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INTERACTIONS OF DEAMINO-6-CARBA-OXYTOCIN ANALOGUES IN RAT KIDNEY AND LIVER MEMBRANE SYSTEMS

Tomislav Barth, Michal Lebl, Karel Jošt
Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia

Bernard Cantau, Daniel Butlan, Gilles Guillon, Serge Jard
Laboratoire de Physiologie Cellulaire, Collège de France, Paris 5, France

Introduction

The fact that a number of oxytocin analogues derived from deamino-6-carba-oxytocin have specific natriuretic activity (1,2) led us to investigate the character of the interaction of these compounds with the adenylate cyclase system in the mammalian kidney and with receptors in liver cell membranes. The binding to the membrane fraction of the kidney medulla and the activation of adenylate cyclase is in correlation with the antidiuretic (natriuretic) action of the compounds, while the binding to the liver membranes can serve as a measure of the ability of the analogues to affect blood pressure.

Material and Methods

Oxytocin (I), deamino-6-carba-oxytocin (II) (ref. (3)), [2-p-methylphenylalanine]deamino-6-carba-oxytocin (III), [2-p-ethylphenylalanine]deamino-6-carba-oxytocin (IV), [2-phenylalanine]deamino-6-carba-oxytocin (V), [2-p-ethyl-tyrosine]deamino-6-carba-oxytocin (VI), [2-p-aminophenylalanine]deamino-6-carba-oxytocin (VII), [2-p-nitrophenylalani-

ne]deamino-6-carba-oxytocin (VIII) were prepared at the Prague Institute (2).

[2-[^{3}H]Tyrosine-8-lysine]vasopressin (^{3}H-LVP) was prepared and purified according to (4,5). The binding of analogues to liver membranes (6) was followed by determining the ability of the individual analogues to compete for binding sites with ^{3}H-LVP (7). The method for measuring the binding of analogues to the membrane fraction of the rat kidney medulla and the activation of adenylate cyclase was the same as described in papers (8) and (9).

Results

In a number of oxytocin analogues, deamination and carba-substitution of the bridge leads to an increase of the natriuretic effect. The modification of the p-position of tyrosine resulted not only in an absolute change of the natriuretic effect but also affected other biological responses. As can be seen in Table I, the modifications performed in the p-position of a series of analogues of deamino-6-carba-oxytocin resulted in a decrease of the affinity to the liver receptor system. Although the decrease in affinity was not dramatic - at the most by 1.5 order as compared with oxytocin - the compounds were found to be only weak antagonists of ^{3}H-LVP binding (pK_D = 8.33) in this system.

In accordance with these data, the pressor action of the analogues was also lower than that of oxytocin (2). The character of the dependence of the binding and the adenylate cyclase activation on the molecular structure is somewhat different in the renal system. The substitution of the hydroxyl group by methyl or ethyl groups increased the affinity and activation ability of both compounds (III, IV), whereas the other modification decreased the affinity ten times and the activation ability in some cases even more (VI and VIII). The anti-

Table I

Characteristics of binding and activation constants of oxytocin analogues

Compound	Liver membranes	Renal system		Natriuresis
		Binding	Adenylate cyclase	
	pK_D	pK_D	pK_D	%
I	7.11	7.47	7.21	100[a]
II	7.71	7.49	7.43	298
III	6.48	7.80	7.54	326
IV	6.25	7.60	7.48	255
V	5.37	6.28	6.72	131
VI	5.86	6.39	5.70	31
VII	5.36	6.62	6.72	87
VIII	5.86	6.57	5.96	66

[a] The natriuretic potency of oxytocin was taken as a basis for calculations.

diuretic potency is in agreement with the tendency of changes of the activation constants for the adenylate cyclase system; compounds III and IV have potencies in the range of tens of antidiuretic units per mg, the other compounds have a lower activity than that of oxytocin. As can be seen from the results presented in Table I, compounds II-IV have 2.5-3 times higher natriuretic potency than oxytocin (I), the others have 30-130% of the activity of oxytocin. In most of the structural modifications performed the lipophilic properties of the analogue molecule were enhanced; this effect was most pronounced in the case of compounds II-IV. Although these changes result in higher affinity to renal receptors and more pronounced activation of adenylate cyclase (compare the pK_D and pK_A values for oxytocin and compounds II and IV and

their natriuretic action), the higher natriuretic and antidiuretic potency (2) of these compounds is apparently brought about by altered distribution and elimination leading to the prolongation of the individual responses, rather than by increased affinity and by the activation of adenylate cyclase in kidneys.

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