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RENAL SODIUM EXCRETION IN RATS. EFFECT OF STRUCTURAL MODIFICATION IN POSITION 2 OF DEAMINO-6-CARBA-OXYTOCIN

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INTRODUCTION

Some attemps were made to account for the mechanism of renal natrium excretion. A hypothesis assuming the regulatory action of a "natriuretic hormone" could explain certain states, whether natural of experimentally evoked. This substance has so far eluded isolation and characterization and its precise mechanism of action at the molecular level is at present largely unknown. In fact, it was suggested that oxytocin acts either directly as the natriuretic hormone[1], or indirectly by causing the liberation of another low molecular weight substance from some intracranial structure, which is responsible for increasing the rejection fraction of sodium[2]. While further separation work is under way[3], a program of studying the structure-activity relation of synthetic oxytocin analogues is also in progress, aiming at specifying which structural features of the peptide molecule are effectual in producing a natriuretic response. Chan[4] assumed that modifications in position 4 of the oxytocin molecule could result in natriuretically potent analogues. This supposition was not confirmed by the experimental data obtained so far, on the contrary, modifications of the S-S bridge, i.e. carba-substitution, combined with the replacement of the primary amino group by hydrogen have been found promisable. Among the analogues of this type, deamino-6--carba-oxytocin had the highest natriuretic potency[5]. However, the higher natriuretic activity was accompanied by increased uterotonic and pressor activites[6]. As it was found that certain modifications in position 2 led to a decrease of the uterotonic and pressor activities[7,8], further synthetic work was aimed in this direction. A number of analogues modified in position 2 were

produced; their natriuretic potency and specificity are described in this paper.

MATERIALS AND METHODS

Oxytocin and its analogues were prepared at the Department of Organic Synthesis of this Institute. The following analogues were studied: deamino-6-carba-oxytocin[9], [2-isoleucine]deamino-6-carba--oxytocin[10], [2-methionine]deamino-6-carba-oxytocin[10], [2-O-methyltyrosine]deamino-6-carba-oxytocin[10], [2-phenylalanine]deamino--6-carba-oxytocin[11], [2-O-ethyltyrosine]deamino-6-carba-oxytocin[11] [2-p-methylphenylalanine]deamino-6-carba-oxytocin[11],[2-p-benzyloxycarbonylaminophenylalanine]deamino-6-carba-oxytocin[11], [2-p--ethylphenylalanine]deamino-6-carba-oxytocin[11], [2-p-N,N-dimethylaminophenylalanine]deamino-6-carba-oxytocin[11], [2-p-aminophenylalanine]deamino-6-carba-oxytocin[11], [2-p-nitrophenylalanine]deamino-6-carba-oxytocin[11].

Natriuresis was assayed using a modification of Burn`s test described elsewhere[12] performed on conscious rats. The compounds were administered s.c. in doses of 0.2, 1, 5 and 25 ug/kg of body weight in 1 ml of saline/kg after receiving a water load (4% of body weight). Urine was collected for 4 hours and the sodium content was determined by means of a flame photometer. Natriuretic activity was expressed as $U_{Na}V$ (meq/kg of body weight in 4 hours) and its dependence on the peptide dosage was evaluated statistically.

The uterotonic activity of the compounds was assayed on isolated rat uterine strips according to Holton[13] in Munsick`s modification.

RESULTS

The modifications performed in position 2 of deamino-6-carba-oxytocin can be divided into three groups.

The substitution of tyrosine by phenylalanine, isoleucine and methionine led to a decrease of natriuretic activity. The analogue containing phenylalanine in position 2 had the highest natriuretic activity of this group; it had almost half the natriuretic potency of deamino-6-carba-oxytocin (Figure 1, Table I).

Substitution of the hydrogen of tyrosine hydroxyl by an alkoxy

group. The most active analogue of this group was [2-O-methyl-tyrosine]deamino-6-carba-oxytocin with approximately half the natriuretic activity of deamino-6-carba-oxytocin. The activity abruptly decreased when the size of the substituent was increased; the analogue containing the benzyloxycarbonylamino group was without any activity (Table I).

