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CONFORMATIONAL AND STRUCTURAL CONSIDERATIONS IN OXYTOCIN-RECEPTOR BINDING AND BIOLOGICAL ACTIVITY

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INTRODUCTION

The Natural Neurohypophyseal Peptides

Oxytocin (H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2, OT) and Arginine Vasopressin (H-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2, AVP) and their naturally occurring analogs are nonapeptide hormones found throughout the animal kingdom. They are synthesized in neurons of the supraoptic and the paraventricular nuclei within the hypothalamus from larger precursor proteins. They are transported along with their carrier proteins, the neurophysins (which are derived from the same genes), via axonal processes to the posterior lobe of the pituitary gland where they are stored pending release into the blood circulation. These hormones have also been found elsewhere in the brain. These peptides have been at the forefront of studies in molecular

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biology and endocrinology, and in efforts to develop a chemical-physical basis for understanding peptide hormone and neurotransmitter actions and functions. Two major treatises on the biology and chemistry of these hormones have recently appeared (1, 2). This review briefly discusses the interactions of the neurohypophyseal hormones in humans and other mammals from the perspective of their conformational and structural properties as an example of how chemical/physical principles can be used to examine pharmacological and physiological properties. We specifically emphasize the place of chemical design in our efforts to understand peptide structure-biological function relationships.

Conformation, Peptide Design, and Biological Activity

Considerable evidence suggests that the conformation of a peptide hormone or neurotransmitter is directly related to its biological activities. Nonetheless, efforts to establish a rational approach to the design of peptide receptor ligands have been difficult, partly because of the complexity of peptide structure, their conformational flexibility, their ready biodegradation, and often their multiple biological effects. Thus, any rational approach must include state-of-the-art chemical and biophysical methodology *and* multiple biological studies (3, 4). Indeed, many biological binding and bioassay systems provide the most sensitive means of assessing the effects of structural and conformational change on the properties of a ligand, and it is now possible, if one has a conformationally stable template, to design precise conformational and topographical structure into a peptide ligand (e.g. see 5, 6). However, the use of conformational constraint has generally been a successful approach in the design of peptide hormone and neurotransmitter analogs (7–9). Conformational constraint when properly applied can provide peptide analogs with the desired conformational, structural, and biological properties necessary for further design. These include: (*a*) high biological potency; (*b*) antagonist (inhibitor) analogs; (*c*) high specificity and selectivity for a particular biological receptor; (*d*) a specific conformation or class of conformations that can be determined by physical methods in conjunction with molecular mechanics and molecular dynamics calculations; (*e*) sufficient information to suggest a possible "biologically active" conformation whose validity can be tested by specific structural and/or topographical modification; (*f*) stability against enzymatic degradation; and (*g*) ability to cross biological membranes.

In this review we selectively examine conformational and structural considerations that have gone into the design of oxytocin and vasopressin agonist and antagonist analogs with important biological activities. We emphasize studies that appear to provide insights into the differential conformational and structural requirement of the different neurohypophyseal receptors. In addi-

tion, we point to emerging structure-activity relationships that provide new opportunities for the future, especially in the central nervous system (CNS).

STRUCTURE-ACTIVITY RELATIONSHIPS OF OXYTOCINS AT THE UTERINE RECEPTOR

Traditionally, the rat uterus (oxytoxic) assay, the avian vasodepressor assay, and the milk ejection assay are all used for examining oxytocin and oxytocin-like biological activities. However, because studies of conformation-activity relationships of oxytocin and its analogs have been mostly related to the rat uterus assay, our discussion primarily refers to this receptor.

Agonist Activity

BACKBONE CONFORMATION Classical structure-activity relationships in oxytocin must be examined before one can consider the "biologically active" conformation that would provide a basis for the more rational design of analogs. Native oxytocin contains a 20-membered disulfide ring and an acyclic tripeptide tail. From earlier fragment studies (Table 1), it was concluded that the 20-membered ring structure is necessary for full agonist activty at the uterus receptor. The ring-opened form of oxytocin has very low potency and a slow-onset biological activity, probably due to the slow oxidation of the linear peptide to the cyclic form (18). Recently it was suggested that the low activity of oxytocin dimer may be due to slowly generated oxytocin (by a transsulfidation reaction), since the dimer of deamino-carba-1-oxytocin did not show any activity (19). Though the tripeptide tail has no biological activity itself, it affects the binding efficiency to a great extent, especially from the terminal $CONH_2$ group. Replacement of the C-terminal $CONH_2$ by COOH leads to oxytocinoic acid, which has only about 1/400th activity of oxytocin (Table 1). It is also generally believed that the tripeptide side chain and the 20-membered disulfide ring of oxytocin work cooperatively in the process of interacting with the uterine receptor, and perhaps for transduction in certain cases. Oxytocin possesses numerous conformations both in its backbone and in its side chain groups. Thus, the question arises, "What is (are) the conformation(s) responsible for the biological activity at the uterus receptor?" To answer this question, the relationships of conformation to biological activities for oxytocin and its agonist analogs have been extensively examined with a wide variety of physical methods (for a comprehensive review, see 20). The conclusion from those studies is that oxytocin and its agonist analogs have considerable conformational flexibility in solution. Although the active conformation for oxytocin agonists is not yet known, some specific constraints have been suggested for agonist analogs either in side chain groups (21, 22) or in the backbone (23, 24). Based on classical structure-biological activity analysis and ^1H NMR studies in DMSO,

Table 1 Biological activities of oxytocin and oxytocin fragments

Compound	Rat Uterus IU/mg	Reference
Oxytocin	546	10, 11
[Ser^1, Ser^6]OT	inhibitor + agonist	12
H-Pro-Leu-Gly-NH_2	Nil	Hruby et al (unpublished)
Tocinamide	3.2	13–15
Tocinoic Acid	0.25	16
Oxytocinoic Acid	1.3	17

Walter et al (25) proposed a biologically active conformation for oxytocin in which 2 β-turns possessing intramolecular hydrogen bonds (Asn^5 peptide NH to Tyr^2 carbonyl and Gly^9 peptide NH to Cys^6 carbonyl) were present. However, Brewster & Hruby (26) showed that the temperature dependence of the chemical shift of Gly^9 peptide NH was near normal, suggesting that this proton probably does not form an intramolecular hydrogen bond in DMSO. Thus, the initially proposed H-bond between Gly^9 peptide NH and Cys^6 carbonyl was not retained in the modified co-operative model (27). Some aspects of this model and related suggestions are summarized in Table 2. Subsequent studies in which a pseudopeptide Leu^8-Gly^9 bond was incorporated into oxytocin (see Table 3), further supported the proposition that the intramolecular H-bond due to Gly^9 peptide NH is not necessary for high biological activity. Analogs 3, 6 and 8 (Table 3) have no available peptide bond for hydrogen bonding, yet all are more potent than analog 5, which retains the hydrogen-bonding source in its pseudopeptide bond. However, a proper orientation of the terminal $CONH_2$ group is still important because analog 8 (Table 3), which contains a *trans* double bond that can place the terminal $CONH_2$ group to a similar position as it is in the regular peptide bond, is more active than analog 6 which has a *cis* configuration of this pseudopeptide bond.

