SYNTHETIC ANALOGS OF OXYTOCIN AND THE VASOPRESSINS

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Vincent du Vigneaud and his associates synthesized oxytocin and the vasopressins almost twenty years ago. These were the first polypeptide hormones prepared by total synthesis. Their pharmacological properties were identical with those of the purified natural hormones. This provided elegant proof that the molecular structures determined by previous analyses were indeed correct.

Once the structures of the mammalian neurohypophysial hormones were established several groups began preparing synthetic analogs. These were not synthesized at random, but with several general purposes in mind. These included:

(*a*) Definition of the importance of specific molecular structures to the varied biological activities of the hormones (1, 2).

(*b*) Illumination of the intimate natures of hormone-receptor interactions, of stimulus-response coupling, and peptide inactivation (3).

(*c*) Development of analogs with selectively altered properties as potentially useful therapeutic agents, both as agonists and antagonists (4). Chemists and pharmacologists have had some success in pursuing these general goals and have obtained much detailed information on the pharmacological consequences of many structural alterations in the hormone molecules. No structural analogs have yet become established as generally useful clinical agents, but this is an area that holds promise.

The number of structural analogs of the neurohypophysial hormones that could be synthesized is, of course, almost infinite. The number already reported is quite formidable. Recent application of the Merrifield solid-phase method to the synthesis of analogs (5) has further accelerated the pace at which these may be prepared for pharmacological studies. We do not have space in this review to list all analogs that have been prepared and pharmacologically characterized. Fortunately this has been attempted by others. Schwartz & Livingston (6) reviewed the properties of 120 analogs in 1964. Schröder & Lübke (7) listed about 110 synthetic analogs and their pharmacological properties in their 1966 book. Four years later Branda & Ferrier (8) added 90 more to these lists. They did not report on their biological properties but did provide references to the sources of such information.

Additional references to analogs synthesized during 1968–1970 are given by Jones (9–11) in the Chemical Society Specialist Periodical Reports on Amino-acids, Peptides and Proteins. Berde & Boissonnas' review (12) provides the most inclusive and useful source of pharmacological data on synthetic analogs of the

neurohypophysial hormones. They tabulate activities of about 145 peptides on several standard biological assays. Information on more recently reported analogs can be obtained from the references provided by Branda & Ferrier (8) and from Jones (9–11). Limited space prevents us from presenting pharmacological data on all of these. Instead, we plan to discuss a few general principles that have emerged from studies on the pharmacology of synthetic analogs, and to concentrate on a few particular aspects of these studies that we find most interesting. We will not attempt to provide references to individual reports on synthetic peptides that are listed in the inclusive bibliographies prepared by Schröder & Lübke (7), Berde & Boissonnas (12), Branda & Ferrier (8), Jones (9–11) and Rudinger (3, 4).

In this review we shall pay particular attention to Nature's role as a synthesizer of analogs. By comparing naturally occurring neurohypophysial peptides with some of the many provided by synthetic chemists we may find clues as to which structural features allow biological properties that are adaptable to hormonal use.

Nine biologically active peptides have been isolated from vertebrate neurohypophyses (13) (Table 1). Nature appears to have been much more conservative in producing structural analogs during some 400 million years of evolution than have the synthetic chemists during the last two decades. Only a few substitutions in the basic octapeptide molecule appear to have had adaptive value and have survived among the living vertebrates. These substitutions occur only in positions 3, 4, and 8. The remaining amino acids in the molecule appear to occupy favored positions. This suggests that their presence in these positions is essential if peptides are to function as useful hormones. This assumption may be misleading, however, since all these peptides were discovered because they were active on biological assays designed to detect the mammalian hormones. Other related peptides may have arisen during vertebrate evolution by mutations affecting the apparently inviolate positions in the neurohypophysial hormones. Such molecules may have acquired other functions yet unknown. By virtue of their loss of activities detected by routine biological assays they may have escaped detection.

