ACTIVATION OF ADENYLATE CYCLASE FROM RAT RENAL MEDULLA BY SOME OXYTOCIN ANALOGS

Věra KLENEROVÁ[a], Miroslav MUNCLINGER[a], Sixtus HYNIE[b], Tomislav BARTH[c] and Karel JOŠT[c]

[a] *Institute of Pathological Physiology,*
[b] *Institute of Pharmacology,*
Faculty of General Medicine, Charles University, 128 00 Prague 2 and
[c] *Institute of Organic Chemistry and Biochemistry,*
Czechoslovak Academy of Sciences, 166 10 Prague 6

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The ability of carba-analogs of oxytocin to activate adenylate cyclase was followed on the crude enzyme preparation from rat renal medulla. Their stimulating effects on adenylate cyclase activity were in a correlation with their antidiuretic potency. There is discussed the dependence of the affinity of carba-analogs of oxytocin to stimulate adenylate cyclase and their antidiuretic potency on the character of changes in the primary structure of these drugs.

Antidiuretic effect of [8-arginine]-vasopressin* and its synthetic analogs in mammalian kidney is mediated by cyclic AMP which is formed by adenylate cyclase in renal medulla[2]. The final antidiuretic effect of individual vasopressin analogs is dependent, in addition to the factors which influence the interaction of the particular drug with the hormonal receptor (functional part of the adenylate cyclase system), also on the pharmacokinetic factors which determine the distribution and metabolism of the analogs under study possessing changes in their primary structure. It has been shown by several authors[3-5] that some analogs with higher antidiuretic activity than natural vasopressins reveal lower stimulation of adenylate cyclase than the latter compounds. Thus, it seems that the synthesis of new therapeutically useful derivatives should be directed on the basis of estimation of parameters of interaction of individual analogs with adenylate cyclase system and the length of their antidiuretic effect[6], both in relation to their chemical structure. In this paper we describe the effects of some new synthetic analogs of oxytocin on adenylate cyclase preparation from rat renal medulla, which revealed high sensitivity of the hormonal stimulation, in spite of its relatively simple preparation.

* The nomenclature of amino acids and peptides is given according to published suggestions[1].

EXPERIMENTAL

Materials

[8-Arginine]-vasopressin (*Ia*) was supplied by Koch-Light, England. Deamino-1-carba-oxytocin[7] (*Ib*), [2-isoleucine]-deamino-1-carba-oxytocin[8] (*Ic*), [2-phenylalanine]-deamino-1-carba-oxytocin[8] (*Id*), [2-O-methyltyrosine]-deamino-1-carba-oxytocin[8] (*Ie*), deamino-dicarba-oxytocin[9] (*If*), [1-6-deaminolanthionine]-oxytocin[10] (*Ig*), [1-6-α-aminopimelic acid]-oxytocin[10] (*Ih*), N^{α}-acetyl-[2-O-methyltyrosine]-oxytocin[11] (*Ii*) and triglycyl-[8-lysine]-vasopressin[12,13] (*Ij*) were prepared in the Department of Organic Synthesis, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences.

Phospho(enol)pyruvate tricyclohexylamine salt, pyruvate kinase from rabbit muscle, type II, myokinase from rabbit muscle, grade III, and N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid (HEPES) were supplied by Sigma, U.S.A. Adenosine 3′,5′-cyclic phosphate was a product of SERVA, U.S.A. and adenosine 5′-triphosphate was purchased from LOBA-Chemie, Austria.

[α^{32}P]-ATP (500—3000 mCi/mmol) was a product of The Radiochemical Centre, Amersham, England, adenosine [^{3}H] (G)-3′,5′-cyclic phosphate (spec. activity 24 Ci/mmol) was supplied by New England Nuclear, Boston, U.S.A. Neutral aluminium oxide for chromatographic adsorption analysis according to Brockmann (act. II) was a product of Reanal, Hungary. Other chemicals used were from the commonly available sources.

Enzyme Preparation

White male rats of the Wistar strain (Velaz, Prague), weighing about 180 g and fed *ad libitum*, were used in all our experiments. After killing the rats by decapitation the kidneys were dissected and frozen on dry ice. After removing the renal cortex and papilla, 100 mg of the medulla was homogenized with 5 ml of 25 mM-$MgCl_2$ in a Dounce, all glass tissue grinder. This fraction was used both directly for protein determination[14] and after mixing 1 : 1 with the solution containing 150 mM-HEPES-NaOH buffer (pH 8·0), 5 mM-EDTA and 0·5% human albumin as the enzyme preparation for adenylate cyclase activity determination. This procedure was used because the presence of HEPES interferes with protein determination[14]. In order to preserve the highest enzyme activity, albumin was added to enzyme preparation, the activity was estimated 15—30 min after homogenization of the tissue, and all manipulations with enzyme preparation were performed at 0—4°C.

