WEAK OXYTOCIN ANTAGONISTS FROM MINOR MODIFICATION OF THE CYCLIC PORTION OF AGONISTS

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[(OMe)Ser^5]-oxytocin (*I*), [(OMe)Thr^5]-oxytocin (*II*) and [Aib^3]-oxytocin (*III*) were synthesized by solid phase techniques. The three analogs were found to be relatively weak antagonists with pA_2 values 5.7 (for *I*), 5.8 (for *II*) and 5.8 (for *III*), respectively, in the rat uterotonic in vitro assay. They were all inactive in the pressor and galactogonic assays.

Oxytocin (OT) analogs with inhibitory properties are valuable tools for the study of the mechanism of action of this hormone and can be utilized in clinical practice for the treatment of preterm abortion. Inhibitors of this type have been obtained by drastic modifications of the oxytocin molecule which affect backbone conformation and impose rigidity of structure[1]. Among these, a potent antagonist (Pen^1, D-Phe^2, Thr^4, Thr^5, Orn^8]OT was recently reported as result of previous systematic studies[2]. We hypothesized that minimal changes in the cyclic portion of the proposed "biologically active" model of oxytocin[3] may provide keen information concerning the structure–activity relationships of antagonists and may facilitate the design of strong inhibitors. Along this line two kind of modifications leading to the synthesis of [(OMe)Ser)^5]OT, [(OMe)Thr^5]OT and [(Aib)^3]OT was undertaken.

EXPERIMENTAL

Synthesis was accomplished by the solid phase method on a *p*-methylbenzhydrylamine (MBHA) resin with Boc-strategy. For the side-chain protection we have used: 4-methylbenzyl(Cys) and 2-bromo carbobenzoxy(Tyr). (3-*O*-Methyl)Thr and (3-*O*-methyl)Ser were prepared according to Chimiac and Rudinger[4]. Protected amino acids were coupled by DCC and HOBt in dimethylformamide. Boc-Aib, DCC and HOBt (3 molar excess, 1 : 1 : 1) were shaken with the resin until a negative ninhydrin test was obtained (5 h). Side-chain protecting groups were cleaved simultaneously with the peptide from the resin by liquid HF. Yields of fully protected peptide-resins were in excess of 90%. Sulfhydryl group oxidation was performed by 1,2-diiodoethane. The analogues were purified by gel chromatography on Sephadex G-15 in 50% acetic acid followed by partition chromatography on Sephadex G-25 using the system 1-butanol–1.5% pyridine in aqueous 3.5% acetic acid (1 : 1 v/v)

and by gel chromatography on Sephadex G-15 using 0.2 M acetic acid. The purity was checked by thin-layer chromatography in three solvent systems, reversed-phase high performance liquid chromatography (solvent system: 50% acetonitrile (A) and 0.1 M sodium dihydrogenphosphate. Gradient 30% A to 60% A in 30 min) and amino acid analysis. Aib emerged 29.5 ml before Val from the long column of the analyzer (Beckman 119 CL) and had a colour value which was 7% of that of Leu. In order to obtain a reasonable peak size, large amount of the peptide was hydrolyzed.

RESULTS AND DISCUSSION

Walter's[5] "cooperative model" for oxytocin proposes a key role for the Asn[5] residue in the induction of the biological message. Substitution of the Asn[5] by any other amino acid leads to almost loss in agonistic potency, while modifications of the amide group in the side chain of Asn[5], e.g. [5-N^4,N^4-dimethylasparagine]OT reduces dramatically the potency (4.60 + 0.5 U/mg), but more important, it retains identical intrinsic activity compared with oxytocin[6]. However when the alkyl substituent is made larger as in [5-N^4,N^4-diisopropylasparagine]OT, a nearly inactive analog was obtained[7]. Thus, the carbonyl group in the γ-position of the side chain of the Asn[5] residue of oxytocin appears to be an important factor for its oxytocin-like activities. Based upon this consideration, we assumed that alkylation of the side-chain hydroxyl group of the weak agonist [Ser[5]]OT with a 0.7 ± 0.2 U/mg of uterotonic potency[8], it would increase the electronegativity of the oxygen atom and consequently its hydrogen bond accepting character, which might play a role in determining potency. Surprisingly, the weak agonist, by mere methylation, was transformed to a relatively weak antagonist [(OMe)Ser[5]]OT with a pA_2 value of 5.7 in the in vitro rat uterotonic assay. Accordingly, [(OMe)Thr[5]]OT exhibited a pA_2 value of 5.8. Both analogs were found also inactive in the pressor and galactogonic assays. Another modification implied the replacement of Ile[3] at the one corner of the ring portion of oxytocin with Aib, which is known to introduce considerable constraints on the conformation. Obviously, this modification would affect the proper alignment between the CO of Asn[5] and the hydroxyl group of Tyr[2], the later acting cooperatively for initiation of the oxytocic activity. Interestingly, the analog [Aib[3]]OT was found to be a weak antagonist with a pA_2 value of 5.9 and not detectable pressor and galactogonic activity. The accumulation of these minor modifications in one molecule is in progress.

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