NATRIURETIC EFFECTS OF OXYTOCIN ANALOGUES

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INTRODUCTION

Apart from studies concerning the character of the natural natriuretic hormone, another research program is in progress aiming at elucidating the renal sodium excretion evoked by peptides related to oxytocin. Most attention was paid to oxytocin analogues modified in positions 2 and 4 and to vasotocin analogue modified in position 4 (refs. 1-4). Machová and Jošt[5] reported that carba analogues of deamino-oxytocin had high natriuretic activity.

This communication describes the preparation and some biological activities of analogues of deamino-6-carba-oxytocin (a compound which has 3times higher natriuretic effect than oxytocin[5,6]) modified in position 2. By altering the amino acid in position 2, we attempted to prepare compounds with selective natriuretic potency.

EXPERIMENTAL

Materials. The following peptides were used in the study: Oxytocin, deamino-6-carba-oxytocin[7], [2-isoleucine]deamino-6-carba-oxytocin[8], [2-methionine]deamino-6-carba-oxytocin[8], [2-O-methyltyrosine]deamino-6-carba-oxytocin[8], [2-phenylalanine]deamino-6-carba-oxytocin[9], [2-O-ethyltyrosine]deamino-6-carba-oxytocin[9], [2-p-methylphenylalanine]deamino-6-carba-oxytocin[9], [2-p-ethylphenylalanine]deamino-6-carba-oxytocin[9], [2-p-N,N-dimethylaminophenylalanine]deamino-6-carba-oxytocin[9], [2-p-aminophenylalanine]deamino-6-carba-oxytocin[9] and [2-p-nitrophenylalanine]deamino-6-carba-oxytocin[9]. The carba analogues were prepared by a stepwise method of peptide synthesis in solution, mainly by using

Nps-aminoprotecting groups and active esters for the formation of the peptide bond. Cyclization of the linear octapeptides was also performed by the active ester method. The analogues were purified by HPLC with a reversed phase.

Methods. The uterotonic activity of the compounds was assayed on isolated rat uterine strips according to Holton[10] in Munsicks modification[11]. The pressor activity was determined using pithed male rats[12]. Natriuresis ($U_{Na}V$) was evaluated as total amount of sodium (mmol kg^{-1}) excreted during the experimental period (4h) by rats[6]. The compounds were administered s.c. in doses 0.2, 1.0, 5.0 and 25 ug/kg^{-1} of body weight of conscious rats. The water load (4% of the body weight) was administered by a stomach catheter. Urine was collected for 4h and the sodium content was determined by flame photometry. The dose-response relationship was evaluated statistically. Relative natriuretic effect is based on the comparison of the amount of excreted sodium after an individual dose of various analogues and deamino-6-carba--oxytocin (5 ug kg^{-1}).

RESULTS

The pharmacological data are summarized in Table 1. Natriuretic potency is expressed in % of activity of deamino--6-carba-oxytocin, uterotonic and pressoric activities are given in I.U./mg.

The replacement of tyrosine in position 2 by phenylalanine, isoleucine or methionine led to a decrease of all the activities studied. The introduction of phenylalanine into position 2 resulted in a 50% decrease of natriuretic potency and in a significant lowering of the uterotonic and pressoric potencies. The specificity of the natriuretic activity of the other two analogues is even higher as far the uterotonic and pressoric effects are concerned but the absolute values of natriuresis are low.

In the group of analogues in which the hydrogen of the tyrosine hydroxyl was substituted by an alkoxy group, [2-O--methyltyrosine]deamino-6-carba-oxytocin deserved special attention. This compound has approximately 50% of the natriuretic activity of deamino-6-carba-oxytocin but has no

Table 1

Selected pharmacological data of analogues of deamino-6--carba-oxytocin

Peptide	Natriur.[a] potency, %	Uterot.[b] potency, I.U./mg	Pressor. potency, I.U./mg	Ref.
Deamino-6-carba-oxytocin	100	929	1.5	7
Oxytocin	34	450	3.0	
[2-Phenylalanine]deamino--6-carba-oxytocin	47	75	0.9	9
[2-Isoleucine]deamino-6--carba-oxytocin	19	3.1	<0.02	8
[2-Methionine]deamino-6--carba-oxytocin	10	4.7	<0.02	8
[2-O-Methyltyrosine]deamino--6-carba-oxytocin	43	3.1	hypo	8
[2-O-Ethyltyrosine]deamino--6-carba-oxytocin	10	<0.05	<0.02	9
[2-p-Methylphenylalanine]-deamino-6-carba-oxytocin	105	70	1.0	9
[2-p-Ethylphenylalanine]-deamino-6-carba-oxytocin	86	27	<0.2	9
[2-p-Aminophenylalanine]-deamino-6-carba-oxytocin	30	13	<0.2	9
[2-p-N,N-Dimethylaminophe-nylalanine]deamino-6--carba-oxytocin	25	<0.05	<0.2	9
[2-p-Nitrophenylalanine]-deamino-6-carba-oxytocin	22	<0.05	<0.2	9

[a] % of natriuretic effect of deamino-6-carba-oxytocin

[b] method *in vitro*

detectable pressor activity.

In the last group of analogues, in which the tyrosine hydroxyl was replaced by different groups we found [2-p-methylphenylalanine]deamino-6-carba-oxytocin to be the

natriuretically most potent compound; it still has some of pressoric and uterotonic potency of the parent compound. The analogue containing an ethylgroup was also highly active as far natriuresis is concerned. In this case the natriuretic effect was rather specific, because the pressoric potency was almost negligible and uterotonic (in vivo 38.5 I.U./mg) potency represented only 2% of that of the deamino-6-carba-oxytocin.

REFERENCES

1. Chan,W.Y. and du Vigneaud, V., J. Pharmacol. Exptl. Therap. 174 (1970) 541.
2. Chan, W.Y., Endocrinology 77 (1965) 1097.
3. Hruby, V.J., Flouret, G. and du Vigneaud, V., J. Biol. Chem. 244 (1969) 3890.
4. Cort, J.H., Strub, K.M., Häuzler, G. and Rudinger, J., Experientia 29 (1973) 173.
5. Machová, A. and Jošt, K., Endocr. Exptl. 9 (1975) 269.
6. Hrbas, P., Barth, T., Škopková, J., Lebl, M. and Jošt, K., Endocr. Exptl. 14 (1980) 151.
7. Jošt, K. and Šorm, F., Coll. Czechoslov. Chem. Commun. 36 (1971) 234.
8. Lebl, M., Barth, T. and Jošt, K., Coll. Czechoslov. Chem. Commun. 45 (1980) 000.
9. Lebl, M. et al., Coll. Czechoslov. Commun. in press.
10. Holton, P., Brit. J. Pharmacol. 3 (1948) 328.
11. Munsick, R.A., Endocrinology 66 (1960) 451.
12. Krejčí, I., Kupková, B. and Vávra, I., Brit. J. Pharmacol. Chemotherap. 30 (1967) 497.