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Communications to the Editor

[5-Aspartic acid]-oxytocin: First 5-Position Neurohypophyseal Hormone Analogue Possessing Significant Biological Activity[1]

Sir:

After 25 years of extensive structure-activity studies,[2] starting with the original synthesis of oxytocin,[3] the first 5-position neurohypophyseal hormone analogue to retain a high degree of potency in the oxytocin-like activities is reported. The asparagine residue of oxytocin has been replaced by aspartic acid to yield [5-aspartic acid]-oxytocin.

Early structure-function correlations with oxytocin and vasopressin demonstrated that chemical modifications of the asparagine residue, present in position 5 of all nine naturally occurring neurohypophyseal peptides,[4] virtually abolished biological activity (see ref 5 for a recent review) and, in fact, it was not possible with analogues of such low specific activities to determine dose-response relationships.[6] The preferred solution conformation of oxytocin[7] offered an explanation of these results by assigning to the asparagine residue not only a key role in maintaining the preferred three-dimensional backbone structure, but, moreover, by assigning to the asparagine side chain an important function in the biologically "active site" of the hydrophylic surface of the hormone.[8,9] On the basis of this model it was speculated that an aspartic acid residue in position 5 could retain much of the hydrophilicity, appropriate steric requirements, and hydrogen-bonding capabilities of the asparagine residue (Figure 1).

The synthesis of [5-aspartic acid]-oxytocin has been achieved by stepwise solution techniques beginning with Z-Cys(Bzl)-Pro-Leu-Gly-NH_2[10] (2.5 g, 4.0 mmol). The benzyloxycarbonyl (Z) group was removed by treatment with 2 M HBr/AcOH and Boc-Asp(OBzl)-OH was coupled with dicyclohexylcarbodiimide[11] (DCC) mediated by 1-hydroxybenzotriazole (HBT)[12] in dimethylformamide/glyme (1:1). In this and succeeding steps the *tert*-butyloxycarbonyl (Boc) group was removed by CF_3CO_2H; glutamine was incorporated as Boc-Gln-ONp; Boc-Ile-OH, Boc-Tyr(Bzl)-OH, and Z-Cys(Bzl)-OH were coupled by preactivating a solution of the amino acid in glyme with DCC (1 equiv) in the presence of HBT (2 equiv). Completeness of coupling reactions was monitored by the semiquantitative ninhydrin test.[13] Each protected peptide intermediate was isolated and characterized by melting point, optical rotation, thin-layer chromatography (TLC), and elemental or amino acid analysis; the synthesis yielded 1.5 g (0.98 mmol) of Z-Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asp(OBzl)-Cys(Bzl)-Pro-Leu-Gly-NH_2.[14] All of the protecting groups were simultaneously removed by adding the nonapeptide (307 mg, 0.20 mmol) to a solution of Na in liquid NH_3;[15] cyclization of the dithiol intermediate was accomplished by oxidative disulfide bond formation with ICH_2CH_2I.[16] The product was purified by gel filtration on Sephadex G-15 (fine) in 50% AcOH and by partition chromatography[17] on Sephadex G-25 (block polymerisate, 100-200 mesh) in the system 1-BuOH/C_6H_6/H_2O containing 1.5% pyridine and 3.5% AcOH (6:1:7). Lyophilization gave 76 mg of [5-aspartic acid]-oxytocin: $[\alpha]^{25}{}_D$ − 14° (*c* 0.47, 1

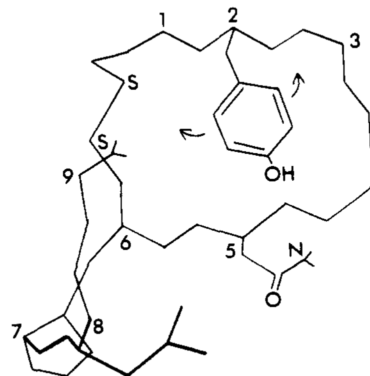

Figure 1. Schematic representation of the conformation of oxytocin thought to be optimal for the interaction of the hormone with the uterine smooth muscle receptor. Residues in positions 3, 4, 7, and 8, which are thought to contain "binding elements", are located at the corner position of the two β turns proposed for the hormone. The "active elements" are the hydroxy group of Tyr[2] and the carboxamide group of Asn.[5] For details see ref 9.

