defined as the minimum effective dose (MED). For inactive compounds, the highest inactive dose tested was reported.

Prevention of *d*-amphetamine-induced stereotyped behavior in rats was used as an index of antipsychotic activity. The test was a modification of that described previously.[8] Male Sprague–Dawley rats, 100–130 g, from Russell Roberts Farms were medicated orally with graded doses of test drugs 4 h before subcutaneous injection of *d*-amphetamine sulfate, 3.7 mg/kg of the free base. Oral dosing began at 32 mg/kg of the free base. One-minute observations were conducted 90, 100, and 110 min after injection of amphetamine. A rat was scored as affected by amphetamine if it exhibited licking, gnawing, biting, or repetitive head movements during any of the three observation periods. The ED_{50} values and confidence limits for antagonism were calculated.[9]

Pilot work was performed to obtain supplementary indexes of antipsychotic activity. To determine whether the compounds prevented apomorphine-induced emesis in dogs, a modification of a method described previously was used.[10] Compounds were injected intravenously 30 min before intravenous injection of apomorphine hydrochloride, 0.05 mg/kg of the free base.

Rhesus monkeys were medicated orally to determine whether the compounds produced a chlorpromazine-like pattern of activity consisting of catalepsy, ptosis, and taming.[6] Although accurate quantitative estimates of activity were not obtained in monkeys and dogs, results were consistent with those reported for the amphetamine test.

References and Notes

(1) W. C. Cutting, "Handbook of Pharmacology", 4th ed, Meredith Corp., New York, N.Y., 1969, pp 736–739.
(2) D. M. Gallant, M. P. Bishop, and R. Guerrero-Figueroa, *Curr. Ther. Res.*, 14, 61 (1972).
(3) N. A. Nelson and G. M. Mortimer, *J. Org. Chem.*, **22**, 1146 (1957).
(4) (a) H. J. Teuber and D. Cornelius, *Justus Liebigs Ann. Chem.*, **671**, 127–134 (1964); (b) G. E. A. Coombes and D. J. Harvey, *J. Chem. Soc.*, 325 (1970).
(5) E. C. Kleiderer and E. C. Kornfeld, *J. Org. Chem.*, **13**, 455 (1948).
(6) D. W. Wylie and S. Archer, *J. Med. Chem.*, **5**, 932 (1962).
(7) M. D. Aceto and L. S. Harris, *Toxicol. Appl. Pharmacol.*, **7**, 329 (1965).
(8) A. Randrup, I. Munkvad, and P. Udsen, *Acta Pharmacol. Toxicol.*, **20**, 145 (1963).
(9) D. J. Finney, "Statistical Methods in Biological Assay", 2nd ed, Hafner Publishing Co., New York, N.Y., 1964.
(10) P. A. J. Janssen, C. J. Niemegeers, and K. H. L. Schellekens, *Arzneim.-Forsch.*, **15**, 1196 (1963).

Combined High Oxytocic with Negligible Antidiuretic and Pressor Activities in Multisubstituted Oxytocins

Glenn L. Stahl and Roderich Walter*

Department of Physiology and Biophysics, University of Illinois at the Medical Center, Chicago, Illinois 60612.
Received August 24, 1976

Oxytocin analogues which combine high oxytocic activities with negligible antidiuretic and pressor activities have been studied. [4-Threonine,7-glycine]oxytocin, [1-(L-2-hydroxy-3-mercaptopropionic acid),4-threonine,7-glycine]oxytocin, and [1-(L-2-hydroxy-3-mercaptopropionic acid)]oxytocin were found to possess the following specific biological activities respectively: rat uterotonic, 270 ± 10, 337 ± 23, 1542 ± 18; avian vasodepressor, 8.6 ± 0.2, 50 ± 4, 1778 ± 25; rat pressor, mixed depressor/pressor, mixed depressor/pressor, 23.7 ± 0.4; rat antidiuretic, 0.002 ± 0.0008, 0.048 ± 0.005, 40.3 ± 2.4. The results are analyzed from a conformation–activity viewpoint in a continued attempt to evaluate the scope and limitations of this approach in comparison to structure–activity studies.