Table 2 Proposed relationships of conformation of oxytocin to biological activity at uterine receptor

Residue	Proposed conformational property	Biological consequence
Tyr-2	Side-chain oriented over 20-membered disulfide ring of hormone	Essential for full efficacy
Ile-3	At corner of a β-turn of C_7 turn	Affects potency only
Gln-4	At corner of a β-turn or C_7 turn	Affects potency only
Asn-5	Side chain oriented to 'interact' with side chain of Tyr-2	Essential for full efficacy
Pro-7	At corner of a reverse turn	Affects potency only
Leu-8	At corner of a reverse turn	Affects potency only

Table 3 Biological activities of oxytocin analogs containing α modified Leu^8---Gly^9 peptide bond

Analog	Replacement of CONH	Possibility of H-bonding to Cys^{6}CO	Rat uterus (IU/mg)	Reference
1 [Mpa^1]OT		+	803	28
2 [Leuψ(CH_2S)Gly]OT	CH_2S	−	10.2	29
3 [Mpa^1, Glc^9]OT	COO	−	134	30, 31
4 [Sar^9]OT	$CON(CH_3)$	−	36	32
5 [Mpa^1,Leuψ(CH_2NH)Gly$^{8-9}$]OT	CH_2NH	+	22.3	33
6 [Mpa^1,Leuψ(1,5-CN_4)Gly$^{8-9}$]OT	[a]	−	104	34
7 [Mpa^1,Leuψ(2,5-CN_4)Gly$^{8-9}$]OT	[b]	−	24.8	34
8 [Mpa^1,Leuψ(CH = CH)Gly$^{8-9}$]OT	trans CH = CH	−	200	34

[a] 1,5-substituted tetrazole ring; fixes peptide bond as cis
[b] 2,5-substituted tetrazole ring

A recent X-ray crystal structure of deamino-oxytocin (35); showed that in the crystalline state it is flexible and exists in at least two defined and closely related conformations with disulfide bridges of different chirality. Three intramolecular hydrogen bonds were observed in the crystal structure of deamino-oxytocin: (*a*) Tyr^2 CO–Ans^5 NH, (*b*) Tyr^2 NH–Asn^5 CO, and (*c*) Cys^6 CO–Gly^9 NH. These features are similar to conformations proposed on the basis of spectroscopic studies in DMSO, except that the Cys^6 CO–Gly^9 NH H-bond may not be retained in solution (26, 36–38). The chirality of the disulfide bridge in solution has been extensively discussed (for review see 39). Interestingly, in the crystal structure, one deamino-oxytocin conformer has a disulfide torsional angle of +76° (right-handed helicity), whereas the other has a torsional angle of −101° (left-handed helicity). Based on this and the observation that the antagonist [1-*L*-penicillamine]oxytocin is much more conformationally restricted in the ring and has a preferred disulfide torsion angle of +110°−115° (40), it was suggested (35, 41) that agonist activity may be enhanced by lowering the barrier to inconversion from right- to left-handed helicity, or by designing a constrained analog in which the left-handed conformation in the disulfide bridge is more favored.

Thus, in spite of the high flexibility of oxytocin agonists, conformation studies have provided important insights into its conformation-agonist activity relationships. However, reduced flexibility consistent with agonist activity will probably be needed in order to obtain further information. Introducing appropriate conformational restrictions into some of the more potent oxytocin agonists (e.g. 4–threonine oxytocin or deamino-oxytocin), should provide a fruitful starting point.

SIDE CHAIN CONFORMATIONS AND EFFECTS OF SUBSTITUENTS Since the first total synthesis of oxytocin (42), hundreds of oxytocin analogs have been

prepared. The interested reader is referred to recent comprehensive reviews (43–45). Only a few were designed for conformational studies. However, some substituents have provided interesting insights into conformation or agonist activity, and are briefly summarized here.

In 20-membered ring, [1-carba]OT (analog 9, Table 4), in which Cys^1 sulfur is replaced by a methylene group, is more flexible than oxytocin, and also has higher potency than OXT. This supports the concept that the oxytocin agonist activity may be enhanced by reducing the energy barrier between a right- and left-handed helicity of the disulfide bridge (41).

Clearly the Tyr^2 residue is important both for binding and transduction. Correct orientation of the aromatic ring of Tyr^2 (over the 20-membered ring) is critical for agonist activity (23). Changing stereochemistry of Tyr to *D*-Tyr, produces [*D*-Tyr^2]OT (analog 10, Table 4), which has only partial agonist activity. There also is considerable evidence that Tyr^2 side chain aromatic π electrons interact with Cys^6 sulfur (58–61). In the crystal structure of deamino-OT, the conformer with left-handed helicity in the disulfide bridge has the conformation in which the Tyr^2 side chain could interact with the Cys^6 sulfur. This may explain why deamino-1-carba-OT with a Cys^6 sulfur (analog 11), is more potent than deamino-6-carba-OT, with no Cys^6 sulfur (analog 12). However, the increased flexibility of the bridge region alone could explain the enhanced potency, because both analogs 11 and 12 are more potent than deamino-OT (analog 13, Table 4). The importance of Tyr hydroxy group is evident from the fact that [Phe^2]OT (analog 17) has only about 1/15th the potency of oxytocin (Table 4). Two conformational-constraining amino acids, *L*-tetrahydroisoquinoline carboxylic acid (*L*-Tic) and cycloleucine (c-Leu), have been introduced into position 2 in oxytocin (Table 4). Interestingly, both substitutions led to very poor activity. Con-

Table 4 Biological activity of oxytocin agonists

Analog	Rat uterus IU/mg	Reference
9 [1-carba]OT	734	46
10 [D-Tyr^2]OT	p.a.	47, 48
11 Deamino-[1-carba]OT	1898	49
12 Deamino-[6-carba]OT	929	50
13 Deamino-OT	803	28
14 Deamino-[1,6-dicarba]OT	100	51
15 [Tic^2]OT	Weak antagonist	52
16 [c-Leu^2]OT	4.9	53a,b
17 [Phe^2]OT	30	54
18 [Thr^4]OT	900	55
19 [1-Acc^7]OT	9.8	56
20 [Gly-$NH(CH_3)^9$]OT	~15	57

formational studies of these two analogs (52, 53a,b, 62) have shown that the *L*-Tic^2 or c-Leu^2 analogs possess different conformations from that of oxytocin in aqueous solution.

The side chain moieties in position 3 and 4 of OT are important residues for binding of OT to the uterine receptor. Agonist activity is best maintained with a lipophilic amino acid at position 3 (63) and an amino acid with hydrogen-bond donating and/or hydrogen-bond accepting properties at position 4, e.g. Thr (analog 18, Table 4). The Thr^4 residue also can influence prolongation of the uterotonic response (64). Other amino acids with a variety of properties can replace Gln^4 (for reviews see 43, 44) without causing backbone conformational changes. Although Asn^5 is known to be critical in transduction, very few modifications have been made in this position, probably because replacement of Asn^5 usually leads to analogs with very low potency (43, 44). More work is needed to further our understanding of this residue.

The half-cystine residue in position 6, together with the covalently attached half-cysteine residue in position 1, play a very important role in oxytocin biological activity. Replacement of either sulfur in the disulfide bridge by a methylene group gives analogs with higher agonist potency (analogs 11, 12, Table IV). However, replacement of both sulfurs by two methylene groups leads to the analog, deamino-[1,6-carba]OT (analog 14) which is less potent than oxytocin.

The tripeptide tail of OT (positions 7, 8, and 9) is only important for binding, and Pro^7 and Leu^8 can be replaced by a variety of residues and still maintain high potency (43, 44). However, when the highly conformationally constrained amino acid 1-amino cyclopropane-1-carboxylic acid is substituted into position 7, an analog with greatly reduced potency is obtained (analog 19, Table IV). This suggests that some flexibility of the tripeptide tail is important for effective binding. Modification of the terminal $CONH_2$ usually leads to a large decrease in biological potency (e.g. analog 20, Table 4).

