PROLONGED ANTIDIURETIC ACTION OF VASOPRESSIN ANALOGUES IN RELATION TO THEIR PRIMARY STRUCTURE

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The effectiveness of vasopressin analogues with prolonged antidiuretic action was related to their primary structure. Prolonged action was primarily due to the absence of the amino group of cysteine in position 1 of the peptide chain. The carba substitution of the disulphide bridge and introduction of basic homologous amino acids into position 8 contributed in differing degrees to the prolonged action of the analogues. [8-L-Norarginine]deamino vasopressin was the most potent of the analogues compared; its action was 10 times more prolonged than that of dDAVP.

Since the preparation of [8-D-arginine]deamino vasopressin[1] (dDAVP), an analogue of natural vasopressin with prolonged antidiuretic action, a number of similar analogues synthetized later on have been described as very active or specifically active. However, the first pharmacological studies of dDAVP (ref. 2) showed that it was not simple to express its antidiuretic potency accurately. The difficulty was caused by the fact that its prolonged effect was compared with the short-term response to natural vasopressins. The situation was further complicated by the synthesis of new analogues. Eventually it became clear that the potency of this type of analogues must be estimated by taking into account the duration of their antidiuretic action[3,4]. For determining the duration of the response, Burn's[5] experimental arrangement proved to be more suitable than the method of Jeffers and coworkers[6] which used anaesthetized animals. The potency of a number of analogues was compared with regard to the duration of antidiuresis[8,9] or on the basis of the so-called half-time of antidiuresis (T/2), *i.e.* the time in which half of the water load was excreted[7,20]. In our previous paper[11], we confirmed the results of Vávra and coworkers[2] by establishing the fact that the regression of the half-time of antidiuresis on log dose for dDAVP did not parallel that for LVP. We also suggested that it would be appropriate to use dDAVP as a standard for testing vasopressin analogues with prolonged antidiuretic action. In the present work, we investigated the influence of the primary structure of vasopressin analogues on the duration of their antidiuretic action.

EXPERIMENTAL

Material: [8-L-arginine]deamino vasopressin[12,13], [8-L-homoarginine]deamino vasopressin[14,15], [8-L-γ-guanido- α-aminobutyric acid]deamino vasopressin[16,17] ([8-L-norarginine]deamino vasopressin), [8-L-β-guanido-α-aminopropionic acid]deamino vasopressin[18], [8-D-arginine]deamino vasopressin[1], [8-D-homoarginine]deamino vasopressin[15], [8-D-γ-guanido-α-aminobutyric acid]deamino vasopressin[17] ([8-D-norarginine]deamino vasopressin), [8-D-β-guanido-α-aminopropionic acid]deamino vasopressin[18], [8-D-arginine]vasopressin[19], [8-L-arginine]deamino-6-carba-vasopressin[7], [8-L-arginine]deamino-1-carba-vasopressin[20] were supplied by the Department of Organic Synthesis of this Institute and by Léčiva, Prague.

Methods: The antidiuretic activity and $T/2$ were determined by means of the modified Burn assay using conscious rats, as described in our previous paper[11]. The dependence of $T/2$ on doses was subjected to regression analysis. On the basis of the regression lines, calculations were made of the dose corresponding to $T/2 = 200$ min (the $T/2$ value after the application of approximately 0·1 ng/kg of dDAVP) and $T/2 = 68$ min ($T/2$ after an injection of saline).* The antidiuretic potency of the individual analogues was expressed in terms of the ratio between the dose of peptide corresponding to the $T/2$ value of 200 min and the equipotent dDAVP dose. The regression lines were compared by variation analysis.

RESULTS AND DISCUSSION

We compared the antidiuretic potency of a number of vasopressin analogues. The comparison was based on two criteria: *1*) the dose of peptide that resulted in a $T/2$ value of 200 min; *2*) the dose of peptide corresponding to the $T/2$ value obtained after the administration of saline (extrapolated threshold dose). In both cases, as in the work of Cort and coworkers[21], the activity of dDAVP was placed equal to 1.

