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Biological and Chiroptical Sequelae of Graded Alkyl Substitutions in the Vasopressin Ring

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SUMMARY

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Using 1-deamino-[8-D-arginine]-vasopressin (dDAVP) as a reference substance because of its practically pure antidiuretic activity with very little smooth muscle agonism, substitutions for 4-glutamine were made in the following order: serine, glycine, alanine, α-aminobutyric acid, valine, isoleucine, and leucine. These substances were assayed for antidiuretic activity in trained, unanesthetized, water-loaded rats and for antagonism to the pressor action of arginine-vasopressin and angiotensin II amide in ganglion-blocked, urethane-anesthetized rats. Some of the 4-substituted analogues were also tested in normal, consenting human volunteers in water diuresis. Circular dichroic spectra of the series were measured in 0.02 M phosphate buffer, pH 7.1, and in hexafluoroacetone. In the order given, antidiuretic activity decreased from the natural glutamine at position 4 through serine to practically nil at glycine, increased again to a peak at valine, and then decreased through isoleucine to leucine. Antagonistic action to arginine-vasopressin on vascular smooth muscle became evident first with 4-α-aminobutyric acid substitution and increased gradually through valine and isoleucine to leucine. There was no evidence of competition of any of these molecules with dDAVP or arginine-vasopressin at the antidiuretic receptor, or with angiotensin II amide at the vascular smooth muscle receptor. This activity spectrum appeared to be related to both bulk and hydrophobicity of the side chain at position 4. Analysis of the circular dichroic spectra showed that while the basic conformation was not qualitatively altered by substitution at position 4 (with the possible exception of 1-deamino-[4-α-aminobutyric acid,8-D-arginine]-vasopressin) there were quantitative differences in the separate bands. There was a suggestive correlation between antidiuretic activity and the amplitude of the positive band at 225 nm.

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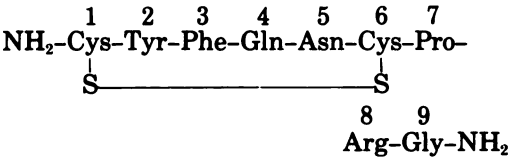
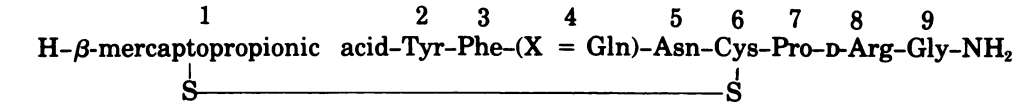
INTRODUCTION

The cyclic neurohypophysial nonapeptide hormone arginine-vasopressin



has one of the widest spectra of biological actions, the lowest threshold for receptor interaction, and one of the highest potencies of any natural or synthetic substance (see ref. 1). Because of the approximate nature and lack of specificity of so many biological assays, this wide spectrum is a disadvantage for studies of structure-activity relationships. A clinically useful AVP[6] analogue results when the NH_2 terminus is deaminated (to slow 1–2 C–N cleavage) and D-arginine is substituted for the L isomer in the COOH-terminal tripeptide [which theoretically should render the 7–8 and 8–9 C–N bonds relatively uncleavable and, in fact, practically eliminates triggering interaction with all smooth muscle receptors (1-3)]. This analogue, 1-deamino-[8-D-arginine]-vasopressin,



has a much higher peak antidiuretic potency than its parent hormone, AVP, and because of the above structural changes has (a) an activity persistence for 7–10 hr after single doses as compared with less than 1 hr for AVP and (b) no smooth muscle side effects either for the patient or as a complicating factor in the present studies. It has become the treatment of choice for central diabetes insipidus (4–7). Recently it has been reported that if polar 4-glutamine is replaced by hydrophobic 4-valine in dDAVP, the antidiuretic potency and response persistence are still further increased (1, 8, 9). This latter site is one of the "least crowded" in the molecule (see DISCUSSION) in terms of COOH-terminal tripeptide interactions with the ring side chains (10).