Antagonist Activity

Although the conformational flexibility of most oxytocin agonists has limited our ability to deduce conformation-activity relationships for oxytocin agonist activity, the situation for oxytocin antagonists is more favorable. Since the discovery of [Pen^1]oxytocin (65), a large number of β,β-dialkyl half-cystine-1-substituted oxytocin antagonist analogs have been prepared (43, 44, 82). Somewhat later it was recognized that the penicillamine residues constrain the conformations of the rings in which they appear, and extensive conformational studies of [Pen^1]oxytocin and some of its analogs using NMR, CD, and laser Raman spectroscopies (23, 24, 40, 66–71) provided considerable insight as to how the introduction of geminal β-methyl groups into the Cys^1 residue restricted the oxytocin molecule. These include restriction of the C-S-S-C to dihedral angles greater than 110° (right-handed helicity), as well

as the conformation of the 20-membered ring via transannular effects. Some of the physical evidence supporting a defined conformational family for these analogs is summarized in Table 5. These studies led to the proposal (66–68, 70) that the backbone conformations of [Pen^1]OT, [Pen^1, Leu^2]OT, [Pen^1, c-Leu^2]OT, and [Pen^1, Phe^2, Thr^4]OT contain either two or a single C_7-turn (Asn^{5}NH to Ile^{3}CO and/or Ile^{3}NH to Pen^{1}CO).) These results, the implications from the X-ray crystal structure of deamino-oxytocin (41), the right-handed disulfide helicity, and the constrained backbone conformations that would limit access to low energy interconversion of a right- to a left-handed disulfide bridge in the Pen^1-containing OT analogs are all consistent with the model for potent antagonist activity at oxytocic receptors (66, 67). Introduction of a carbabridge into the deamino-penicillamine oxytocin led to the relatively potent agonist, which was converted to an antagonist only after modification of the tyrosine side chain hydroxyl group (72). These latter findings also support the idea that the inhibitory activity of penicillamine-containing analogs are caused by conformational constraints in the vicinity of the disulfide bridge. It is important to note that the conformations of side-chain groups in these analogs were also severely limited, especially position 2 and/or position 5 (66–71). These constraints may also be directly related to their antagonist activities, as the aromatic ring could not be properly placed over the 20-membered ring as is apparently needed for agonist activity.

In addition to the β,β-dialkyl half-cystine-1-substituted oxytocin antagonist analogs, certain 2-substituted oxytocin analogs also have antagonist activities (Table 6). Aromatic amino acids of *D*-configuration with lipophilic substituents in the p-position possess high inhibitory potency (76, 77). It was recently shown that replacement of *D*-Phe by *D*-Tic in position 2 does not cause a significant difference in the analog's conformation (52). This result suggests that these analogs can bind to the OT receptor, but will not transduce

Table 5 Physical evidence consistent with a defined conformation for [Pen^1]oxytocin analogues

Method	Evidence
NMR	Large (>8 Hz) and very small (≤3 Hz) $^3J_{NH\text{-}\alpha\text{-}CH}$) values in 20-membered ring.
NMR	Very large (>10 Hz) and very small (>3 Hz) $^3J_{\alpha CH\text{-}\beta CH}$ values for side chain groups of residues in the 20-membered ring.
NMR	Near zero temperature dependence for one or two N$^\alpha$ amide protons in the 20-membered ring residues.
NMR	Reduced ^{13}C spin-lattice relaxation times (T_1) in side chains relative to oxytocin and other agonist analogs
Raman	Disulfide bond similar to penicillamine not cystine
CD	Cotton effects for disulfide σ-transitions consistent with a right-handed helicity
CD	Absorption maxima consistent with a dihedral angle of about 115–120°
CD	Peptide backbone quite rigid—intense amide absorptions

the biological message due to an improper fit of the aromatic side-chain due to local steric effects. The same consideration may also explain the antagonist activities of halogenated tyrosine analogs (analogs 26 and 27, Table 6).

Most interesting are Jošt and coworkers' analogs (80, 81), which have minimal conformational and steric restrictions. The only change in these analogs is the substitution of Tyr^2 residue by either an O-alkylated tyrosine residue or a p-alkylphenylalanine derivative. Their antagonist activity may be a result of their binding differently to oxytocin uterine receptor than agonists, such that the conformation for transduction cannot occur (82).

Substitutions of β,β-dialkyl derivatives of Cys^1 by D-amino acids, O-alkylated tyrosine, or p-alkylphenylalanines in position 2, in conjunction with other modification in the oxytocin structure (particularly positions 4 and 8) have been used in designing potent antagonists (for reviews see 43–45). Some analogs possess prolonged action, which may make them clinically useful as tocolytic agents (83, 84). An interesting highly conformationally constrained bicyclic oxytocin antagonist, [β-Mpa^1, Glu^4, Lys^8]OT, has been recently prepared by Hruby et al (85). A striking design feature is that introducing a second lactam ring by cyclizing Glu^4 and Lys^8 side chains to further constrain the molecule [β-Mpa^1, Glu^4, Lys^8]OT, a weak *agonist,* results in an *antagonist* [β-Mpa^1, Glu^4, Lys^8]OT with a pA_2 of 8.2. Further definition of the conformational requirements for oxytocin antagonists can be expected from detailed conformational studies of this new class of compounds.

CONFORMATION-ACTIVITY RELATIONSHIPS OF VASOPRESSINS AT V_1 AND V_2 RECEPTORS

Conformational Considerations

AGONISTS Progress in conformational studies of [8-arginine]vasopressin (AVP) and [8-lysine]vasopressin (LVP) has been much slower than for

Table 6 Antagonist activities of 2-substituted oxytocin analogs

Analog	Rat Uterus (pA_2)	Reference
10 [D-Tyr^2]OT	partial agonist	48
21 [D-Phe^2]OT	6.0	73
22 [D-Trp^2]OT	weak	74
23 [D-Tic*^{2}]OT	6.50	52
24 [$Tyr(OMe)^2$]OT	6.79	25
25 [D-$Phe(4\text{-}Et)^2$]OT	8.15	76, 77
26 [2-Br-Tyr^2]OT	7.05	78
27 [3-I-Tyr^2]OT	7.05	79

D-Tic*: D-Tetrahydroisoquinoline carboxylic acid

oxytocin, although they were both begun almost at the same time using NMR and other biophysical methods (86–89). One important reason is that vasopressins have a more complicated proton NMR spectrum, especially in the β-protons region. Information obtained to date is insufficient to suggest a specific conformation for LVP (or AVP) either in DMSO or in aqueous solution (90–93). In fact, vasopressins appear to have more extended conformations than oxytocin, and are highly conformationally flexible even in DMSO. However, the evidence indicates that both LVP and AVP have very similar overall conformational properties as oxytocin, but with some differences as a result of interactions of the Cys^1, Tyr^2, and Phe^3 residues (92–94). In addition, the relationship of the tripeptide tail to the 20-membered ring moiety, which appears to be important for the biological activities of vasopressins, may be different from that in oxytocin. Actually, Walter et al (95) have proposed a biologically active conformation for vasopressin at the kidney receptor. The conformation has a β-turn in its ring moiety, and the key elements for transduction were suggested to be the Asn^5-carboxamide group and the basic group on the side chain of Arg^8 or Lys^8. The binding residues were suggested to be the lipophilic side-chain groups of Phe^3, Gln^4, Pro^7 and Arg^8 (or Lys^8). Some aspects of this model were supported by subsequent studies (96). In particular, the recently reported X-ray crystal structure of pressinoic acid confirms the possible presence of a β-turn (residue 2–5) in these molecules (97). Further studies involving more conformationally constrained agonist analogs of AVP and LVP are needed for detailed investigation of the proposed conformation.