Substitution of the tyrosine hydroxyl by nitro-, amino-, dimethylamino-, methyl- and ethyl-groups. In this series, the most active analogue was [2-p-methylphenylalanine]deamino-6-carba-oxytocin; its activity was even higher than that of the parent molecule. The analogue containing an ethyl group was also active, whereas the activity of the other analogues containing polar groups was pronouncedly lower (Table I, Figure 2).

Table I presents the relative natriuretic potency expressed in % of activity of deamino-6-carba-oxytocin and the uterotonic activity.

Natriuresis evoked by the neurohypophysial hormones is a complex response involving tubular and vascular components, and there is an ongoing discussion as to whether the natriuretic activity of oxytocin and vasopresin is in direct relation to their pressor-vascular and antidiuretic effects. The synthesis of analogues the activities of which are dissociated can be seen as a prerequisite for analysing the mechanism of natriuresis evoked by neurohypophysial hormones.

CONCLUSION

The modifications of the oxytocin molecule mentioned in the introduction (i.e. carba substitution and deamination) led to an increase of natriuresis, but also to an increase of the uterotonic and pressor activities[6]. The alterations performed in position 2 of deamino-6-carba-oxytocin suppressed these undesirable effects. All the analogues studied had significantly lower uterotonic activity (cf Table I). The pressor effect was also decreased (with the exception of [2-phenylalanine]deamino-6-carba-oxytocin); the analogues studied did not influence the systemic blood pressure [10,11] in doses up to 200 ug/kg of body weight. In the first group of analogues in which the whole amino acid had been substituted, the decrease of these activities was accompanied

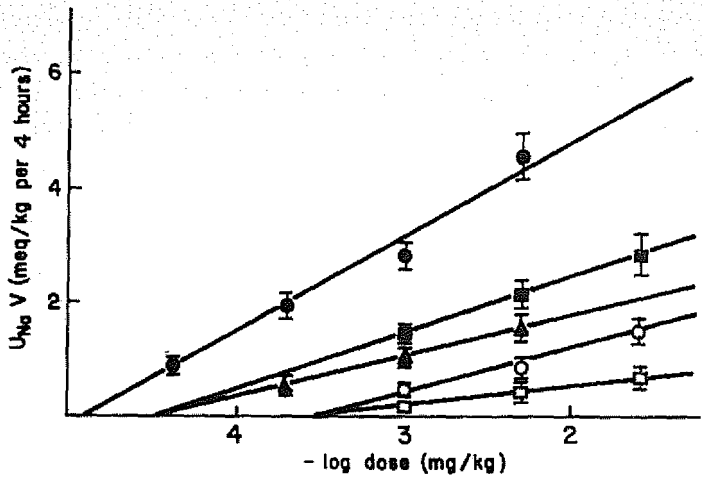

Fig. 1. Log dose - natriuretic response curves for deamino-6--carba-oxytocin ● , oxytocin ▲ , [2-phenylalanine]deamino-6-carba--oxytocin ■ ,[2-isoleucine]deamino-6-carba-oxytocin O and [2-methionine]deamino-6-carba-oxytocin □ .

