SYNTHESIS AND SOME ACTIVITIES OF [1-PENICILLAMINE]-DEAMINO-6-CARBA-OXYTOCIN

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Introduction

Analogues of oxytocin having the properties of uterotonic inhibitors of oxytocin could find use in the clinical practice as tocolytics in the cases of menacing miscarriage or preterm delivery[1]. An effective inhibitor must be able to block direct action of oxytocin on myometrial contractions as well as to prevent prostaglandin release by oxytocin stimulated uterus[2]. From the structural features leading to the oxytocin inhibitors in the in vivo uterotonic test we can name the following :

1) Dialkylation of cysteine β-carbon[3] in position 1 (this modification in the case of two methyl groups and molecule without primary amino-group is not sufficient for inhibitory action[4]).

2) Change of configuration of amino-acid in position 2 connected with the lipophilization of tyrosine moiety[5] by substituting the OH group by a more liopophilic one[6].

3) Alkylation of tyrosine hydroxyl, which again as single substitution does not lead to the in vivo acting inhibitor, but which can strikingly increase the inhibitory properties of the analogue, as well as acylation of the amino-group in position 1 (for review see[7]).

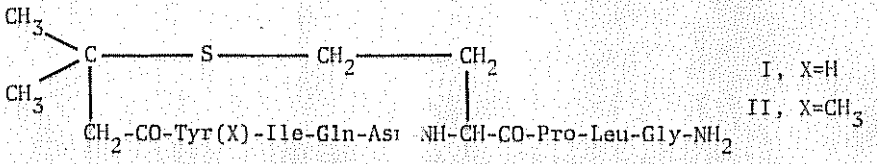
Further modifications leading to the increase of inhibitory activities of analogues are : substitution of threonine for glutamine, dehydroproline for proline and basic amino acid for leucine (for review see[8]).

The modification which we have found[6] extremely useful in the design of inhibitors with D-amino acid in position 2 and which has not been tested up to now in combinations with other structural features given above is carba substitution of disulfide bridge[9]. This substitution has striking

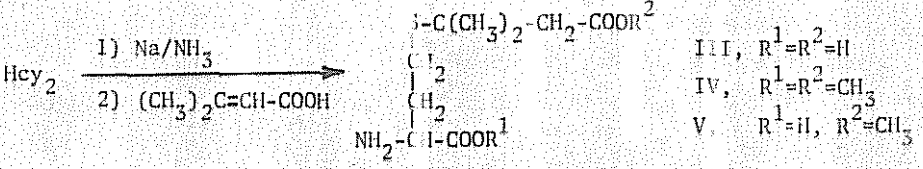
influence on the agonistic activities of prepared analogues[10].

Results and Discussion

We have prepared two analogues (I and II) containing in position 1 the deaminopenicillamine residue and carba-6 modification of the S-S bridge.



The first (I) differs from [1-penicillamin]deamino-oxytocin only by the exchange of methylene for sulfur and the second in addition contains O-methyl-tyrosine in position 2. For their synthesis we have chosen the stepwise elongation of the peptide chain using Nps protecting group and active esters for the coupling reaction. The critical step in the synthesis was the preparation of a suitably modified homocysteine derivative V, which is shown on scheme.



Due to the impossibility of selective esterification of ω-carboxyl of compound III, it was necessary to use selective hydrolysis of diester IV. We have performed the cleavage of Nps group either in presence of mercaptoethanol or by the thiosemicarbazide hydrochloride[11] to overcome the splitting of the S-alkyl group. Cyclization was performed by means of active ester according to Krojidlo et al.[12], and the product was purified by reverse-phase HPLC.

The results of biological tests are given in Table I. As can be seen, in comparison with [Pen^1]dOT, analogue I has a very high degree of its own uterotonic activity in vivo and has very weak inhibitory activity in the pressor test. On the other hand analogue II is one of the most potent inhibitors of oxytocin uterotonic activity in vivo, described up to now.

Table I

Uterotonic (UT), galactogogic (GA), antidiuretic (AD) and pressor (BP) activities of prepared analogues determined in rats (I.U./mg). Values in parenthesis mean antagonistic activity given as pA_2.

Compound[a]	UT in vivo	UT in vitro	GA	AD	BP	Ref.
OT	450	450	450	3	3	10
dOT	900	795	536	19	1.4	10
dCOT-6	2792	929	456	118	1.5	10
[Pen^1]dOT	P.A.[b]	(7.14)	Agonist	-	(6.27)	3,15
[Pen^1,Tyr(Me)^2]dOT	(6.86)	(7.76)	(6.94)	0.02	(7.59)	15,16
I	279.8	16.2	6.9	4.81	(6.82)	
II	(7.13)	(8.43)	0[c]	0.52	(7.43)	

a) OT oxytocin, dOT deaminooxytocin, dCOT-6 deamino-6-carba-oxytocin ; b) partial agonist ; c) inactive up to 2×10^{-2} mg.

The high uterotonic activity of compound I can be explained on the basis of Hruby's view on the importance of rigidization of the molecule for the inhibitory activity of analogue[13], as the CH_2-S group has a substantially higher degree of mobility than the S-S grouping. In the case of analogue II either the combination of dimethyl group in position 1 with O-methyltyrosine in position 2 leads to the increased rigidity or the elimination of free OH group (part of the active site of oxytocin[14]) from tyrosine connected with the increased lipophilicity of the analogue is sufficient for the inhibitory activity of the resulting molecule (the reason could provide also an explanation of the inhibitory activity of the D-amino acid in position 2 containing analogues[6]).

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