In the present work a series of 4-substituted dDAVP analogues was prepared and studied, in which the polar extreme was the parent molecule (dDAVP, X = glutamine) and the substituents at 4-X varied through a shorter polar side chain (serine), no side chain (glycine), and side chains of increasing hydrophobicity, bulk, and length (alanine, α-aminobutyric acid, valine, isoleucine, and leucine). Biological studies involved estimates of antidiuretic activities of the entire series in the rat and of some of the series in man, and measurements of agonist and antagonist smooth muscle actions in the rat. With the exception of 1-deamino-[4-serine,8-D-arginine]-vasopressin, where sufficient material was not available, circular dichroism was measured in 0.02 M phosphate buffer, pH 7.1, and in hexafluoroacetone trihydrate.

MATERIALS AND METHODS

Materials. dDAVP and AVP were commercial products of Ferring, Ltd., Malmö, and were further purified by continuous free-flow electrophoresis. [5-Valine]-angiotensin II amide was Hypertensin from Ciba, Basel. [4-Abu]-dDAVP was prepared by one of us (D. G.) in Basel; the remaining analogues were prepared by L. C. in Malmö. The syntheses and analytical data on these analogues will be published elsewhere. Dr. K. Jošt of this Academy Institute supplied three oxytocin antagonist analogues: [2-tyrosine-*O*-methyl]-, *N*-acetyl-[2-tyrosine-*O*-methyl]-, and *N*-sarcosyl-[2-tyrosine-*O*-methyl]-oxytocin (11–13). With the exception of AII, all peptides were available as highly purified freeze-dried powders, with no dimer chromatographically demonstrable, and kept in sealed tubes at −40°. For biological assays stock solutions of 100 μg/ml were made up in sterile 0.9% NaCl at pH 3.5 (added HCl) from weighed amounts at frequent inter-

[6] The abbreviations used are: AVP, arginine-vasopressin; dDAVP, 1-deamino-[8-D-arginine]-vasopressin; [4-Abu]-dDAVP, 1-deamino-[4-α-aminobutyric acid, 8-D-arginine]-vasopressin; AII, [5-valine]-angiotensin II amide.

vals. These stock solutions were further diluted with neutral sterile NaCl freshly for each assay and used within 20 min, and the unused remainder was discarded.

Methods. CD spectra were measured at room temperature in a Roussel-Jouan Dichrographe CD 185, model II, as molar ellipticites [θ] (degrees per square centimeter per decimole, based on total molecular weight) under conditions standard for this laboratory (14) over the spectral range 185–300 nm. The dry powders were dissolved in a concentration range of 0.2–0.3 mg/ml in either 0.02 M phosphate buffer, pH 7.1, or hexafluoroacetone trihydrate. Peptide concentration was taken as weight per volume.

Antidiuretic rat assays were carried out by a modification of the Burn assay on large numbers of trained, unanesthetized Wistar male rats (190–250 g) under conditions specified in detail elsewhere (1). All results were expressed as activities relative to the parent dDAVP (1.00).

Antidiuresis in the human was tested under conditions of mild water diuresis on five volunteers who gave their informed consent (including two of us, J. H. C. and J. Š.) by procedures described elsewhere (1). Increments in $(U/P)_{osm}$ and $(U/P)_{creatinine}$ [where (U/P) = the ratio of concentrations in urine to plasma] were taken as measures of peak antidiuretic response (all related to dDAVP and control water diuresis tests without peptides in the same subjects).

Pressor assays were carried out on male Wistar rats (250–300 g) under urethane anesthesia, ganglion-blocked with pentolinium (Ansolysen Wyeth) under conditions standard for this laboratory (15). The animals were not nephrectomized. The reference standards were AVP and AII, selected because these two highly pressor hormones apparently have molecule-specific receptors in vascular smooth muscle (16, 17).

All measured and calculated data were analyzed for statistical significance by covariance analysis and Student's *t*-test.

RESULTS

Circular dichroism. Two media were used for measurements of CD spectra: 0.02 M phosphate buffer (pH 7.1) and hexafluoroacetone, which serves to weaken intramolecular interactions (18). Figure 1 shows only a few selected CD spectra to illustrate the basic bands and the nature of the changes produced by either substitution at position 4 or a shift to hexafluoroacetone. The amplitudes of the main bands below 250 nm are given for the entire series (except for [4-Ser]-dDAVP) in Table 1. All the changes were relatively small, so that no qualitative change in conformation resulted from substitution at position 4. [θ] values of the positive band at about 225 nm were used as the basis for comparison of analogues. This band, resulting from a superposition of the B_{1u} aromatic band of tyrosine (about 228 nm) and the amide $n - \pi^*$ band (at about 220 nm) is sensitive to changes in conformation of the tyrosine side chain and can serve as an index of its interaction with other parts of the molecule. In both media the amplitude of this band was a function of the side chain at position 4, increasing from glycine through alanine to valine, then decreasing again in the order leucine > isoleucine. [4-Abu]-dDAVP was not included in the