ANTAGONISTS Although antagonist analogs of AVP at the kidney receptors, which are usually conformationally constrained relative to AVP, have been developed for some time (98), only a few reports have examined their conformational properties. Again, NMR-based conformational analysis of some antagonists are complicated by conformational flexibility (99). The presence of γ-turns was suggested based on the CD spectra of some antagonists (100), and other studies propose that antagonists and agonists might share similar conformational features about position 4 (101). Other recent studies have further supported the concept that the antagonists may bind to receptor in a different manner from the agonists (82). For example, the conformational constraint imposed by an N-alkyl residue at position 7 is not necessary for binding of antagonist to the antidiuretic receptor (102, 103), but it is critical for agonist activities. Also, recently reported linear antagonists (104a,b) showed that the 20-membered ring structure, which appears to be important for biological activities in agonists, is not necessary for binding in antagonists.

Structure-Activity Relationships

AGONISTS Though conformational analysis of vasopressins at various receptor systems has been more limited than that of oxytocin, the extensive structure-activity studies at both the kidney receptor (V_2 receptor; antidiuretic effect) and the vascular receptor (V_1 receptor; pressor effect) have been very fruitful. Examination of these activities for the naturally occurring neurohypophyseal hormones suggests that high potency at either receptor apparently requires a basic residue (Arg or Lys) in position 8. Higher potency is usually obtained for analogs with an Arg^8 residue, and an aromatic residue in position 3 is preferable to an aliphatic residue. It also appears that the pressor activities are much more sensitive to structural changes. In this section, we briefly summarize the effect of substitutions on antidiuretic and pressor activity residue by residue. (For detailed reviews see 43, 105, 210).

(a) Position 1 and position 6 The extremely weak biological activities of [Ala^1, Ala^6]AVP and [Ala^1, Ala^6]LVP clearly demonstrate that the maintenance of the 20-membered ring is essential for efficient binding at both antidiuretic and pressor receptors (analogs 28 and 29, Table 7). Deamino analogs 30 and 31 showed different effects at the V_1 receptor than have been observed at other neurohypophyseal hormone receptors. [β-Mpa^1]AVP is almost three times more potent than its parent hormone at the antidiuretic receptor (analog 30), but less potent in the prcssor assay compared to AVP (Table 7). Similarly, the greatly enhanced activities at antidiuretic receptor by 1-carba or 6-carba analogs 32 and 33 werc not observed at prcssor receptors (Table 7). Thus, although the ring structure is important for binding both at V_1 andV_2 receptors, the two receptors probably have different stereoelectronic requirements (especially the Cys^1 residue).

(b) Position 2 The most interesting modification in position 2 of AVP and LVP involved the p-hydroxy group of tyrosine. Quite consistent results were observed at the pressor receptor in that either removing or alkylating this group (analogs 34–36) leads to analogs with diminished activities (Table 7). However, unpredictable changes in activity were observed at the antidiuretic receptor. For example, [$Tyr(OEt)^2$]LVP (analog 36) is actually a weak inhibitor at the antidiuretic receptor (Table 7), which may indicate that the steric effect of the para substituent is incompatible with transduction after it binds to V_2 receptor. The Tic^2 analog of AVP (analog 37) was inactive at both receptors either as an agonist or an antagonist.

(c) Position 3 Though the 3 position is considered important for binding to V_1 and V_2 receptors, only a relatively few modifications have been made

(analog 37; 105). Biological results for [Ile^3]AVP (analog 38) are quite variable (Table 7). An aromatic residue is preferable to an aliphatic residue both at V_1 and V_2 receptors.

(d) Position 4 Binding requirements of agonists in position 4 at V_1 and V_2 receptors are apparently quite distinct, because totally different substituent effects are observed. Substitution of Gln^4 either by Abu^4 (analogs 39, 40) or Val^4 (analog 41) enhanced the antidiuretic activities, but drastically reduced activity at the pressor receptor (Table 7). Comparison of activities of the analogs [Val^4]AVP (analog 41) and [Thr^4]AVP (analog 42) at the V_2 receptor indicate that the interaction of the hormone with the antidiuretic receptor at position 4 involves a lipophilic rather than a more hydrophilic interaction. The opposite might be true for the pressor receptor.

(e) Position 5 As for all the oxytocic receptors, the Asn^5 appears to be critical for both binding and transduction at the V_1 and V_2 receptors, though not very much work has been done because modification of Asn^5 usually leads to low potency (analogs 43, 44, Table 7).

(f) Position 7 As the first residue of the tripeptide tail, Pro^7 is considered to be important in correctly positioning the "tail" portion relative to the ring portion in the molecule. As Table 7 shows, analogs without the conformational constraint possessed by the pyrrolidine ring in position 7 [e.g. [Gly^7]LVP, analog 45 and [Leu^7]AVP, analog 46], have very low activity at both the V_1 and V_2 receptors. Reinstalling the conformational constraints by putting a methyl group on the amide nitrogen in position 7 ([N-MeAla^7]AVP, analog 47) enhances potency, especially at the antidiuretic receptor, but the pressor activity remains low (Table 7). Interestingly, incorporation of a conformational constraint on the pyrrolidine ring either by introducing a double bond (analog 48) or a hydroxy group (analogs 49–51) to the pyrrodine ring greatly enhanced potency of the antidiuretic receptor, but diminished pressor activity (Table 7). Thus the selectivity of V_2/V_1 is increased, especially in the hydroxyproline^7 analogs. Clearly, fundamentally different interactions are occurring at the V_1 and V_2 receptors.

(g) Position 8 As mentioned, Arg^8 (or Lys^8) may be important for transduction at antidiuretic receptor. However, a wide variety of substitutions, (both D- and L-amino acids), can be used to replace Arg^8 without a substantial decrease in antidiuretic activity potency. Notable are substitutions by unusual amino acids or peptides (see 134). In some cases, the activity was even greatly enhanced (for reviews see 43, 105). In contrast, the pressor receptor is much more sensitive to structure change. Almost all the D-amino acid-

Table 7 Biological activities of vasopressin analogs

Analog	Antidiuretic IU/mg	Pressor IU/mg	Reference
AVP	503	487	106
LVP	203	243	107
28 [Ala^1, Ala^6]AVP	0.08	0.025	108
29 [Ala^1, Ala^6]LVP	0.05	0.012	108
30 [β-Mpa^1]AVP (deamino-AVP)	1400	395	108
31 [β-Mpa^1]LVP (deamino-LVP)	301	126	109
32 Deamino-[1-carba]AVP	15–23,000	555	110
33 Deamino-[6-carba]AVP	1569	223	111
34 [Phe^2]LVP	21	57	112
35 [Tyr(OMe)^2]LVP	1.5–3	79	113
36 [Tyr(OEt)^2]LVP	inh.	5	113
37 [Tic^2]LVP	<0.1	<0.05	114
38 [Ile^3]AVP	250	245	115
[Ile^3]AVP	231	160	116
[Ile^3]AVP	24	130	117
39 [Abu^4]AVP	760	38	118
40 [Abu^4]LVP	707	10.2	118
41 [Val^4]AVP	738	32	119
42 [Thr^4]AVP	231	104	119
43 [Asn(Me$_2$)^5]LVP	2.55	5.55	120
44 [Ala^5]LVP	0.2	0.15	118
45 [Gly^7]LVP	1.0	0.15	121
46 [Leu^7]AVP	19	—	122
47 [N-MeAla^7]AVP	343	10.6	123
48 [$\Delta^{3,4}$Pro^7]AVP	1260	255	124
49 [(4-trans-hydroxy)Pro^7]AVP	712	4	125
50 d[4-trans-hydroxy)Pro^7]AVP	780	1	125
51 d[(4-cis-hydroxy)Pro^{7}AVP	614	8	125
52 [β-Mpa^1, D-Arg^8]AVP (dDAVP)	870	0.96	129, 130
	955	0.5	131
	1200	0.39	132
53 [Ala^9]AVP	104	0.4	126
54 [D-Ala^9]AVP	189	7.6	126
55 [des-Gly-NH$_2^9$]AVP	0.03	Nil	127
56 pressinoic acid	5.6	>0.02	128
57 desGlydDAVP	149	26	133b
58 desGly(NH$_2$)dDAVP	73	298	133b
59 desGlydVDAVP	321	389	90, 133b
60 desGly(NH$_2$)dVDAVP	321	190	133b
61 [Mpa^1, Val^4, *D*-Arg^8]AVP(dVDAVP)	1230	pA$_2$ = 7.03	132

substituted analogs in this position have very low pressor activity. In fact [β-Mpa^1, *D*-Arg^8]AVP (analog 52, dDAVP) is a highly selective V_2/V_1 agonist (Table 7), and is now widely used in human medicine for treatment of diabetes insipidus.

