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ANALOGUES OF OXYTOCIN ACTING AS IRREVERSIBLE INHIBITORS OF OXYTOCIN

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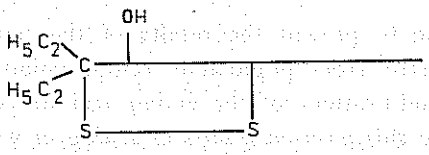
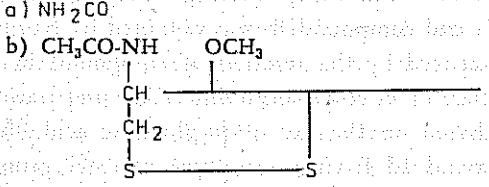
INTRODUCTION

The inhibitors of the uterotonic effect of oxytocin synthesized so far are characterized by a synthetic modification in position 1 of the peptide chain resulting in a bulky N-terminal (Fig. 1) or by cumulated modifications in positions 1 and 2. The typical representatives are N^{α}-acetyl or N^{α}-carbamoyl [2-O-methyltyrosine] oxytocins. The compounds behaved like typical competitive inhibitors, even in the cases (Fig. 2) when the group in position 1, like bromoacetyl or maleoyl had a highly reactive character [6,7]. This fact, together with the finding of tissue specificity of the inhibitors studied [2], stimulated the formula-



[ββ' diethyl mercaptopropionic acid] oxytocin [12]



a) N^{α} – carbamoyl – [2 – O-methyltyrosine] oxytocin [3]

b) N^{α} – acetyl – [2 – O-methyltyrosine] oxytocin [5]

Fig. 1. Oxytocin inhibitors — the structure of the N-terminus

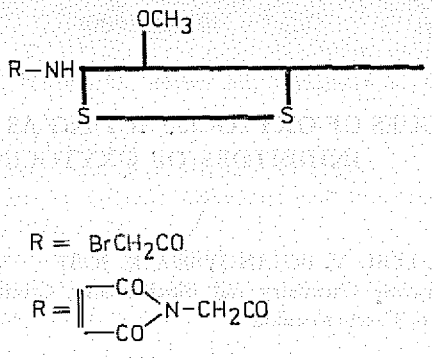


Fig. 2. Inhibitors of oxytocin with the reactive groups in position 1 of the peptide chain

tion of criteria valid for potential irreversible inhibitors with the specific effects on the receptors in the individual target tissues. Such criteria can be summarized as

a) adequate affinity of inhibitor to the receptor,

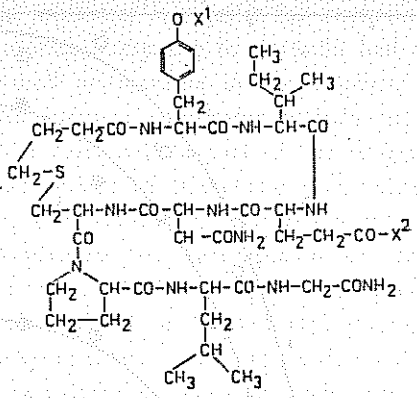
b) suitable position of reactive group in the peptide chain of the inhibitor molecule,

c) adequate reactivity and stability of reactive groups.

RESULTS AND DISCUSSION

In this lecture we should like to present the results of the preparation of irreversibly acting inhibitors derived from [4-glutamic acid]deamino-1-carba-oxytocin [8]. We concentrated on modifications of the amino acid in position 4 of the peptide chain. Modifications in this position results in analogues with a certain level of biological activities [11, 9]. Different reactive groups were introduced (Fig. 3) in this position.

The reaction of [4-glutamic acid]deamino-1-carba-oxytocin and methyl ester of S-benzylcysteine by means of dicyclohexylcarbodiimide and 1-hydroxybenzotiazole result in an intermediate from which the protecting benzyl group was split off by sodium in liquid ammonia and compound Ib was obtained by lyophilization. The mixed anhydride Ic was prepared by the reaction of compound Ia with sec. butyl chloroformate in the presence of N-ethylmorpholine. The preparation of the three following analogues was based on the use of [4-glutamic acid-γ-hydrazide] deamino-1-carba-oxytocin. Compound Id having a reactive maleoyl group in position 4 was prepared by the reaction of the above-mentioned hydrazide with maleoylglycine in the presence of 1-hydroxybenzotriazole. Compound Id was prepared by treating hydrazide with bromoacetyl bromide. The derivative If, an azide, was prepared from the hydrazide by means of butyl nitrite. The active esters, 1-hydroxybenzotriazolyl and p-nitrophenyl esters are the most effective



Ia	$X^1 = H$	$X^2 = OH$
Ib	$X^1 = H$	$X^2 = NH\text{-}CH(CH_2SH)OCH_3$
Ic	$X^1 = H$	$X^2 = O\text{-}COOCH(CH_3)C_2H_5$
Id	$X^1 = H$	$X^2 = NH\text{-}NH\text{-}CO\text{-}CH_2N$ (CO-CH, CO-CH)
Ie	$X^1 = H$	$X^2 = NH\text{-}NH\text{-}CO\text{-}CH_2Br$
If	$X^1 = H$	$X^2 = N_3$
Ig	$X^1 = H$	$X^2 = O\text{-}C_6H_4N_3$
Ih	$X^1 = H$	$X^2 = O\text{-}C_6H_4NO_2(p)$
Ii	$X^1 = H$	$X^2 = NH(CH_2)_5\ COO\text{-}C_6H_4NO_2(p)$
Ij	$X^1 = CH_3$	$X^2 = O\text{-}C_6H_4N_3$
Ik	$X^1 = CH_3$	$X^2 = O\text{-}C_6H_4NO_2$