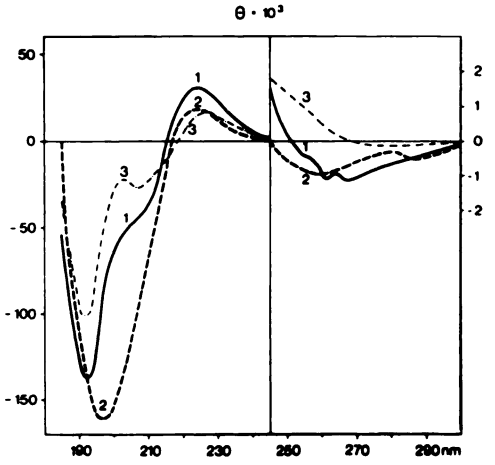


FIG. 1. *Sample spectra of analogue series 1-deamino-[4-X,8-D-arginine]-vasopressin to illustrate basic bands and changes due to substitution at X and transition from 0.02 M phosphate buffer (pH 7.1) to hexafluoroacetone trihydrate*

Ordinate, calculated molar ellipticities [θ] in thousands (note the scale expansion from 245 to 300 nm); abscissa, wavelength in nanometers. Curve *1*, X = valine (buffer); curve *2*, X = valine (hexafluoroacetone); curve *3*, X = glycine (buffer).

TABLE 1

Peak wavelengths and [θ] values of calculated molar ellipticities (in parentheses) of CD spectra of the analogue series 1-deamino-[4-X,8-D-arginine]-vasopressin

X	ΔH_S	Positive complex		Amide $\pi - \pi^*$		Short-wavelength aromatic	
		Buffer	Hexafluoroacetone	Buffer	Hexafluoroacetone	Buffer	Hexafluoroacetone
		nm	*nm*	*nm*	*nm*	*nm*	*nm*
Glycine	0.0	226 (+16,000)	224 (+9,100)	207[a] (−26,900)	205[b] (−59,000)	192 (−101,000)	190 (−159,000)
Alanine	+1.0	224 (+23,000)	223 (+17,900)	205[b] (−45,000)	202.5[b] (−52,000)	193 (−135,000)	190 (−102,000)
α-Aminobutyric acid	+2.0	227 (+17,000)	227 (−10,000)	207.5[b] (−60,000)	201 (−89,000)	194 (−153,000)	
Valine	+4.4	224 (+30,900)	224 (+19,200)	205[b] (−49,000)		192 (−139,000)	197 (−160,000)
Isoleucine	+5.2	223.5 (+20,200)	222 (+13,600)	203[b] (−38,000)	202[b] (−72,000)	192.5 (−87,000)	194.5 (−109,000)
Leucine	+5.9	224.5 (+26,900)	222.5 (+17,100)	205[b] (−50,000)		194.5 (−145,000)	192 (−154,000)
Glutamine	−2.7	227.5 (+17,100)	224 (+11,500)	203.5[b] (−50,000)	202.5[b] (−45,000)	193.5 (−91,000)	190 (−83,000)

[a] Minimum at 202.5 nm (−21,000).
[b] Shoulder.

above comparison because of its exceptional properties. In contrast with the other analogues, it showed a positive, long-wavelength tyrosine band, and its positive complex band disappeared in hexafluoroacetone, as did the short-wavelength tyrosine band at 193 nm (Table 1).