The amino acid residues in positions, 3, 4, 7, and 8 of neurohypophyseal hormones, oxytocin and vasopressin, comprise the corner positions of the two β turns which are important features of the peptide backbone structure in the proposed solution conformation of the hormones.[1] Consistent with the proposal that modifications of these corner positions would lead to the most dramatic as well as selective alterations of the biological activity profile of the hormones,[2] oxytocin analogues with substitutions in position 4 (e.g., ref 3 and 4) or 7 (ref 5a and references cited therein) show a marked dissociation of the smooth muscle and antidiuretic activities. Particularly noteworthy is the high ratio of rat uterotonic to antidiuretic activities characteristic of these analogues and also of [4-threonine]oxytocin[6] ([Thr^4]oxytocin)[7] which possesses an enhanced specific uterotonic activity as compared to oxytocin. This trend in the biological activity profile not only suggests that such analogues might be superior to oxytocin for the induction of labor in women ([Asu^{1,6},Gly^7]oxytocin is already being used under the name Statocin in Japan for this purpose[8]) but also raises expectations for the use of such analogues as contraceptive agents. The latter suggestion assumes, first, that the capacity of oxytocin to selectively stimulate the contractility of the human Fallopian tube in vivo at concentrations not affecting the nongravid uterus[9,10] can be extended to oxytocin analogues and, second, that the enhanced tubal muscular activity, which will increase the rate of ovum transport, will decrease the probability of successful fertilization and implantation.[11] Furthermore, any potentially useful analogue must totally lack or exhibit only negligible antidiuretic activity in order to prevent overhydration upon prolonged administration.

From a conformation–activity viewpoint, substitutions at more than one position in oxytocin—each substitution involving only a corner position of the β turns in the hormone—should result in selective modifications of the biological activity profile that reflect a summation of the changes seen with the individual monosubstituted compounds. However, substitutions at noncorner positions should not show this kind of selective additivity. In light of this assumption, with the high oxytocic activity of [Thr^4]oxytocin[6] and the negligible antidiuretic activity of [Gly^7]oxytocin,[5b] the synthesis and pharmacological study of [4-threonine,7-glycine]oxytocin ([Thr^4,Gly^7]oxytocin, **2**) appeared worthwhile. It should be noted that Manning had proposed earlier the synthesis of this compound on the basis of structure–activity studies.[12] Should the conformation–activity viewpoint be valid, [1-(L-2-hydroxy-3-mercaptopropionic acid),4-threonine,7-glycine]oxytocin ([Hmp^1,Thr^4,Gly^7]oxytocin, **4**), which has one additional structural change in a noncorner position (the hydroxyl group in position 1 when substituted into oxytocin causes an increase in all biological activities tested),

Table I. Biological Activities of Multisubstituted Oxytocins Compared with Pertinent Monosubstituted Oxytocins

Peptide	Oxytocic (rat)	Avian vasodepressor	Antidiuretic (rat)	Pressor (rat)
Oxytocin	546 ± 18[a]	507 ± 15[b]	2.7 ± 0.2[b]	3.1 ± 0.1[b]
[Thr⁴,Gly⁷]oxytocin (2)	270 ± 10, 175[c]	8.6 ± 0.2	0.002 ± 0.0008, 0.001[c]	Depressor/pressor
[Hmp¹,Thr⁴,Gly⁷]oxytocin (4)	337 ± 23	50 ± 4	0.048 ± 0.005	Depressor/pressor
[Hmp¹]oxytocin (6)	1542 ± 18, 1607[d]	1778 ± 25	40.3 ± 2.4	32.7 ± 0.4, 32[d]
	1275 ± 51[e]		16.6 ± 1.3[e]	14.7 ± 0.3[e]
[Thr⁴]oxytocin	923 ± 95[e]	1480 ± 40[f]	1.8 ± 0.3[e]	0.43 ± 0.01[e]
[Gly⁷]oxytocin[g]	65 ± 2	224 ± 15	0.01	0.3

