Bicyclization of a Weak Oxytocin Agonist Produces a Highly Potent Oxytocin Antagonist

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Abstract: [Mpa[1],Glu[4],Cys[6],Lys[8]]oxytocin was prepared and found to be a very weak agonist in the oxytocic assay with about 1/1400 the potency of the native hormone. Bicyclization of this compound via a lactam bridge between the Glu[4] δ-carboxyl group and the ε-amino group of Lys[8] led to the bicyclic analogue 4,8-cyclo[Mpa[1],Glu[4],Cys[6],Lys[8]]oxytocin. This bicyclic peptide acts as a very potent antagonist of oxytocin in the rat uterus assay with an in vitro pA_2 value of 8.2. In the in vivo oxytocic assay the pA_2 value was 6.45. The peptide displays mixed agonist/antagonist character in the in vivo galactogogic assay. The implications of these results to hormone agonist and antagonist activity are discussed with respect to earlier studies on constrained oxytocin antagonists and the results from the X-ray crystal structure of deaminooxytocin.

Oxytocin (H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2)[2] is the neurohypophyseal peptide hormone generally accepted as physiologically important for its milk-ejecting and uterine-contracting activities in mammals. Oxytocin has been widely studied since the early 1950's, when the structure of oxytocin was first elucidated and proven by total synthesis.[3] Oxytocin possesses a 20-membered disulfide-containing ring and an acyclic tripeptide tail. Numerous analogues of oxytocin have been synthesized in an effort to define structure-activity relationships for the hormone.[4] Detailed biophysical studies of oxytocin and several agonist and antagonist analogues have been made to determine the conformational properties of these peptides in solution and their possible relationships to biological activity.[5] These studies have led to proposed models for oxytocin–uterine receptor interaction. In the "cooperative model"[6] it was proposed (see ref 4a for a review) that the lipophilic side chain groups of amino acids in positions 3, 4, 7, and 8 (which lie at the corners of proposed reverse turns) are important for the binding of oxytocin to this receptor, whereas the Tyr[2] and Asn[5] positions are considered to be critical for initiation of the biological response (transduction). The complementary "dynamic model"[7] has emphasized the importance of conformational flexibility for agonist activity and relative conformational rigidity and steric constraints for antagonism. In the latter model the topological relationships of the Cys[1], Tyr[2], and Asn[5] side-chain moieties of the residues in the 20-membered disulfide ring and the relationship of this ring to the more flexible acyclic C-terminal tripeptide moiety are emphasized as critical to binding and transduction. Evidence supporting this latter model has come from extensive biophysical studies of the conformational and dynamic properties of [1-penicillamine]oxytocin and other related conformationally constrained oxytocin antagonist (inhibitor) analogues[7,8] and corresponding biological activity studies.

Recently the crystal structure of the potent oxytocin agonist deaminooxytocin has been reported.[9] Two distinct conformations were observed for this peptide in the solid state, differing in disulfide chirality and several other conformational features. This study has demonstrated that although deaminooxytocin possessed conformational features in the crystal related to those suggested from conformational studies in solution such as the cooperative model of Walter et al.,[6] even in the crystal form it retains significant conformational flexibility, lending further support to the dynamic model of hormone–receptor interaction for oxytocin.[7]

Examination of the crystal structure of deaminooxytocin and of the three-dimensional relationships possible for the distorted 20-membered disulfide ring conformation and the C-terminal acyclic tripeptide extended away from this ring but parallel to it and considerations from structure-function analysis led us to hypothesize that suitable side-chain-to-side-chain cyclization[10] between the amino acids in the 4 and 8 positions of deaminooxytocin might be compatible with a strong hormone–receptor interaction. We report here that formation of the bicyclic analogue

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(2) Symbols and abbreviations are in accord with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977). All optically active amino acids are of the L variety unless otherwise stated. Other abbreviations include the following: N^{α}-Boc, N^{α}-*tert*-butyloxycarbonyl; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; FAB-MS, fast atom bombardment mass spectrometry; TFA, trifluoroacetic acid; HOBt, *N*-hydroxybenzotriazole; DMF, *N*,*N*-dimethylformide; *p*-MBHA, *p*-methylbenzhydrylamine; DCM, dichloromethane; TEA, triethylamine; DPPA, diphenylphosphorylazide.

