SYNTHESIS AND SOME BIOLOGICAL PROPERTIES OF AMIDES DERIVED FROM [4-GLUTAMIC ACID]DEAMINO-1-CARBA-OXYTOCIN*

Michal LEBL, Anastasia DIMELI, Vera BOJANOVSKA**, Jiřina SLANINOVÁ, Tomislav BARTH and Karel JOŠT

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received July 14th, 1978

A number of amides of [4-glutamic acid]deamino-1-carba-oxytocin, as well as certain derivatives of amino acids, and a protected hydrazide of the above-mentioned peptide were prepared by a reaction with the amino-component, using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. The analogues were checked for uterotonic and galactogogic activity.

The synthesis of [4-glutamic acid]deamino-1-carba-oxytocin[1] (*I*) initiated the preparation of a series of analogues containing a glutamic acid*** residue modified in the γ-carboxyl. The synthesis of this type of compounds has not been described so far; the only pharmacological results were obtained in the assay of the uterotonic activity of [4-glutamic acid γ-methyl ester]oxytocin and a similar derivative of deamino-oxytocin[3]. [4-Aspartic acid β-methylamide]oxytocin[4] and its deamino-derivative[4], which have the modified amide bond nearer to the backbone of the molecule, had approximately 10% of the uterotonic activity of oxytocin.

In the preparation of amides we used a reaction[1] that had been applied successfully for proving the structure of analogue *I* by transforming it to deamino-1-carba-oxytocin[5], whose biological activities are known. Compound *I* reacts with the amino component in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. The reaction mixture was treated in such a way that the procedure was independent of the physical properties of the product obtained and could always be performed under the same conditions. The reaction takes place in dimethylformamide and it is therefore neccessary for the product to be sufficiently soluble in this medium (at least 2 mg in 1 ml). After the termination of the reaction the peptide material was separated from the surplus of reagents by filtration through a column of Sephadex

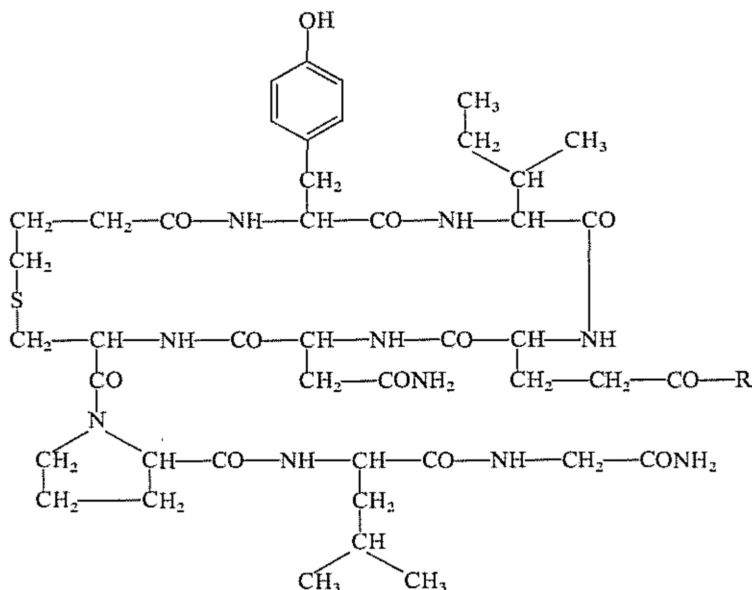
* Part CLVI in the series Amino Acids and Peptides: Part CLV: This Journal *44*, 2447 (1979).

** Predoctoral fellow from the Institute of Infectious and Parasite Diseases, Bulgarian Medical Academy of Sciences, Sofia, Bulgaria.

*** The amino acids used in this work were of L-configuration. The nomenclature and symbols of amino acids and peptides are used according to published suggestions[2].

LH-20 and purified by gel filtration on Bio-Gel P–4 in 3M acetic acid. The analogues *III–XIII* were characterized by elemental analysis, thin-layer chromatography and amino-acid analysis. The amines used could not be determined by amino-acid analysis because the colour yield of their reaction with ninhydrin was very low.

In most cases a side product was formed which was separated by gel filtration on Bio-Gel P—4. This substance was prepared from analogue *I* by reaction with dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in the absence of the amino component. The possibility that this compound has the structure of a lactone as a result of inter- or intramolecular esterification of the hydroxyl of the tyrosine residue was disproved by infra-red spectroscopy (no band corresponding to an ester group was found in the spectrum), and also by ultraviolet and circular dichroism spectra (in both cases the dependence of the spectrum on the pH of the solution gave evidence of a product with a free tyrosine hydroxyl). The reaction product was chromatographically identical with compound *IV* and had similar pharmacological properties. It was therefore concluded that its structure is that of dimethylamide *IV*. The dimethylamine necessary for this reaction is apparently present in the dimethylformamide used as solvent[6].