Table 8 Biological multisubstituted vasopressin analogs

Analog	Antidiuretic IU/mg	Pressor IU/mg	Reference
62 [Val^4,*D*-Arg^8]VP	653	0.037	119
63 [β-Mpa^1,Val^4,*D*-Arg^8]VP	1230	<0.01	131
64 [β-Mpa^1,Asn^4,*D*-Arg^8]VP	10,750	Nil	136
65 [Phe^2,$\Delta^{3,4}$-Pro^7]AVP	13,000	Nil	124
66 [*D*-Arg^8]VP(DAVP)	257	1.1	137
67 [Tyr(Me^2)]DAVP	309	pA_2 = 6.74	133
41 [Val^4]AVP(VAVP)	738	32	119
68 [Tyr(Me)^2]VAVP	443	pA_2 = 6.63	133a
69 [Val^4,*D*-Arg^8]VP(VDAVP)	653	0.037	119
70 [Tyr(Me)^2]VDAVP	35	pA_2 = 6.63	133a
71 [Tyr(Et)^2]VDAVP	106	pA_2 = 6.54	133a
72 dTyr(Me)AVP	830	4.1	138
73 dTyr(Me)VAVP	789	pA_2 = 7.26	138
74 dTyr(Me)VDAVP	1740	pA_2 = 7.01	138
75 [Mpa^1, *D*-Tyr^2, Val^4, *D*-Arg^8]carba-1-VP	[a]	pA_2 = 7.0	139

[a]Ten times more potent than dDAVP

(h) Position 9 There are numerous analogs made with modification in this position, and most have very low pressor potency (Table 7). On the other hand, the antidiuretic receptor is much more accessible to changes in this position. For example, [Ala^9]AVP (analog 53) or [*D*-Ala^9]AVP (analog 54) are quite potent at the V_2 receptor. Any removal of the Gly^9-$CONH_2$ residue leads to analogs such as 55 and 56 with low or no agonist activity at both receptors (Table 7). These studies suggest that the terminal Gly-NH_2 is necessary for transduction at both receptors. However, contrasting results were found in the subsequent structure-activity studies on multisubstituted analogs. For example, analogs like desGly(NH_2)dDAVP (analog 58), desGlydVDAVP (analog 57), and desGly(NH_2)dVDAVP (analog 59) all retain good agonist potency (Table 7). Thus, a terminal Gly-NH_2 is apparently not required for transduction at V_1 and V_2 receptors. However, recently reported ^{1}H NMR studies of [Ala^9]LVP and [*D*-Ala^9]LVP indicated a possible interaction between the Ala^9 (or *D*-Ala^9) and the Phe^3 residues (135). This result may suggest that the terminal $CONH_2$ group is indirectly involved in transduction in some analogs through interaction with the Phe^3 residue.

(i) Multiple substituted vasopressin analogs As discussed above, substitutions at the 1, 2, 4, 7, and 8 positions have quite different effects at V_1 and V_2 receptor. Usually highly potent analogs at the antidiuretic receptor are obtained. Appropriate multiple substitutions at these positions should provide more potent and more specific analogs at the antidiuretic receptor. This concept is well supported by some of the multisubstituted analogs prepared

(Table 8). For example, alkylation of the Tyr^2 in some of these potent V_2/V_1 agonists resulted in (*a*) retention of appreciable antidiuretic agonist activity, and (*b*) abolishment of pressor agonism with concomitant conversion of the parent agonists into moderately potent antagonists at the V_1 receptor. Thus, nearly all the new O-alkylated analogs exhibit high antidiuretic/pressor selectivity (Table 8). Multisubstitution led to the most potent and selective antidiuretic agonist described—[*D*-Tyr^2, Val^4, *D*-Arg^8]deamino-carba-1-vasopressin (analog 75, Table 8) with ten times higher antidiuretic activity than dDAVP, and no pressor activity. Its activity is even more prolonged then the activity of dDAVP and it is orally active.

ANTAGONISTS *(a) V_1 receptor* Simple modifications in position 1 of vasopressins (generally substituted with β,β-dialkyl-β-mecaptopropionic acids) invariably provide potent V_1 receptor antagonists (Table 9; for reviews see 43, 105). This conversion to antagonists probably results from a conformation change in the 20-membered ring, including the disulfide bridge caused by imposing bulky β,β-dialkylated residues in position 1. Monosubstitution in other positions of the molecule usually does not produce potent antagonists. Thus the design of V_1 receptor antagonists traditionally has concentrated on making multisubstituted analogs of [β,β-dialkyl-β-mecaptopropionic $acid^1$]AVP, particularly in various combinations with the 2, 4, and 8 positions.

Substitutions such as *D*- or *L*- O-alkyltyrosine in position 2 also leads to potent antagonists (Table 8). Not much work has been done on position 3. However, introduction of tetrahydroisoquinoline carboxylic acid moiety into positions 2 or 3 completely eliminated any activity (114). Many L-amino acids (in conjunction with *D*-Phe^2 or *D*-Ile^2) can apparently be substituted at position 4 and good antagonist activity is retained. Among those amino acids, valine is used most extensively in the design of antagonists (analog 81, Table 9). Recently, modification of C-terminal of antagonists was found not to affect the potency of the analog. For example, des-Gly and des-Gly(NH_2) analogs of [$(CH_2)_5$-β-Mpa^1, $Tyr(Me)^2$]AVP (analogs 88, 89, Table 9) leads to antagonists with pA_2 = 8.44 and 8.46, respectively. Substitution of $Gly(NH_2)^9$ by $Gly(NH(CH_2)_2NH_2$ (analog 92), $Gly(NHCH_3)$ (analog 93), or $Ala(NHCH_3)$ (analog 94) all provide potent analogs (Table 9). Thus, although the C-terminal Gly(NH_2) is important in preserving V_1-agonistic activity, it is not as important in determining V_1-antagonist activity. Some investigators thought that the 20-membered ring structure was required for antagonist activity, but it was recently found not to be an essential requirement for binding to both V_1 and V_2 receptors (104a,b). Since some linear peptides were prepared and displayed antagonist activities (with the highest pA_2 = 8.42) (analog 97, Table 9). Interesting developments in vasopressin antagonists can be expected by following up this discovery.

(b) V_2 receptor Though the search for antagonists at the antidiuretic (V_2) receptors was occurring simultaneously with those for other neurohypophyseal hormone receptors, success has been much more recent. Some of the most potent V_1 antagonists, such as [Et_2-β-Mpa^1]AVP (analog 77) and [$(CH_2)_5$-β-Mpa^1]AVP (analog 79), were still weak agonists at the V_2 receptor, clearly demonstrating significantly different requirements at the V_1 and V_2 receptors. Finally, by using a combination of changes in several different positions in vasopressin (particularly the 1, 2, 4, and 8 positions) antagonists at the V_2 receptor were obtained (141). A key feature of almost all V_2 antagonists is the presence of the bulky β,β-cyclopentamethylene-β-mercaptopropionic acid group at position 1. However, β,β-diethyl-β-mercaptopropionic acid at position 1 of *D*-Tyr(Et)^2 analogs can also lead to antagonists (151).