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of deaminooxytocin prepared by side-chain-to-side-chain amide bond cyclization of the very weak monocyclic agonist analogue [Mpa^1,Glu^4,Cys^6,Lys^8]oxytocin gives the bicyclic analogue [Mpa^1,Glu^4,Cys^6,Lys^8]oxytocin which has highly potent antagonist activities in the classical rat uterus assay for oxytocin.

Experimental Section

General Synthetic and Analytical Procedures. Capillary melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on silica gel G plates with use of the following solvent systems: (A) 1-butanol–acetic acid–water (4:1:5, upper phase only); (B) 1-butanol–acetic acid–pyridine–water (15;3;10;12); and (C) 1-pentanol–pyridine–water (7:7:6). Detection was made by UV, iodine, ninhydrin, or Cl_2/*o*-tolidine. Single symmetrical spots were observed for all purified materials. Purity was also confirmed by analytical RP-HPLC performed on a Spectra Physics Model 8700 instrument equipped with a Spectra Physics Model 8400 variable wavelength detector. A reversed phase C_{18} Vydac column (17 μm, 4.5 mm × 25 cm) was used with a mobile phase consisting of acetonitrile (CH_3CN) in aqueous 0.1% trifluoroacetic acid (TFA). Amino acid analyses were obtained by the method of Spackman, Stein, and Moore[11] on a Beckman 120C amino acid analyzer following hydrolysis of peptide material for 22–48 h at 110 °C with either 6 M HCl or 4 M 2-methanesulfonic acid (MSA) and subsequent neutralization with an equivalent volume of 3.5 N NaOH.[12] No corrections were made for destruction of amino acids during hydrolysis. Optical rotation values were measured at the mercury green line (547 nm) by using a Rudolph Research Auto-Pol III polarimeter. Molecular weights of the peptides were determined by fast atom bombardment mass spectrometry (FAB-MS) by using a Kratos MS-50 triple analyzer equipped with a Kratos DS-55 data system. Mass spectral determinations were performed by either the University of Arizona, Department of Pharmacology, or by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Instrumentation Facility (Grant No. CHE 8211164).

The general procedures used in the solid phase syntheses were similar to those previously used to synthesize [1-penicillamine]oxytocin and other oxytocin analogues.[13,14] The *p*-methylbenzhydrylamine (*p*-MBHA) resin (1% divinylbenzene cross-linked polystyrene) was purchased from Peptides International (Louisville, KY) or was prepared by previously reported methods[15] with an amine substitution as indicated. N^{α}-Boc amino acids and amino acid derivatives were purchased from Vega Biotechnologies, Chemical Dynamics Corporation, Bachem, and Aldrich Chemical Company. All N^{α}-Boc amino acids not purchased were synthesized by the literature procedure of Tarbell et al.[16] Purity for each amino acid was established by the ninhydrin test,[17] melting point, and TLC on Baker-flex silica gel 1B-F plates in three solvent systems: (D) chloroform–methanol (95;5); (E) chloroform–methanol (1:1); and (F) acetone–acetic acid (98:2). Detection was with UV (254 nm), iodine, and ninhydrin. All Boc amino acids were coupled by dicyclohexylcarbodiimide and *N*-hydroxybenzotriazole (HOBt), except asparagine, which was coupled as its *p*-nitrophenyl ester with HOBt as a catalyst in the solid phase coupling procedure.[13] Progress in the condensation reaction was followed by the ninhydrin test.[17]

