photoaffinity-labeled derivatives for use as probes of V_2 and V_{1a} receptors.

Conclusion

In this manuscript we have attempted to explore the usefulness of a novel approach to the design of AVP V_2/V_{1a} antagonists. This approach is based on an earlier observation from these and other laboratories that ethylenediamine (Eda) can be used to replace the entire side chain or a part of the side chain but especially the C-terminal Gly-NH_2 in potent AVP V_2/V_{1a} antagonists and in V_{1a} antagonists with partial or full retention of antagonistic potencies.[8,11,15,16,18,27,39] The object of the current study was to expand on these earlier observations and to determine whether attaching one or more amino acids in retrolinkage to V_2/V_{1a} antagonists possessing a C-terminal Eda substituent would result in molecules possessing equal or enhanced anti-V_2/V_{1a} potencies. Thus this study has focused on two interrelated goals: (i) the synthesis and examination of a variety of side chain shortened and side chain extended AVP V_2/V_{1a} antagonists possessing a C-terminal Eda residue and (ii) the synthesis of selected retro-modified derivatives of these peptides. We have reported here the properties of six C-terminal Eda peptides (1, 13, 17, 19, 21, and 23) (Tables I–III). Peptide 13 has been independently reported.[21] These all exhibit V_2 and V_{1a} antagonism. Four of these (1, 13, 17, and 19) have the ring of d(CH_2)$_5$D-Tyr(Et)[2]VAVP[3] (A, Table II). Their abbreviated structures and corresponding anti-V_2 pA_2 values are as follows: [ring]Eda, 7.07 (1); [ring]-Pro-Eda, 7.64 (13); [ring]Pro-Arg-Eda, 7.79 (17); [ring]-Arg-Gly-Eda, 8.03 (19). Peptides 21 and 23 are analogues of d(CH_2)$_5$[D-Tyr(Et)[2]Ile[4]]AVP[11] (D) and d(CH_2)$_5$[D-Phe[2],Ile[4]]AVP[5] (E) in which the C-terminal Gly-NH_2 has respectively been replaced by Eda and extended by Eda. Their anti-V_2 pA_2 values are 8.00 and 8.10, respectively. These findings clearly confirm that the Eda modification is very well tolerated at a variety of positions in the AVP V_2/V_{1a} antagonists studied here. In the second part of the study reported here we have presented the synthsis and antagonistic potencies of analogues of these six C-terminal Eda peptides having a variety of amino acids coupled in retrolinkage to the Eda residue in each case. With an anti-V_2 pA_2 = 7.54, the [ring]Eda←Arg analogue 2 (Table I) with D-Tyr(Et) at position two is clearly the most potent of the five position 2 modified peptides presented in Table I. Thus the [ring]Eda←Arg peptide 2 was the logical choice for the studies reported in Table II aimed at exploring the effect of replacing the retro-Arg in 2 by D-Arg (7), Gly (8), Orn (8), D-Orn (10), D-Lys (11), or Arg-Arg (12) in a retrofashion. Apart from the Gly and D-Orn substitutions, all the remaining analogues, 7, 9, 11, and 12, exhibit excellent retention of V_2 antagonism. With an anti-V_2 pA_2 = 7.95 the retro-D-Arg derivative 7 is over twice as potent as its L-Arg counterpart 2 (anti-V_2 pA_2 = 7.54). Likewise, with anti-V_2 pA_2 of 7.92 the retro-D-Orn (14) and retro-D-Arg (16) analogues exhibit significant enhancements in V_2 antagonism relative to their [ring]-Pro-Eda parent 13, which has an anti-V_2 pA_2 = 7.64. With anti-V_2 pA_2 values of 8.10, 8.10, and 8.09, the retro-Arg, retro-Val, and retro-D-Val analogues 22, 24, and 25 (Table V) are among the most potent V_2 antagonists reported to date. The enhancements in anti-V_2 potencies observed for the Ile[4] analogue 22 relative to the Val[4] analogue 18 and for many of the retro-D-amino acid analogues offer promising clues to the design of more potent V_2 antagonists. A number of the peptides reported here may be orally active.

It should also be noted that with only a few exceptions (5 and 6, Table I; 24, Table III) virtually all of the C-terminal Eda peptides and the Eda-linked retrosubstituted amino acid analogues reported here are also potent vasopressor antagonists with anti-V_{1a} pA_2 values in the range 7.64 (4, Table I) to 8.33 (21, Table III). Thus this approach clearly also has merit for the design of potent and selective AVP V_{1a} antagonists.