Recent reports (14, 15) that prolylleucylglycinamide may serve as a hypothalamic hormone, inhibiting hypophysial release of melanocyte-stimulating hormone, could provide an example of such a phenomenon. This is the terminal tripeptide amide found in oxytocin. It has no intrinsic oxytocic activity and would not be detected by biological assays designed to detect neurohypophysial hormones. Yet it is conceivable that this tripeptide originated from oxytocin by mutation, or that it was produced from oxytocin enzymically, and that it has been put to a new use quite distinct from any of the previously recognized functions of neurohypophysial peptides. Thus our failure to identify naturally occurring analogs with substitutions or deletions involving the "inviolate" positions may simply reflect the fact that such changes usually result in peptides that are quite incompatible with the receptors in the few mammalian tissues that we exploit in our routine biological assays. We must not assume that such peptides would be necessarily biologically useless.

TABLE 1. Naturally occurring active neurohypophysial peptides (13), their structures, biological activities, and phyletic distribution.

Structures				Biological Activities (Units/mg)					Where Found
1 2 3 4 5 6 7 8 9 CyS-Tyr-(X)-(Y)-Asn-CyS-Pro-(Z)-Gly-NH_2									
	X	Y	Z	Rat uterus	Fowl depressor	Milk ejection	Vaso-pressor	Anti-diuretic	
Vasopressor Peptides:									
Arginine vasopressin	Phe	Gln	Arg	14[a]	105	70	370	320	Mammals
Lysine vasopressin	Phe	Gln	Lys	10[b]	50	60	270	280	Pig-like mammals
Arginine vasotocin	Ile	Gln	Arg	130[a]	490	200	160	230	Nonmammals, fetal mammals.
Oxytocin-like ("Neutral") Peptides:									
Oxytocin	Ile	Gln	Leu	520[a]	550	475	4	4	Mammals, holocephalian fish.
Mesotocin	Ile	Gln	Ile	390[c]	830	300	6	6	Birds, reptiles, amphibians, lungfishes.
Valitocin	Ile	Gln	Val	265[a]	280	370	17	5	Sharks
Aspartocin	Ile	Asn	Leu	108[d]	201	300	0.1	0.04	Sharks
Isotocin	Ile	Ser	Ile	130[c]	380	375	0.04	0.7	Ray-finned fishes
Glumitocin	Ile	Ser	Gln	10[a]	30	50	0.4	0.5	Rays

[a] Activities of peptides synthesized by Manning and associates (34, 52, and unpublished) and assayed against USP Posterior Pituitary Reference Standard.
[b] Activities of a solution provided by Sandoz Ltd calculated on the assumption that the peptide had absolute activity of 270 vasopressor U/ml (12).
[c] Assays of peptides synthesized by Rudinger et al (53).
[d] Value from Berde & Boissonnas (12). All other assays reported were performed in the authors' laboratories.

Certain general features of the basic octapeptide molecule do appear essential if it is to manifest those pharmacological properties that we measure. One is the intact pentapeptide ring. Early experiments showed that reduction of the disulfide bond between the two half-cystines destroyed biological activities. Linear analogs containing alanine in positions 1 and 6 are essentially devoid of activities (16). This does not indicate that the disulfide bond itself is needed, merely that a closed ring is essential. If one or both sulfur atoms are replaced by selenium or methylene groups, keeping the ring structure intact, biological activities are well maintained. In fact, "deamino-carba^6-oxytocin", in which the sulfur of the half-cystine in position 6 is replaced by a methylene group, has about twice the oxytocic activity of deamino-oxytocin, the corresponding analog containing the natural disulfide bridge (17).