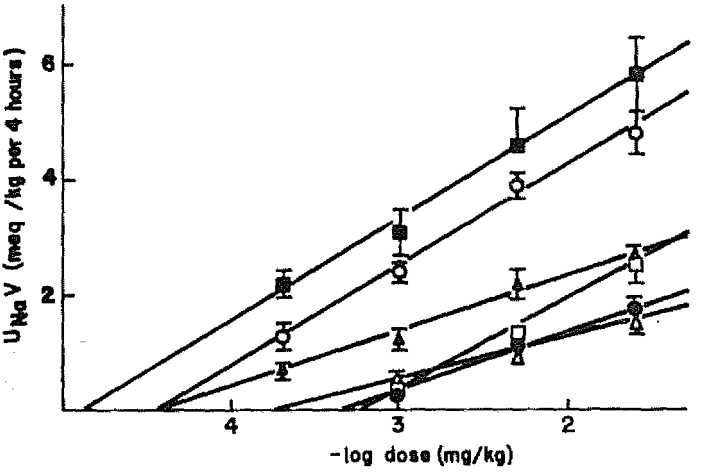

Fig. 2. Log dose - natriuretic response curves for [2-O-methyltyrosine]deamino-6-carba-oxytocin ▲ , [2-p-methylphenylalanine]-deamino-6-carba-oxytocin ■ , [2-p-ethylphenylalanine]deamino-6--carba-oxytocin O , [2-p-aminophenylalanine]deamino-6-carba-oxytocin □ , [2-p-N,N dimethylaminophenylalanine]deamino-6-carba--oxytocin ● and [2-p-nitrophenylalanine]deamino-6-carba-oxytocin .

TABLE I

COMPARISON OF UTEROTONIC AND NATRIURETIC ACTIVITIES OF DEAMINO--6-CARBA-OXYTOCIN AND ITS DERIVATIVES

Compound	Natriuretic activity, NA $U_{Na}V$	%	Uterotonic activity, UA I.U./mg	NA/UA ratio
Deamino-6-carba-oxytocin	4.54 ± 0,36*	100	929	0.005
Oxytocin	1.53 ± 0.26	34	450	0.003
[2-Phenylalanine]deamino--6-carba-oxytocin	2.15 ± 0.38	47	75	0.029
[2-Isoleucine]deamino-6--carba-oxytocin	0.87 ± 0.11	19	3.1	0.279
[2-Methionine]deamino-6--carba-oxytocin	0.43 ± 0.07	10	4.7	0.091
[2-O-Methyltyrosine]deamino--6-carba-oxytocin	0.18 ± 0.21	48	3.1	0.694
[2-O-Ethyltyrosine]deamino--6-carba-oxytocin	0.45 ± 0.09	10	< 0.05	9.020
[2-p-Benzyloxycarbonylamino-phenylalanine]deamino-6-carba-oxytocin	0.15 ± 0.05	**	0.07	2.086
[2-p-Methylphenylalanine]-deamino-6-carba-oxytocin	4.75 ± 0.48	105	70	0.068
[2-p-Ethylphenylalanine]-deamino-6-carba-oxytocin	3.92 ± 0.18	86	27	0.145
[2-p-Aminophenylalanine]-deamino-6-carba-oxytocin	1.34 ± 0.13	30	< 0.05	26.880
[2-p-N,N-Dimethylaminophe-nylalanine]deamino-6-carba-oxytocin	1.15 ± 0.11	25	< 0.05	23.100
[2-p-Nitrophenylalanine]-deamino-6-carba-oxytocin	1.00 ± 0.14	22	< 0.05	20.060
Controls	0.08			

*Arithmetic mean values ± SE

**The dose-response relation is not statistically significant

with a pronounced decrease of natriuretic activity, as compared with deamino-6-carba-oxytocin. The substitution of hydrogen in the hydroxyl group of tyrosine brought about a further decrease of the uterotonic and pressor activities. The substitution of the whole hydroxyl group of tyrosine resulted in a slight decrease of the uterotonic activity and in a pronounced decrease of the pressor activity (lower than 0.2 IU/mg). On the other hand, the natriuretic activity of the ethyl derivate was only slightly lower and that of the methyl derivative was even higher than the parent molecule. The most advantageous ratio of natriuretic and pressor activities, with insignificant pressor activities (0.2 IU/mg) was obtained in the case of [2-p-aminophenylalanine]deamino-6-carba--oxytocin. This group of analogues therefore yielded compounds with specific natriuretic action without systemic pressor activity and with very low uterotonic activity. At present, we do not know whether the analogues change the local hemodynamic parameters in the kidney, which part of the nephron it effects, or to what degree it influences renal Na,K-ATPase. Further studies should decide whether therapeutical application is promissable.

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