THE EFFECT OF SOME STRUCTURAL CHANGES IN THE MOLECULE OF VASOPRESSIN ON THE DURATION OF ANTIDIURETIC ACTION

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The effect of two structural variations in the molecule of [8-D-arginine]deamino-vasopressin on the prolongation of the antidiuretic action of the analogues in non-anesthetised rats was studied. The replacement of glutamine in position 4 of the peptide chain with a suitable amino acid has prolonged the half-life of the antidiuretic effect. A more prolonged antidiuretic effect was obtained when the stabilization of the cyclic structure of [8-D-arginine]deamino-vasopressin was performed by replacement of the disulfidic bridge by the monocarba bridge. The binding and activating constants (adenylate cyclase) in a membrane fraction of the rat kidney were compared with the factors influencing the metabolic stability of the molecule of the individual analogues.

In our preceding papers we studied the dependence of the protractiveness of the antidiuretic response on the structure of vasopressin analogues[1,2]. The prolongation of the biological effect is firmly linked to the absence of the primary amino group of cysteine in position 1. Other alterations performed such as the replacement of the sulphur atoms in the bridge by methylene groups, stereoreplacement in position 8 or homologization of amino acid in the same position alter the magnitude of protractiveness together with the specificity of the biological effect. In this study we present the results of experiments where we compared the protractiveness of the antidiuretic response of several structural analogues of [8-D-arginine]deamino-vasopressin bearing modifications which could be reflected either in the affinity to the kidney receptor or in the metabolic stability.

EXPERIMENTAL

Materials

[8-Arginine]vasopressin (AVP)* and [8-D-arginine]deamino-vasopressin (dDAVP[4]) were syntheti-

* Unless otherwise stated all the chiral amino acids are of the L-series. The nomenclature and symbols of the amino acids and peptides obey the published recommendations[3].

zed by one of us (M. F.), [8-D-arginine]deamino-6-carba-vasopressin[5] was prepared in the Department of Organic Synthesis, Institute of Organic Chemistry and Biochemistry. The remaining three analogues of [8-D-arginine]deamino-vasopressin at position 4 (valine, isoleucine, or leucine) were supplied by H. Vilhardt[6,7].

Methods

The modified Burn's method[1,8] using non-anesthetized rats was chosen for the estimation of antidiuretic potency of the individual analogue. The dependence of the half-times of the antidiuretic effect on the log of dose was calculated by 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 administration of 0·1 mg of dDAVP per kg body weight) and $T/2 = 68$ min a treshold dose. 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 (or 68 min) and the equipotent dDAVP dose (A_{200} and A_{68}; the potency of dDAVP being expressed as 1). The regression lines were compared by analysis of variance.

RESULTS AND DISCUSSION

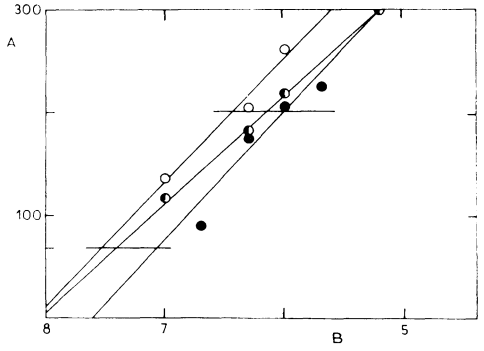
The dependence of $T/2$ values of the antidiuretic effect of the analogues on the logarithm of the dose is shown in Figs 1*a*–*c*. The values A_{200} and A_{68} are included in Table I. The analogue with the most protracted antidiuretic effect is [8-D-arginine]-deamino-6-carba-vasopressin, considering both parameters A_{68} and A_{200}. [4-Valine, 8-D-arginine]deamino-vasopressin has longer duration of the antidiuretic effect compared to dDAVP. The other analogues having the lipophilic amino acid leucine or isoleucine in position 4 are less potent than dDAVP. In the system of rat kidney medullary membranes [4-valine, 8-D-arginine]deamino-vasopressin revealed higher binding affinity in comparison to AVP, but its activation of adenylate cyclase was lower than that of AVP or dDAVP (ref.[9]). The antidiuretic potency of dDAVP with the amino acid substitution in position 4 has previously been investigated by Sawyer and coworkers[10], who found [4-valine, 8-D-arginine]deamino-vasopressin more active than dDAVP. The higher activity for this compound has also been claimed by Cort and coworkers[7] who showed moreover the decrease in potency for the dDAVP analogue having glutamine replaced by isoleucine or leucine, but the drop of activity was not so dramatic as indicated in our results.

