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TWO ANALOGUES OF OXYTOCIN WITH MODIFIED PROLINE CYCLIC STRUCTURE

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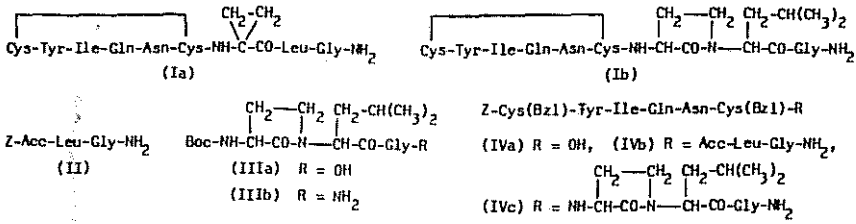
Introduction

It has been established (1) that there exist several different mechanisms, by which neurohypophysial hormones can be inactivated. Nevertheless, it seems probable that the enzymic cleavage of the linear tripeptide chain (2, 3) is of major importance in the living organism. In order to obtain metabolically stable analogues of oxytocin with protracted biological effects, it is therefore expedient to modify the structure of this part of the molecule.
The proline residue is considered to have an important role in forming such a spatial arrangement of the molecule that could be responsible for high biological activity. We prepared two analogues of oxytocin modified in position 7 and investigated the influence of the structural change on the biological activity. In one of the analogues (Ia), the proline residue was substituted by 1-aminocyclopropanecarboxylic acid (Acc: cf. (5)) and in the other (Ib) the amino acids in positions 7 and 8 (proline and leucine) were substituted by a 2-(2-oxo-3-amino-1-pyrrolidinyl)-4-methylpentanoic acid residue.

Results

Both analogues were prepared from the protected hexapeptide acid (6) which was condensed with carboxyterminal peptides. In the preparation of analogue (Ia), tripeptide (II) was obtained by carbodiimide condensation of 1-benzyloxycarbonylaminocyclopropanecarboxylic acid (7) with leucyl-glycine ethyl ester, followed by ammonolysis. In the preparation of compound (IIIb), the initial material was terc-butyloxycarbonylmethionyl-leucyl-glycine ethyl



ester which was transformed to a sulphonium salt by means of methyl iodide. The action of sodium hydride resulted in the formation of the five-membered cycle and the hydrolysis of the ester group (IIIa). A similar procedure has already been used for preparing analogues of luliberin and enkephalin modified in this way (8). The structure of the product was confirmed by mass, infrared and ^{1}H-NMR spectroscopy. After esterification with diazomethane, the resultant ester was transformed to an amide (IIIb) by ammonolysis. The aminoprotective group of both carboxyterminal peptides were removed and the peptide were condensed with compound (IVa) in the presence of an excess of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. The protecting groups of compound (IVc) were removed by sodium in liquid ammonia. In the case of nonapeptide (IVb), liquid hydrogen fluoride in the presence of anisole had to be used because sodium in liquid ammonia caused the opening of the ring of 1-aminocyclopropanecarboxylic acid. After oxidation and desalting, the analogues were purified by means of HPLC. Biological activities of the analogues are stated in Table I.

Discussion

Both the analogues have lower biological activities than oxytocin and the activities are dissociated in favour of the galactogogic effect. This finding supports the assumption that the galactogogic receptor does not have such strict structural requirements as the other receptors for oxytocin. The analogues of oxytocin modified in position 7 (we take into account only those oxytocin analogues that have just one structural modification; cf. review (9)) can be divided into two groups according to their uterotonic activity:

Table I

Biological activities determined in experiments on rats (I.U./mg)

Compound	Uterotonic (in vitro)	Galactogogic (in vivo)	Pressor
Oxytocin	450	450	3
Tocinamide[a]	2.7	5.9	0
Ia	9.8	62	<0.2
Ib	1.6	8	<0.2

[a]Ref. (15)

1. analogues with higher activity than tocinamide: [7-(thiazolidine-4-carboxylic acid)]oxytocin, [7-(3,4-dehydroproline)]oxytocin, analogues containing azetidine-2-carboxylic acid, alanine, D-proline, glycine, sarcosine (10) or methylalanine (10) in position 7 and analogue Ia.
2. those with lower activity than tocinamide: analogues containing D-leucine (11), norvaline (12), hydroxyproline (13,14) and analogue Ib.

In analogues of the second group, various factors (e.g. lipophilic and steric interactions, the possible formation of an "unsuitable" hydrogen bridge) may influence the spatial arrangement of the cyclic part of the molecule (this cannot occur in the case of tocinamide).

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