I, R = OH
II, R = NH_2
III, R = $NHCH_3$
IV, R = $N(CH_3)_2$
V, R = $NHC_{10}H_{15}$[a]
VI, R = $NHC_6H_4CH_3$-*p*
VII, R = NHNHBoc
VIII, R = $NHNH_2$
IX, R = $GlyNH_2$
X, R = GlyOMe
XI, R = Pro-Leu-$GlyNH_2$
XII, R = $NH(CH_2)_5COOBu^t$
XIII, R = $NH(CH_2)_5COOH$

[a] 1-Adamantyl.

The new analogues *III*–*XIII* were tested for uterotonic activity *in vitro*[7,8] and galactogogic activity *in vivo*[9,10]; the results are stated in Table I together with those of reference analogues. Both activities of methylamide *III* are significantly lower than those of amide *II* but their ratio remains virtually unchanged. By contrast, dimethylamide *IV* had higher galactogogic than uterotonic activity and thus resembled acid *I*. A similar specificity of the galactogogic effect was observed in the case of *p*-toluidid *VI*, protected hydrazide *VII* and the glycinamide derivative *IX*. The derivative of glycine methyl ester *X* had even higher specificity, whereas the analogue lengthened by tripeptide amide *XI* showed a decrease in the specificity of the galactogogic effect, as well as in the absolute value of the activities. Both derivatives containing ε-aminocaproic acid with a free or protected carboxyl group had ten times higher galactogogic activity than uterotonic. On comparing analogues *I* and *XIII* it can be seen that the greater the distance of the free carboxylic group from the backbone of the molecule, the higher the uterotonic activity of the analogue. On the other hand, adamantylamide *V* and free hydrazide *VIII* have higher uterotonic than galactogogic effects. The comparison of three pairs of compounds, namely *I* and *II*, *III* and *IV*, *VII* and *VIII*, indicates that the receptors specific for the uterotonic and galactogogic response may differ in the region that is near the glutamic acid residue at the moment of the binding of the peptide to the receptor. The decrease of biological activities

TABLE I

Uterotonic and Galactogogic Activities (I.U./mg) of Analogues of Deamino-1-carba-oxytocin

Compound	Uterus *in vitro* (A)	Mammary gland *in vivo* (B)	B/A
I	1·0	39·0	39
II	1 898	604	0·32
III	40·7	12·2	0·30
IV	4·26	34·5	8·1
V	9·2	8·2	0·89
VI	21·2	100	4·7
VII	3·24	21·6	6·7
VIII	19·4	13·8	0·71
IX	5·3	36·5	6·9
X	10·2	98·0	9·6
XI	0·15	0·29	1·9
XII	2·36	23·3	9·85
XIII	4·78	42·7	8·90

could also be attributed to a partial change in the secondary structure caused by the presence of a substituent in position 4; in this case various modifications in this part of the molecule would have a similar effect on the alteration of the configuration. The fact that various substitutions do not result in total loss of activity is advantageous and makes it possible to synthetize compounds containing reactive groups[11,12] in place of the glutamic acid residue and have sufficient affinity to the receptors, thus decreasing the possibility of non-specific binding. Moreover, each substitution in this position influences the uterotonic and galactogogic activity of the resultant compound to a different degree (Table I). This approach enables us to obtain compounds with specific galactogogic activity[13].

However, the fact that these analogues are active is not in agreement with the assumption that the so-called "active site" of the oxytocin molecule contains the γ-carboxamide group of glutamine. The substitution of glutamine, for example, by the adamantyl residue (*V*) or the *p*-tolyl residue (*VI*), alters the character and possibly even the accessibility of the hypothetical active site (*cf.*[14]).

EXPERIMENTAL

Samples for elemental analysis were dried for 24 h at room temperature and 150 Pa. Thin-layer chromatography was carried out on ready-for-use plates (Silufol, Kavalier) in the solvent systems: 2-butanol–98% formic acid–water (75 : 13·5 : 11·5) (S1), 2-butanol–25% ammonia–water (85 : 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3) and pyridine–1-butanol–acetic acid–water (10 : 15 : 3 : 5) (S4). Detection was performed by means of chlorination method. Samples for amino acid analysis were hydrolyzed for 20 h at 105°C in 6M-HCl (in ampoules sealed at 150 Pa). The analyses were carried out on an automatic analyzer (Development Workshops, Czechoslovak Academy of Sciences, Prague, type 6020). Dimethylformamide was dried over P_2O_5, distilled *in vacuo*, shaken for several days with BaO and redistilled. Water and acetic acid in elemental analyses were calculated for the best fit with values determined and were not estimated analytically.