[a] See ref 36. [b] See ref 37. [c] See ref 38. [d] See ref 13. [e] See ref 15. [f] See ref 6. [g] See ref 5.

should not selectively alter the oxytocic–antidiuretic ratio. Therefore, the resynthesis of [Hmp¹]oxytocin (6)[13] was undertaken in order to determine the oxytocic–antidiuretic ratio of this analogue and compare it to compound 4.[14]

Experimental Section

All melting points were determined in open capillary tubes and are reported uncorrected. Thin-layer chromatograms were done on E. Merck silica gel GF-254 plates using the following systems: A, 1-butanol–acetic acid–water (4:1:1, v/v/v); B, 1-butanol–acetic acid–water–pyridine (15:3:6:10, v/v/v/v). The compounds were revealed by chlorine–tolidine reagent[16] or ninhydrin. Aliquots of the analogues were hydrolyzed in 6 N HCl for either 16 or 22 h and the hydrolysate was subjected to amino acid analysis as outlined by Moore[17] on a Durrum D-500 amino acid analyzer. For the preparation of cysteic acid peptides, aliquots were oxidized with performic acid prior to hydrolysis.[18] Elemental analyses were performed by Robertson Laboratory, Florham Park, N.J.

Boc-Cys(Bzl)-Tyr(Bzl)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Gly-Leu-Gly-NH_2 (1). Boc-Gly-O-resin[19] (4.04 g) containing 0.51 mmol of glucine/g of substituted resin (polystyrene 1% cross-linked with divinylbenzene) was used with the cycles of deprotection, neutralization, and coupling previously described.[20] After each coupling procedure with the appropriately protected *tert*-butyloxycarbonylamino acid, a ninhydrin test[21] was done to check for unreacted primary amines and coupling was repeated where necessary. Upon completion of the peptide chain, a weight gain of 1.78 g was observed (89% of theory). An aliquot of the protected peptide-resin (4.9 g) was subjected to amminolysis.[22] The crude protected peptide, which was extracted from the resin with hot DMF (20 mL), was purified by precipitation on the addition of water (100 mL). After washing with DMF–H_2O (1:5), H_2O, C_2H_5OH–H_2O (1:1), and CH_3OH and drying in vacuo, the yield of protected peptide was 1.33 g (67%): $[\alpha]^{25}D$ −20.0° (*c* 1, DMF); mp 248–249 °C. Amino acid analysis of a hydrolyzed sample gave the following molar ratios: Asp, 1.00; Thr, 1.00; Gly, 1.98; Ile, 0.96; Leu, 1.02; Tyr, 0.89; Cys(Bzl), 1.95; NH_3, 2.02.

[4-Threonine,7-glycine]oxytocin (2). A sample of the protected nonapeptide (108 mg, 77 μmol) was partially deprotected with trifluoroacetic acid (2 mL) for 30 min. After removal of the trifluoroacetic acid in vacuo, and lyophilization from acetic acid, the resulting material was debenzylated by sodium in liquid ammonia.[23] The ammonia was removed under reduced pressure and the residue dissolved in 50% methanol–water (previously deaerated and saturated with nitrogen). Over 90% of the theoretical amount of thiol was revealed by the Ellman method.[24] Oxidation to the disulfide was performed with 1 equiv of 1,2-diiodoethane.[25] Following the desalting of the product by gel filtration on Sephadex G-15 (117 × 2.5 cm) with 50% acetic acid[26] (elution volume ≈180 mL), final purification was afforded by partition chromatography[27] on Sephadex G-25 with the solvent system 1-butanol–benzene–water containing 3.5% acetic acid and 1.5% pyridine (9:1:10, v/v/v), R_f 0.28, followed by gel filtration on Sephadex G-25 using 0.2 M acetic acid. The yield after lyophilization from 0.2 M acetic acid was 33 mg (48%). Thin-layer chromatography gave a single spot: R_f(A) 0.36, R_f(B) 0.63; $[\alpha]^{23}D$ −16.7° (*c* 0.5, 1 N CH_3CO_2H). Amino acid analysis: Asp, 1.00; Thr, 1.00; Gly, 2.00; Leu, 1.03; Ile, 0.96; Tyr, 0.87; $^1/_2$Cys, 2.07 (determined as cysteic acid); NH_2, 2.10. Anal. ($C_{39}H_{61}N_{11}O_{12}S_2 \cdot CH_3CO_2H \cdot 3H_2O$) C, H, N.