To our knowledge the use of Eda as a C-terminal linker for the incorporation of retro-amino acids into biologically active peptides has not been previously reported. The novel findings reported here clearly illustrate the usefulness of this approach for the design of cyclic AVP V_2/V_{1a} antagonists. In a separate study, to be reported elsewhere, we have also demonstrated the usefulness of this approach in the design of potent linear antagonists of vasopressin.[12,13] The findings reported here offer promising clues to the design of (i) more potent and selective AVP V_{1a} and V_2 antagonists; (ii) orally active AVP V_2 antagonists; and (iii) photoaffinity and radioiodinated ligands for use as probes of AVP V_2, V_{1a} and V_{1b} (pituitary) receptors. They may also be of value in the design of antagonists of other biologically active peptides.

Experimental Section

Amino acid derivatives were purchased from Bachem Inc. or from Chemalog. Boc-L-Tyr(Et), Boc-D-Tyr(Et),[51] and β-(benzylthio)-β,β-pentamethylenepropionic acid[43] were synthesized by previously published procedures. The protected peptides terminating in ethylenediamine (I, XIII, XVII, XIX, XXI, XXIII, and IIIA–VIA, Table IV) were synthesized by the manual solid-

(51) Kolodziejczyk, A. M.; Manning, M. A convenient method for O-alkylation of N-substituted, tyrosines using a crown ether. *J. Org. Chem.* 1981, *46*, 1944–1946.

phase method.[2-6,35-37] Chloromethylated resin (Bio-Rad Bio-Beads SX-1) was esterified with Boc-Cys(Bzl), Boc-Pro, Boc-Arg(Tos), and Boc-Gly to an incorporation of 0.5–0.65 mmol/g by the cesium salt method.[52] Boc amino acids (except Boc-asparagine) and β-(benzylthio)-β,β-pentamethylenepropionic acid were coupled by the DCC/HOBt[40,41] procedure in CH_2Cl_2 or in CH_2Cl_2/DMF (9:1, v/v). Boc-asparagine was coupled as its nitrophenyl ester[42] in DMF. Aminolysis with ethylenediamine and purification of the protected C-terminal Eda peptides was carried out as previously described.[38,39] All the retrocouplings were carried out in dimethylformamide (DMF) anhydrous (99+%), Aldrich Chemical Co. Other solvents and reagents were of analytical grade. For the retroadditions the following amino acid derivatives were coupled to the appropriate protected C-terminal Eda peptide by the DCC/HOBt[40,41] or active ester[42] procedures to give the protected retromodified peptides II–XII, XIV–XVI, XVIII, XX, XXII, XXIV, and XXV (Table IV): Z-Arg(Tos), Boc-D-Arg(Tos), Z-Gly-ONp, Z-Orn(Z) Boc-D-Orn-(Tos), Boc-D-Lys(Z), Boc-D-Val, Z-Val-ONp. The choice of protected amino acid derivative was dictated solely by commercial availability. For the Boc-containing retroprotected peptides, the Boc group was removed with TFA prior to total deblocking with sodium liquid ammonia[44] as previously described.[2-6] The protected retropeptides III–VI were prepared from the appropriate protected Eda peptide IIIA–VIA as for II. The physicochemical properties of the purified protected peptides (I–XXV and IIIA–VIA) are given in Table IV. All protected precursors (I–XXV) were converted to the required free cyclic peptides (**1–25**) by deblocking with Na/liquid NH_3,[2-6,44] oxidative cyclization with $K_3[Fe(CN)_6]$,[45] desalting, and purification in a two-step procedure using gel filtration on Sephadex G-15 as previously described.[46] The physicochemical properties of the free peptides (**1–25**) are given in Table V. Thin-layer chromatography (TLC) was performed on silica gel precoated plates (0.25 mm, Merck). The following solvent systems were used: (A) butan-1-ol–acetic acid–water (4:1:5, v/v/v, upper phase), (B) butan-1-ol–acetic acid–water (4:1:1, v/v/v), (C) butan-1-ol–acetic acid–water–pyridine (15:3:3:10, v/v/v/v), (D) chloroform–methanol (7:3, v/v). Loads of 10–50 μg were applied and chromatograms were a minimum length of 10 cm. The chlorine gas procedure for the KI–starch reagent was used for detection.[37] Optical rotations were measured with a Rudolph Autopol III polarimeter. For amino acid analysis[53] peptides (approximately 0.5 mg) were hydrolyzed with constant boiling hydrochloric acid (500 μL) containing a 1% solution of phenol in water (10 μL) in evacuated and sealed ampules for 24 h at 110 °C. The analyses were performed on a Beckman System 6300 amino acid analyzer. Molar ratios were referred to Phe or Gly = 1.00. All peptides gave the expected amino acid ratios ±3%. Melting points of the protected peptides are uncorrected.