The exact size of the pentapeptide ring also appears important. In the natural hormones it consists of a chain of 20 atoms. If the ring of [1-deamino]-oxytocin ([1-β-mercaptobutyric acid]-oxytocin) is enlarged by introducing an additional methylene group, as in [1-γ-mercaptobutyric acid]-oxytocin, oxytocic activity is reduced by about 99% (18). Reducing ring size by one methylene group, as in [1-mercaptoacetic acid]-oxytocin, reduces activity somewhat less, by about 97% (19). Rather remarkably, deletion of one amino acid, removing three atoms from the ring, as in [4-deglutamine]-oxytocin, does not abolish activity. This analog retains about 1% of the activity of oxytocin on the rat uterus (Manning & Sawyer, unpublished). Thus the 20-membered ring is important, but not absolutely essential, for a peptide to manifest biological activities.

Cystine is the only natural amino acid that contains a covalent bridge that can close the pentapeptide ring. Thus no other natural amino acid in position 1 or 6 could be substituted for these half-cystines in a biologically active analog.

Structural alterations of the 1-half-cystine have produced some analogs with interesting properties. If substitutions are made on the terminal amino group most activities are drastically reduced. If the peptide chain is elongated by additions to the N-terminal of oxytocin or the vasopressins, the resultant analogs are relatively inactive but, in vivo, can be hydrolized to release active peptides. Such "hormonogens" can, therefore, produce sustained responses (4).

If two methyl groups are added to the β-carbon of the half-cystine in the 1-position, as in [1-penicillamine]-oxytocin, the activities characteristic of oxytocin are destroyed but the analog can inhibit competitively the effects of oxytocin on the rat uterus in vivo and vitro (20).

Removal of the terminal amino group actually enhances oxytocic, fowl vasodepressor, and antidiuretic activities of oxytocin and the vasopressins, while reducing vasopressor activities. Although more potent, [1-deamino]-oxytocin would not appear to offer a therapeutic advance over oxytocin, since its antidiuretic activity is enhanced relatively more than its oxytocic activity (12). On the other hand, [1-deamino]-arginine vasopressin is a potent and highly specific antidiuretic substance. Instead of a ratio of antidiuretic to vasopressor activities of 1.0, as in arginine vasopressin, this ratio is increased to about 4. If D-arginine is substituted in the 8-position of [1-deamino]-arginine vasopressin, antidiuretic

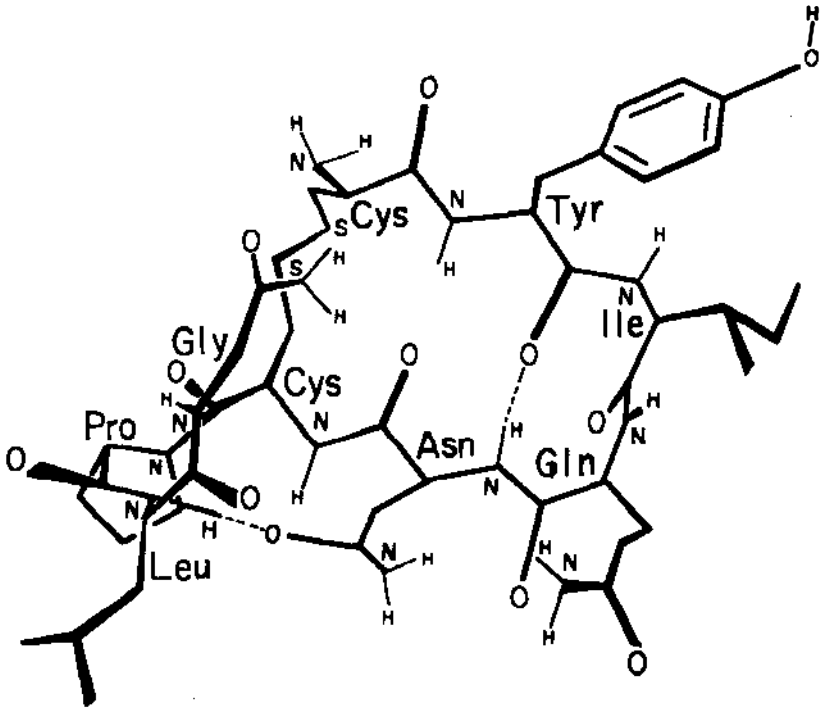


FIG. 1. Proposed molecular conformation of oxytocin in solution based largely on studies of nuclear magnetic resonance by Urry & Walter (35). Hydrogen bonds are indicated by dashed lines. Slightly modified from (51) and reprinted by kind permission of Dr. Roderich Walter and the Excerpta Medica Foundation.

activity remains high, but vasopressor activity is further diminished. The ratio of activities rises to about 80 (4), making this a much more specific and active antidiuretic agent than the natural antidiuretic hormone, arginine vasopressin.