L-Hmp(Bzl)-Tyr(Bzl)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Gly-Leu-Gly-NH_2 (3). The protected octapeptide-resin was prepared in the same way as 1 starting from 2 g of Boc-Gly-O-resin. The L-Hmp residue was introduced into the chain as 2-acetoxy-*S*-benzyl-3-mercaptopropionic acid[13] (1.5 equiv) with 1 equiv of DCCI[28] and 3 equiv of 1-hydroxybenzotriazole.[29] The weight gain on the resin of 0.87 g represented 85% of the theoretical incorporation. In the course of amminolysis from the resin, the acetoxy group was removed.[13] After purification similar to that of 1, 767 mg of protected precursor was collected: mp 255–260 °C; $[\alpha]^{23}D$ −15.4° (*c* 1, DMF). Amino acid analysis: Asp, 1.00; Thr, 0.97; Gly, 1.97; Ile, 0.93; Leu, 1.01; Tyr, 0.86; Cys(Bzl), 0.86; NH_3, 2.09.

[1-(L-2-Hydroxy-3-mercaptopropionic acid),4-threonine,7-glycine]oxytocin (4). A sample of 3 (74 mg) was deprotected, oxidized, and desalted as in 2 yielding 39 mg of crude product. Final purification was afforded by partition chromatography on Sephadex G-25 using the solvent system 1-butanol–benzene–water containing 3.5% acetic acid and 1.5% pyridine (1:1:2): R_f 0.36; yield 30.0 mg (50%). Single spots were revealed on TLC, R_f(A) 0.48, R_f(B) 0.65; $[\alpha]^{23}D$ −43° (*c* 0.5, 1 N CH_3CO_2H). Amino acid analysis: Asp, 1.00; Thr, 0.96; Gly, 1.97; Leu, 1.02; Ile, 0.94; Tyr, 0.89; $^1/_2$Cys, 1.03 (as cysteic acid); NH_3, 2.17. Anal. ($C_{39}H_{60}N_{10}O_{13}S_2 \cdot CH_3CO_2H \cdot 2.5H_2O$) C, H, N.

L-2-Acetoxy-*S*-benzyl-3-(mercaptopropionic acid)-Tyr-(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (5). This intermediate was prepared by two routes. (a) The partially protected C-terminal octapeptide of oxytocin[30] (334 mg, 0.31 mmol), dissolved in dimethylformamide (3 mL), was acylated with 2-acetoxy-*S*-benzyl-3-mercaptopropionic acid (120 mg, 0.45 mmol) with the aid of DCCI (63 mg, 0.31 mmol) and 1-hydroxybenzotriazole (140 mg, 0.9 mmol). After 3 days, TLC no longer showed the presence of the ninhydrin-positive octapeptide. The reaction mixture was worked up as described[13] and the product had the physical characteristics described therein: yield, 320 mg; mp 233–234 °C; $[\alpha]^{23}D$ −40.5° (*c* 1, DMF) [lit.[13] mp 231.5–233 °C; $[\alpha]^{23}D$ −42.5° (*c* 1, DMF)].

(b) The procedure described by Hope and Walti was repeated:[13] yield 160 mg, 71%; mp 234–235 °C; $[\alpha]^{23}D$ −43° (*c* 1, DMF). Amino acid analysis: Asp, 1.01; Glu, 1.02; Pro, 1.02; Gly, 1.00; Ile, 0.95; Leu, 1.02; Tyr, 0.96; Cys(Bzl), 0.93; NH_3, 3.05.

