CROSS-REACTIVITY OF VASOPRESSIN ANALOGUES WITH PORCINE ANTIBODIES TO [8-ARGININE]VASOPRESSIN

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The immunochemical specificity of porcine antivasopressin antibodies was studied using a set of new vasopressin analogues. Homological substitution of the amino acid in position 8 of the peptidic chain (using either L- or D-stereoisomers of norarginine, arginine and homoarginine) had a negligible effect on the affinity of the analogues to antibodies. The affinity decreased 50 times when the hydrogen atom on the N-atom of the peptidic bond in position 8 was substituted by a methyl group (N-methylarginine). In comparison with the monomer of [8-D-arginine]deamino-vasopressin, the dimer had even 30 times lower binding affinity. The replacement of the sulphur atom in the disulphidic bridge of deamino-vasopressin by a methylene group (carba-1 or carba-6) resulted in a 5-10 fold decrease of the affinity to antibodies.

The complementarity of the antibody binding site and the antigenic determinant is the prerequisite for an immunological reaction between the antigen and antibody. The investigation of cross-reactions between peptide hormone analogues and specific antibodies aims at determining the structural requirements for the binding of antigens to antibodies and the character of the antigenic determinant. Immunochemical studies may also help to elucidate the conformation of the compounds concerned. Finally such studies may serve as models for the interaction of a biologically active substance with its receptor. Numerous immunochemical studies were performed with a series of bradykinin[1] and angiotensin[2] analogues. The results obtained by Vallotton[3] supported the concept of the three-dimensional model of angiotensin as proposed by Smeby[4]. The immunochemistry of neurohypophysial hormones has been studied to a smaller extent yet[5-8].

As shown in our previous paper[8], we prepared highly specific porcine antibodies which were suitable for immunochemical experiments. In this paper we present data on the binding of newly synthesized analogues of [8-arginine]vasopressin to the antibodies. The analogues investigated had the following structural features in common: absence of the primary amino group in position 1 of the peptidic chain, modification of the basic amino acid in position 8 and changes in the area of the disulphidic bridge.

EXPERIMENTAL

Materials. [8-Arginine]vasopressin* was isolated from neurosecretory material according to Prusík and coworkers[9] and its biological activity corresponded to that of the fully active hormone. The other analogues (Table I) were prepared in the Department of Organic Synthesis of our Institute. Porcine antiserum against [8-arginine]vasopressin binding 50 per cent of labelled [8-arginine]vasopressin (in the order of pg) at the dilution 10 000 times we obtained as described elsewhere[8].

Methods. The preparation of radioactively labelled [8-arginine]vasopressin, the conditions of the radioimmunoassay and of inhibitory experiments were the same as those stated in the paper of Vaněčková and coworkers[8]. The labelling of the hormone by [^{125}I] was performed according to Greenwood and coworkers[10]; the labelled hormone was purified by chromatography on columns of Dowex 1X8 and Sephadex G-25. Its specific radioactivity was 400 Ci/mmol. Inhibitory experiments were performed in 0·1M-Tris-HCl buffer, pH 7·8, containing 0·2% bovine serumalbumin. The concentration of [8-arginine]vasopressin and its analogues in the reaction was $10^{-4}-10^{4}$ pmol/ml. The incubation was run for 24 h at 4°C. The free hormone was separated from the bound one using dextrane coated charcoal. All the experiments (done

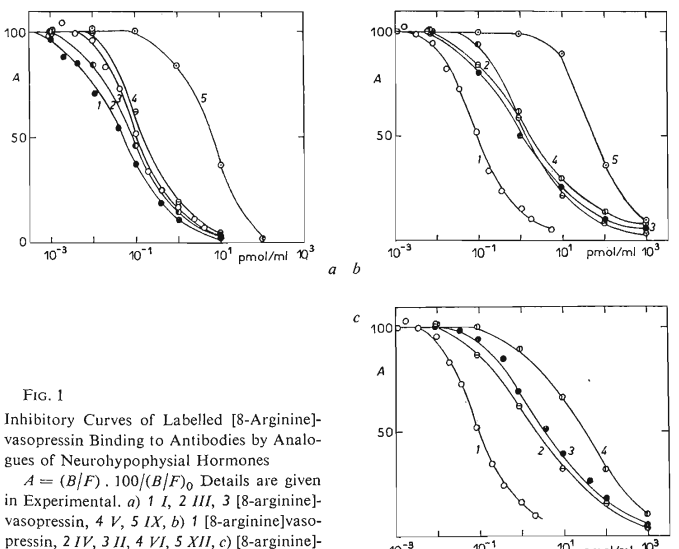


FIG. 1

Inhibitory Curves of Labelled [8-Arginine]-vasopressin Binding to Antibodies by Analogues of Neurohypophysial Hormones

$A = (B/F) \cdot 100/(B/F)_0$ Details are given in Experimental. *a*) *1 I*, *2 III*, *3* [8-arginine]-vasopressin, *4 V*, *5 IX*, *b*) *1* [8-arginine]vasopressin, *2 IV*, *3 II*, *4 VI*, *5 XII*, *c*) [8-arginine]-vasopressin, *2 IV*, *3 VII*, *4 VIII*.

* All the amino acids were of L-configuration unless stated otherwise.

in double or triple series) were performed under such conditions that the maximal binding of labelled hormone to the antibody in the absence of unlabelled hormone was equal to 50 ± 3%, *i.e.* the ratio of bound and free hormone $[B/F]_0$ was 1 ± 0·1. The value of $[B/F]_0$ was laid equal to 100% and all the values (B/F) obtained for various concentrations of vasopressin analogues were related to this value. The values of $[B/F] \cdot 100/[B/F]_0$ were plotted against the log of analogue concentration in the reaction. The concentration of the analogue resulting in 50% inhibition of binding was determined graphically.