As can be seen in Fig. 1*a* and Table 1, prolonged antidiuretic action was mainly due to the absence of the primary amino group of cysteine in position 1, whereas the substitution of L-arginine in position 8 by D-arginine was of lesser importance. When comparing the antidiuretic potency of [8-D-arginine]deamino vasopressin and [8-L-arginine]deamino vasopressin, we did not find a statistically significant difference ($P > 0{\cdot}20$ for the slope, $P > 0{\cdot}25$ for the elevation). The replacement of L-arginine by D-arginine in position 8 of vasopressin did not influence the antidiuretic potency of the resultant analogue to a great extent; nevertheless, the specificity of the antidiuretic activity increased. The substitution of the sulphur atom of cysteine in position 6 by a methylene group led to a prolongation of the antidiuretic response, as compared with the duration of the response to dDAVP. As can be seen in Fig. 1*b*, the response to [8-L-arginine]deamino-6-carba-vasopressin

* We based our calculations of the antidiuretic potency of the analogues studied on the average value of the regression of $T/2$ on dDAVP doses, obtained by measurements performed during several seasons. Therefore, the potency of some analogues (*e.g.* [8-L-arginine] deamino--1-carba-vasopressin[20]) stated in the present paper is somewhat lower than values published earlier.

(in doses higher than 1 ng/kg) exceeds that to dDAVP. The substitution of the sulphur atom of cysteine in position 1 did not cause a statistically significant increase of the duration of the antidiuretic response as compared with dDAVP ($P > 0{\cdot}20$ for slopes, $P > 0{\cdot}10$ for elevations). However, the dose-response curves were not parallel; when the doses were compared on the basis of extrapolated threshold doses, the carba analogues were less potent than dDAVP.

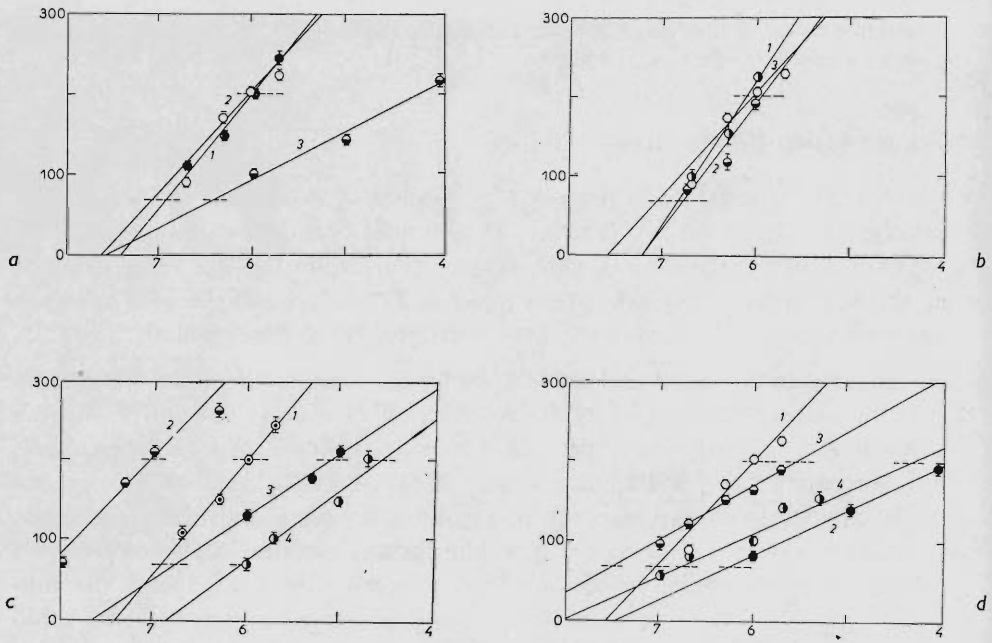

FIG. 1*a*, *b*, *c*, *d*

Plot of the half-time of antidiuresis against the peptide dose. Abscissa — half-time of antidiuresis in min, ordinate — [—log] dose of peptide in mg of peptide/kg of body weight. *a* *1* — [8-L-arginine]deamino vasopressin. *2* — [8-D-arginine]deamino vasopressin, *3* — [8-D-arginine]vasopressin; *b* *1* — [8-L-arginine]deamino-6-carba-vasopressin, *2* — [8-L-arginine]deamino-1-carba-vasopressin, *3* — [8-D-arginine]deamino vasopressin; *c* *1* — [8-L-arginine]deamino vasopressin, *2* — [8-L-norarginine]deamino vasopressin, *3* — [8-L-homoarginine]deamino vasopressin, *4* — [8-L-β-guanido-α-aminopropionic acid]deamino vasopressin; *d* *1* — [8-D-arginine]deamino vasopressin, *2* — [8-D-homoarginine]deamino vasopressin, *3* — [8-D-β-guanido-α-aminopropionic acid]deamino vasopressin, *4* — [8-D-norarginine]deamino vasopressin