Purification of peptides was accomplished by a combination of gel filtration and reversed phase high performance liquid chromatography. Gel filtrations were performed on a Sephadex G-15 column (2.65 × 100 cm). Fractions were monitored for peptide material with a Guilford UV–vis spectrophotometer at 280 nm. Preparative RP-HPLC was performed on a Perkin-Elmer Series 3 B liquid chromatograph equipped with an LC-75 spectrophotometric detector and an LCI-100 laboratory computing integrator. A Vydac C_{18} semipreparative (10 μm, 1.0 × 25 cm) column was used with a mobile phase of CH_3CN in aqueous 0.1% TFA. Detection of peptide material was made by monitoring the UV absorbance of the peptide backbone at 214 nm and the tyrosine side chain at 280 nm. Solvents used for gel filtration, TLC, and RP-HPLC were purified by redistillation and filtration prior to use.

Dimethylformamide was purified by distillation[18] and bubbled with N_2 for 45–60 min prior to use.

***S*-4-Methylbenzyl-β-mercaptopropionic acid** was prepared by a modification of the procedure of Hope and co-workers[19] for *S*-Bzl-β-mercaptopropionic acid. From β-mercaptopropionic acid (5 mL, 57.4 mmol) there was obtained 11.9 g (99%) of the crystalline title compound: mp 74–76 °C, lit.[19b] 74–76 °C; NMR (60 MHz, $CDCl_3$) 2.3 (3 H, s), 2.6 (4 H, t), 3.7 (2 H, s), 7.1 (4 H, s).

[β-Mpa^1,Glu^4,Cys^6,Lys^8]oxytocin (I). The synthesis of the title peptide was accomplished starting with 0.93 g of *p*-MBHA resin (1.1 mM/g titratable amine, 1.0 mM). The N^{α}-Boc amino acids were coupled to the resin by using a 2-fold excess of amino acid, DCC, and HOBt in CH_2Cl_2/DMF. The *p*-nitrophenyl ester of asparagine was added in 4-fold excess and allowed to couple in the presence of a 4-fold excess of HOBt in DMF. After each amino acid residue was coupled, the reaction was monitored for completion of coupling by the ninhydrin test,[17] and complete coupling was indicated at each step. Following these procedures, the protected peptide resin β-Mpa(*S*-4-MeBzl)-Tyr(*O*-BrZ)-Ile-Glu(*O*-Bzl)-Asn-Cys(*S*-4-MeBzl)-Pro-Lys(2,4-Cl_2-Z)-Gly-MBHA resin (2.06 g) was obtained. The peptide resin was washed with DCM, EtOH, and DCM and dried in vacuo. A portion (0.69 g, 0.33 mM) was treated with anhydrous HF (10 mL) and anisole (3 mL) (45 min, 0 °C) in order to cleave the peptide from the resin and to remove all remaining protecting groups. After evaporation of the HF and anisole in vacuo, the residue was washed with deaerated EtOAc (3 × 30 mL). The resin was then extracted with deaerated glacial acetic acid (50 mL), and three 30-mL portions each of 30% HOAc, 0.2 N HOAc, and H_2O. The aqueous extracts were combined and lyophilized to yield the crude deprotected peptide. The white powder was dissolved in 0.1% HOAc (1000 mL) which had been deaerated and bubbled with N_2 for 90 min. The pH of the solution was adjusted to 8.5 with 3 N NH_4OH, and the peptide sulfhydryl groups oxidized to the disulfide by the addition of an excess of 0.01 N $K_3Fe(CN)_6$ (40 mL) as indicated by the persistence of a yellow color. The solution was allowed to stir for 30–60 min. The pH was then adjusted to 4.8 with 30% HOAc and anion exchange resin (Amberlite IRA-45; Chemical Dynamics Corporation) was added (30 mL settled volume) to remove excess ferro- and ferricyanide. The suspension was stirred for 1–2 h, and the resin was removed by filtration and washed (3 × 25 mL) with 20% HOAc. The filtrate was reduced in volume by rotary evaporation and then lyophilized to yield the crude cyclized peptide. The crude peptide was initially purified by two gel filtrations on a Sephadex G-15 (Pharmacia Fine Chemicals) column (2.65 × 100 cm). The first utilized 50% HOAc and the second utilized 0,2 N HOAc as eluent. The fractions (3.5 mL) were monitored for peptide material (280 nm), and the fractions containing the major peak were pooled and lyophilized to yield a fluffy white powder (132.30 mg). The peptide was further purified by preparative RP-HPLC by using a linear gradient of 20–24% acetonitirle (CH_3CN) in aqueous 0.1% trifluoroacetic acid (TFA) (0.4%/min). The title peptide was obtained (97.8 mg) as its trifluoroacetate salt: $[\alpha]^{22}_{547} = -92.5°$ (*c* 0.20 in 1 M HOAc). The purified peptide gave single, uniform spots (UV, iodine, Cl_2/*o*-tolidine) on TLC: R_f 0.03 (A), 0.36 (B), and 0.14 (C). Analytical HPLC of the title peptide under isocratic elution (0.1% aqueous TFA/CH_3CN, 78:22) gave a k' value of 0.66. Amino acid analysis (hydrolysis for 48 h at 100 °C in 4 M MSA) resulted in the following molar ratios: Mpa-Cys 0.93 (1.0), Tyr 0.94 (1.0), Ile 0.93 (1.0), Glu 1.00 (1.0), Asp 1.03 (1.0), Pro 0.95 (1.0), Lys 0.97 (1.0), Gly 0.95 (1.0). FAB mass spectrum: MH^+ calcd, 1008; found, 1008.