Though a very bulky lipophilic group in position 1 is required, changes of the disulfide bridge are tolerated because substitution of the disulfide bridge by a dimethylene bridge seems not to affect antagonist activity (152). Linear antagonists at V_2 receptor were also prepared. The most powerful antagonists are apparently substituted with a combination of $(CH_2)_5$-β-Mpa^1, a *D*-aliphatic or *D*-aromatic residue in position 2, and a lipophilic amino acid at position 4 (Table 9). A variety of substitutions and deletions can be made in the terminal tripeptide tail with retention of good V_2 antagonist activity (analogs 101–103. Table 9). Actually, it was shown that all that is necessary for retention of antagonist affinity and potency at antidiuretic receptor is a basic functional group, amine or guanidine, extended an optimal distance from the hexapeptide ring. The conformational requirements for antagonist activity at the V_2 receptor have not been examined. (For recent comprehensive reviews see 213, 214).

CNS ACTIVITIES OF NEUROHYPOPHYSEAL HORMONES AND ANALOGS

Introduction

Immunohistochemical studies have shown the presence of parvocellular OT and VP-producing neurons both inside and outside the hypothalamus (153); these do not project to the neurohypophysis but to various central locations (154, 155). A combination of immunohistochemistry and radio-immunoassay methods has been used to identify an array of oxytocin and vasopressin fibres throughout the mammalian central nervous system (CNS) (153, 156). These areas vary considerably from centers involved in nociception in the brain stem and spinal cord, to forebrain areas such as the hippocampus, septum, amygdala and mediodorsal thalamus, which are thought to be involved in memory and behavioral processes. The latter areas are especially significant, because

Table 9 Biological activities of vasopressin antagonists

Analog		pA_2 Antidiuretic	pA_2 Antipressor	Reference
76	[Me_2-β-Mpa^1]AVP	weak agonist	7.45	140
77	[Et_2-β-Mpa^1]AVP	0.38 (agonist)	8.36	141
78	[Et_2-β-Mpa^1]LVP	[redacted]	7.15	142
79	[$(CH_2)_5$-β-Mpa^1]AVP	0.033 (agonist)	8.35	143
80	[$(CH_2)_5$-β-Mpa^1,*D*-Phe^2]AVP	7.21	8.35	141
81	[$(CH_2)_5$-β-Mpa^1,Val^4]AVP	0.32 (agonist)	7.97	141
82	[$(CH_2)_5$-β-Mpa^1,*D*-Tyr^2,Val^4]AVP	7.51	8.41	98
83	[$(CH_2)_5$-β-Mpa^1,Tyr(Me)^2]AVP	0.31 (agonist)	8.62	143
84	[$(CH_2)_5$-β-Mpa^1,Tyr(Me)^2,Val^4]AVP	7.35	8.32	98
85	[$(CH_2)_5$-β-Mpa^1,*D*-Tyr(Me)^2,Val^4]AVP	7.77	8.48	141
86	[$(CH_2)_5$-β-Mpa^1,*D*-Arg^8]AVP	0.31 (agonist)	8.52	98
87	[$(CH_2)_5$-β-Mpa^1,Tyr(Me)^2, Val^4,*D*-Arg^8]AVP	6.68	8.44	98
88	desGly[$(CH_2)_5$-β-Mpa^1,Tyr(Me)^2]AVP	6.10	8.44	133b
89	desGly(NH_2)[$(CH_2)_5$-β-Mpa^1,Tyr(Me)^2]AVP	5.38	8.46	133b
90	desGly(NH_2)[*D*-Phe(Et)^2]LVP	[redacted]	8.80	76
91	[$(CH_2)_5$-β-Mpa^1,*D*-Tyr(Et)^2,Val^4]AVP	7.81	8.22	142
92	[$(CH_2)_5$-β-Mpa^1,*D*-Tyr(Et)^2,Val^4, Gly(NH$(CH_2)_2NH_2)^9$]AVP	7.82	8.33	144
93	[$(CH_2)_5$-β-Mpa^1,*D*-Tyr(Et)^2,Val^4, Gly(NHCH$_3)^9$]AVP	7.53	7.71	144
94	[$(CH_2)_5$-β-Mpa^1,*D*-Tyr(Et)^2,Val^4, Ala(NHCH$_3)^9$]AVP	7.89	8.08	144
95	[$(CH_2)_5$-β-MPA^1,Tyr(Me)^2,Tyr($NH_2)^9$]AVP	6.0	8.47	145
96	[$(CH_2)_5$-β-Mpa^1,Tyr(Me)^2,I-Tyr($NH_2)^9$]AVP	<6.3	8.17	145
97	C_6H_5-CH_2CO-*D*-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-Arg-NH_2	7.99	8.42	104a,b
98	[$(CH_2)_5$-β-Mpa^1,*D*-Ile^2,Abu^4]AVP	8.22	6.73	146
99	[$(CH_2)_5$-β-Mpa^1,*D*-Phe^2,Ile^4]AVP	8.24	7.86	147
100	[$(CH_2)_5$-β-Mpa^1,*D*-Tyr(Et)^2,Val^4]AVP	11*	[redacted]	103
101	des-Pro-[$(CH_2)_5$-β-Mpa^1,*D*-Tyr(Et)^2, Val^4]AVP	23*		103
102	[$(CH_2)_5$-β-Mpa^1,*D*-Tyr(Et)^2[redacted]al^4, N-MeAla^7]AVP	19*		102
103	($CH_2)_5$-β-Mpa-*D*-Tyr(Et)-Phe-Val-Asn-Cys-X			
X =	Pro-Arg-Gly-NH_2	11*		148
=	Pro-Arg-NH_2	9.2*		148
=	Pro-Lys-NH_2	22*		148
=	Arg-NH_2	58*		149
=	Arg-Arg-NH_2	7.2*		149
=	Pro-NH-$(CH_2)_2$-NH_2	108*		150
=	Pro-NH$(CH_2)_4$-NH_2	19*		150
=	Pro-NH$(CH_2)_5$-NH_2	27*		150

* Dose required to lower urine osmolality to 300 mOsm/kg; in μg/kg

OT and VP have been shown to be intimately involved in behavioral responses. Interestingly, the abundance of OT and VP in these fibers varies considerably with location, with very dense innervation found in limbic brain areas such as the lateral septum and the amygdala, and only single fibers seen in the neocortex. The proportion of OT- and VP-containing fibres also differs with location (157).

Over the last decade both peptides have been shown to exhibit many biological effects in the CNS, all involved in behavioral, learning, and memory processes. Both induce grooming behaviors in male and female rats (158–162); affect sexual receptivity in female rats, with OT facilitating this activity (163–166); affect other sexual responses (167–168); influence active and passive avoidance behavior (169, 170); and produce nonopiate-dependent analgesia (171–175). OT is known to induce maternal behavior (176) and VP has been shown to induce flank-marking in golden hamsters (177) and to block amnesia (170). In the remaining review we discuss structure-activity relationships of neurohypophyseal hormones at various receptor sites in the CNS.