Antidiuretic activity in rats. Figure 2 presents a series of parallel log dose–response ($T_{0.5}$) curves for most of the given series of analogues. Because of the crowded, vertical nature of the curves, the standard error distribution lines have been omitted, but statistical comparison of the relative activities [taken from comparison of doses required for a corrected $T_{0.5}$ of excretion of a 2% (v/w) oral water load of 200 min] is given in Table 2. The order of activities from Fig. 2 is: [4-Val]-dDAVP > dDAVP > [4-Abu]-dDAVP > [4-Ile]-dDAVP = [4-Ala]-dDAVP > [4-Leu]-dDAVP > [4-Ser]-dDAVP ≧ [4-Gly]-dDAVP. This takes on a somewhat different appearance when the relative activities are plotted against relative hydrophobicities (ΔH_S) of the separate substituent side chains in Fig. 3 (19). With ΔH_S = O at

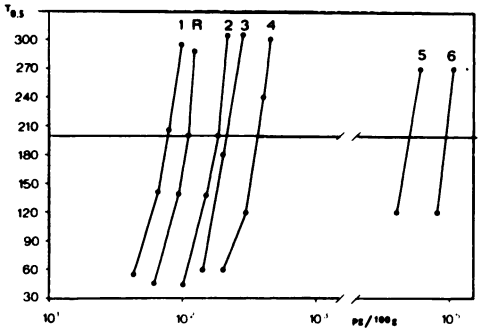


FIG. 2. *Log dose (abscissa)–antidiuretic response ($\Delta T_{0.5}$ in minutes, ordinate) curves for analogue series 1-deamino-[4-X,8-D-arginine]-vasopressin*

$\Delta T_{0.5}$ = corrected (minus control values) time for 50% excretion of a 2% (v/w) oral water load in trained rats (see ref. 1). X residues for the separate curves are: *R* (reference dDAVP) = glutamine; *1* = valine; *2* = α-aminobutyric acid; *3* = combined data for isoleucine, alanine, and AVP; *4* = leucine; *5* = serine; *6* = glycine.

glycine (no side chain) the curve has its minimum inflection, equivalent to practically no antidiuretic activity. With increasing length or bulk of polar side chains to the left of glycine—in the order serine >

TABLE 2

Relative antidiuretic activities of [4-X]-dDAVP, referred to dDAVP = 1.00

For calculation from log dose-response curves, see ref. 1. n = degrees of freedom.

X	Relative activity	n
Glutamine (dDAVP)	1.00 ± 0.12	20
(AVP)	0.52 ± 0.08	20
Serine	0.02 ± 0.01	10
Glycine	0.01 ± 0.01	8
Alanine	0.48 ± 0.05	10
α-Aminobutyric acid	0.65 ± 0.06	12
Valine	1.47 ± 0.13	20
Isoleucine	0.49 ± 0.04	10
Leucine	0.24 ± 0.03	10

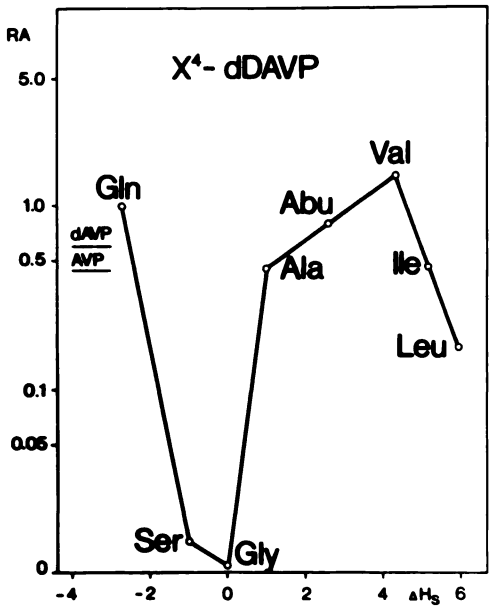


FIG. 3. *Relative antidiuretic activities (RA, ordinate; see Table 2) vs. relative hydrophobicities of 4-side chain (ΔH_s, abscissa; see ref. 19) for analogue series 1-deamino-[4-X,8-D-arginine]-vasopressin*

X residues are identified at each point in the curve. Two reference values for relative activity not belonging to the analogue series are given on the left: AVP and deamino-AVP (dAVP) (20).

glutamine—there is increasing activity. With increasing hydrophobicity and side chain length to the right of glycine—in the order alanine > α-aminobutyric acid > valine > isoleucine > leucine—there is first an increase in activity, then a maximum inflection point at valine, followed by a decrease through isoleucine to leucine.

