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ANALOGUES OF NEUROHYPOPHYSIAL HORMONES CONTAINING A D-AMINO ACID IN POSITION 2

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Introduction

Several modifications of the oxytocin molecule are known, leading to analogues with antagonistic activity. The resulting effect can be increased by combining some of them (for review, see (1)). Analogues with inhibitory properties not only are valuable tools for the evaluation of the action mechanism of hormones, but they can also be utilized in clinical practice. Inhibitors obtained by introducing a D-amino acid in position 2 were first studied by Soviet authors (2). In the present paper, we describe the preparation and properties of a series of analogues containing a hydrophobic D-amino acid in this position.

Results

Table I shows the structure of the compounds studied and states their uterotonic activity (I.U./mg) or inhibitory potency (pA_2).The analogues were prepared by stepwise synthesis of the peptide chain. Cyclization was achieved by means of the active ester method (in the case of carba-analogues) or the oxidation of sulfhydryl groups (compounds XVI-XVIII). In some cases, a racemic amino acid for position 2 was used and the resulting diastereoisomeric peptides were separated by reversed phase HPLC. Analogue XXb was prepared by acylation of the compound XVIb with o-nitrobenzenesulfenylglycyl-glycyl-glycine N-hydroxysuccinimide ester. Analogue XIXb was prepared by the action of chymotrypsin on compound XIVb. Sulfoxides were obtained by periodate treatment and the diastereoisomers were separated by means of HPLC (3).

Table 1

In vitro uterotonic activities of some oxytocin analogues

Compound[a]	Activity[b] of "L"	Ref.	Activity[b] of "D"	Ref.
Oxytocin (I)	450	4	6.6	4
Deamino-oxytocin (II)	745	4	1	4
Deamino-1-carba-oxytocin (III)	1898	5	42.6	c
Sulfoxide of III	0.13	3	pA_2=4.56	c
	7.2	3	pA_2=5.94	c
Deamino-6-carba-oxytocin (IV)	929	5	28	c
Sulfoxide of IV	455	3	22.5	c
[Leu^2]oxytocin (V)	0.44	4	pA_2=5.23	2
[Phe^2]oxytocin (VI)	32	4	pA_2=6.00	2
[Tyr(Et)^2]oxytocin (VII)	0.15	4	2.6	2
[Trp^2]oxytocin (VIII)	0.24	2	pA_2=6.87	2
[Phe(F_5)^2]oxytocin (IX)	0.01	2	pA_2=6.27	2
[Tyr(3-NO_2)^2]oxytocin (X)	1.1	2	pA_2=6.28	2
[Val^2]deamino-6-carba-oxytocin (XI)	-		4.9	c
[Phe^2]deamino-6-carba-oxytocin (XII)	70	6	pA_2=8.06	c
[Tyr(Et)^2]deamino-6-carba-oxytocin (XIII)	0.001	6	pA_2=7.45	c
[Phe(Me)^2]deamino-6-carba-oxytocin (XIV)	127	6	pA_2=8.73	c
[Phe(Et)^2]deamino-6-carba-oxytocin (XV)	27	6	pA_2=8.73	c
Sulfoxide of XV	17	6	pA_2=7.91	c
[Phe(Et)^2]oxytocin (XVI)	6.5	4	pA_2=8.15	c
[Phe(Et)^2]deamino-oxytocin (XVII)	-		pA_2=8.06	c
[Pen^1,Phe(Et)^2]oxytocin (XVIII)	pA_2=7.78	c	pA_2=8.09	c
Des-GlyNH_2-[Phe(Me)^2]deamino-6-carba-oxytocin (XIX)	-		pA_2=8.80	c
Triglycyl-[Phe(Et)^2]oxytocin (XX)	-		pA_2=6.14	c

[a] Pen=penicillamine; [b] activities of analogues containing either L-amino acid (index a in the text) or a D-amino acid (index b in the text) in position 2; [c] this contribution.

Discussion

Apart from our results concerning analogues containing a D-amino acid in position 2, Table I includes the activities of compounds prepared by Soviet authors and analogues containing a L-amino acid in position 2. As can be seen, analogues XIVb and XVb with position 2 occupied by D-phenylalanine substituted in the para position by a hydrophobic group and a carba bridge instead of a disulfide one, have the highest inhibitory activity. Compounds with an unmodified bridge have a lower inhibitory potency; this is in agreement with the assumption that compounds with a carba bridge are more compatible with the uterotonic receptor and thus have higher affinity. The combination of the substitution of the β-carbon of cysteine in position 1 with the inclusion of a D-amino acid in position 2 did not produce an analogue with a corresponding cumulative increase of inhibitory potency, as can be seen by comparing compounds XVIIIb and XVIb. The activities of compounds XVIa, XVIb and XVIIIa give evidence that the inversion of the configuration on the α-carbon of the amino acid in position 2 is more important for obtaining an inhibitor than the introduction of penicillamine in position 1. However, inversion by itself is not sufficient. The removal of the tyrosine hydroxyl group is of major importance because compounds containing D-tyrosine (Ib-IVb) have intrinsic uterotonic activity. The oxidation of the 1--carba analogue IIIb to the sulfoxide stage produced two diastereoisomers with weak inhibitory properties. The decrease of the activity of sulfoxides was more pronounced in the 1-carba than in the 6-carba series; in both series the activity of the compounds containing L-amino acids was decreased, whereas the 1-carba analogue with D-tyrosine in position 2 had weak inhibitory potency. Oxidation of compound XVb to the sulfoxide stage produced an analogue with lower inhibitory activity, possibly due to an increase in the polarity of the portion of the molecule responsible for the binding to the receptor. The alkylation of D-tyrosine just as the introduction of a D-aliphatic amino acid in position 2did not always produce inhibitors (cf. compounds Vb, XIb, VIIb and XIIIb). The presence of the C-terminal glycine amide is apparently not a prerequisite for inhibition, as documented by the inhibitory potency of compound XIXb. Acylation of compound XVIb by triglycine resulted in analogue XXb with "inhibitorogen" (inhibitor-generating) properties that had protracted inhibitory action in vivo. Apart from the

oxytocin analogues mentioned in Table I, an analogue of 8-lysine-vasopressin containing D-p-ethylphenylalanine in position 2 was also synthetized. Analogues of vasopressin containing O-alkylated D-tyrosine in position 2 have been studied recently by American authors (7). However, from their results it is not clear what influence has inversion of tyrosine configuration on the inhibitory activity because all the analogues studied were modified in two or three more positions. The analogue of vasopressin, containing D-tyrosine in position 2, exhibits agonistic activity in all common tests (8). On the contrary, [2-D-p-ethylphenylalanine, 8-lysine]vasopressin prepared by us, showed in preliminary experiments an inhibitory activity in the pressor assay (pA_2=7.05).

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