Solid-Phase Synthesis. Chloromethylated resin (Chemalog 1% cross-linked S-DVB 200–400 mesh, 0.7–1.00 mmol/g) was esterified with either Boc-Gly, Boc-Pro, Boc-Cys(Bzl), or Boc-Arg(Tos) to an incorporation of approximately 0.5 mmol/g by the cesium salt method.[54] Five, six, seven, or eight cycles of deprotection, neutralization, and coupling were carried out for the synthesis of the required 10 protected peptidyl resins. Aminolysis with ethylenediamine (Eda) in MeOH[38] was used to split the protected peptides (I, XIII, XVII, XIX, XXI, XXIII, and IIIA–VIA) from the resin. All of the protected precursors were purified by the same general method: extraction with hot DMF followed by reprecipitations with H_2O and EtOH/Et_2O until adjudged pure by TLC.

[(β-Benzylthio)-β,β-pentamethylenepropionyl]D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-NH(CH$_2$)$_2$NH$_2$ (I, Table IV). Aminolytic Cleavage.[38,39] Boc-Cys(Bzl) resin (1.05 g, 1.0 mmol) was converted to protected acyl pentapeptidyl resin in five cycles of deprotection, neutralization and coupling (mediated by DCC/HOBt or active ester) with Boc-Asn-ONp, Boc-Val, Boc-Phe, Boc-D-Tyr(Et), and β-(benzylthio)-β,β-pentamethylenepropionic acid, respectively. The resulting protected peptidyl resin (1.74 g, 1.0 mmol) was suspended in anhydrous methanol (30 mL) and ethylenediamine (30 mL), freshly distilled from Na) was added; the suspension was stirred at room temperature for 48 h. The solvents were evaporated, and the protected peptide was extracted with hot DMF (ca. 30 mL) and reprecipitated by the addition of hot water (ca. 500 mL). Following overnight storage at 4 °C, the product was collected, dried in vacuo over P_2O_5, and reprecipitated from hot DMF (5 mL) with EtOH (50 mL) and Et_2O (ca. 300 mL). The solid was collected and dried in vacuo over P_2O_5 to give the required protected acyl pentapeptide Eda (I, Table IV). With the appropriate Boc amino acid substituted resin as starting material similar procedures were used for the synthesis and purification of the related C-terminal Eda substituted protected peptides (Table IV) required for the synthesis of all of the retrosubstituted protected peptides. The physicochemical properties of the protected C-terminal Eda peptides I, XIII, XVII, XIX, XXI, XXIII, and IIIA–VIA are given in Table IV.

[(β-(Benzylthio)-β,β-pentamethylenepropionyl]D-Tyr-(Et)-Phe-Val-Asn-Cys(Bzl)-NH(CH$_2$)$_2$NH←Z-Arg(Tos) (II, Table IV). Retrosynthesis 1. To a cooled (0 °C) solution of Z-Arg(Tos)-OH (370 mg, 0.8 mmol) and HOBt (185 mg, 1.2 mmol) dissolved in 3 mL of DMF was added 165 mg (0.8 mmol) of DCC. The reaction mixture was stirred for 1 h, whereupon the dicyclohexylurea (DCU) was removed by filtration. The filtrate was added to a solution of $(CH_2)_5C$(S-Bzl)CH_2-CO-D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-NH$(CH_2)_2NH_2$ (I) (210 mg, 0.2 mmol) in DMF (2 mL). Et_3N was added to give a pH ~7.5. After the mixture was stirred for 18 h at room temperature, MeOH (20 mL) was added followed by Et_2O (250 mL). The precipitated product was collected after storage overnight at 4 °C. Washing with warm MeOH gave the required protected peptide II, 204 mg, yield 72.0% (II, Table IV). With minor variations this procedure was followed to prepare the protected retromodified peptides III–VI, IX, XVIII, XX, and XXII (Table IV) from the corresponding Eda peptide and the appropriate Z protected amino acid. The protected retropeptides VIII and XXIV were synthesized in DMF using HOBt-assisted active ester couplings of Z-Gly-ONp and Z-Val-ONp, respectively, to the protected Eda peptides I and XXII.