The above refers to triggering interactions with the antidiuretic receptor. Interaction without triggering—antagonism—can be reliably tested only when the intrinsic agonist activity of a potential anti-AVP molecule is less than 0.2 (referred in this case again to dDAVP = 1.00). The two least active members of the present series, the 4-serine and 4-glycine analogues, were tested with AVP and dDAVP in the molar ratio range 10:1–1000:1. In addition, the three oxytocin antagonist analogues, which also show crossover antagonism to smooth muscle actions of vasopressin (21, 22), were combined with AVP and dDAVP in the same molar range as above. Each assay contained three groups: agonist alone, "antagonist" alone, and the same doses of both together in the same 0.1-ml volume. There was no evidence of inhibition of the antidiuretic action of AVP or dDAVP by any of these analogues.

Antidiuretic activity in humans. Four dDAVP analogues (X = valine, α-aminobutyric acid, isoleucine, and leucine) were tested in a group of five healthy human volunteers, along with reference dDAVP, with both subcutaneous and intranasal administration [the latter doses were 10 times the former because absorption from the nasal mucosa is about 10% (2)]. Table 3 shows that with subcutaneous administration the same order of potencies was observed as in the rat. Of interest was the observation that with intranasal administration the two most hydrophobic analogues, isoleucine and leucine at position 4, showed a far lower antidiuretic response than was expected after the subcutaneous route.

Rat pressor assays. The absolute pressor activity of dDAVP is only about 0.5 IU/mg, and hydrophobic substitutions at position 4, such as valine and leucine, are known to suppress this action still further in vasopressin (8, 23). Thus all 8-D-arginine analogues have such low activities that the error of measurement is high and large amounts of peptide are lost. Of greater interest than agonist actions (which have

TABLE 3

Comparison of 5-μg intranasal and 0.5-μg subcutaneous doses of dDAVP analogues in five healthy control subjects, average age 39 ± 2 years

D = values at height of water diuresis before peptide; MA = maximal antidiuresis; $(U/P)_x$, concentration ratio of x between urine and plasma; Cr = endogenous creatinine; OSM = osmolarity.

Analogue	$(U/P)_{Cr}$	D:MA	$(U/P)_{OSM}$	D:MA
Control[a]				
D	15.03 ± 2.4	0.89	0.34 ± 0.04	0.81
MA	16.85 ± 3.5		0.42 ± 0.08	
dDAVP				
Intranasal D	15.5 ± 3.1	0.28	0.40 ± 0.03	0.29
Intranasal MA	55.2 ± 5.7		1.39 ± 0.43	
Subcutaneous D	14.8 ± 2.1	0.25	0.38 ± 0.05	0.25
Subcutaneous MA	60.3 ± 4.6		1.54 ± 0.54	
[4-Val]-dDAVP				
Intranasal D	18.2 ± 3.8	0.13	0.44 ± 0.01	0.19
Intranasal MA	136.1 ± 15.2		2.38 ± 0.13	
Subcutaneous D	14.7 ± 0.6	0.12	0.36 ± 0.05	0.17
Subcutaneous MA	125.4 ± 10.3		2.13 ± 0.20	
[4-Abu]-dDAVP				
Intranasal D	16.3 ± 2.2	0.38	0.32 ± 0.05	0.37
Intranasal MA	43.2 ± 2.1		0.86 ± 0.11	
Subcutaneous D	13.7 ± 1.6	0.35	0.44 ± 0.07	0.48
Subcutaneous MA	40.6 ± 0.9		0.92 ± 0.12	
[4-Ile]-dDAVP				
Intranasal D	19.3 ± 3.3	0.83	0.48 ± 0.06	0.85
Intranasal MA	23.2 ± 1.4		0.56 ± 0.03	
Subcutaneous D	16.3 ± 2.4	0.42	0.34 ± 0.04	0.47
Subcutaneous MA	38.5 ± 3.6		0.72 ± 0.20	
[4-Leu]-dDAVP				
Intranasal D	13.4 ± 1.2	0.65	0.40 ± 0.05	0.64
Intranasal MA	20.7 ± 1.8		0.62 ± 0.10	
Subcutaneous D	15.8 ± 2.2	0.51	0.36 ± 0.04	0.53
Subcutaneous MA	31.0 ± 3.0		0.68 ± 0.06	

[a] Control runs with no peptide administered. MA values were taken from samples collected at same time as in runs with peptide. For all details, see ref. 1.

