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4-Proline and 4-hydroxyproline analogs of arginine vasopressin: Role of the proline substitution in the two β-turns of vasopressin

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Summary. [4-L-Proline]arginine vasopressin, [4-D-proline]arginine vasopressin, [4-hydroxyproline]arginine vasopressin and [4-proline, 7-hydroxyproline] arginine vasopressin were synthesized and found to have antidiuretic activities of 91 ± 4, 1.7 ± 0.2, 1.0 ± 0.1 and 4.4 ± 1.0 units/mg, respectively. None of these analogs exhibited a significant level of rat pressor activity. The observed activities of these and other analogs with substitutions at position 4 and/or 7 are discussed on the basis of hypotheses and data bearing on the solution conformation of vasopressins.
Key words. Vasopressin; position 4 and 7 analogs; structure-activity analysis; solid phase peptide synthesis.

The results of solution studies by NMR spectroscopy[1,2] and solid state studies by X-ray crystallography[3] suggest that the two major structural features of oxytocin and arginine vasopressin (AVP) are two β-turns. One β-turn involves positions 2–5 (the sequence -Tyr-Phe-Gln-Asn-) and the other, positions 6–9 (the sequence -Cys-Pro-Arg-GlyNH$_2$). Proline, the amino acid which is classically inserted into a sequence in order to 'force' it into a β-turn, occurs naturally in position 7 (in the second β-turn). Hydroxyproline (Hyp), which may hydrogen bond to receptors, improves antidiuretic activity[4] but destroys pressor activity when substituted for proline in position 7. In the first β-turn, Tyr-Phe-Gln-Asn, glutamine is thought to play an important role in maintaining the β-turn structure[5]. In the present study we have sought to compare this role of glutamine with the role of proline by substituting proline and hydroxyproline, for glutamine in position 4. We here report the synthesis and biological activities of the following four compounds: [4-L-proline] arginine vasopressin, [4-D-proline] arginine vasopressin, [4-hydroxyproline] arginine vasopressin and [4-proline, 7-hydroxyproline] arginine vasopressin.

Materials and methods. Antidiuretic activities were assayed on water-loaded ethanol-anesthetized rats according to the procedures of Sawyer[6]. Pressor activities were assayed on urethane-anesthetized, phenoxybenzamine-treated rats as described by Dekanski[7].

The preparation of analogs I, II, III and IV was performed by solid phase techniques[8] according to a well established procedure used for the synthesis of many AVP analogs[9]. 1 g of 1% cross-linked benzhydrylamine-HCl resin (0.67 mequiv. amine/g of resin) was the solid support for the stepwise incorporation of the N^a and side-protected amino acids Boc-Gly, Boc-N^G-Tos-L-Arg, Boc-L-(D)-Pro, Boc-trans-L-Hyp(OBzl), Boc-Cys(MeBzl), Boc-Asn, Boc-Phe, and Boc-Tyr. An individual cycle for each amino acid included deprotection of the Boc-group with 50% trifluoracetic acid in CH_2Cl_2, neutralization with 5% diisopropyl-ethylamine in CH_2Cl_2 and acylation with 5-fold excess of the protected amino acids. Between each operation, several extensive washings were performed with CH_2Cl_2-isopropyl alcohol and dimethylformamide. Boc-N^G-L-Tos-Arg, Boc-Hyp(O-Bzl) and Boc-Asn were coupled by the BtOH/DCC method[10]. Boc-Tyr was used unprotected and coupled with DCC in the same way as Boc-Gly (attachment to the resin), Boc-Cys(MeBzl), Boc-Phe and Boc-L-(D)-Pro. The completion of the acylation was determined by the ninhydrin test[11] and repeated couplings were undertaken if necessary. The fully protected peptides were cleaved from the resin with HF/anisole 9:1 for 1 h at 0 °C and the extracted crude peptides oxidized with $K_3Fe(CN)_6$.

The peptides were purified by partition chromatography and gel-filtration as previously described[9]. The overall yield was between 36–40% based on the initial 0.67 mequiv. amine groups/g resin. Analytical data for these analogs are shown in table 1.

Results and discussion. Antidiuretic and pressor activities of the 4- and 7-substituted proline and hydroxyproline analogs and some related compounds are shown in table 2. [4-L-Proline]AVP shows fairly high antidiuretic activity. [4-D-proline]AVP and [trans-4-hydroxyproline] AVP have little antidiuretic activity. The introduction of hydroxyproline into position 7, a substitution which when made alone increases antidiuretic activity to 712 U/mg[4], decreases the 90 U/mg activity of [4-proline]AVP to almost nothing. All four analogs have negligible pressor activities.

In a proposed model[17] for AVP receptor binding and activation, the amino acid side chains in position 3 and 4 (first β-turn) and 7 and 8 (second β-turn) are considered to be binding elements, free for intermolecular interactions with the receptor and having a limited effect in stabilizing backbone conformation. In this view these side chains can be more variable than the 'active' elements (Arg^8 in conjunction with GlyNH$_2$ and Asn^5) changes in which could destabilize the 3-dimensional integrity of the molecule.

