NEUROPHYSIN BINDING OF SOME ANALOGS OF NEUROHYPOPHYSIAL HORMONES

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The effect of certain structural alterations of oxytocin and vasopressin on their ability to bind to bovine neurophysin was studied by gel filtration with synthetic analogs of the hormones. None of the analogs studied shows a significantly decreased binding ability.

The binding to neurophysin of hormones of the posterior pituitary has been studied both as a suitable model of molecular interaction between a peptide and a protein and also in an effort to cast light on the physiological role of the hormone–neurophysin complex.

One of the hypotheses[1] postulating that the function of neurophysin in the organism is that of a precursor of the neurohypophysial hormones has been negated by the determination of the complete structure[2,3] of neurophysin: enzymatic cleavage of neurophysins cannot give rise either to oxytocin or vasopressin. The theory generally accepted at present regards neurophysins as carriers of neurohypophysial hormones. Attention has therefore been focused on a detailed elucidation of the binding of these hormones to neurophysin. Evidence has been adduced[4–6] showing that the necessary condition of the binding of the hormones is the presence of the α-amino group of cysteine* in position 1 and of an aromatic amino acid in position 2. We have demonstrated in our earlier study[8] that the distance between the free α-amino group and the aromatic residue is also critical. Lysine-vasopressin analogs with the amino group of cysteine in position 1 acylated by an amino acid residue or by a short peptide chain (the so-called hormonogens) do not bind to neurophysin. Neither does the binding ability of vasopressin analogs decrease in their molecules which have been shortened by 1 to 2 amino acid residues at the carboxyl terminus. In the present study, we examined the influence of other structural alterations of the molecules of neurohypophysial hormones, namely of the size of the side chain of the amino acid in position 2, the role of the disulfide bond, and the consequences of elimination of a linear

* All amino acids used in this study are of L-configuration. The nomenclature and symbols of amino acids and peptides follow suggestions published elsewhere[7]. All vasopressin analogs were derived from 8-lysine-vasopressin.

tripeptide tail. The aim of our work was to investigate great differences in binding constants, as manifested by the all or none binding on gel permeation chromatography, rather than an exact determination of complexity constants. The interpretation of the exact values of the constants is not possible without a physico-chemical investigation of the equilibrium of the neurophysin monomer–dimer system; this equilibrium is readily attained in solutions[9–11] and depends-besides pH and protein concentration – perhaps also on the method of preparation[12]. In our opinion, the importance of the complexity constants, determined without a thorough physico-chemical characterization of the association degree of the preparation, lies only in the comparison of their values for the given neurophysin preparation.

EXPERIMENTAL

[2-O-Methyltyrosine]oxytocin[13], *Ia*, [2-O-ethyltyrosine]oxytocin[14], *Ib*, and 1-carba-oxytocin [6,1-cystathionine]oxytocin[15], *Ic*, were synthetized by methods reported earlier and showed the same characteristics as the products described in literature. [2-Phenylalanine]vasopressin (Octapressin), *Id*, was a product of Sandoz and contained 5 I.U./1 ml. The content of the ampules was lyophilized and the analog isolated by descending electrophoresis[16] at pH 5·6. Amino acid analysis: $Asp_{1.00}$, $Cys_{1.74}$, $Glu_{1.05}$, $Gly_{1.00}$, $Lys_{0.94}$, $Phe_{1.82}$, $Pro_{0.94}$. Pressinamide, *Ie*, was a gift of Ferring Ltd., Malmö. Manitol was removed from the preparation by electrophoresis[16] at pH 5·6. Amino-acid analysis: $Asp_{0.98}$, $Cys_{1.72}$, $Glu_{1.00}$, $Phe_{1.00}$, $Tyr_{0.90}$. [1-S-Methylcysteine, 6-S-methylcysteine]vasopressin, *II*, was prepared from synthetic lysine-vasopressin as follows. β-Mercaptoethanol (100 μl) was added to a solution of 10 mg of lysine-vasopressin in 2 ml of water. The pH of the reaction mixture was adjusted to 8·3 by 1M-NaOH and the mixture was set aside for 1 h in an atmosphere of nitrogen. Methyl iodide (120 μl) was then added, the mixture kept at constant pH (8·3) for 30 min and subsequently acidified by acetic acid to pH 2·8. The product was isolated on Amberlite CG-50.

The product was homogeneous on electrophoresis at pH 1·9 and 5·6 and its mobility was the same as that of lysine-vasopressin. Amino-acid analysis: $Asp_{1.00}$, $Cys(Me)_{1.92}$, $Glu_{1.00}$, $Gly_{0.94}$, $Phe_{1.00}$, $Pro_{0.98}$, $Tyr_{0.84}$, Lys not determined. A product of identical properties was obtained even when methyl iodide had been replaced by *p*-nitrobenzenesulfonic acid methyl ester.

Samples for amino-acid analysis were hydrolyzed 20 h at 105°C in constant-boiling hydrochloric acid. The analyses were carried out on Model 120 B Beckman-Spinco Amino Acid Analyzer according to Benson and Patterson[17].

Neurophysin *II* was prepared according to Hollenberg and Hope[18].