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M AcOH); TLC on Merck silica gel F-254, R_f 0.23 (oxytocin, R_f 0.19), in 4:1:1 1-BuOH/AcOH/H_2O, R_f 0.57 (oxytocin, R_f 0.61) in 15:3:10:6 1-BuOH/AcOH/pyridine/H_2O, R_f 0.51 (oxytocin, R_f 0.55) in 20:10:11 1-BuOH/pyridine/H_2O. Anal. ($C_{43}H_{66}N_{11}O_{13}S_2 \cdot CH_3CO_2H \cdot 2\frac{1}{2}H_2O$), C, H, N. Amino acid analysis[18] gave the following molar ratios: Cys(O_3H),[19] 2.04; Asp, 0.90; Glu, 1.03; Pro, 1.00; Gly, 1.00; Ile, 0.96; Leu, 1.01; Tyr, 0.89; NH_3, 2.09.

In the in vitro rat uterotonic assay[20] [5-aspartic acid]-oxytocin possesses a potency of 20.3 ± 0.8 (mean ± SEM) units/mg in the absence of added Mg^{2+}; the comparable value for oxytocin is 546 ± 18 units/mg.[21] Dose-response studies on the isolated rat uterus with [5-aspartic acid]-oxytocin and oxytocin using the individual injection technique[22] in the presence of either 0.5 mM or 1.0 mM added Mg^{2+} in the bathing medium or without added Mg^{2+} and in the presence of either 0.5 mM Ca^{2+} (standard assay conditions) or reduced Ca^{2+} levels (0.3 and 0.15 mM) revealed identical intrinsic activities for the analogue compared with the hormone. In fact, the activity of [5-aspartic acid]-oxytocin is potentiated to such a degree by added Mg^{2+} that the dose-response relationships of the analogue and oxytocin are virtually identical in the presence of 1.0 mM added Mg^{2+}. In addition, [5-aspartic acid]-oxytocin exhibits 41 ± 2 units/mg avian vasodepressor[23] and 0.14 ± 0.02 units/mg rat antidiuretic activities;[24] these values should be compared with 507 ± 15[25] and 2.7 ± 0.2,[25] respectively, for oxytocin.

Thus, [5-aspartic acid]-oxytocin retains not only a high affinity for the uterotonic receptor, but, more importantly, it exhibits an intrinsic activity identical with that of oxytocin under various experimental conditions. This result is significant in view of the proposed biologically active model of oxytocin where the side chain of the 5-position residue was assigned to contain an "active element" responsible for the intrinsic activity of the hormone when bound to the uterine receptor (Figure 1).[9]

The synthesis of [5-aspartic acid]-vasopressin is warranted in order to test the proposed biologically active model of vasopressin when bound to its antidiuretic receptor.[5]

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Roderich Walter,* Gerald Skala, Clark W. Smith
Department of Physiology and Biophysics
University of Illinois Medical Center
Chicago, Illinois 60612

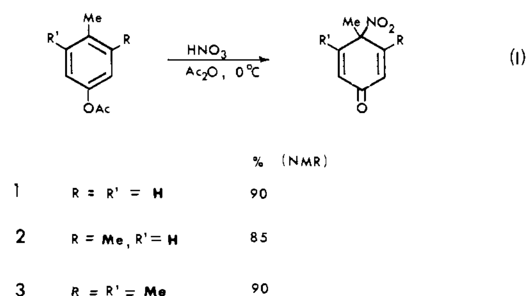
Received September 30, 1977

Ipso Nitration. Characterization of Nitro Group Shifts in 4-Methyl-4-nitrocyclohexa-2,5-dienones[1]

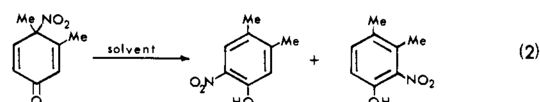
Sir:

Aromatizations of 4-nitrocyclohexa-2,5-dienones by 1,3 shift of the nitro group are documented.[2,3] These observations stand in marked contrast to 1,2 shifts of nitro groups that have been observed with 4-alkyl-4-nitrocyclohexadienyl cations.[4,5] We report here some studies that aid differentiation and mechanistic characterization of these nitro group shifts with migration order [1,3].

Nitration of 4-methylphenyl acetate, 3,4-dimethylphenyl acetate, or 3,4,5-trimethylphenyl acetate with nitric acid in acetic anhydride at 0 °C yields the corresponding 4-methyl-4-nitrocyclohexadienone (**1**, **2**, or **3**) in good yield, eq 1. In each



case the crystalline dienone may be isolated by low temperature precipitation and purified by low temperature crystallization. Earlier reports indicate that such dienones decompose in acidic or basic media to yield *o*-nitrophenols.[2,3] We find that **1**, **2**, or **3** rearomatize in all solvents tested to yield products of a formal 1,3 shift of a nitro group, eq 2, and that reaction rates are actually much slower in aqueous solvents than in nonpolar hydrocarbon solvents.



Rates of the reactions, eq 2, could be followed spectrophotometrically. Well-behaved first-order kinetics were observed for product formation and reactant disappearance. Table I lists