[β-(Benzylthio)-β,β-pentamethylenepropionyl]D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-NH(CH$_2$)$_2$NH←Boc-D-Arg(Tos) (VII, Table IV). Retrosynthesis 2. To a cooled (0 °C) solution of Boc-D-Arg(Tos) (360 mg, 0.85 mmol) and HOBt (190 mg, 1.25 mmol) dissolved in 3 mL of DMF was added 175 mg (0.8 mmol) of DCC. The reaction mixture was stirred for 1 h followed by the removal of the precipitated DCU. The resulting filtrate was added to a solution of $(CH_2)_5C$(S-Bzl)-CH_2-CO-D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-NH$(CH_2)_2NH_2$ (I) (262 mg, 0.25 mmol) following the procedure used for the protected analogue II to give VII (284 mg, yield 79.9%). Protected peptides X, XI, XIV–XVI, and XXV (Table IV) were prepared in the same way from the corresponding protected Eda peptide and the appropriate Boc-protected amino acids. The Boc group was removed as follows prior to the sodium liquid ammonia reduction. VII was placed in a small tube with a stopper, and 2 mL of TFA was added. The solution was stirred for 30 min followed by the addition of Et_2O and stirring was continued for 15 min. The solid crystals were collected by filtration, washed with Et_2O, and dried in vacuo over KOH and P_2O_5 (160 mg, yield 80.4%). This procedure was used for the deblocking of the Boc group from each of the remaining protected peptides X, XI, XIV–XVI, and XXV (Table IV).

[(β-Benzylthio)-β,β-pentamethylenepropionyl]D-Tyr(Et)-Phe-Val-Asn-Cys(Bzl)-NH(CH$_2$)$_2$NH←Arg(Tos)←Z-Arg-(Tos) (XII, Table IV). Retrosynthesis 3. The double retromodified protected peptide XII was obtained from I in two coupling steps. The first coupling step was made following the procedure utilized for VII using the following intermediates: Boc-Arg(Tos) (525 mg, 1.2 mmol), HOBt (243 mg, 1.8 mmol), DCC (248 mg, 1.2 mmol), and compound I (315 mg, 0.3 mmol). The yield from this first coupling was 393 mg (89.9%). Following deblocking of Boc-Arg(Tos) with TFA as described for VII the resulting TFA salt (390 mg (0.26 mmol) was used directly in the second coupling step as described for II with Z-Arg(Tos) (555

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mg, 1.2 mmol), HOBt (243 mg, 1.8 mmol), and DCC (248 mg, 1.2 mmol) to give XII (330 mg), yield 61%.

[(β-Mercapto-β,β-pentamethylenepropionyl)]D-Tyr(Et)-Phe-Val-Asn-Cys-NH(CH$_2$)$_2$NH←Arg (2, Tables I and V). A solution of the protected peptide (II, Table II) (160 mg) in sodium-dried ammonia (ca. 500 mL) was treated at the boiling point and with stirring with sodium from a stick of 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 nitrogen was passed through the flask. After 5 min, the residue was dissolved in degassed aqueous acetic acid (50%, 80 mL) and quickly poured into ice-cold water (ca. 1000 mL). The pH was adjusted to approximately 7.0 with concentrated ammonium hydroxide. Following neutralization, an excess of a solution of potassium ferricyanide (0.01 M, 14 mL) was added gradually with stirring. The yellow solution was stirred for an additional 20 min, and after acidification with 0.2 N AcOH to pH 4, for 10 min with anion-exchange resin (Bio-Rad AG-3, Cl^- form, 10 g damp weight). The suspension was slowly filtered through a bed of resin (10 g damp weight), and the bed was washed with 0.2 M AcOH (3 × 100 mL); 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 eluate 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 analogue (**2**, wt 27 mg (40.3%) Table V). With minor modifications this procedure was utilized to give the remaining 24 free peptides **1**, **3–25**) in Table V.

Acknowledgment. This work was supported in part by research grants from the National Institute of General Medical Sciences (No. GM-25280; M.M.), the National Institute of Diabetes, Digestive and Kidney Diseases (No. DK-01940; W.H.S.), and the National Institute of Child Health and Human Development (HD-20839; W.Y.C.). We thank Ms. Ann Chlebowski, Ms. Lorrie Newman, and volunteer Mr. Brian Manning for their expert assistance in the preparation of the manuscript.