Hope & Wälti (21) recently reported that substitution of a hydroxyl for the terminal amino group in oxytocin results in a peptide ([1-L-2-hydroxy-3-mercaptopropanoic acid]-oxytocin) with about twice the oxytocic activity of [1-deamino]-oxytocin and three times that of oxytocin. Unfortunately other pharmacological properties of this surprising analog have not yet been reported.

Tyrosine appears to have remained unchanged in the 2-position throughout evolution. It is unclear why this should be. [2-phenylalanine]-oxytocin has about 7% of the oxytocic activity of oxytocin, while [2-phenylalanine]-vasopressins have 20–30% of the vasopressor activities of the corresponding vasopressins (12). The presence of an aromatic ring in the side chain in the 2-position does not appear critical since [2-isoleucine]-oxytocin is approximately as active as [2-phenylalanine]-oxytocin (22). Inspection of the proposed conformation of oxytocin (Fig. 1) does not explain why the tyrosine side-chain should be so important to

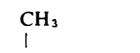
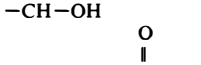
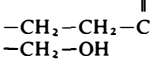
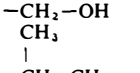
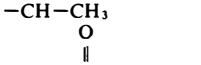
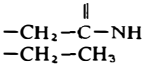
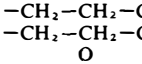
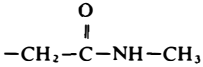
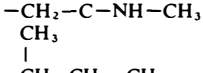
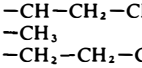
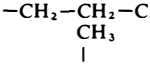
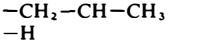
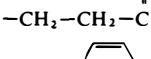
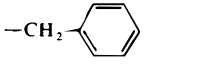
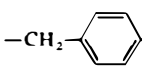
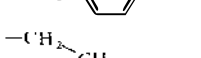
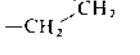
biological activities, although the relatively high activity of 2-phenylalanine and 2-isoleucine analogs suggests that a hydrophobic side chain in this position favors both affinity and intrinsic activity.

The most interesting analogs that have been prepared with changes in the 2-position are the series in which substitutions have been introduced on the para-carbon of phenylalanine or on the tyrosine hydroxyl. These analogs exhibit the properties of partial agonists and can also antagonize many of the responses to oxytocin (3, 4, 23). The degree to which these analogs manifest agonistic and antagonistic oxytocic activities is highly dependent upon the exact ionic milieu in which they are studied and they are relatively ineffective as antagonists in vivo. When the N-terminal amino group is carbamylated (24) and the tyrosine hydroxyl is methylated, the resultant analog ([1-*N*-carbamyl-hemicystine, 2-*O*-methyl-tyrosine]-oxytocin) is a weak but effective antagonist of oxytocin when tested in vivo in milk ejection and rat uterus assays (25).

Nature has been rather conservative concerning the 3-position. The presence of isoleucine in this position is critical to oxytocic activity. Substitution by valine or phenylalanine reduces potency to 1/10 or less (12), while substituting leucine results in an analog with about 1/100 the oxytocic activity of oxytocin (26). It is evident, from Table 1, that the introduction of phenylalanine in place of the isoleucine of position 3 of arginine vasotocin, to produce arginine vasopressin, served mainly to suppress oxytocic and milk-ejecting activities, with the increase in vasopressor and antidiuretic potencies being relatively small. When phenylalanine is introduced in the 3-position of oxytocin (as in [3-phenylalanine]-oxytocin or "oxypressin") (27) there is a modest increase in antidiuretic activity but vasopressor activity is reduced, and oxytocic potency falls to about 1/25 that of oxytocin. Thus the role of phenylalanine appears largely to suppress activities on the uterus and mammary gland, its influence on vasopressor and antidiuretic activities being moderate.

