Effect of Some Oxytocin Analogues on Natriuresis in Rats

P. HRBAS, T. BARTH, J. ŠKOPKOVÁ, M. LEBL, K. JOŠT

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

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Non-anaesthetized rats were used for studying the relationship between the amount of sodium excreted and structural modifications of oxytocin molecule. Any change performed in position 4 (i.e. the glutamine residue) resulted in a decrease of natriuretic activity as compared to that of oxytocin. The analogues with modifications in the amino-terminal part of the molecule (e.g. substitution of the amino group in position 1 by hydrogen, or of the disulfide bond by a thioether group) resulted in a higher natriuretic effect than oxytocin.

The neurohypophysial hormone, oxytocin, is known for its typical uterotonic and galactogogic activities. Its other effects are more and more becoming the object of intesive studies. The participation of oxytocin in the regulation of renal sodium excretion is studied from the viewpoint of possible therapeutical application. Although the natriuretic effect is often masked by the antidiuretic effect, it can be observed under various experimental conditions, differing in the mode of application of the hormone [Chan 1965; Machová 1971; Škopková et al. 1973; Chan 1976]. A number of oxytocin analogues[1] with higher natriuretic effect than the parent hormone were prepared and, in some cases, they even showed diuretic activity. These properties were observed mainly in the case of analogues containing amino acids with lipophilic side chains in positions 2 and 4 [summaried by Chan 1976; Walter et al. 1977], but also in other cases (for instance [2-0-methyltyrosine] oxytocin [Machová 1971] or deamino-analogues [Chan 1965; Cort et al. 1972[). Significantly higher natriuretic activity was observed in the case of so-called carba-analogues

[1] The nomenclature of the analogues is based on the published suggestion: Biochemistry 6, 362—364, 1967.

that have a sulfur of the disulfide bridge substituted by a methylene group [Machová and Jošt 1975].

The present paper describes the effect of several oxytocin analogues on total sodium excretion of water-loaded non-anaesthetized rats as compared with the effect of oxytocin.

Materials and Methods

The natriuretic action of oxytocin and its analogues was determined in experiments on non-anaesthetized male Wistar rats. The experiment itself was preceded by one week of adaptation to the experimental procedure, i.e. to cathetrization with a polyethylene cannula, injections in the inguinal region and to the stay in metabolic cages. This treatment, as well as the experiment itself, was performed at three-day intervals. At the end of adpatation period the average weight of rats was 190 g. For 18 h before the experiment, the animals received no food but had free access to water. The compounds tested were applied subcutaneously in doses of 1—300 $\mu g\ kg^{-1}$ in saline after the administration of a mild (4%) water load using a stomach tube. The control groups of animals received saline. The rats were observed for 4 h, samples of urine being taken at 30 min intervals and analyzed for the content of sodium

Tablo 1

Structural modifications of oxytocin analogues

Symbol	Compound	X^1	X^2	R	Synthesis[1)]
Ia	Oxytocin	S-S	NH_2	Gln	—
Ib	Deamino-oxytocin	S-S	H	Gln	[3)]
Ic	Deamino-1-carba-oyxtocin	CH_2-S	H	Gln	[3)]
Id	1-Carba-oxytocin	CH_2-S	NH_2	Gln	[4)]
Ie	[Leu^4]oxytocin	S-S	NH_2	Leu	[5)]
If	[Glu^4]deamino-1-carba-oxytocin	CH_2-S	H	Glu	[6)]
Ig	[Glu(NHNH$_2$)^4]deamino-1-carba-oxytocin	CH_2-S	H	Glu(NHNH$_2$)	[7)]
Ih	[Glu(GlyNH$_2$)^4]deamino-1-carba-oxytocin	CH_2-S	H	Glu(GlyNH$_2$)	[7)]
Ii	[Glu(OCH$_3$)^4]deamino-1-carba-oxytocin	CH_2-S	H	Glu(OCH$_3$)	[8)]
Ij	[Glu(GlyOCH$_3$)^4]deamino-1-carba-oxytocin	CH_2-S	H	Glu(GlyOCH$_3$)	[7)]

[1)] The compounds used had the same chemical, physico-chemical and biochemical properties as stated in the individual references;

[2)] du Vigneaud et al. [1960]; [3)] Jošt 1971]; [4)] Jošt et al. [1973]; [5)] Hrubý et al. [1969]; [6)] Lebl and Jošt [1978]; [7)] Lebl et al. [1979a]; [8)] Lebl et al. [1979b].

by means of a flame photometer. Natriuresis (U Na V) was evaluated as total amount of sodium (mmol kg^{-1}) excreted during experimental period (4 h). The rats were submitted three times to the experiment and no group received the same compound twice.

