PROBING THE MOLECULAR BASIS OF ANTIDIURETIC SPECIFICITY AND DURATION OF ACTION WITH SYNTHETIC PEPTIDES

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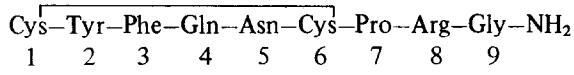
1. Introduction

The study of a number of synthetic analogs of arginine vasopressin (AVP) has helped to delineate those structural changes of the AVP molecule which give rise to enhancement of antidiuretic/vasopressor (A/V) specificity [1–9]. AVP has the following structure in which the numbers indicate the position of the individual amino acid residues.



The individual structural alterations of AVP which bring about enhancement of A/V specificity are: (a) deamination of position one [3]; (b) enhancement of lipophilicity at position 4 [1,8] and (c) substitution of D- for L-arginine at position 8 [5]. When all three changes were incorporated into one molecule the resulting peptide [1-deamino-4-valine]-8-D-arginine vasopressin (dVDAVP) was found to be the most highly specific antidiuretic peptide ever reported [10]. dVDAVP has undetectable vasopressor activity, antidiuretic activity about four times that of AVP and an A/V ratio of over 123 000 compared to 0.9 for AVP. It also exhibits a markedly prolonged duration of action similar to that previously observed for [1-deamino]-8-D-arginine vasopressin (DDAVP) [7].

The present communiation summarizes our subsequent separately reported findings on the relative effects of these structural changes in contributing to (1) enhanced antidiuretic specificity [11] and (2) prolonged antidiuresis [12]. We have found that antidiuretic specificity is influenced in the following order of decreasing effectiveness (c) >> (b) > (a). Duration of antidiuresis is due solely to (a) and is not at all influenced by (c). Structural change (b) alone has no effect on duration of action but in combination with (a) it appears to further prolong the persistence brought about by (a) alone. These and related studies may provide new insight into the structural specifications of AVP receptors, into the mechanisms that terminate antidiuretic responses and to the design of AVP peptides possessing desired biological properties of potential clinical value.

2. Materials and methods

The antidiuretic peptides used in our studies [11,12] are listed in table 1. Some were donated [12] but most were synthesized in our laboratories with the use of the solid phase method [13] following the procedure used for the synthesis of oxytocin [14] AVP [1] and analogs [2,10,11]. The bioassay procedures are described in detail elsewhere [11,12].

3. Results and discussion

3.1. *Antidiuretic specificity*

The data in table 1 support the following conclusions in regard to the relative effects of the three structural changes in bringing about enhancement of antidiuretic specificity.

Table 1
Structural changes of AVP which bring about enhanced antidiuretic specificity

Peptide	Abbreviation	N-Terminal group	Amino acid in positions 3	4	8	Activities* Antidiuretic (A)	Vasopressor (V)	Activity ratio A/V
A. *Deamination of 8-L-arginine analogs:*								
Arginine vasopressin+	AVP	NH_2	Phe	Gln	L-Arg	332	376	0.9
1-Deamino-AVP	dAVP	H	Phe	Gln	L-Arg	1390	(370)	3.8
Arginine-vasotocin	AVT	NH_2	Ile	Gln	L-Arg	231	160	1.4
Deamino-AVT	dAVT	H	Ile	Gln	L-Arg	890	256	3.5
B. *Introduction of lipophilic amino acids in the 4-position & deamination of resultant peptides*								
[4-Valine]-AVP	VAVP	NH_2	Phe	Val	L-Arg	738	32	23
1-Deamino-[Val^4]-AVP	dVAVP	H	Phe	Val	L-Arg	1150	90	13
[4-Threonine]-AVP	TAVP	NH_2	Phe	Thr	L-Arg	231	104	2.2
1-Deamino-[Thr^4]-AVP	dTAVP	H	Phe	Thr	L-Arg	758	30	25
[4-α-Aminobutyric acid]-AVP	AAVP	NH_2	Phe	Abu	L-Arg	(760)	(38)	20
1-Deamino-[Abu^4]-AVP	dAAVP	H	Phe	Abu	L-Arg	(1020)	(11)	95
C. *Introduction of 8-D-arginine: Deamination and Val^4 substitution of resultant peptides*								
[8-D-arginine]-vasopressin	DAVP	NH_2	Phe	Gln	D-Arg	257	1.1	238
1-Deamino-[D-Arg^8]-VP	dDAVP	H	Phe	Gln	D-Arg	955	0.47	2000
[Val^4, D-Arg^8]-VP	VDAVP	NH_2	Phe	Val	D-Arg	653	0.037	17 650
1-Deamino[Val^4, D-Arg^8]-vasopressin	dVDAVP	H	Phe	Val	D-Arg	1230	< 0.01 ≠	> 123 000 ≠

* Activities in units/mg. Values shown in parentheses are from other reports, the remainder are based on our own assays [12].

\+ Arginine vasopressin = Cys–Tyr–Phe–Gln–Asn–Cys–Pro–Arg–Gly–NH_2.
1 2 3 4 5 6 7 8 9

≠ No vasopressor effect could be elicited. Higher doses are vasodepressor [11].