Three amino acids have been found in the 4-position of natural neurohypophysial hormones (Table 1). These all have nonionized but hydrophilic side-chains. Du Vigneaud and colleagues (28) prepared a series of analogs of oxytocin with alkyl side chains (Table 2) and found that the optimal length of the side chain was two carbons (as in [4-α-aminobutyric acid]-oxytocin). Activity fell off rather sharply if the chain was longer or shorter. If the chain branched, as in [4-valine]-oxytocin, oxytocic activity was doubled over that of [4-α-aminobutyric acid]-oxytocin. Comparison of the activities of [4-serine]- and [4-glutamine]-oxytocins with those of analogs with alkyl chains of similar length, however, indicates that a hydrophilic carboxamide or hydroxyl group adds substantially to oxytocic activity. As part of a program in which we were attempting synthesis of possible evolutionary intermediate hormone analogs, Manning et al (29) made [4-threonine]-oxytocin and [4-threonine]-mesotocin. These analogs are considerably more active than oxytocin on rat uterus and fowl vasodepressor assays; in fact, they are the most active analogs known that contain only naturally occurring amino acids. It is surprising, perhaps, that the combination of a methyl group and a hydrophilic hydroxyl on the β-carbon of the side chain should result in an

TABLE 2. Oxytocic activities of analogs of oxytocin and of [1-deamino]-oxytocin containing substituted amino acids in the 4-position.

Amino acid in 4-position	Structure of side chain	Activity on rat uterus in vitro (no Mg^{++}) in U/ mg	
		Oxytocin analog	Deamino-oxytocin analog
Threonine		920[a]	150[a]
Glutamine		520[a]	803[b]
Serine		197[c]	37[c]
Valine		140[b]	322[b]
Asparagine		108[a]	
α-Aminobutyric acid		82[b]	93[b]
Norvaline		61[b]	56[b]
Ornithine		58[c]	
N^4-methyl-asparagine		41[c]	
Isoleucine		37[b]	67[b]
Alanine		36[d]	
Norleucine		21[b]	13[b]
Leucine		13[b]	37[b]
Glycine		3[d]	
None (deglutamine)		3[c]	0.5[c]
Glutamic acid		1.5[d]	
Phenylalanine		1[c]	
Tyrosine		0.8[c]	
Proline		0.007[f]	

[a] Manning et al (31).
[b] Flouret & du Vigneaud (28).
[c] Manning and Sawyer, unpublished.
[d] Berde & Boissonnas (12).
[e] Havran et al (54).
[f] Sawyer et al (55).

analog with higher affinity for oxytocin-receptors than the natural hormones containing glutamine, asparagine, or serine in the 4-position (Table 2). One can speculate as to why such an apparently improved oxytocin did not arise during the course of vertebrate evolution. Several possible explanations have been discussed elsewhere (30). The 4-threonine analogs appear to be more specific in their oxytocic activities as well as being more potent, as antidiuretic and vasopressor activities are actually reduced as compared to those found in oxytocin and mesotocin.