Oxytocin (Fig. 1) was purchased from LÉČIVA (Praha). The other compounds (Tab. 1) were synthetized in this Institute.

Table 2

Comparison of total amount of excreted sodium during the experimental period (4 h) after an individual dose of various analogues (5 μg kg^{-1}) (n = 15); oxytocin activity = 100 %

Compound	$U_{Na}V$ (a. m ± S.E.)	Activity % of Ia
Ia	1.530 ± 0.261	100
Ib	2.024 ± 0.492	132
Ic	2.996 ± 0.289	194
Id	2.283 ± 0.300	149
Ie	0.185 ± 0.076	12
If	0.206 ± 0.083	14
Ig	1.273 ± 0.107	83
Ih	0.503 ± 0.191	33
Ii	0.319 ± 0.204	21
Ij	0.693 ± 0.220	45
Control	0.084	

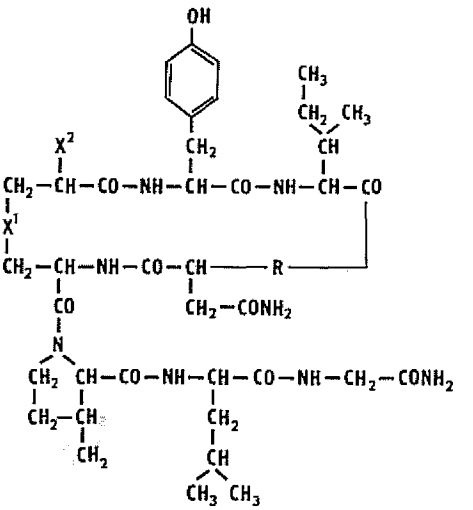

Fig. 1. Structural formula of oxytocin.

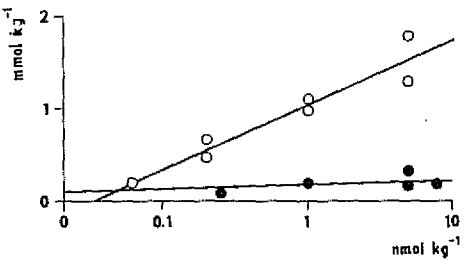

Fig. 2. Dependence of natriuresis ($\Sigma U_{Na}V$ [mmol Na kg^{-1}]) on the log dose of oxytocin (○) and Ie (●). The corresponding regression lines are $y = 0.703x - 0.366$; $r^2 = 0.86$ (oxytocin), $y = 0{,}045x + 0.062$; $r^2 = 0.22$ (Ie).

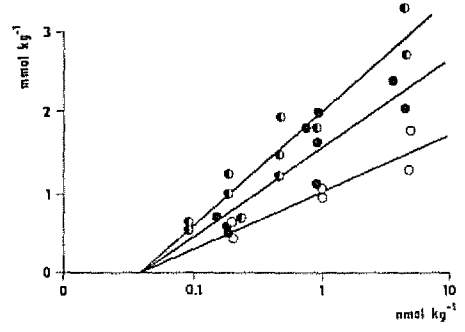

Fig. 3. Dependence of natriuresis ($\Sigma U_{Na}V$ [mmol Na kg^{-1}]) on the log dose of Ia (○), Id (●), Ic (×). The regression lines for Id and Ic are: $y = 0.956x - 0.480$; $r^2 = 0.070$ and $y = 1.282x - 0.636$; $r^2 = 0.74$, resp.

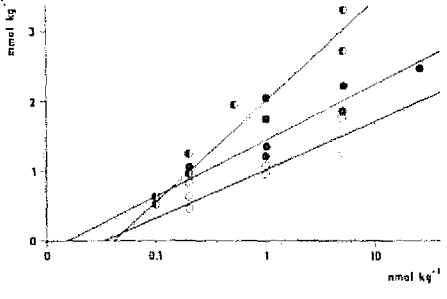

Fig. 4. Dependence of natriuresis ($\Sigma U_{Na}V$ [mmol Na kg^{-1}]) on the log dose of Ia (○), Ib (●) and Ic (×). The regression line for Ib is $y = 0.827x - 0.120$; $r^2 = 0.35$.