[β-Mpa^1,Glu^4,Cys^6,Lys^8]oxytocin (II). Two similar methods were utilized to prepare the bicyclic analogue. The one reported here gives more consistent and reproducible results. [β-Mpa^1,Glu^4,Cys^6,Lys^8]-oxytocin·HOAc (I) (100 mg, 0.1 mM) was dissolved in 5% aqueous hydrochloric acid (HCl (aqueous)) (2 mL) and applied to a dimethylaminoethylcellulose ion exchange column previously equilibrated with 5% HCl (aqueous). The column was eluted with 5% HCl (aqueous) and the eluant was monitored continuously at 280 nm. The main peak was pooled and lyophilized to yield the hydrochloride salt of I which was used directly in the next step.

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Table I. Biological Activities of Oxytocin and Its Analogues

compound	biological activity		
	rat uterotonic (IU/mg)	rat pressor (IU/mg)	galactogenic (IU/mg)
oxytocin	546	3.1	410,[a,b] 450[c]
[β-Mpa^1]oxytocin (deaminooxytocin)	803	1.44	541[b]
[Glu^4]oxytocin	1.5	<0.1	11[b]
[Mpa^1,Glu^4]oxytocin	13.3	ND	34.2[b]
[Lys^8]oxytocin	80	133	185[b]
[Mpa^1,Lys^8]oxytocin	20	ND	ND
[Mpa^1,Glu^4,Cys^6,Lys^8]oxytocin (I)	0.4 ± 0.2 (n = 4)	0.10 ± 0.03 (n = 4)	9.1 ± 1.6[c]
[Mpa^1,Glu^4,Cys^6,Lys^8]oxytocin (II)	pA_2 = 8.2 ± 0.2[d] (n = 5)	0.10 ± 0.02 (n = 5)	pA_2 = 6.0
[Pen^1]oxytocin	pA_2 = 6.86	ND	ND

[a] The biological assays for the oxytocin analogues I and II were determined as outlined in the text (see refs 20–23). The biological activities of the other analogues reported here are taken from the literature: for oxytocin, [Mpa^1]oxytocin, [Mpa^1,Glu^4]oxytocin, [Mpa^1,Lys^8]oxytocin, and [Pen^1]oxytocin see the reviews in ref 4, for [Glu^4]oxytocin see ref 29, and for [Lys^8]oxytocin see ref 30. [b] In rabbit. [c] In rat. [d] In the presence of 0.5 mM Mg^{2+} pA_2 = 7.8.