Deamination of the 1-hemicystine had been found to enhance the oxytocic activity of oxytocin and many of its analogs with alkyl side-chains on the amino acid in the 4-position (28). Since substitution of threonine in this position resulted in increased oxytocic activity, Manning et al (31) prepared deamino derivatives of [4-threonine]-oxytocin and [4-threonine]-mesotocin. Instead of having increased oxytocic activity these analogs showed diminished activity. In order to see whether this unexpected event was characteristic of peptides containing amino acids with hydroxyl groups in their side chains [1-deamino, 4-serine]-oxytocin was prepared. Again, this showed decreased oxytocic activity when compared to [4-serine]-oxytocin (32) (Table 2). It is difficult to explain this phenomenon, since removal of the amino group from oxytocin increases its activity, and so does introduction of threonine in position 4. The proposed conformation of oxytocin (Fig. 1) places the β-carbon of the side chain of the amino acid in position 4 about as far from the α-carbon of the half-cystine in position 1 as possible. This might suggest that direct interactions between substituents in these positions would be unlikely. A possible explanation for the low oxytocic activities of deamino, 4-threonine analogs has been suggested (31) based on their lipophilic characteristics. On chromatography, the 4-threonine analogs of oxytocin and mesotocin appear more lipophilic than either oxytocin or mesotocin, respectively (31), as are [4-serine]-oxytocin and [4-serine]-mesotocin (33, and Sawyer, unpublished). The deamino, 4-threonine derivatives are much more lipophilic than their parent peptides (31). One may suggest, therefore, that moderately increased lipophilic properties enhance oxytocic activity, but that the additive lipophilic effects of a hydroxyl amino acid in the 4-position, and deamination of the 1-half-cystine, may result in too great a change in solubility characteristics and that this diminishes oxytocic activities. The deamino, 4-threonine derivatives of oxytocin and mesotocin are only about 1/3 as active as oxytocin and mesotocin in stimulating the rat uterus.

Similar changes in oxytocic activity were observed when 4-threonine and deamino, 4-threonine analogs of the vasopressins were studied (34). Both deamination and 4-threonine substitution diminish vasopressor activities, and these effects are additive, the deamino, 4-threonine derivatives having less than 10% of the vasopressor activities of the parent vasopressins. Deamination tends to increase antidiuretic activities, while 4-threonine substitution decreases them. The deamino-4-threonine derivatives of arginine and lysine vasopressin actually turn out to have greater antidiuretic activities than the corresponding vasopressins. The ratios for antidiuretic to vasopressor activities are about 10 and about 50 for

[1-deamino, 4-threonine]-arginine vasopressin and [1-deamino, 4-threonine]-lysine vasopressin. These are, then, like the D-arginine analogs of vasopressin and deamino-arginine vasopressin, extremely active and specific antidiuretic agents. Whether or not such analogs can offer a therapeutic advantage by having less vasoconstrictor properties than the natural antidiuretic hormones, they do serve to demonstrate strikingly that the structural specificities of antidiuretic and vasopressor receptors are quite different.

Asparagine in the 5-position appears critical for biological activity. Substitution of closely related amino acids, such as glutamine, or D-asparagine, reduces activity by factors of 500 or more (12). Urry & Walter's (35) studies on the conformation of oxytocin in solution may offer an explanation for the importance of this asparagine residue (Fig. 1). These suggest that asparagine plays a critical role in stabilizing the secondary structure of oxytocin. Its peptide NH forms a hydrogen bond with the peptide CO of the tyrosine to stabilize a β-turn involving the pentapeptide ring; its side chain CO forms a hydrogen bond with the NH of the leucine to form a second β-turn in the tripeptide " tail ". These authors suggest that oxytocin must assume a specific conformation in order to interact with uterine receptors. It thus appears reasonable that any change in the side chain of an amino acid such as asparagine, that stabilizes the shape of the molecule, could destroy oxytocic activity.

The proline residue also remains unchanged among the neurohypophysial hormones (Table 1), although other amino acids in this position would be compatible with the assumption of the conformation proposed by Urry & Walter (35). This position has not received much attention. [7-Hydroxyproline]-oxytocin has been synthesized by two separate groups. In one case it was found to have about 6% of the oxytocic potency of oxytocin (36) whereas in the other it was shown to be inactive (37). The reason for this discrepancy is obscure. [7-Pipecolic acid]-oxytocin, in which the 5-membered pyrrolidine ring of proline is replaced by a 6-membered ring, is reported to have about 40% of the activity of oxytocin (36). [7-D-Proline]-oxytocin (38) and [7-valine, 8-lysine]-vasopressin (Boissonnas, personal communication cited by Morel & Bastide, 39) are quite inactive. [7-Glycine]-oxytocin does, however, retain about 15% of the oxytocic activity of oxytocin (4). In the absence of information on other substitutions in this position, however, one cannot decide how important structural peculiarities of the proline residue are to the biological properties of neurohypophysial peptides.