The results obtained so far indicate that the position of the basic group in the side chain of the amino acid in position 8 is of major importance for the biological activity of the analogue. Fig. 1*c* and 1*d* and Table I show the influence of the replacement of L- or D-arginine by the corresponding L- and D-homologues. In the L-series, [8-L-norarginine]deamino vasopressin was the most active and [8-L-β-guanido--α-aminopropionic acid]deamino vasopressin the least active analogue. In the D-stereoisomer series, the situation was somewhat different. The fact that the regression of duration of antidiuretic responses on log dose for dDAVP does not parallel that for individual analogues is more obvious. When the responses to the analogues were compared on the basis of $T/2 = 200$ min, [8-D-arginine]deamino vasopressin was the most active, whereas [8-D-homoarginine]deamino vasopressin the least active analogue (Fig. 1*d*). On the other hand, when the calculations were based on extrapolated threshold doses, [8-D-β-guanido-α-aminopropionic acid]deamino vasopressin appeared to be more potent than dDAVP.

Our findings lead to the conclusion that when two analogues are compared, the ratio of their potencies can be influenced by the criteria on which the comparison

TABLE I

Activity of antidiuretically potent analogues of vasopressin. *A* 200-activity expressed as the ratio between doses of dDAVP and the compound studied that resulted in $T/2 = 200$ min; *A* 68-activity expressed as the ratio of doses corresponding to the $T/2$ value obtained after the administration of saline (extrapolated threshold dose)

Compound	*A* 200	*A* 68	Regression coefficients ($Y = a + bx$)		
			a	*b*	R^2
[8-D-Arginine] deamino vasopressin	1·000	1·000	− 52·20	126·74	0·60
[8-L-Arginine] deamino vasopressin	0·973	0·739	− 86·51	143·13	0·77
[8-D-Arginine] vasopressin	0·017	0·232	− 27·64	60·40	0·81
[8-L-Arginine] deamino-1-carba-vasopressin	0·829	0·520	−125·94	157·35	0·82
[8-L-Arginine] deamino-6-carba-vasopressin	1·294	0·677	−126·01	173·61	0·83
[8-L-Homoarginine] deamino vasopressin	0·130	0·579	− 24·67	78·17	0·73
[8-L-Norarginine] deamino vasopressin	10·465	11·574	82·00	121·63	0·94
[8-L-β-Guanido-α-aminopropionic acid]-deaminovasopressin	0·035	0·102	−101·32	87·34	0·82
[8-D-Homoarginine] deamino vasopressin	0·007	0·158	− 28·80	55·33	0·92
[8-D-Norarginine] deamino vasopressin	0·022	0·564	3·66	53·75	0·59
[8-D-β-Guanido-α-aminopropionic acid]-deamino vasopressin	0·350	2·922	35·55	67·23	0·63

is based. Moreover, if the compounds compared cannot be assayed in a reasonable range of doses (if their potencies differ greatly, or their action is accompanied by side-effects), the evaluation, based on extrapolated doses is necessarily biassed. Therefore, the course of the dose-response curve must be carefully investigated in each case and the results obtained in assays using an extreme range of doses should be judged with the utmost caution.

In view of the duration of the antidiuretic response, it is not possible to determine the antidiuretic potency using anaesthetized rats according to the method of Jeffers and coworkers[6], which requires the repeated administration of the standard and the compound studied within a reasonable period. Moreover, it was found that adenylate cyclase in the renal medulla was desensitized after the administration of dDAVP[22]. Our method of determining the dose-response dependence proved to be suitable for comparing the effect of a number of antidiuretically highly potent analogues of vasopressin. Together with studies concerning the kinetic parameters of the interaction of vasopressin analogues with kidney receptors, the investigation of their prolonged antidiuretic action makes it possible to assess the way in which modifications of the primary structure influence the distribution and elimination of the peptides in the receptor compartment.

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