A solution of I·HCl (0.1 mM) in DMF (100 mL) was cooled to −25 °C with stirring before altering the pH to 7.2 (as determined by damp pH Hydrion narrow range paper) with TEA. Diphenylphosphoryl azide (DPPA) (26 μL, 0.12 mM) in DMF (80 μL) was added, and, after stirring at −25 °C for 1 h, the reaction mixture was left to stand for 2 days at −25 °C and 4 days at 4 °C whereupon TLC and the qualitative ninhydrin test showed the reaction to be complete. The pH was maintained throughout at 7.0–7.5 by periodic addition of TEA.

Water (12 mL) and mixed bed ion-exchange resin (AG 5018) (12 mL settled volume) were added, and the mixture was stirred for 6 h. The resin was filtered off, solvent was removed under a reduced pressure, and the residue was dissolved in 3 M aqueous acetic acid (AcOH (aqueous)) (2 mL) before being applied to a gel filtration column (Sephadex G 25, 100 × 25 cm). The column was eluted with 3 M AcOH (aqueous) at a flow rate of 10 mL/h, and the eluant was continuously monitored at 280 nm. Fractions of 3.3 mL were collected, and the product was found in tubes 79–90 which were pooled and lyophilized to yield 39 mg of the title peptide (>98% pure as judged by HPLC). A sample (20 mg) was further purified by semipreparative HPLC with a linear gradient of 15–31% CH_3CN in aqueous 0.1% TFA over 16 min. The title peptide was obtained as a fluffy white powder (13 mg), $[\alpha]^{22}_D$ = −69° (c 0.2, 1 M HOAc); the purified peptide gave single uniform spots (UV, iodine, Cl_2/o-tolidine) on TLC R_f 0.20 (A), R_f 0.57 (B), R_f 0.58 (C); HPLC k' = 1.44 (isocratic, 0.1% aqueous TFA/CH_3CH_3CN (78:22)). Amino acid analysis (hydrolysis for 48 h at 110 °C in 4 M MSA) resulted in the following molar ratios: Tyr 1.01 (1.00), Ile 0.93 (1.00), Glu 0.95 (1.00), Asp 1.00 (1.00), Mpa-Cys 1.08 (1.00), Pro 1.01 (1.00), Lys 0.99 (1.00), Gly 1.02 (1.00). FAB mass spectrum: MH^+ calcd, 990; found 990.

Biological Assay Methods

Wistar rats were used in all experiments. Rat uterotonic test in vitro was performed according to Holton[20] in Munsick's solution.[20] In some experiments 0.5 mM magnesium was also used. Cumulative dose-response curves were constructed. Rats were estrogenized 24–48 h before the experiment. The galactogogic test was performed on ethanol anaesthetized lactating rats 5–15 days after delivery.[21] Synthetic oxytocin was used as standard in these tests. Pressor activity was determined on pithed rat preparation[22] by using synthetic arginine vasopressin as a standard. A detailed description is given by Slaninova.[23]