[1-(L-2-Hydroxy-3-mercaptopropionic acid)]oxytocin (6). Samples from both preparations of 5 (50 mg, 37 μmol) were individually deacetylated with ammonia in methanol,[13] deprotected by sodium in liquid ammonia, and oxidized by 1 equiv of 1,2-diiodoethane. After partial purification by gel filtration using 50% acetic acid on Sephadex G-15, final purification was accomplished by partition chromatography on Sephadex G-25 using the systems 1-butanol–benzene–water containing 3.5% acetic acid and 1.5% pyridine (2:1:3), R_f 0.31; and 1-butanol–benzene–acetic acid–water containing 1.5% pyridine (5:5:4:6), R_f 0.34. The final products were found to be identical: yield, 50% from protected peptide (23 mg, 18.5 μmol); TLC, R_f(A) 0.38 and R_f(B) 0.63; $[\alpha]^{23}D$ −90.4° (*c* 0.5, 1 N CH_3CO_2H). Amino acid analysis: Asp, 1.03; Glu, 1.00; Pro, 1.01; Gly, 0.97; Ile, 0.99, Leu, 1.05; Tyr, 0.92; $^1/_2$Cys (as cysteic acid), 1.03; NH_3, 3.03. Anal. ($C_{43}H_{65}N_{11}O_{13}S_2 \cdot CH_3CO_2H \cdot 2H_2O$) C, H, N.

Compounds 2, 4, and 6 were tested for biological activity as follows. Rat uterine assays were performed on isolated horns from rats in natural estrus.[31] Avian vasodepressor assays were performed on conscious chickens.[32] Pressor[33] and antidiuretic[34] assays were carried out on anesthesized male rats. Either the four-point design[35] or matches were used to obtain specific activities as compared to U.S.P. posterior pituitary powder reference standard.

Results and Discussion

Table I summarizes the biological activities found for [Thr⁴,Gly⁷]oxytocin, [Hmp¹,Thr⁴,Gly⁷]oxytocin, and

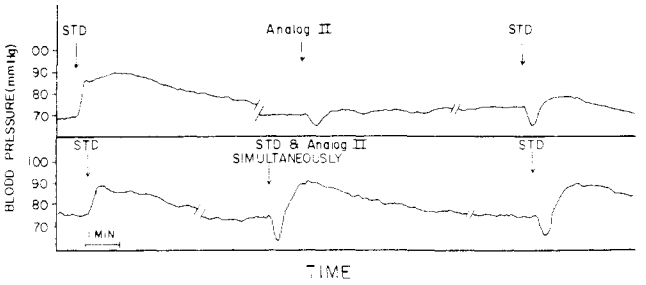

Figure 1. The effect of [Hmp^1,Thr^4,Gly^7]oxytocin (**4**) on rat blood pressure response. Injections of the solutions were made at 15-min intervals. The sequence and amounts administered were as follows: (upper tracing) standard (std), 0.05 mL, 5.25×10^{-2} u/mL; **4**, 0.1 ml, 2.6×10^{-9} M; and again std; (lower tracing) std; std and **4** simultaneously, 0.05 mL of std solution plus **4**, 0.05 mL, 5.2×10^{-9} M; and again std.

[Hmp^1]oxytocin and compares them with those of oxytocin, [Thr^4]oxytocin, and [Gly^7]oxytocin. Analogues **2** and **4** had protracted actions in the rat uterotonic and pressor assays. This required numerous treatments with the standard before reproducible values could be obtained. Once the uterus preparation had been in contact with either **2** or **4**, several administrations of standard were required before the latter's potency was consistent in between injections of the analogue; nevertheless, the standard error for the oxytocic activity of **4** remained high. Similar effects were previously noted for [Thr^4]oxytocin.[39] In the rat pressor assay, both **2** and **4** caused a depressor prior to a pressor response (illustrated for **4**, Figure 1). Even the subsequently administered standard gave a *depressor* response. No specific values could be obtained for the pressor activity. The protracted effects may be partially explained by the fact that [Gly^7]oxytocin is resistant to enzymatic degradation;[8,40] both **2** and **4** would be expected to show the same enzymatic stability. Peptide **4** would, in addition, be resistant to aminopeptidase action.