Broad side chain alterations can be made in position 4 with retention of appreciable biological activity (see table 2). The carboxamide moiety of Gln seems not to be necessary. Amino acids with side chains of shorter than that of Gln, and with mostly lipophilic branched groups attached to the β-carbon, enhance activity. Such hydrophobic amino acids, although they do not 'force' β-turns[18,19], are thought to sta-

Cys-Tyr-Phe-X-Asn-Cys-Y-Arg-GlyNH$_2$

	Arginine vasopressin	X = Gln	Y = Pro
I	L-Pro^4-AVP	X = L-Pro	Y = Pro
II	D-Pro^4-AVP	X = D-Pro	Y = Pro
III	Hyp^4-AVP	X = Hyp	Y = Pro
IV	Pro^4, Hyp^{7}AVP	X = Pro	Y = Hyp

Amino acid sequences of arginine vasopressin and proline and hydroxyproline substituted analogs.

Table 1. Analytical data for 4 and 7-substituted vasopressin analogs containing proline and hydroxyproline

Peptide	$[\alpha]_D^{20}$ c = 0.1, 1N AcOH	Amino acid analysis	K' HPLC*
I	21.4°	Asp, 1.01, Pro 2.04, Gly 1.05, Cys 1.95, Tyr 1.00, Phe 1.04, Arg 1.01	5.56
II	13.4°	Asp 0.93, Pro 2.10, Gly 0.95 Cys 1.80, Tyr 0.99, Phe 1.05, Arg 1.02	6.6
III	25.0°	Asp 0.99, Pro 1.08, Gly 0.99, Hyp 1.05, Cys 1.70, Tyr 1.00, Phe 0.95, Arg 0.99	4.46
IV	22.4°	Asp 1.07, Pro 1.05, Gly 1.03, Hyp. 1.10, Cys 1.81, Tyr 0.87, Phe 0.95, Arg 1.03	6.50

* High pressure liquid chromatography: solvent system 6% isopropanol in 0.1% trifluoracetic acid for petpides I, III, IV and 8% isopropanol in 0.1% trifluoracetic acid for peptide II.

Table 2. Biological activities of 4, 7 substituted AVP analogs

Analog	Antidiuretic activity (U/Mg)	Pressor activity (U/mg)	Reference
AVP	323	369	22
[4-L-Pro]AVP	91 ± 4	0.1	
[4-D-Pro]AVP	1.7 ± 0.2	0	
[4-L-Hyp]AVP	1.0 ± 0.1	0	
[4-L-Pro-7-Hypro]AVP	4.4 ± 1.0	0	
[7-Hyp]AVP	712	4	4
[4-Val]AVP	738	33	12
[4Thr]AVP]	231	104	13
[4α-Abu]AVP	760	38	15
[4-Arg]AVP	430	168	16
[4-Phe]AVP	17	0.5	14

bilize them by influencing their degree of conformational freedom.

The arginine residue, apparently a β-turn breaker[20], seems in position 4 to potentiate the β-turn in association with its adjacent amino acid residue[18,19]. However, when phenylalanine (also considered to be a β-turn breaker[20]) is substituted in position 4, substantial loss of activity occurs, possibly, as suggested by the CD studies of Brtnik et al.[14], as a consequence of additional conformational changes and interactions within the ring moiety. Proline is a strong reverse turn promoter and although it is not placed in its preferred i+1 position[18,19] in the first β-turn of the 4-proline AVP analog, this analog still possesses remarkable activity. On the contrary, hydroxyproline in the same position gives an analog with very low activity. The [D-proline]AVP analog also shows weak antidiuretic activity. Although in this analog the type II β-turn[21] is maintained, the D-configuration changes the orientation of the peptide moiety (-CO-N-) in the β-turn. Comparing the activities of these analogs and that of phenylalanine, the only other analog with a cyclized side chain in position 4, it seems possible that rigid ring structures make the β-turn in the cyclic part of AVP more compact and consequently reduce the required degree of conformational freedom as in the case of the β-turn in the acyclic tripeptide. However, vasopressin in solution is a highly flexible molecule[23] and modifications at side chains could change potencies by altering direct interactions with the receptor or by affecting flexibility with consequent kinetic effects. Since 4-proline AVP and 7-hydroxyproline AVP both have substantial antidiuretic activity and both, presumably, retain the integrity of their two β-turns, the surprising thing is that the doubly-substituted analog, [4-proline, 7-hydroxyproline]AVP has very little antidiuretic activity. This finding should be explored further, both in chemical and physical studies to see the effect of the hydroxy substitution on the orientation of the two β-turns. Finally, the diminished pressor activities of all of the analogs synthesized for this study are in agreement with the intolerance of the pressor receptor to conformational changes, an observation encountered in studies on many other synthetic analogs.

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