Results and Discussion

Synthesis of the protected precursor peptide was accomplished by the solid phase method of peptide synthesis[24] on a p-methylbenzhydrylamine (p-MBHA) resin. N^{α}-*tert*-butyloxycarbonylglycine was attached to the resin and the N^{α}-*tert*-butyloxycarbonyl (Boc) protecting group was removed by 50% trifluoroacetic acid in dichloromethane. The protected peptide was extended in a stepwise manner employing N^{α}-Boc protected amino acids with standard side-chain protection; coupling was mediated by dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole. The protected peptide resin, β-Mpa(*S*-4-MeBzl)-Tyr(*O*-BrZ)-Ile-Glu(*O*-Bzl)-Asn-Cys(*S*-4-MeBzl)-Pro-Lys(2,4-Cl_2-Z)-Gly-*p*-MBHA resin, was obtained in high yield. The peptide was cleaved from the resin, and the side chain protecting groups were removed by treatment with anhydrous hydrofluoric acid.[13] After extraction of the peptide into an aqueous acid phase and lyophilization, the bissulfhydryl compound was oxidized to the disulfide by using potassium ferricyanide in dilute aqueous solution at pH 8.5. The monocyclic disulfide product, [β-Mpa^1,Glu^4,Cys^6,Lys^8]oxytocin, was purified by gel filtration on Sephadex G-15 in 30% aqueous acetic acid to remove salts and polymeric byproducts followed by preparative reversed phase high performance liquid chromatography. The lactam ring was formed by coupling the glutamic acid δ-carboxyl group with the lysine ε-amino group by using diphenylphosphoryl azide (DPPA)[25] as the coupling agent at high dilution in dimethylformamide solution. Bicyclic 4,8-cyclo-[Mpa^1,Glu^4,Cys^6,Lys^8]oxytocin was purified by gel filtration on Biorad P-4 or Sephadex G-25. Final purification was by preparative RP-HPLC. The purity of both the bicyclic peptide and the monocyclic precursor peptide was checked by analytical HPLC in two solvent systems, by thin-layer chromatography on silica gel G using three solvent systems, and by amino acid analysis. Their identifies were further verified by fast atom bombardment mass spectrometry.

The monocyclic and bicyclic oxytocin analogues were tested for their potency in two oxytocic biological assays, the classical in vitro uterotonic assay on isolated rat uterine strips and the galactogogic test in vivo. In addition, the rat pressor activity in vivo,[25] an activity generally associated with vasopressin was also examined because of the lysine in position 8 of the monocyclic analogue I. The results of these assays are shown in Table I along with the activities of the parent hormone oxytocin, closely related monocyclic analogues having single substitutions in their structure, and the classical oxytocin antagonist analogue [Pen^1]oxytocin (Pen = penicillamine).

The monocyclic precursor, [Mpa^1,Glu^4,Cys^6,Lys^8]oxytocin (I), was found to be a very weak agonist in the uterotonic assay with a potency approximately 1/1400th that of the native hormone and about 1/2000th that of deaminooxytocin (Table I), its more closely structurally related oxytocin agonist analogue. Reduced potency also was seen in the galactogogic assay and very low potency also was observed in the rat pressor assay. Upon bi-

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Table II. Amide Proton Temperature Dependencies for the Bicyclic Analogue II

residue	$-d^\delta/dT \times 10^3$ (ppm/K)	residue	$-d^\delta/dT \times 10^3$ (ppm/K)
Cys[1]		Cys[6]	4.40
Tyr[2]	2.15	Pro[7]	
Ile[3]	1.20	Lys[8]	2.48
Glu[4]	2.48	Gly[9]	3.48
Asn[5]	0.23	side chain amide (lactam)	2.26

cyclization, however, this very weakly potent agonist analogue of oxytocin was converted to a highly potent antagonist analogue in the in vitro rat uterine assay, having a pA_2 value of 8.2. Bicyclic analogue II is thus over 30 times more potent as an antagonist than the classical oxytocin antagonist analogue [1-penicillamine]oxytocin ([Pen[1]]oxytocin) ($pA_2 = 6.86$) (Table I). This makes the bicyclic analogue [β-Mpa[1],Glu[4],Cys[6],Lys[8]]oxytocin among the most potent oxytocin antagonist analogues for the oxytocin uterine receptor known.[4] In the galactogogic test, however, the analogue displayed mixed agonist/antagonist character. In general it possesses low agonist activity which is followed by a short lasting inhibitory period. The character of the activity depends on the sensitivity of the animal toward oxytocin. If the animal responds to low doses of standard oxytocin, the agonist activity can be seen. If the animal is less sensitive, only the antagonist action can be observed. Interestingly, the compound's weak agonist activity in the rat pressor assay was unaffected in the bicyclic analogue II relative to the monocyclic analogue I (Table I).