Pressor activity was determined on despinalized rats according to Dekanski[19] and using the modification of Krejčí and coworkers[20]. Uterotonic activity was assayed on isolated strips of rat uterus[21] according to Munsick[22].

Neurophysin and the analog examined at a weight ratio of 10 : 1 (usually 5—10 mg of neurophysin was used) were dissolved in 1 ml of 0·05M pyridine–acetate buffer at pH 5·8 and applied onto a column of Sephadex G-25 (0·9 × 75 cm). The column was eluted by the same buffer at a rate of 6·8 ml/h. The course of the elution was checked spectrophotometrically at 280 nm. In all experiments, UV-absorbing material (neurophysin–peptide complex) emerged in the hold-up volume of the column. This material was pooled and lyophilized. The lyophilisate was dissolved in 1 ml of 0·1M-HCOCOH and applied onto a column of Sephadex G-25 of the dimensions given above. The column was eluted by 0·1M-HCOOH at the same rate. Neurophysin was separated by

gel chromatography from the analog studied whose quantity was determined either by amino acid analysis or by the biological activity (pressor, uterotonic) test.

RESULTS AND DISCUSSION

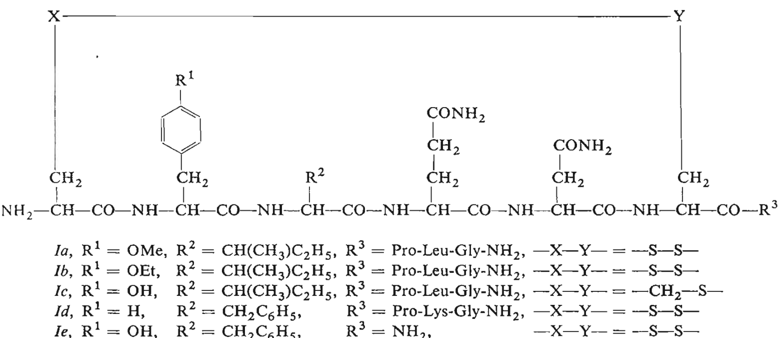
The analog released by dissociation of the neurophysin-analog complex was determined by the corresponding method. The results obtained are summarized below; the quantity of the peptide bound is given in % of the original quantity of peptide taken for the binding experiment. It is obvious that none of the analogs of neurohypophysial hormones shows a markedly decreased binding ability.

Analog	Method of determination	Quantity of bound peptide in % of original quantity
Ia	uterotonic activity	82
Ib	amino acid analysis	75
Ic	pressor activity	84
	amino acid analysis	90
Id	amino acid analysis	88
Ie	amino acid analysis	84
II	amino acid analysis	93

We have shown in the preceding paper that the removal of two amino acid residues from the carboxyl terminus of the peptide chain of vasopressin does not affect significantly the neurophysin binding of the peptide. We demonstrated in this study that the cyclic portion itself of the vasopressin molecule, too, is capable binding to neurophysin. It has been observed earlier with oxytocin[5] that the replacement of tyrosine by phenylalanine does not bring about a loss of binding ability. We demonstrated here that the same is true for a vasopressin analog (*Id*).

We also examined the effect of the enlargement of the substituent in *p*-position of tyrosine in oxytocin (analogs *Ia*, *Ib*); this alteration did not affect the binding ability either. It has been reported recently[23] that neither the presence of a bulky group in meta position leads to a loss of the ability of [2-(3-nitrotyrosine)]-oxytocin to bind to neurophysin.

We investigated the importance of the disulfide bond and of the cyclic arrangement of the hormone molecule with two other analogs, *Ic* and *II*. The disulfide bond of the former was replaced by a thioether group (1-carba-oxytocin, *Ic*). According to our expectation, this replacement did not affect any substantially the formation of the complex of derivative *Ic* with neurophysin. Similarly, the formation of the complex of derivative *II* [1-S-methylcysteine, 6-S-methylcysteine]-vasopressin with neurophy-



Cys(Me)-Tyr-Phe-Gln-Asn-Cys(Me)-Pro-Lys-Gly-NH_2

II

sin was not influenced by the reduction of the disulfide bond and methylation of the sulfhydryl groups of lysine-vasopressin.

It has been demonstrated[24] that the complexity constant of linear di- and tripeptides with intact N-terminal sequence of the native hormone and methylcysteine in position 1 is decreased compared to the native hormones. This difference can be explained by the conformation of the native hormone. Our data show that this conformation is determined by the primary structure of the peptide chain rather than by the presence of the disulfide bond. On the other hand, however, the absence of biological activities of analog *II* shows that a change in conformation must have taken place as a result of the interruption of the disulfide bond. The structural requirements of the receptors of biological effects are obviously more strict than the structural requirements necessary for binding of neurohypophysial hormones to neurophysin.

In view of this fact the problem arises whether neurophysins act exclusively as carrier proteins for neurohypophysial hormones. We may expect that neurophysin will bind also certain hormone fragments resulting from processes of inactivation of these hormones by enzymes, including those fragments whose participation in the metabolic processes already has been determined[25,26].

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