The natural neurohypophysial hormones are most variable in the 8-position. As far as oxytocic activity is concerned, the nature of the side-chain in this position does not seem critical. Analogs with valine, isoleucine (12), or threonine (Manning & Sawyer, unpublished) in this position are at least half as active as oxytocin itself. Side chains as different as those of serine (40), citrulline (41), phenylalanine (42), alanine, ornithine, and arginine (12) are still compatible with oxytocic activity at least 20% that of oxytocin. [8-Glutamine]-oxytocin is about 10% as active as oxytocin (43), while [8-glycine]-oxytocin is considerably weaker (12). Thus uterine receptors appear to tolerate considerable freedom as far as the side chains of the 8-amino acids are concerned, and it makes relatively little dif-

ference whether they are hydrophobic or hydrophilic, aromatic or aliphatic, neutral or basic.

Receptors that mediate antidiuretic and vasopressor responses are much more discriminating. The presence of a basic amino acid in the 8-position is essential for peptides to manifest strong vasopressor and antidiuretic activities. Arginine, lysine, or ornithine in the 8-position confers high vasopressor and antidiuretic potency on oxytocin or vasopressin (12). On the other hand, citrulline (41), histidine, or other neutral amino acids in this position yield relatively weakly active analogs (12). It was noted earlier that substitution of D for L-arginine in arginine vasopressin has been reported to reduce antidiuretic activity much less than vasopressor activity; the 8-D-arginine analogs of arginine vasopressin and of [1-deamino]-arginine vasopressin are thus highly specific in possessing high antidiuretic activity and little vasopressor activity (44). The absence of a terminal amino group, and the presence of D-arginine in the 8-position appear to protect this peptide from destruction in the vicinity of the antidiuretic receptors (3). This results in significant prolongation of its antidiuretic action in both animals and man (45). The combination of prolonged effects on diuresis and weak vasopressor activity in [1-deamino, 8-D-arginine]-vasopressin holds promise that this can be a highly useful preparation for chronic treatment of patients with hypothalamic diabetes insipidus.

All naturally-occurring neurohypophysial hormones contain a terminal glycinamide. Few amino acid substitutions have been reported in this position. Methylation of the amino NH of glycinamide, as in [9-sarcosine]-oxytocin, reduces the oxytocic activity of oxytocin by about 99% (46). [9-β-Alanine]-oxytocin and [9-alanine]-oxytocin have been synthesized (37) and found to be weakly active on the isolated rat uterus. Lengthening the C-terminal end of oxytocin by inserting an NH between the CO of leucine and the NH of glycine, or adding a glycine-NH in this position, essentially destroys biological activity (47).

The amido group on the terminal glycinamide is also important. If it is removed, as in [9-deamido]-oxytocin, or if the carboxamide group is removed, as in [9-decarboxamido]-oxytocin, activity essentially disappears. Recent studies on the ring structures of oxytocin and vasopressin ("tocinoic" and "pressinoic" acids) further emphasize the importance of the terminal amide. These pentapeptides have little or no oxytocic activity, but their amides are clearly more active (48, 49).