Preparation of Analogues of Deamino-1-carba-oxytocin Having a Modified Glutamine Residue in the Position 4

Procedure A: To the solution of [4-glutamic acid]deamino-1-carba-oxytocin[1] (*I*) (5 mg) in dimethylformamide (250 μl), 1-hydroxybenzotriazole (13·5 mg) and an appropriate amine (50 μM) were added. The mixture was cooled to 0°C and dicyclohexylcarbodiimide (20·6 mg) was added. After standing for 1 h at 0°C and 20 h at room temperature the mixture was diluted with dimethylformamide (1·5 ml), separated dicyclohexylurea was removed by filtration and the solution was transferred onto a column (100 × 1 cm) of Sephadex LH-20 in dimethylformamide. The peak containing peptide material was evaporated *in vacuo* using an oil pump at room temperature. The residue was dissolved in a mixture of methanol (0·2 ml) and 3M acetic acid (2 ml) and placed on a column (100 × 1 cm) of Bio-Gel P—4. The peptide was recovered by lyophilization. The yields were in the range of 50—70%. Properties and analytical data are given in Table II.

TABLE II

Characteristics of the New Analogues

Analogue Method	R_F S1 S2	S3 S4	Formula (m.w.)	Calculated/Found % C	% H	% N	Amino-acid composition Asp Glu	Pro Gly	Ile Leu	Tyr Cys($C_4H_7O_2$)	
I	0·35	0·44			[a]				[a]		
	0·06	0·64									
II	0·21	0·26			[b]				[b]		
A	0·16	0·66									
III	0·24	0·28	$C_{45}H_{69}N_{11}O_{12}S$.	53·10	7·00	13·90	1·01	1·02	0·99	0·92	
A, B	0·18	0·65	.2 $C_2H_4O_2$	53·26	7·11	13·84	1·01	1·04	1·04	0·98	
			(1 108)								
IV	0·22	0·26	$C_{46}H_{71}N_{11}O_{12}S$.	51·65	7·31	13·80	1·03	1·00	1·00	0·98	
B	0·18	0·66	.$C_2H_4O_2$.3 H_2O	51·87	7·04	13·69	1·01	1·04	1·05	0·91	
			(1 116)								
V	0·47	0·53	$C_{54}H_{81}N_{11}O_{13}S$.	54·11	7·02	11·70	1·00	1·03	0·94	0·98	
B	0·42	0·76	.2 $C_2H_4O_2$.2 H_2O	54·36	7·39	12·02	0·96	1·04	1·01	1·04	
			(1 282)								
VI	0·43	0·55	$C_{51}H_{73}N_{11}O_{12}S$.	53·34	7·08	12·44	0·99	1·00	0·97	1·00	
A	0·56	0·77	.2 $C_2H_4O_2$.3 H_2O	53·21	6·76	12·47	0·95	1·00	1·02	1·08	
			(1 238)								
VII	0·39	0·49	$C_{49}H_{76}N_{12}O_{14}S$.	51·88	7·20	14·82	1·05	0·92	0·99	0·99	
A	0·37	0·75	.2·5 H_2O	51·76	7·51	15·04	0·99	1·02	1·05	1·04	
			(1 134)								
VIII	0·10	0·47	$C_{44}H_{68}N_{12}O_{12}S$.	50·08	7·12	15·23	1·00	0·97	0·97	0·99	
	0·11	0·71	.$C_2H_4O_2$.3 H_2O	49·86	6·94	15·39	0·97	1·00	1·01	1·07	
			(1 103)								
IX	0·14	0·22	$C_{46}H_{70}N_{12}O_{13}S$.	51·66	6·90	14·75	1·01	0·98	0·99	0·98	
B[c]	0·09	0·64	.1·5 $C_2H_4O_2$.H_2O	51·84	6·72	14·72	1·03	1·92	1·00	1·08	
			(1 139)								
X	0·28	0·32	$C_{47}H_{71}N_{11}O_{14}S$.	50·72	7·04	13·28	0·97	1·00	1·00	1·02	
B	0·18	0·68	.$C_2H_4O_2$.3 H_2O	50·62	6·84	13·50	0·97	2·01	1·03	1·10	
			(1 160)								
XI	0·20	0·25	$C_{57}H_{88}N_{14}O_{15}S$.	50·92	7·12	15·23	0·98	2·03	1·02	0·99	
A	0·18	0·67	.$C_2H_4O_2$.5 H_2O	50·96	6·94	15·39	0·98	1·96	2·00	1·03	
			(1 392)								
XII	0·41	0·51	$C_{54}H_{85}N_{11}O_{14}S$.	54·53	7·62	12·95	1·05	1·00	0·96	0·99	0·94[d]
A	0·38	0·75	.2·5 H_2O	54·29	7·33	13·20	0·99	1·03	1·03	1·00	
			(1 189)								
XIII	0·34	0·44	$C_{50}H_{77}N_{11}O_{14}S$.	50·86	6·98	12·82	1·01	0·96	0·97	1·00	1·02[d]
	0·06	0·67	.$C_2H_4O_2$.4 H_2O	51·18	7·35	12·62	0·99	1·05	1·02	0·98	
			(1 220)								