not been compared here) is the finding that the more hydrophobic members of the present series (starting from 4-α-aminobutyric acid and progressing to 4-leucine) proved to be partial antagonists to the pressor action of AVP, but not to AII. In these assays the potential antagonist was given intravenously 30 sec before the standard pressor peptides (AVP and AII) in the molar ratio range 10:1–1000:1 (all in 0.1 ml), or both substances were administered together in the same volume. Each animal first received four repeated doses of AVP and AII (2, 5, 10, and 20 ng/rat), and when repeatable dose-response curves were established, the 5- or 10-ng standard doses were combined with increasing molar ratios of potential antagonist. The same standards were tested between each pair of combined injections, and the dose-response curves were repeated at the end of the experiment. Table 4 presents a summary of the molar ratio ranges of the various 4-X analogues of AVP which resulted in 50% inhibition of the action of the latter. No inhibitory action of any of the molecules to the pressor action of AII was observed. Antagonist to agonist ratios of 3000:1 were tested to find maximal inhibition, which never exceeded 75% against AVP.

If responses to the two standards were compared on an equimolar basis, peak response to AVP was 1.3–3.0 times that to

TABLE 4

Molar ratios of [4-X]-dDAVP to AVP necessary for 50% inhibition of pressor response to 5 or 10 ng of AVP per rat

X	*n*	Ratio giving 50% inhibition	Maximal inhibition at 3000:1 ratio
			%
Glutamine (dDAVP)	5	—[a]	0
Serine	5	—[a]	0
Glycine	5	—[a]	0
Alanine	5	—[a]	0
α-Aminobutyric acid	8	362 ± 34	65
Valine	10	210 ± 22	68
Isoleucine	15	105 ± 14	70
Leucine	15	85 ± 8	75

[a] No inhibition.

AII, and in all cases persistence of the AVP response was greater than AII persistence. Comparison of mean integral responses gave an AVP:AII ratio of 5.3 ± 1.4.

DISCUSSION

The antidiuretic receptor has been characterized as having an extremely high affinity for vasopressin but very low stereochemical requirements, as compared with the vascular smooth muscle receptor (1, 16). This view of the triggered receptor-vasopressin interaction has proved useful in explaining, for example, why 8-D-arginine-vasopressin analogues have a high antidiuretic potency and persistence but very little agonist smooth muscle activity. The biological data on antidiuretic activity in rats and humans for the present analogue series confirm and considerably extend previous reports that two alkyl substitutions at position 4 in vasopressin and dDAVP—valine and leucine—result in an increase and decrease, respectively, in antidiuretic activities (1, 8, 9, 23). The complete series of [4-X]-dDAVP analogues shows that at 4-valine there is maximum receptor interaction, possibly with increased triggering affinity (1). It was not possible to show that binding without triggering, or competitive antagonism, occurs in the interaction of any cyclic neurohypophysial nonapeptide tested here with the antidiuretic receptor in the renal medulla. The human data further suggest that with sufficient side chain bulk or hydrophobicity (formally, the number of carbon atoms in the δ position of the aliphatic side chain) at this same site, the absorption of these molecules across the mucosal membrane may be retarded.

As previously reported (16), the vascular smooth muscle receptor for vasopressin behaves quite differently, with higher stereochemical requirements. Binding without triggering at this receptor has long been known to be associated with decreases in polarity in the ring side chains, involving the *p*-hydroxyl group of 2-tyrosine in particular (see ref. 22). In the present 4-X series of dDAVP analogues, the glycine and alanine forms showed neither antagonist nor agonist activity to any degree, but, starting with 4-α-aminobutyric acid and increasing through valine and isoleucine to leucine, there was increasing antagonist potency. Rather than a peak at valine, a plateau was formed at leucine or beyond. In other words, these latter changes resulted in a continuous increase in receptor binding without triggering. The effect was specific for vasopressin in the sense that it did not cross-react with AII.