Adenylate Cyclase Assay

The activity of adenylate cyclase was estimated by a procedure using [α^{32}P]-ATP, as described earlier[15], with a subsequent chromatographic isolation of the product on aluminium oxide[16]. In all experiments was the activity of enzyme (20—40 μg protein/20 μl) estimated in a total incubation volume of 50 μl in the presence of 0·1 mM ATP (0·5—0·8 μCi per sample) and ATP regeneration system containing 5 mM phospho(enol)pyruvate, phospho(enol)pyruvate kinase (40 μg/ml) and myokinase (20 μg/ml). The final incubation mixture also contained 30 mM-HEPES-NaOH buffer (pH 8·0), 0·1 mM cyclic AMP, 1 mM-EDTA, 5 mM-Mg^{2+}, 0·1% albumin and 1 mM papaverine. The addition of papaverine which inhibits the activity of phosphodiesterase and the addition of HEPES buffer secured the sufficient hormonal stimulation of adenylate cyclase activity[17]. The enzymatic reaction was stopped by the addition of 1 ml 0·05M-HCl with cyclic AMP (5 mg/100 ml) as a carrier. After 5 min boiling on a water bath the samples were transferred on the chromatographic columns. The elution of cyclic AMP was performed by 2·5 ml

of 0·1M Tris-HCl buffer (pH 7·4). The product is collected directly into the scintillation vials in which the radioactivity of ^{32}P is measured by the Cerenkov's radiation[18]. The recovery of cyclic AMP was determined on the basis of optical density measurement at 260 nm and time from time checked by [^{3}H]-cyclic AMP. This separation procedure provides a very constant recovery (75—80%). The results are expressed in pmol of cyclic AMP produced per mg protein per min.

RESULTS AND DISCUSSION

The above described adenylate cyclase assay in rat renal medulla is a suitable method for the quantitative comparison of the effect of individual oxytocin analogs. Table I shows the apparent values of affinity constants (A_{50}) for adenylate cyclase activation by [8-arginine]-vasopressin and seven oxytocin analogs. A_{50} represents a molar concentration of a drug producing 50% of the maximal effect of [8-arginine]-vasopressin, which is taken as a standard. At the same time, in the Table I there are given literary data concerning the antidiuretic potency (the ultimate antidiuretic effect dependent on the affinity, intrinsic activity and pharmacokinetic properties of the particular compound) which are expressed in I.U./mg drug studied. Data in this table show that there is a very good correlation between relative antidiuretic potencies and relative affinity constants for adenylate cyclase activation of [8-arginine]-vasopressin and analogs studied. If these two parameters for all compounds tested were plotted on the graph (Fig. 1), we can distinguish two different groups of drugs: *A*) Four of the basic compounds showed a very good correlation between antidiuretic potency and adenylate cyclase activation; *B*) When the primary structure of the most potent from these drugs, deamino-1-carba-oxytocin (*Ib*), was modified by the exchange of tyrosine for isoleucine (*Ic*), or phenylalanine (*Id*), or methyltyrosine (*Ie*), then the newly formed analogs revealed substantially lower antidiuretic effects. These findings are probably possible to explain in such a way that the substitution of tyrosine by more lipophilic amino acid in the position 2 changes the distribution properties of these new drugs in the organism that results in their decreased concentration in the vicinity of receptors in the target tissue.

On the other hand, some of the vasopressin analogs reveal a higher antidiuretic potency than corresponds to their ability to activate adenylate cyclase. This finding is possible to interpret in such a way that the analogs discussed are only in the organism transformed into the active hormonal substances which are only then able to activate adenylate cyclase. In our experiments (data not shown) we have followed the effects of triglycyl-lysine-vasopressin (Glypressin) whose activity for adenylate cyclase stimulation was relatively weak at the standard experimental conditions. By the prolongation of incubation time of this compound with preparations of adenylate cyclase from renal medulla its activity to stimulate adenylate cyclase activity gradually increased and reached the maximum after 15—20 min incubation of the drug tested. This finding points out on the ability of renal tissue to generate [8-lysine]-vasopressin, most probably by the action of aminopeptidase[19]. From these findings

it comes out that in experiments with antidiuretically active drugs, which are metabolized in more potent derivatives, it is necessary to estimate adenylate cyclase activity under experimental conditions using only very short incubation time or to use more purified preparations of this enzyme.

TABLE I

The Characterization of Antidiuretic Effect and Activation of Rat Renal Medullary Adenylate Cyclase by [8-Arginine]-vasopressin (*Ia*, AVP) and some Oxytocin Analogs

Analog	Affinity constant A_{50}[a], μM	Antidiuretic potency[b] (ref.)	Relative affinity constant $\frac{A_{50}\ \text{AVP}}{A_{50}\ \text{analog}} \cdot 100$	Relative antidiuretic potency $\frac{\text{analog}}{\text{AVP}} \cdot 100$
Ia	0·05	450	100	100
Ib	2·0	25 (21)	2·5	5·55
Ic	4·4	2 (8)	1·1	0·44
Id	10·0	0·6 (8)	0·5	0·13
Ie	7·0	0·9 (8)	0·71	0·20
If	12·0	3·0 (21)	0·44	0·66
Ig	126·0	0·2 (21)	0·04	0·044
Ih	400·0	0·05 (21)	0·01	0·011

[a] A_{50} corresponds to that molar concentration of drug that produces 50% of maximal adenylate cyclase activation of [8-arginine]-vasopressin (*Ia*, AVP); [b] I.U./mg.

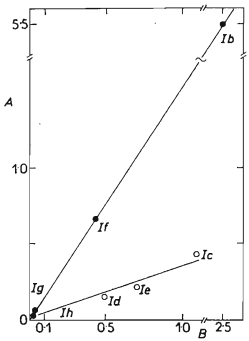

FIG. 1

Relationship between Relative Antidiuretic Potencies and Relative Affinity Constants for Adenylate Cyclase Activation of Several Oxytocin Analogs

A Relative antidiuretic potency; *B* relative affinity constant (see explanation in Table I).

The demonstration of stimulatory effects of various oxytocin analogs on the activity of adenylate cyclase prepared by our simplified procedure has shown that our findings are in good correlation with the results published[20] on adenylate cyclase preparation from pig kidneys which were using far more complicated experimental procedures. It is possible to conclude that we have succeeded to characterize the effects of various oxytocin analogs on a simple adenylate cyclase preparation from rat renal medulla whose activity is possible to determine in any regular laboratory.

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