3.1.1. Deamination at position 1

This change usually but not always leads to a modest enhancement of antidiuretic specificity primarily by increasing antidiuretic activity in all the analogs studied to date. The effect of deamination on vasopressor activity is not at all constant. It varies from peptide pair to peptide pair; it may remain unchanged, increase or decrease.

3.1.2. Introduction of lipophilic amino acids in the 4-position

Antidiuretic specificity is increased in all cases when compared to the 4-glutamine compound. This is due primarily to a reduction of vasopressor activity and in the case of the valine and α-amino butyric acid analogs by either an increase in antidiuretic activity or a retention of the same level of activity as the parent 4-glutamine containing peptide.

3.1.3. D-Arginine substitution

This is by far the single most important structural change leading to antidiuretic specificity. Earlier reports in the literature tended to obscure this fact [6,7]. The A/V values for DAVP and dDAVP were reported as only 4 and 79, respectively [6]. These low values probably resulted from the presence of minor but critical (from the biological standpoint) amounts of L-arginine in the synthetic peptides. The enhancement of antidiuretic specificity is brought about in all cases of D-arginine substitution by a dramatic reduction is vasopressor activities with the antidiuretic potencies, remaining virtually unchanged.

That enhancement of lipophilicity at position 4 plays a greater role than deamination at position 1 in leading to increased specificity can be ascertained from series (c) (table 1). Thus the A/V value of 238 for DAVP is increased to 17 650 in VDAVP as compared to only 2000 for dDAVP. The cumulative effect of all three structural modifications is further demonstrated by the essentially infinite A/V value for dVDAVP. From peptides studied to date the relative contributions of the changes in the AVP molecule which give rise to enhanced antidiuretic specificity are in the following decreasing order: 8-D-Arginine substitution >> lipophilicity at position 4 > deamination at position 1. Perhaps the most striking feature to emerge from these specificity studies is the contrast in discriminatory characteristics between the antidiuretic and vasopressor receptors. The former can tolerate the aforementioned changes in three widely separated areas of the molecule i.e. in positions 1, 4 and 8 while the latter, although tolerating the changes in positions 1 and 4 to a somewhat lesser degree, are extremely sensitive to the change of optical configuration of the arginine residue at position 8. The vasopressor receptors have thus a definite requirement for an L-amino acid at position 8 in AVP.

3.2. *Duration of antidiuresis*

Studies on DI rats show clearly that deamination of vasopressin analogs which have arginine at position 8 is the single molecular change that has the greatest effect on duration of antidiuretic action (fig. 1). Substitution of a more hydrophobic amino acid residue for the 4-glutamine in deamino analogs of AVP appears to prolong action further (fig. 1). Such substitution in the absence of deamination, however, appears to have little influence on duration of action. The peptides among those tested that have the longest action are dTAVP, dAAVP, dVAVP and dVDAVP (fig. 1). Substitution of D-arginine for the 8-L-arginine has little influence on the duration of action in DI rats (fig. 1).

The influence of deamination on the persistence of antidiuretic action is evidently dependent, at least in part, on the nature of the amino acid in the 8-position. Prolongation of action by deamination is

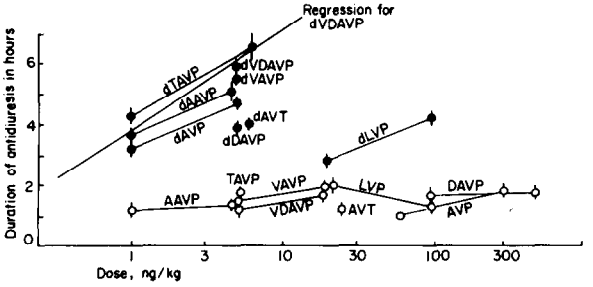


Fig. 1. Duration of antidiuretic responses by DI (diabetes insipidus) rats to 8-arginine and 8-lysine analogs of vasopressin. The 'duration' was considered the number of hours during which urine flow remained less than 50% of the control rate for each rat [12]. Mean control, $\bar{V}$=3.26±0.47 (SD) ml/100 g/hr. Closed circles indicate mean responses to deaminated analogs. Vertical lines represent SE's. See table 1 for definitions of abbreviations.

marked and highly consistent among all the analogs tested containing D- or L-arginine but it is less pronounced and more variable if other basic amino acids are substituted in the 8-position [12]. Deamino-lysine-vasopresin, deamino-homoarginine-vasopressin, and deamino-homolysine-vasopressin have more prolonged effects than their aminated counterparts but the antidiuresis they produce decays more rapidly than that seen in response to deamino-8-arginine analogs [12]. Deamino-mesotocin does have a prolonged action when given in massive doses, while the response to deaminooxytocin does not persist [12]. We have speculated [12] that the persistent action of deamino AVP peptides derives primarily from their resistance to metabolic degradation. This contention has been supported by the observation that such analogs are resistant to enzymatic degradation by soluble renal enzymes in vitro [15].

Further studies on the correlation between the persistance of action of AVP analogs in vivo with their susceptibility to enzymatic inactivation in vitro can provide a useful approach to the localization and characterization of the enzymes that terminate the actions of antidiuretic hormones. Furthermore the study of these and related analogs of AVP may lead to the design of peptides possessing (a) anti-AVP characteristics and (b) peptides possessing enhanced vasopressor specificity.

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