The conformation of the basic cyclohexapeptide ring is roughly the same for all neurohypophysial hormones. Relatively drastic changes in the amino acid sequence of oxytocin, vasotocin, and vasopressin are reflected by only small changes in the 220-MHz ^{1}H NMR spectra (24). Even deamination of the NH_2 terminus results in relatively small changes (25). The COOH-terminal tripeptide appears to have greater conformational freedom than the ring [particularly in AVP (10, 24, 26)] and interacts mainly with the side chains at positions 1, 2, 5, and 6, rather than 3 and 4. ^{1}H NMR spectroscopy of lysine-vasopressin has suggested a stacking position of the adjacent 2-tyrosine and 3-phenylalanine aromatic side chains (27). These data indicate that position 4 in the ring, in the region of the β turn (28), is the "least

crowded site." Substitution of the 2-carbamoylethyl side chain of glutamine need not, therefore, result in excessive steric strain which would alter the basic conformation. From Walter's models (10, 24) we can estimate approximate torsion angles ϕ and ψ of residues 3 and 4 (ϕ_3 about +30°, ψ_3 about −90°, ϕ_4 about −90°, ψ_4 about +30°), which, according to quantum chemical calculations (29), are in the permissible range (including energy minima) in conformation maps of dipeptides of all the residues involved here and would correspond to type II' (β turn) of Lewis *et al.* (28).

The CD measurements presented here are in general agreement with the above, since in almost the entire series we observed only quantitative changes in $[\theta]$ of some of the bands. Since these bands involve electron transitions of aromatic nuclei, the changes measured will reflect local changes in interaction (of the buttressing effect type) of the side chain at position 4 with the benzyl side chains positions at 3 and 2. The hydrophobic character of the side chains at positions 3 and 4 is important for receptor contact. With increasing hydrophobicity of residue 4 (glycine to leucine) (30, 31), antidiuretic activity was optimal at 4-valine, which indicates that this factor is not the only one involved, and that bulk and length of the side chain must also be taken into consideration. The presence of δ-carbon atoms (1 in isoleucine and 2 in leucine) would appear to be a steric blockage of peptide complexing with antidiuretic receptors.

Comparison of the biological data with Table 1 suggests that some rough correlations may exist for separate bands of the CD spectra. Antidiuretic activity showed a peak at [4-Val]-dDAVP, and the same molecule also showed the highest positive $[\theta]_{n-\pi^*}$ values at about 225 nm, in a spectral region where differences were great enough to be considered significant (see Fig. 4). Antagonism to the smooth muscle action of AVP showed its highest biological value at 4-leucine, and there is the suggestion that this same analogue had the highest $[\theta]_{\pi-\pi^*}$ shoulder of the [4-X]-dDAVP series. [4-Abu]-dDAVP was the CD exception, despite the fact that

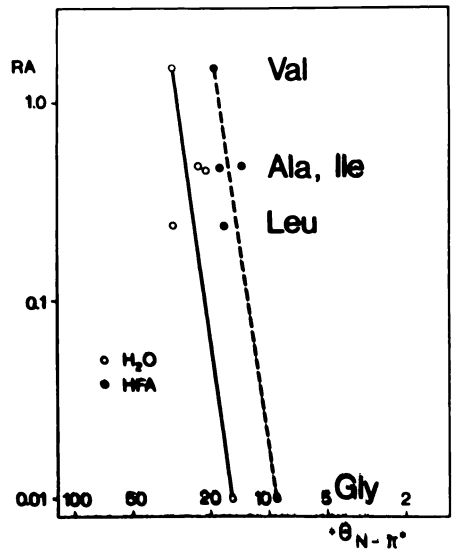


FIG. 4. *Log-log relation between relative antidiuretic activity (RA, ordinate; see Table 2) vs. positive amplitude of complex band at 225 nm (abscissa, + $\theta_{n-\pi}$ in thousands; see Table 1) in CD spectra for analogue series 1-deamino-[4-X,8-D-arginine]-vasopressin*

X residues are identified at each point. O——O, CD spectra in buffer; ●---●, CD spectra in hexafluoroacetone (HFA).

biologically it fit into a niche between the 4-alanine and 4-valine analogues. In terms of the effect of hexafluoroacetone on both the positive complex and short-wavelength (193 nm) bands, compared with all other deamino cyclononapeptides with a basic residue at position 8 (14, 26, 32), [4-Abu]-dDAVP behaved more like a deamino-oxytocin analogue than a deamino-vasopressin derivative. We have no explanation for this difference. The analytical data for the analogue were in excellent agreement with theoretical values, and repeated chromatography at different pH values failed to show the presence of dimer.

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