The result of chemically changing two "corner" residues of the same oxytocin analogue can be seen as a linear combination of the biological activity values of the relevant monosubstituted analogues. Using oxytocin as a reference and calculating the percent of a biological activity for a monocorner substituted analogue and then linearly combining it with that of another analogue one obtains, at least in this series, a first-order approximation of the activity for the multisubstituted compound. [Thr^4]-oxytocin has 169% of the uterotonic activity of oxytocin and [Gly^7]oxytocin has 12%; therefore, [Thr^4,Gly^7]oxytocin would be expected to have approximately 20% of the oxytocic activity (or 110 u/mg) of oxytocin. Similarly, a fowl depressor activity of 16 u/mg and an antidiuretic activity of 0.007 u/mg would be expected for **2**. As can be seen from Table I this trend is observed. Indeed, not only are the effects additive, but they appear to be synergistic as well.

As anticipated analogue **2** has an oxytocic to antidiuretic ratio orders of magnitude larger than either monosubstituted material (see Table II). However, the introduction of a hydroxyl group in place of the primary amino group of the cysteinyl moiety in position 1, a residue not in a corner position of the proposed β turns for oxytocin, results in a nonspecific increase of all biological activities measured.

These preliminary results may provide further impetus toward the development of a series of analogues derived on the basis of stepwise chemical modifications of corner positions of β turns of the peptide backbone. The results of the series compared in this study (oxytocin, [Thr^4]-oxytocin, [Gly^7]oxytocin, and [Thr^4,Gly^7]oxytocin) seem to indicate, to a first-order approximation, that the biological activities are additive. In contrast, stepwise modifications of residues which are not in corner positions result in nonlinear biological relationships.

Table II. Approximate Oxytocic-Antidiuretic Ratios of Oxytocin and Analogues

Peptide	Oxytocic/antidiuretic ratio[a]
Oxytocin	200
[Thr^4]oxytocin	500
[Gly^7]oxytocin	6500
[Thr^4,Gly^7]oxytocin (**2**)	135000
[Hmp^1,Thr^4,Gly^7]oxytocin (**4**)	7000
[Hmp^1]oxytocin (**6**)	40

[a] Ratios are based on the potencies reported in Table I.

Acknowledgment. This work was supported by U.S. Public Health Service Grant AM-18399. We also wish to thank Dr. C. W. Smith for helpful discussion and Ms. A. Formento and Ms. S. Chan for the bioassays.