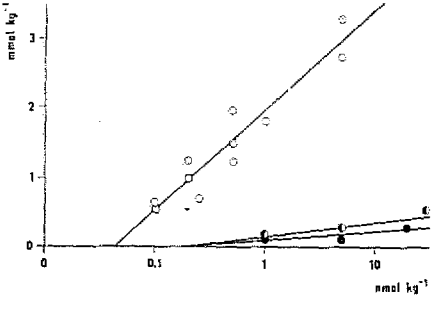

Fig. 5. Dependence of natriuresis ($\Sigma U_{Na}V$ [mmol Na]) on the log dose of compounds Ic (○), If (●) and Ig (×). The regression lines for If and Ig are: $y = 0.145x - 0.186$; $r^2 = 0.56$ and $y = 0.290x - 0.254$; $r^2 = 0.68$, resp.

In order to verify the effect of introducing an amino acid with alifatic side chain into the oxytocin molecule (Fig. 1; Ia) on natriuretic potency [cf. Chan 1976] under our experimental conditions, we tested an oxytocin analogue (Ie) in which the glutamine residue in position 4 had been replaced by leucine. As shown in Fig. 2, this modification results in a considerable decrease of the amount of sodium excreted. This confirms the previous data [Machová and Jošt 1975] showing that the substitution of the disulfide bond by thioether group in combination with the replacement of α-amino group of cysteine in position 1 by hydrogen (analogue Ic) considerably increases the natriuretic activity (Fig. 3). Two structural modifications of the oxytocin molecule showed a cumulative effect. This is demonstrated by the fact that both the analogue Ib (Fig. 4) and Id (Fig. 3) showed a higher natriuretic potency than oxytocin (Ia) under the experimental conditions used, but lower than that of compound Ic. All the derivatives of deamino-1-carba-oxytocin (Ic), where the side chain of glutamine in position 4 had been modified in various ways (If and Ig), had lower natriuretic potency than oxytocin (Fig. 5). The same holds for the analogues Ih — Ij. The activities of oxytocin and its analogues are given in Tab. 2.

Discussion

The oxytocin molecule (Ia) has amino acids with hydrophilic side chains in positions 2, 4, and 5 (Tyr, Gln, Asn), and isoleucine with a markedly hydrophobic side chain in position 3. It was assumed [Chan and du Vigneaud 1970] that an enhancement of the hydrophobic character of this part of the molecule is a structural prerequisite for obtaining an oxytocin analogue with higher natriuretic activity than the parent hormone. However, further experiments showed [Chan 1976] that during mannitol diuresis the oxytocin shows at least three times higher natriuretic activity than analogue Ie. This led to the conclusion that the structural modification did not cause any increase of natriuretic activity, but eliminated the structural features of the oxytocin molecule that were responsible for the antidiuretic effect. Even during water diuresis, the natriuretic activity of analogue Ie is lower than that of oxytocin by almost one order of ten. Moreover, the experimental arrangement used [Chan and du Vigneaud 1970], namely the anaesthesia, operation, high water load, and the criterion for natriuresis, was not very suitable for the evaluation of natriuresis.

In previous experiments [Barth et al. 1973] it was found that the substitution

of disulfide bond by a thioether group in combination with the replacement of α-amino group of cysteine increased oxytocin activities. Recently, we found that the same applies to natriuretic activity [Machová and Jošt 1975]. It seems likely that this type of structural change is more promising for obtaining analogues with high natriuretic potency than the introduction of alifatic amino acids into positions 2 and 4 of the oxytocin molecule. The two analogues described [Machová and Jošt 1975], namely deamino-1-carba-oxytocin and deamino-6-carba-oxytocin, still possess the activities typical for neurohypophysial hormones (i.e. uretonic and antidiuretic), in some cases even higher than the parent hormone. We therefore concentrated on searching for oxytocin analogues that would have a high natriuretic potency with no other activities. Substitutions of position 4 of the prototype analogue Ic by alifatic amino acids are described in another paper [Lebl et al., in press]. In this paper we deal with analogues that have the amide group of glutamine modified in various ways. The presence of a free carboxyl group (in compound If) causes a considerable decrease of all activities [Lebl et al. 1979a, b] including the natriuretic effect. The decrease of natriuresis was significantly smaller in the case of the corresponding (i.e. weakly alkaline) hydrazide Ig. Methyl ester Ii also had a lower natriuretic effect, which could have been due (with regard to the mode its administration) to hydrolysis and the formation of weekly active acid If. Both the analogues that had their peptide chain lengthened by a residue of glycine methyl ester (Ij) or glycine amide (Ih) showed less than half of natriuretic activity of oxytocin. It can be seen that all the modifications in position 4 mentioned in this paper resulted in analogues with lower natriuretic activity than that of the natriuretically effective analogue deamino-1-carba-oxytocin, and even than that of oxytocin.

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