Actions of Neurohypophyseal Agonists in the CNS

ACTIVE AND PASSIVE AVOIDANCE BEHAVIOR Active avoidance behavior has been studied by noting the maintenance of a pole-jumping avoidance response in rats under various experimental conditions (178, 179). Arginine-vasopressin (AVP) is the most potent peptide that increases resistance of pole-jumping avoidance behavior to extinction; lysine-vasopressin (LVP) and OT are less potent at 63% and 13% potency of AVP, respectively (180). The des-glycinamide derivatives of AVP and LVP (DG-AVP and DG-LVP, respectively) and [Ala^8]AVP and LVP-(1–8)-NH_2 retain activity with a slightly reduced potency. These findings suggest that the active behavior response can tolerate considerable structural modification in positions 8 and 9, in contrast to the peripheral endocrine activity of OT and VP where the position 8 residue and/or the C-terminal glycinamide moiety are critical for binding and transduction (43). The 20-membered ring moiety of VP, pressinamide, was found to be almost equipotent with AVP when administered intraventricularly (181). Substitution within positions 1–5 of AVP and LVP (i.e. in the 20-membered covalent ring of the intact hormone), for a variety of amino acid residues, such as [Mpa^1]AVP, [Mpa^1]LVP, [Ala^2]AVP, [Ala^2]LVP, [Ile^3]AVP and [Thi^2]LVP, invariably leads to analogs with diminished potencies (180). The idea that OT and VP may be precursors of fragments with more selective and potent behavioral effects (182, 183) was further supported by the finding that products of in vitro processing of OT and AVP by membrane-bound peptidases from the brain are behaviorally highly potent (184, 185). The two major products were [$pGlu^4$, Cyt^6]AVP-(4–9) and

[pGlu^4,Cyt^6]OT-(4–9), which were as potent as the parent hormones. Removal of the glycinamide functionality from the AVP analog resulted in the most potent compound, yet attenuated the extinction of pole-jumping avoidance behavior. Further shortening of the peptide chain by deleting the *p*-Glu residue resulted in a drop in potency (186).

Passive avoidance behavior can be studied using rats' innate preference for dark over light areas. Such a study would consist of a learning trial in which the rat is placed on a lighted platform surrounded by a dark area and the animal's latency to enter the dark area is measured. This is repeated several times over two days and at the end of the last trial the animal, having entered the dark area, is given an electric foot shock. Retention trials are done 24 and 48 hours later. The rat's latency to enter the dark area is noted. This behavior can be used to examine the influences of neuropeptides on consolidation and retrieval processes. Peptides that are administered immediately after the learning trial and affect the passive avoidance latency 24 hours later in the retention trial are considered to influence consolidation processes. Peptides that are administered 1 hour prior to the retention trial and affect the passive avoidance latency are considered to influence retrieval processes. AVP and LVP increased avoidance latencies whether injected after the acquisition trial or 1 hour before the retention test. AVP-(1-8), AVP-(1-7) and AVP-(1–6) enhanced consolidation processes as did OT-(1–7). The acyclic tripeptide tails of OT and VP facilitated consolidation as well, but these fragments were much less potent (183). Inhibition of consolidation was found with OT, OT-(1–8) and arginine vasotocin-(1–8), [AVT-(1–8)]. Hence, although removal of the glycinamide residue has little effect on the amnesic effects of OT on the consolidation processes, further shortening of the C-terminus facilitates the avoidance latency. Because AVT-(1–8) was an inhibitor, and AVP-(1–9) and AVP-(1–8) were not, the tocin sequence, OT(1–7) with the intact twenty-membered ring (as well as the pressin ring) must have the favored conformation for facilitation of the consolidation processes. Although AVP facilitated retrieval processes, its fragments AVP-(1–8), AVP-(1–7) and OT-(1–6), OT-(7–9) and OT-(8–9) did so with a much lower potency. Attenuation of retrieval was found with OT and AVT (187), and also their fragments OT-(1–7) and AVT-(1–8) (170). Thus, changing a Phe^3 in AVP-(1–8) to Ile, that is AVT-(1–8), selectively changes the activity from facilitation to inhibition of retrieval processes. The possible presence of fragments of OT and AVP within the CNS was further substantiated when [*p*-Glu^4, Cyt^6]-AVP-(4–9) and [*p*-Glu^4,Cyt^6]-OT-(4–9) were found to have potent behavioral effects. In memory consolidation processes (185), [*p*-Glu^4,Cyt^6]-AVP-(4–9) is more potent than AVP. Removal of the glycinamide and *pyro*-glutamic acid residues increases the potency and long-lasting effects and results in [Cyt^6]-AVP-(5–8) as the most potent analog (186). Retrieval processes demonstrated

a similar story except that the glycinamide residue seemed to be required for high potency. Hence, [*p*-Glu^4,Cyt^6]-AVP-(4–9) was more potent than AVP and [Cyt^4]-AVP-(5–9) was the most potent (186). Similarly, OT was always less potent than any of its fragments in all of the passive behavioral tests. [Cyt^6]OT-(5–9) was less potent than [*p*-Glu^4, Cyt^6]OT-(4–9) and [*p*-Glu^4,Cyt^6]OT-(4–8) in inhibiting consolidation after postlearning injection, whereas [Cyt^6]OT-(5–9) was the most potent fragment in attenuating retrieval processes (186). The recent discovery that N-α-acetyl-AVP (AcAVP), which has been found in the brain (187–189), is an antagonist to the passive avoidance behavioral effects of [Cyt^6]AVP-(5–9), but not to those of AVP (190), further suggests that the passive avoidance behavioral responses are mediated through receptors totally unlike the peripheral V_1, V_2, and uterine receptors.

The topolographical and conformational requirements for activity and high potency (strong binding) of central receptors involved in behavioral processes on neurohypophyseal hormones, are largely met by the central elements of the hormones (i.e. in residues 4–8). The 20-membered disulphide ring together with residues in positions 2,3,8, and 9 do not seem to be required, although an intact cystine^6 moiety does. An exception is the need for a C-terminal glycinamide for high potency of the effects on retrieval processes. Although the behavioral effects of OT and VP differ, they have analogous structural requirements for activity and high potency. Does this mean that they mediate their effects through the same receptor?

AMNESIA Vasopressin has been shown to protect against various forms of amnesia including retrograde amnesia induced by electro-convulsive shock (191), by CO_2 inhalation (192), by pentylenetetrazol (PTZ) treatment (193, 194), and puromycin-induced memory loss (195). In contrast, OT attenuates memory processes (187) and can be considered as an amnesic peptide. In studying the effects of oxytocin and vasopressin analogs on puromycin-induced amnesia in mice, Walter et al (196) found AVP and LVP to be essentially equipotent. OT and AVT showed no detectable amount of activity. Substitution within the pressin ring of VP and/or replacement of an Arg^8 for D-Arg in AVP resulted in very little loss in potency. Hence, [Leu^4]LVP, [Mpa^1]LVP, [Asu^{1,6}]LVP and [Mpa^1,*D*-Arg^8]VP were all highly active. However, substitution of Tyr^2 for an Ala, as in [Ala^2]AVP or removal of the C-terminal amide functionality, [Arg^8]vasopressinoic acid, resulted in drastic losses of activity. The *des*-glycinamide derivative of LVP (DG-LVP) was approximately 75% of the activity of LVP but pressinoic acid, in which all of the acyclic tripeptide tail has been removed, is completely inactive. Thus it would seem that the features of the vasopressins, that is the basic residue in position 8 together with an aromatic residue in position 3 of the 20-membered

ring and the influence of these features on the resulting conformation, are required for detectable attenuation of puromycin-induced amnesia. It was therefore surprising to find that the C-terminal tripeptide of OT, Pro-Leu-Gly-NH_2 (PLG), was active in attenuating puromycin induced-amnesia. Structure activity studies on this peptide showed that changing the N- and C-terminal residues drastically eliminated all the activity; substitution of Leu for Lys, on the other hand, resulted in an increase in activity, whereas substitution of Leu for Ile resulted in a drop in activity. Blocking of the N-terminal imino group with a carbobenzoxy group greatly increases the activity, making Cbz-Pro-Leu-Gly-NH_2 the most active fragment of this C-terminal group of compounds. It is far more active than AVP and LVP, but if the formyl group is used as a blocking group a lower level activity occurs. The dipeptide Leu-Gly-NH_2 together with its *D*-isomer, D-Leu-Gly-NH_2, and cyclic analog, -Leu-Gly-, are highly active in blocking puromycin-induced amnesia. Whether these latter C-terminal fragments are acting at the same receptor site as the neurohypophyseal hormones is unclear because much larger doses of the C-terminal peptides had to be used. More interesting is the finding that the larger C-terminal fragments of AVP, which are thought to be the products of peptidase degradation within the brain, are more active than AVP (186). Hence, [*p*-Glu^4,Cyt^6]AVP-(4–9) is more active than AVP, and [Cyt^6]AVP-(5–9) is the most active. Removal of the C-terminal glycinamide residue from both of the above fragments results in a loss of activity. This is analogous to the structure-activity requirements of these peptides on retrieval processes involved in passive avoidance behavior.