References and Notes

(1) D. W. Urry and R. Walter, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 956 (1971).
(2) R. Walter, I. L. Schwartz, J. H. Darnell, and D. W. Urry, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 1355 (1968).
(3) V. du Vigneaud, G. Flouret, and R. Walter, *J. Biol. Chem.*, **241**, 2093 (1966).
(4) W. Chan, V. J. Hruby, G. Flouret, and V. du Vigneaud, *Science*, **161**, 280 (1968).
(5) (a) R. Walter, C. W. Smith, J. Roy, and A. Formento, *J. Med. Chem.*, **19**, 822 (1976); (b) M. Bodanszky and R. Bath, *Chem. Commun.*, 766 (1968).
(6) M. Manning, E. Coy, and W. H. Sawyer, *Biochemistry*, **9**, 3925 (1970).
(7) Nomenclature is in accord with IUPAC–IUB Rules on Biochemical Nomenclature, *Biochem. J.*, **126**, 773 (1972), and *J. Biol. Chem.*, **242**, 555 (1967). All optically active amino acids are of the L configuration. Other abbreviations include Hmp, L-2-hydroxy-3-mercaptopropanoic acid; Asu, L-2-aminosuberic acid; DCCI, dicyclohexylcarbodiimide; DMF, dimethylformamide; TLC, thin-layer chromatography.
(8) R. Walter, T. Yamanaka, and S. Sakakibara, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1901 (1974).
(9) F. E. Guiloff, A. A. Ibarra-polo, and C. Gomez-Rogers, *Fertil. Steril.*, **25**, 946 (1974).
(10) Unpublished results of our laboratory reveal that the isolated Fallopian tube of rabbit responds to oxytocin at significantly lower dose levels than the nongravid uterus.
(11) E. M. Coutinho, *Fertil. Steril.*, **22**, 807 (1971).
(12) M. Manning, J. Lowbridge, and W. H. Sawyer in "Peptides: Chemistry, Structure and Biology", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, pp 737–750.
(13) M. Walti and D. B. Hope, *J. Chem. Soc., Perkin Trans. 1*, 1946 (1972).
(14) Whereas Walti and Hope[13] did not determine the antidiuretic activity of **6**, after the completion of our studies Manning et al.[15] reported a second synthesis of **6** and its antidiuretic activity. However, as can be seen from Table I, there are differences in the biological activities found in the two laboratories.
(15) M. Manning, J. Lowbridge, J. Haldur, and W. H. Sawyer, *J. Med. Chem.*, **19**, 376 (1976).
(16) H. Zahn and E. Rexroth, *Z. Anal. Chem.*, **148**, 181 (1955).
(17) S. Moore in "Chemistry and Biology of Peptides", J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1973, pp 629–653.
(18) L. C. Craig, W. Konigsberg, and T. P. King, *Biochem. Prep.*, 8, 70 (1961).

(19) J. W. M. Baxter, M. Manning, and W. H. Sawyer, *Biochemistry*, **8**, 3592 (1969).
(20) C. W. Smith and M. F. Ferger, *J. Med. Chem.*, **19**, 250 (1976).
(21) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, **34**, 595 (1970).
(22) M. Manning, *J. Am. Chem. Soc.*, **90**, 1348 (1968).
(23) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).
(24) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).
(25) F. Weygand and G. Zumach, *Z. Naturforsch.*, **17**, 807 (1962).
(26) M. Manning, T. C. Wuu, and J. W. M. Baxter, *J. Chromatogr.*, **38**, 396 (1968).
(27) D. Yamashiro, *Nature (London)*, **201**, 76 (1964); D. Yamashiro, D. Gillessen, and V. du Vigneaud, *J. Am. Chem. Soc.*, **88**, 1310 (1966).
(28) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955); J. C. Sheehan, M. Goodman, and G. P. Hess, *ibid.*, **78**, 1367 (1956).
(29) W. Konig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
(30) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, 81, 5688 (1959).
(31) P. Holton, *Br. J. Pharmacol. Chemother.*, **3**, 328 (1948); R. A. Munsick, *Endocrinology*, **66**, 451 (1960).
(32) J. M. Coon, *Arch. Int. Pharmacodyn. Ther.*, **62**, 77 (1939); "The Pharmacopeia of the United States", 18th revision, Mack Publishing Co., Easton, Pa., 1970, p 469; R. A. Munsick, W. H. Sawyer, and A. B. van Dyke, *Endocrinology*, **66**, 860 (1960).
(33) "The Pharmacopeia of the United States", 18th revision, Mack Publishing Co., Easton, Pa., 1970, p 771.
(34) W. H. Jeffers, M. M. Livezy, and J. H. Austin, *Proc. Soc. Exp. Biol. Med.*, **50**, 184 (1942); W. H. Sawyer, *Endocrinology*, **63**, 694 (1958).
(35) H. O. Schild, *J. Physiol. (London)*, **101**, 115 (1942).
(36) W. Y. Chan, M. O'Connell, and S. R. Pomeroy, *Endocrinology*, **72**, 279 (1963).
(37) W. Y. Chan and V. du Vigneaud, *Endocrinology*, **71**, 977 (1962).
(38) M. Manning in "The Pharmacology Symposium on the Comparative Cellular and Pharmacologic Actions of Neurohypophyseal Hormones on Smooth Muscle", *Fed. Proc.*, in press.
(39) W. H. Sawyer, and M. Manning, *J. Endocrinol.*, **49**, 151 (1971).
(40) R. Walter, *Pept., Proc. Eur. Pept. Symp., 12th, 1972*, 363 (1973).