[a] Ref.[1]; [b] ref.[5]; [c] hydrobromide; [d] ε-aminocaproic acid.

Procedure B: When the hydrochloride of an amine was used (50 μM), 7 μl more of N-ethylpiperidine were added to the reaction mixture.

[4-Glutamic Acid γ-Dimethylamide]deamino-1-carba-oxytocin (*IV*)

Compound *I* (5 mg) was subjected to reaction conditions described under procedure *A*, without adding an amine. Isolation and purification were done in the described way and the product of this reaction had the same behaviour as the impurity separated in other cases during gel-filtration.

[4-Glutamic Acid γ-Hydrazide]deamino-1-carba-oxytocin (*VIII*)

The protected hydrazide *VII* (10 mg) was dissolved in trifluoroacetic acid (0·3 ml) and after standing at room temperature for 80 min the solution was diluted with toluene (0·3 ml) and evaporated at room temperature. The residue was precipitated from methanol and ether, yield 8·2 mg. Part of this product (2 mg) was dissolved in methanol (0·1 ml) and 3M acetic acid (1 ml), purified by gel-filtration (Bio-Gel P—4), and used for biological tests.

[4-γ-Glutamoyl-ε-aminocaproic Acid]deamino-1-carba-oxytocin (*XIII*)

Compound *XII* (10 mg) was dissolved in trifluoroacetic acid (0·5 ml) and after standing for 1 h at room temperature the solution was evaporated; yield, 8 mg. Analytical data are given in Table II.

Pharmacological Methods

The uterotonic activity was determined on isolated strips of the rat uterus[7,8] suspended in Mg^{2+}--free solution. The galactogogic activity was assayed using lactating rats (5—10 days after parturition)[9,10].

We wish to thank Mrs H. Kovářová for performing some of the pharmacological tests. Our thanks are due to Mrs H. Farkašová for performing the amino-acid analyses. Elemental analyses were performed at the Analytical Department of our Institute headed by Dr J. Horáček.

REFERENCES

1. Lebl M., Jošt K.: This Journal *43*, 523 (1978).
2. IUPAC-IUB Commission on Biochemical Nomenclature. Biochemistry *6*, 362 (1967); Biochem. J. *126*, 773 (1972).
3. Pliška V., Rudinger J.: Clin. Endocrinol. *5*, 73 (1976).
4. Havran R. T., Schwartz I. L., Walter R.: Mol. Pharmacol. *5*, 83 (1969).
5. Jošt K.: This Journal *36*, 218 (1971).
6. Juillard J.: Pure Appl. Chem. *49*, 885 (1977).
7. Holton P.: Brit. J. Pharmacol. *3*, 328 (1948).
8. Munsick R. A.: Endocrinology *66*, 451 (1960).
9. Bisset G. W., Clark B. J., Haldar J., Harris M., Lewis G. P., Rochae Silva M.: Brit. J. Pharmacol. Chemother. *31*, 537 (1967).

10. Barth T., Jošt K., Rychlik I.: Endocrinol. Exper. *9*, 35 (1975).
11. Lebl M., Bojanovska V., Barth T., Jošt K.: Experientia *34*, 1543 (1978).
12. Lebl M., Bojanovska V., Barth T., Jošt K.: This Journal *44*, 2573 (1979).
13. Lebl M., Barth T., Jošt K.: This Journal *44*, 2563 (1979).
14. Walter R.: Fed. Proc., Fed. Amer. Soc. Exp. Biol. *36*, 1872 (1977).

Translated by L. Servitová.