RESULTS

The typical inhibitory curves for different analogues and labelled [8-arginine]vasopressin are presented in Fig. 1*a* – 1*c*. Table II gives the concentration ratios of analogues and [8-arginine]vasopressin resulting in 50% inhibition of labelled hormone binding to antibodies. The values were determined graphically and present the mean values of three independent experiments.

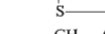
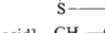
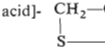
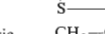
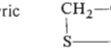
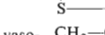
It is evident from Table II that the homological substitution of basic amino acid in position 8 has hardly any effect on the affinity of analogues to antibodies, both in L- and D-stereoisomer series. The replacement of a L-amino acid by a D-stereoisomer in position 8 generally resulted in the lower affinity of the analogue to antibodies. On the average the decrease of affinity was twenty fold. The same level of decrease was observed in the case of a similar modification in the [8-lysine]vasopressin series (compare [8-lysine]vasopressin with [8-D-ornithine]deamino-vasopressin in Table II). When the hydrogen atom on the nitrogen forming the peptide bond of arginine was replaced by a methyl group, the inhibitory capacity decreased 50 times. Both carba analogues of deaminovasopressin (carba-6 and carba-1) also had a lower affinity to the antibodies than [8-arginine] deamino-vasopressin. As expected the dimer of [8-D-arginine]deamino-vasopressin had a very low affinity; it was 500 times lower than that of the natural hormone.

DISCUSSION

The differences in the affinities of individual analogues to antibodies may be explained by changes in the structure of the antigenic determinant or by a change in the structure of another part of the molecule causing an alteration of the antigenic determinant conformation. The results confirm our previous finding[8] that a strong basic character and a specific steric conformation of the amino acid in position 8 of the peptide chain are important factors in the interaction with antibodies. The affinity values show that the sulphur atoms may play a certain role in the conformation of the hormone molecule and its interaction with antibodies (besides the formation of the cycle). The group of the analogues described in this paper may be classified as a L- and D-homological series of [8-arginine]deamino-vasopressin (Table I). The difference in the affinity of analogues caused by stereoreplacement was not

markedly influenced by the other modification, namely by the substitution by homological amino acids. A slight decrease of the affinity was observed in the L-series in relation to the prolongation of the side chain of the amino acid (compare analogues *I*, *III* and *V* in Table II). The substitution of the hydrogen atom in the peptidic

TABLE I
Analogues of [8-Arginine]vasopressin

No	Compound	Structure of N-terminal part of analogue	Amino acid in position 8	Refs
I	[8-Arginine]deamino-vasopressin		L-arginine	11 12
II	[8-D-Arginine]deamino-vasopressin		D-arginine	13
III	[8-Aminoguanidinobutyric acid]-deamino-vasopressin		L-aminoguanidino-butyric acid	14
IV	[8-D-Aminoguanidinobutyric acid]deamino-vasopressin		D-aminoguanidino-butyric acid	14 15
V	[8-Homoarginine]deamino-vasopressin		L-homoarginine	16
VI	[8-D-Homoarginine]deamino--vasopressin		D-homoarginine	16
VII	[8-Lysine]vasopressin		L-lysine	—
VIII	[8-D-Ornithine]deamino-vasopressin		D-ornithine	17
IX	[8-N^g-Methylarginine]deamino--vasopressin		L-N^g-methyl-arginine	18
X	[1,6-α-Deaminocystathionine, 8-arginine]vasopressin		L-arginine	19
XI	[1,6-β-Deaminocystathionine, 8-arginine]vasopressin		L-arginine	20
XII	Dimer of *II*	[a]	D-arginine	21

[a] The spatial arrangement is not known yet.

group in position 8 decreased the affinity to a greater extent; this modification probably alters the conformation of the molecule more than the stereoreplacement of the amino acid. The data on circular dichroism[22] give more evidence of differences among the conformations of L- and D-stereoisomers of [8-arginine] deaminovasopressin and [8-N^{α}-methylarginine] vasopressin (Table I); arginine in position 8 either interacts directly with the tyrosine side chain or indirectly increases the effectiveness of interaction with another part of the tripeptide tail by influencing its conformation[22]. Moreover the different affinities of the compounds *X* and *XI* to antibodies provide evidence either that the disulphidic bridge is an integral part of the antigenic determinant of the hormone or that the sulphur atoms interact with some other part of the hormone molecule.

Bearing in mind that monomer and dimer may have a significantly different conformation features, the low affinity of the dimer of [8-D-arginine] deamino-vasopressin is not surprising. The actual inhibitory action of the compound might be even lower, because in the preparation certain amount of monomer is present which is in equilibrium with the dimer.

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TABLE II

Immunochemical Reactivity of [8-Arginine]vasopressin Analogues

Presented numbers are the concentration ratio of the analogues and [8-arginine]vasopressin resulting in 50 per cent inhibition of binding of labelled [8-arginine]vasopressin; for [8-arginine]-vasopressin the concentration resulting in 50 per cent inhibition is 0·057 pmol/ml.

Compound	Immunochemical reactivity
[8-Arginine]vasopressin	1
I	0·8[a]
II	15·0[a]
III	0·7
IV	20·0
V	1·3
VI	20·0
VII	35·0[a]
VIII	500·0
IX	50·0
X	3·0[a]
XI	10·0
XII	500·0

[a] Values taken from paper of Vaněčková and coworkers[8].

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