Very little work has been done with bicyclic oxytocin analogues. Two bicyclic analogues of oxytocin have previously been prepared; one cyclized between the C-terminal carboxyl and N-terminal amino group in addition to the disulfide ring,[26] the other cyclized between the amino group of a *p*-aminophenylalanine residue in position 2 and the carboxyl group of a glutamic acid residue in position 4 of oxytocin.[27] Both analogues were completely inactive in the rat uterus assay. In the vasopressin series, a bicyclic analogue of vasopressin, cyclized between groups on the side chains of residues in position 5 and 8, has been prepared, and it was found to act as a weak antagonist of antidiuretic activity.[28]

The bicyclic analogue of oxytocin II represents a new class of oxytocin antagonists. Previous oxytocin antagonists[4] have exhibited both local and global conformational constraints due primarily to constraining modifications made at positions 1 and 2. Evidence for conformational rigidity in these earlier antagonists has come from extensive nuclear magnetic resonance studies in both D_2O and DMSO-d_6. Preliminary proton nuclear magnetic resonance spectroscopy studies in DMSO-d_6 conducted with analogue II indicate possible conformational rigidity. Most analogues lacking an amine terminal have been studied in DMSO because of solubility problems. Comparison of the 250 MHz proton spectra of both the monocyclic precursor I and the bicyclic peptide II reveals a number of substantial chemical shift differences in the α region. Three α protons are shifted upfield (Ile[3] by 0.15 ppm, Tyr[2] by 0.35 ppm, and Cys[6] by 0.45 ppm), while two other α protons are shifted downfield; Pro[7] by 0.20 ppm and Glu[4] by 0.6 ppm. The α CH proton shifts for Asn[5], Lys[8], and Gly[9] remain essentially the same. The large number of substantial chemical shift differences argues for a significant degree of conformational change between these two analogues. Also there is a large (0.6 ppm) upfield shift of the Asn[5] amide proton in the bicyclic compound. Determination of amide proton temperature dependences (Table II) suggests that the Asn[5] amide is involved in an intramolecular hydrogen bond. The Ile[3] amide may also be hydrogen bonded and several other amides including Tyr[2], Glu[4], Lys[8] and the bicyclizing lactam amide appear to be quite shielded from solvent as well. The large number of shielded amide protons and the substantial chemical shift differences suggest that the bicyclic molecule is a more rigid molecule, and presumably this rigidity contributes to the antagonist activity of this compound by making it more difficult for the molecule to assume the biologically active conformation needed for transduction to occur.[9b] The high antagonist potency exhibited by this bicyclic analogue may suggest that the lipophilicity and unbranched nature of the 8 position may be important for binding to the receptor in oxytocin antagonists.

Because this bicyclic analogue has no N-terminal amine group and because it appears to be fairly rigid, attempts to obtain crystals suitable for X-ray analysis may be successful. Analogue II thus presents a unique opportunity to compare and contrast two highly potent oxytocin analogues, one a potent agonist, deaminooxytocin (Table I), and the other a potent antagonist, [β-Mpa[1],Glu[4],Cys[6],Lys[8]]oxytocin. In the former anlogue, the binding elements can favorably dispose themselves so they can interact with the uterine receptor and subsequently transduce the biological message to obtain an agonist response.[6,7] The strong antagonist activity of the bicyclic analogue II suggests that the topographical features of the analogue are such that the binding elements are disposed favorably for interaction with the uterine receptor, but at the same time they cannot topologically arrange themselves for initiation (transduction) of the biological response. These results are consistent with the dynamic model of hormone action[7] and with possible relationships of the conformational and dynamic properties based on the recent X-ray crystal structure of deaminooxytocin.[9] The results further suggest that appropriate modifications of the bicyclic structure should lead to even more potent conformationally constrained oxytocin antagonists.

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Registry No. I (free base), 125666-61-7; I·HOAc, 125666-63-9; I·TFA, 125666-64-0; II, 125666-62-8; 4-Me$C_6H_4CH_2$-Mpa-OH, 78981-22-3; H-Mpa-OH, 107-96-0.

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