Pharmacological Effects of Introducing a Double Bond into a Binding Site of Oxytocin. Analogues with L-3,4-Dehydroproline in Position 7[1]

Stanley Moore, Arthur M. Felix, Johannes Meienhofer,*

Chemical Research Department, Hoffman-La Roche Inc., Nutley, New Jersey 07110

Clark W. Smith, and Roderich Walter*

Department of Physiology and Biophysics, University of Illinois at the Medical Center, Chicago, Illinois 60612.
Received August 30, 1976

The side chain of the proline residue in position 7 of oxytocin has been proposed as a binding site of the hormone for the uterotonic receptor. This is the first in a series of studies in which the possibility is explored that amino acid residues located at such sites and bearing unsaturated side chains may contribute more strongly to binding than neutral, aliphatic side chains. To test this hypothesis [7-(L-3,4-dehydroproline)]oxytocin, [1-β-mecaptopropionic acid,7-(L-3,4-dehydroproline)]oxytocin, and [1-L-α-hydroxy-β-mercaptopropionic acid,7-(L-3,4-dehydroproline)]oxytocin were prepared by the solid-phase technique of peptide synthesis. Some of the pharmacological properties of the analogues were determined, and the following specific activities, respectively, were found: rat uterotonic, 1071 ± 59, 1066 ± 95, 880 ± 180; avian vasodepressor, 548 ± 10, 1008 ± 42, 1295 ± 62; rat antidiuretic 5.9 ± 0.2, 23.3 ± 1.1, 76.7 ± 2.3. All analogues possess a lower rat pressor activity than oxytocin. Compared to oxytocin, [7-(L-3,4-dehydroproline)]oxytocin exhibits a parallel displacement of the cumulative uterotonic log dose vs. response curve toward lower concentration (pD_2 = 9.26 vs. 8.63) but elicits the same maximum response. These data would seem to support the hypothesis that the introduction of unsaturation into a binding element of a peptide hormone can enhance the affinity of the hormone for some of its receptors and thereby its selectivity.

A comparison of the primary structure of oxytocin and of the other eight characterized neurohypophyseal nonapeptides found in nature reveals that mutations during evolution have occurred only at positions 3, 4, and 8.[2] The conformation of oxytocin (Figure 1) proposed by Urry and Walter[3] places these residues and the residue in position 7, which is proline in all of the naturally occurring peptides, at the four corner positions of the two β turns in the hormone. Side chains of residues located at corner positions are exposed and possess maximal structural freedom. Therefore, at that time in 1971[4] conformational considerations suggested that modifications at these four positions could yield hormone analogues in which one or more of the biological activities of oxytocin were highly accentuated in terms of potency relative to other activities characteristic of the hormone. Since that time, more specific assignments to individual amino acid side chains of oxytocin have been possible with regard to their roles in the interaction of the hormone with its uterine receptor. It has been proposed that side chains of Ile^3 and Pro^7 are "binding elements" (the specific atoms of a binding site responsible for binding; for details see ref 5) which are involved in the recognition and binding of the hormone by the uterotonic receptor and that the side chain of Leu^8 and the hydrocarbon portion of the side chain of Gln^4 also contribute to binding.[5] In addition to the topological arrangement of the binding elements at the corner positions of the two β turns, which makes them most visible to the receptor, they share a common chemical nature in their lypophilicity.

This is the first of a series of studies in which the possibility is explored that residues bearing unsaturated side chains with their deformable electron clouds and with their ability to undergo π–π interactions may contribute more strongly to binding (provided steric fit at the receptor can be achieved) than neutral, aliphatic side chains. Among aliphatic side chains, a high molecular weight group (again, provided no steric problems are encountered) with a substantial degree of van der Waals binding forces may be more favorable for binding than a low-molecular-weight