Deletion of glycine entirely, as in [9-deglycine]-oxytocin, reduces oxytocic activity by about 99%. It does not effect all biological activities similarly. In fact, [9-deglycine]-oxytocin is more than twice as effective as oxytocin in stimulating sodium transport (the "natriferic" response) across the isolated skin of the frog *Rana esculenta* (39), although vasopressor and antidiuretic activities are much less than those of oxytocin. [8-Deleucine]-oxytocin has rather similar properties, having high natriferic activity on frog skin but being quite inactive by other assays (39). Receptors in skins and bladders of anuran amphibians appear quite unusual in their relative indifference to the presence or absence of the three terminal amino acids. "Tocinamide" ([7-deproline, 8-deleucine, 9-deglycine]-

oxytocin) is nearly as effective as oxytocin itself in increasing water permeability (hydroosmotic response) and sodium transport by isolated toad (*Bufo marinus*) bladders (P.J.S. Chiu and W. Y. Chan, personal communication) despite the fact that it has only about 1/150th the activity of oxytocin on the rat uterus and undetectable fowl vasodepressor, milk ejection, antidiuretic, and vasopressor activities.

CONCLUSIONS

One may rightly ask what useful information has emerged from the synthesis of several hundred analogs of oxytocin and the vasopressins, whether further syntheses are warranted, and, if so, which areas should be explored further.

The elucidation of the structures of oxytocin and the vasopressins nearly twenty years ago raised many questions concerning the relations between molecular structures and biological activities. The answers to some of these, concerning ring size, the importance of the tripeptide side-chain, the significance of functional groups, and stereochemistry, are now fairly clear. We now know that a closed ring is absolutely essential to biological activities, but that the exact size of the ring is not nearly so critical. The optimal length of the side-chain has also been defined, although its exact composition does not appear critical. The amide groups at positions 5 and 9 are essential for activity while the amide at position 4 is not. The primary amino group and the disulfide grouping are clearly nonessential. The natures of the amino acids in positions 3 and 8 are critical for the manifestation of maximum oxytocin-like and vasopressin-like activities, respectively. All D-oxytocin is totally inactive yet it is possible to substitute some individual D-amino acids for their natural L-diastereoisomers in peptides that retain appreciable biologic potencies.

We now have a fairly clear picture of the general structural features that modulate the known individual biological characteristics of the neurohypophysial hormones. It is becoming possible, in many instances, to design analogs with selected properties.

Knowledge of the pharmacological properties of synthetic analogs has been useful in testing the conformational models of oxytocin and lysine vasopressin determined by biophysical methods. Some useful correlations between biological activities and conformation have been drawn (50, 51) but the properties of all analogs cannot be explained on the basis of these models. As an example, it is not possible to explain either the high activity of [4-threonine]-oxytocin or the relatively low activity of [1-deamino, 4-threonine]-oxytocin using the Walter model. The explanation may lie in the fact that conformations in solution may not accurately reflect the state of the peptides at their receptor sites.

One area that has clearly benefited from the synthesis of analogs is the study of the phylogeny of the neurohypophysial peptides. Of the nine known naturally occurring peptides (Table 1) at least four were synthesized in the laboratory before their existence in nature was discovered. Knowledge of the pharmacological properties of synthetic analogs greatly facilitated the subsequent identification of isolated natural principles. The final chapter on the evolution of the neurohypo-

physial hormones has not yet been written but it is certain that studies on synthetic analogs will help unravel this complex story.

It is somewhat disappointing that synthetic analogs of oxytocin and the vasopressins have yet to gain established positions as therapeutic agents clearly superior to the natural hormones. The recent report (45) that [1-deamino, 8-D-arginine]-vasopressin (DDAVP) may represent a real improvement over the natural antidiuretic hormones for the treatment of diabetes insipidus is encouraging. It is also hoped that [4-threonine]-oxytocin may prove to have advantages over oxytocin in some situations where the latter is presently indicated (30).

The search for effective in vivo inhibitors of oxytocin and the antidiuretic hormone will undoubtedly continue. Such inhibitors could be of value to both clinicians and basic scientists. An oxytocin inhibitor could be useful in controlling uterine responses to oxytocin during labor and an inhibitor of the antidiuretic hormone would be a major contribution for the treatment of hyponatremia resulting from its "inappropriate secretion" by certain carcinomas and during the course of some pulmonary and cerebral disorders. For the basic scientist such inhibitors would also provide extremely useful tools for probing the structures and characteristics of tissue receptors.

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