Fig. 3. The chemical structure of [4-glutamic acid] deamino-1-carbaoxytocin and its derivatives

inhibitors. The former was prepared by means of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole, the latter by treating [4-glutamic acid] deamino-1-carba-oxytocin with bis(p-nitrophenyl)sulphite in pyridine. The preparation of another analogue, Ih, was motivated by our interest in the importance of the steric localisation of the reactive group. Analogue Ih was prepared by treating [4-γ-glutamoyl-ε-aminocaproic acid] deamino-1-carba-oxytocin with bis(p-nitrophenyl)sulphite. Furthermore, we prepared two analogues whose hydroxyl groups of tyrosyl residues were replaced by methyl ether. Both active esters, namely Ij, 1-hydroxybenzotriazolyl ester and Ik, p-nitrophenyl ester derivative were prepared as indicated above only using [2-O-methyltyrosine, 4-glutamic acid] deamino-1-carba-oxytocin instead of Ia.

The synthesized analogues were tested by the uterotonic assay using an isolated rat uterus [4, 10], and their inhibition of oxytocin action was investigated. The majority of the analogues prepared had uterotonic activity (Table 1).

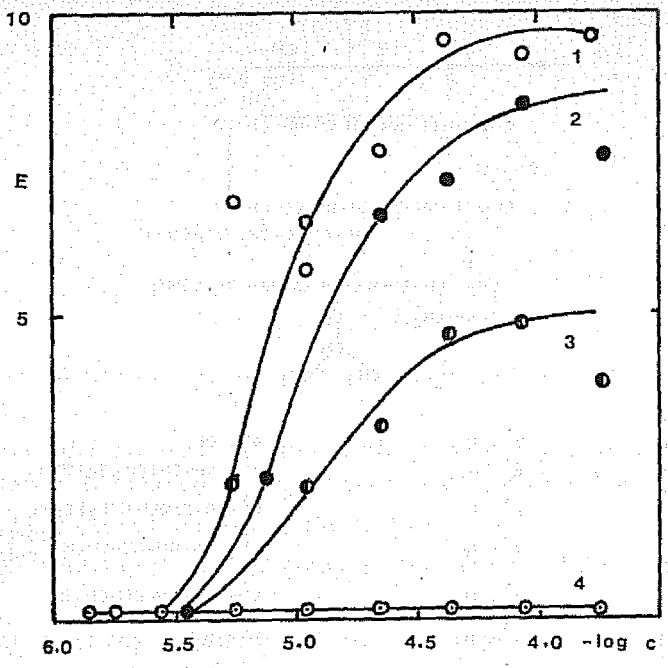


Fig. 4. Inhibition of oxytocin action by compound Ig. Ordinate E-effect, contractions in mm, abscissa: — log of concentration, mM. 1 — Oxytocin, 2 — oxytocin after 5 min action of Ig (2μM), 3 — the same as 2, 4 — the same as 2, but after treatment with 10μM Ig

Table 1. Uterotonic activity of [4-glutamic acid]deamino-1-carba-oxytocin and its derivatives

Compound	Activity IU/mg	Compound	Activity IU/mg
Ia	1.02	Ig	160
Ib	1.8	Ih	not determined
Ic	16.0	Ii	not determined
Id	5.0	Ij	0
Ie	2.0	Ik	not determined
If	0.6		

With the exception of compound Ii, i.e. p-nitrophenyl ester derived from [4-γ-glutamoyl-ε-aminocaproic acid] deamino-1-carba-oxytocin, all the derivatives behaved like irreversible inhibitors [1] compound Ig being the most effective (Fig. 4). The other inhibitors had to be used in 10 times higher concentration (e.g. 20 μg/ml) in order to obtain the same inhibitory effects. The inhibitory properties were also observed in the case of compounds Ij and Ik, in which the hydroxyl group of tyrosine was replaced by the methoxyl group. These analogues had no intrinsic uterotonic effect. The observed inhibitory effect evoked by the

presence of irreversible inhibitors is specific for oxytocin. The addition of $PGF_{2\alpha}$ to the bath after the maximal response to oxytocin had been lowered by the presence of inhibitors caused an increase in the response of the uterus. The fact that the inhibitors had intrinsic uterotonic activity strongly supports the view that the analogues really react with a part of the receptor. The inhibitory effect on the whole was independent of the chemical character of the reactive group inserted into molecule. The quantitative differences among the reactivities of the individual inhibitors indicated the participation or importance of the corresponding groups in the vicinity of the receptor. Our previous results showed that the localisation of maleoylglycyl or bromoacetyl groups in position 1 resulted in compounds acting as competitive inhibitors. Similarly the introduction of reactive groups in position 4 provided the analogues with properties of irreversible inhibitors only when the reactive groups were not too remote from the backbone of the molecule. If the active ester was separated from the backbone by a residue of ε-caproic acid, as in the case of compound Ii, no inhibitory effect was observed.

The results obtained suggest the possibility that radioactively labelled irreversible inhibitors may be successfully used in the future for isolating and studying the properties of the uterine receptor for oxytocin.

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