MORPHINE TOLERANCE OT has been shown to inhibit tolerance to compounds such as morphine (197, 198), heroin (199, 200) and opioid peptides like β-endorphin (201), and enkephalin analog [*D*-Pro^2,Met^5]enkephalinamide (202). Des-Glycin-amide oxytocin (DG-OT) and Cbz-Pro-*D*-Leu also attenuate morphine tolerance but they are less potent than OT (10% and 0.1% the potency of OT, respectively) (203). Interestingly, the attenuating effect of Cbz-Pro-*D*-Leu cannot be blocked by the oxytocic antagonist N-α-Ac[Tyr(Me)^2]OT whereas those of OT and DG-OT can. This would suggest, once again, that the smaller C-terminal derivative mediates its effects through brain processes different from those affected by OT.

ELECTROPHYSIOLOGICAL STUDIES Another method of characterizing neurohypophyseal receptors is to employ an electrophysiological assay where the rate of firing of a class of neurones is measured after treatment with the peptide in question. Using this method one can obtain dose-response curves and therefore various peptides can be compared quantitatively. In the CA1 area of hippocampal slices it has been shown that oxytocin and vasopressin

increase the rate of firing of nonpyramidal neurones (204) with oxytocin being the more potent (205). d[*D*-Arg^8]VP, d[Tyr(Me)^2,Val^4 and *D*-Arg^8]VP, two specific V_2 agonists and [Phe^2,Orn^8]-VT, a specific V_1 agonist, had a much weaker action on hippocampal cell firing, whereas HO[Thr^4,Gly^7]OT, a very specific oxytocic agonist, was found to be as much as twice as potent as OT (204, 205). Also d[Tyr(Me)^2,Val^4,*D*-Arg^8]VP, which is an oxytocic antagonist on uterine smooth muscle, blocked the excitatory actions of OT on hippocampal neurones. These results indicate that the hippocampal response due to oxytocin and vasopressin is probably mediated through an oxytocic receptor not unlike those found in the uterine smooth muscle. A similar story was found in the dorsal motor nucleus of the vagus nerve (DMX) in rat-brain stem slices, where OT and AVP increase the firing rate of neurones present, with OT again being the more potent (206). An effect equal to that of OT was obtained with HO[Thr^4,Gly^7]OT but not with d[Tyr(Me)^2,Val^4,*D*-Arg^8]VP. The latter analog also blocked the excitatory effect of OT. Once again, uterine-like oxytocic receptors are presumed to be involved in stimulating neurones of the DMX. In the dorsal cochlear nucleus (DCN), an area of the auditory brainstem with dense AVP innervation, AVP and OT have an excitatory effect on the firing of neurones, but here AVP is much more potent than OT (207). Treatment of these neurones with a V_1 agonist [Phe^2,Orn^8]VT gave a response similar to AVP treatment, although this could not be repeated using the V_2 agonist d[*D*-Arg^8]VP. Thus, the receptors mediating the firing of neurones within the DCN are similar to V_1 receptors.

ACTIONS OF NEUROHYPOPHYSEAL ANTAGONISTS IN THE CNS

To date, structure-activity studies have not been done to determine the conformational and topographical requirements of central neurohypophyseal receptors for the binding of ligands in such a way that transduction is prevented. However, the vast array of available neurohypophyseal antagonists that are specific for the oxytocic uterine receptors, V_1 receptors or V_2 receptors has led to their use for determining the characteristics of putative central neurohypophyseal receptors.

As mentioned above, OT and AVP are intimately involved in grooming behavior, sexual receptivity, and analgesia in rats and/or mice. Further work on the latter two behaviors has shown that responses induced by central administration of OT can be blocked by [Pen^1,*p*-MePhe^2,Thr^4,Orn^8]OT (PPTO-OT), a specific long-lasting OT antagonist, but this peptide cannot block AVP-induced actions (208, 209). Furthermore, d$(CH_2)_5$[Tyr(Me)^2] AVP, the vasopressor antagonist had no effect on blocking the actions of OT. It is especially surprising that d$(CH_2)_5$[Tyr(Me)^2]AVP had no effect since

this is also a potent oxytocic antagonist on uterine smooth muscle (43). Similarly, grooming behavior induced by OT was blocked by [Pen^1,Phe^2, Thr^4, $\Delta^{3,4}$Pro^7,Orn^8]OT, another uterine antagonist (210). However, AVP-induced grooming could not be blocked.

It has been shown that d[Tyr(Me)^2,Val^4,*D*-Arg^8]VP, an oxytocic antagonist on uterine smooth muscle as well as an antidiuretic agonist, has no effect on the firing rate of neurones in the DMX or in hippocampal slices, but markedly reduces the excitatory effect of OT on neurones. This finding further supports the idea that uterine-like receptors are involved. Recently, ^{125}I-labelled d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH$_2^9$]OT, a highly selective oxytocic antagonist on the rat uterus, was shown to bind to areas within the ventral hippocampus (211).

The attenuating effect of OT and DG-OT on morphine tolerance in mice was shown to be reversed in the presence of N-α-Ac[Tyr(Me)^2]OT, an oxytocic uterine antagonist (203). This antagonist together with [Pen^1,Tyr(Me)^2]LVP, another oxytocic antagonist, did indeed have a facilitatory effect on morphine tolerance when administered alone (203, 212).

CONCLUSION

We have studied the structure-biological activity relationships of the neurohypophyseal hormones at several putative hormone receptor systems within the CNS. Clearly, much more work remains before the complete picture emerges but nevertheless, we can note the existence of several neurohypophyseal receptors with structural and stereochemical requirements for agonist activity that are completely different from those of any known peripheral receptors. Further research is needed to determine structure-activity relationships for antagonist activity, and where OT and VP both effect biological activity, whether they are acting at the same receptor system.

The latter question has been partially answered in the studies of analog that are selective antagonists of oxytoxic, pressor, or antidiuretic responses, to characterize putative receptors. Hence, the grooming behavior induced by OT can be blocked by the specific oxytocic uterine antagonist [Pen^1,Phe^2, Thr^4,$\Delta^{3,4}$Pro^7,Orn^8]OT, but this antagonist cannot block AVP-induced grooming behavior. This would suggest a uterine-like receptor is involved. This approach does not always work since it does not take into consideration that these antagonists are tailored from structure-activity relationships of peripheral receptors, which the studies with agonists show to be possibly very different from structure-activity relationships of central receptors. This may therefore explain why d(CH$_2$)$_5$[Tyr(Me)^2]AVP had no effect on OT-induced analgesia and sexual behavior, even though it is both a potent vasopressor antagonist and a oxytocic antagonist. A more basic, detailed

study of the structure-activity relationships of neurohypophyseal hormone-receptor systems in the CNS should provide a clearer picture of their nature and how they differ from peripheral receptors. This would be useful for the design of specific drugs in the treatment of human and animal diseases.

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