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RENAL SODIUM EXCRETION IN RATS. EFFECT OF AMINO ACID REPLACEMENT IN POSITION 4 OF THE OXYTOCIN MOLECULE

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INTRODUCTION

The effect of oxytocin on the excretion of sodium by mammalian kidneys has been intensively studied lately. The main interest centers on the investigation of the action mechanisms of oxytocin on the one hand, and on the determination of the relation between the primary structure of the hormone or its analogues and sodium excretion on the other. Several oxytocin analogues with pronounced natriuretic action have been prepared by modifying positions 2 and 4 (refs. 1,2), position 1 and the disulfide bridge [3, 4] of the oxytocin molecule. It became apparent that the mentioned higher natriuretic action of the analogues modified in position 4 was dependent on the experimental arrangement[5-7]. The present communication deals with the effect of a number of structural modifications of the parent hormone on its activity to influence the total sodium excretion of conscious rats with standard 4% water load.

MATERIALS AND METHODS

Oxytocin and its analogues were prepared at the Department of Organic Synthesis of this Institute. The following analogues were tested: deamino-oxytocin[8], 1-carba-oxytocin[9], deamino-1-carba-oxytocin[10], deamino-6-carba-oxytocin[11], [4-leucine] oxytocin[12], [4-glutamic]acid deamino-1-carba-oxytocin[13], [4-leucine]-deamino-1-carba-oxytocin[14], [4-isoleucine] deamino-1-carba-oxytocin[14], [4-valine]deamino-1-carba-oxytocin[14], [4-glutamic acid methyl ester]deamino-1-carba-oxytocin[15], [2-O-methyltyrosine]oxytocin[16], [2-O-methyltyrosine]deamino-1-carba-oxytocin[17], [2-isoleucine]deamino-1-carba-oxytocin[17], [2-phenylalanine]deamino--1-carba-oxytocin[17], [9-desglycine]oxytocin[18]. Tocinamide[19] was

supplied by Dr.M. Flegel from Léčiva, Prague.

For the determination of the natriuretic activity of oxytocin and its analogues, we used male rats of the Wistar strain, weighing 180-190 g, adapted for one week prior to the experiment to the experimental conditions. The compounds tested were dissolved in physiological saline and administered s.c. in doses of 1-30 ug/kg of body weight after the water load, corresponding to 4% of body weight, had been applied by means of a stomach cathether. The control group of animals was given s.c. injections of saline. The rats were placed in metabolic cages, urine was collected at 30 min intervals for four hours and analysed for the content of sodium. The natriuretic potency was expressed as $U_{Na}V$(meq/kg of body weight in 4 hours). Each group of 5 rats was submitted three times to the experiment and no group received the same compound twice.

RESULTS

The natriuretic effect of the given compounds was determined for a relatively wide range of doses. Table I presents the natriuretic effect of the individual compounds applied in doses of 5 ug/kg of body weight. The natriuretic potency increased when the primary amino group of cysteine in position 1 of the oxytocin peptide chain was substituted by hydrogen (deamino-oxytocin), the sulfur atom was replaced by a methylene group and when the two modifications were combined. The most effective analogues were deamino-1-carba-oxytocin and deamino-6-carba-oxytocin. In the deamino-1-carba-oxytocin series, an estimation was made of the effect of the substitution of the amino acids in position 2 and 4. The substitution of glutamine in position 4 by glutamic acid, glutamic acid methyl ester, isoleucine and valine decreased the natriuretic potency of the resultant analogues to values lower than those of oxytocin. The most significant decrease was caused by the replacement of glutamine with leucine; in the case of oxytocin, sodium excretion decreased by one order of ten and in the case of deamino-1-carba-oxytocin, natriuretic activity was almost eliminated in the range of doses studied. A 50% decrease of natriuretic potency was observed in the case of analogues of deamino-1-carba-oxytocin that had

TABLE I
NATRIURETIC EFFECT OF OXYTOCIN ANALOGUES

Compound	$U_{Na}V$	Activity,%
Oxytocin	1.53	100.0
Deamino-oxytocin	2.02	132.0
1-Carba-oxytocin	2.28	149.1
Deamino-1-carba-oxytocin	3.00	193.8
Deamino-6-carba-oxytocin	4.56	298.0
[4-Leucine]oxytocin	0.19	12.0
[4-Leucine]deamino-1-carba-oxytocin	0.00	0.0
[4-Isoleucine]deamino-1-carba-oxytocin	1.00	65.4
[4-Valine]deamino-1-carba-oxytocin	0.25	16.0
[4-Glutamic acid]deamino-1-carba--oxytocin	0.21	14.0
[4-Glutamic acid methyl]ester deamino--1-carba-oxytocin	0.32	20.8
[2-O-Methyltyrosine]oxytocin	1.56	102.2
[2-O-Methyltyrosine]deamino-1-carba--oxytocin	1.08	70.8
[2-Isoleucine]deamino-1-carba-oxytocin	1.96	128.1
[2-Phenylalanine]deamino-1-carba--oxytocin	2.00	131.3
Tocinamide	0.44	28.7
[9-Desglycine]oxytocin	0.21	13.7

O-methyltyrosine, phenylalanine or isoleucine instead of tyrosine in position 2. However, the substitution of tyrosine of oxytocin by O-methyltyrosine did not alter the natriuretic activity. The shortening of the oxytocin peptide chain at the C-terminus decreased the natriuretic potency of the resultant analogue.

CONCLUSION

The experiments described in this communication help to clarify the relation between the chemical structure and natriuretic potency of oxytocin analogues. A remarkable decrease of the natriuretic potency was brought about by the substitution of glu-

tamine in position 4 by glutamic acid or by amino acids with hydrophobic side chains; a similar result was obtained when the linear part of the oxytocin molecule was shortened. Although modifications of the amino acid in position 2 did not result in analogues with higher natriuretic activity, several derivatives of deamino-1-carba-oxytocin were obtained that had higher natriuretic activity than oxytocin. The fact that the analogues with modifications in position 2 have relatively high natriuretic effect indicated the possibility of preparing analogues with high specific natriuretic activity. Results of these studies are presented in the following paper.

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