The protractiveness of the antidiuretic response for [8-arginine]vasopressin remains to be solved. Cort and coworkers[7] declared for this compound a potency equal to 50% of that of dDAVP (A_{200} should be compared). This finding is approximately by two orders of ten higher than the value we calculated for the natural hormone (Table I). In the range of doses 60–500 μg/kg of the body weight (diabetes insipidic rats) Sawyer and coworkers[10] did not find a linear dependence of $T/2$ on the administered dose of [8-arginine]vasopressin. $T/2$ values were in the range of 60–120 min, which is in harmony with our findings for the same range of doses (Fig. 1*c*). The effect of higher doses of [8-arginine]vasopressin on the protractiveness of the antidiuretic response was not investigated by Sawyer and coworkers[10].

TABLE I

Activity of antidiuretically potent analogues of vasopressin. Activity expressed as indicated in Experimental

Compound	A_{200}	A_{68}	Regression coefficients ($y = a + bx$)		
			a	b	R^2
[8-D-Arginine]deamino-vasopressin	1·0	1·0	961·72	126·7	0·60
[8-D-Arginine]deamino-6-carba-vasopressin	2·4	2·67	982·35	121·36	0·91
[8-Arginine]vasopressin	0·002	0·21	343·81	43·32	0·75
[4-Valine, 8-D-arginine]deamino-vasopressin	1·33	2·27	857·33	106·61	0·85
[4-Isoleucine, 8-D-arginine]deamino-vasopressin	0·141	1·26	544·22	66·63	0·92
[4-Leucine, 8-D-arginine]deamino-vasopressin	0·016	0·173	468·66	63·60	0·87



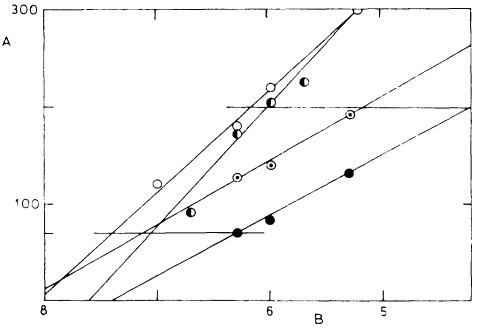
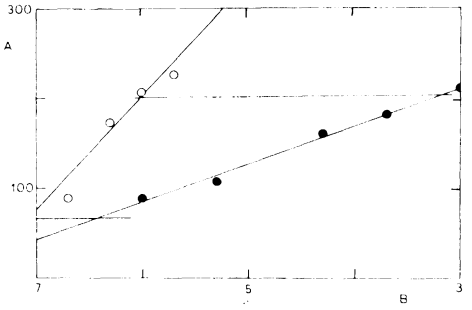


FIG. 1

Plot of the half-time of antidiuresis against the peptide dose. *A* half-time of antidiuresis in min. *B* [−log] of peptide dose in mg of peptide/kg of body weight. *a* ○ [8-D-arginine]deamino-6-carba-vasopressin, ◐ [4-valine, 8-D-arginine]deamino-vasopressin, ● [8-D-arginine]deamino-vasopressin; *b* ○ [4-valine, 8-D-arginine]deamino-vasopressin, ◐ [8-D-arginine]deamino-vasopressin, ⊙ [4-isoleucine, 8-D-arginine]deamino--vasopressin, ● [4-leucine, 8-D-arginine]deamino-vasopressin; *c* ● [8-D-arginine]deamino-vasopressin, ○ [8-arginine]vasopressin



In one of our preceding papers we tried to solve the problem of a suitable standard in Burn's antidiuretic assay when used for measuring analogues of dDAVP. Neither [8-lysine]vasopressin nor [8-arginine]vasopressin – as indicated here – are suitable as standard, because the doses necessary for eliciting a prolonged antidiuretic response comparable to that of dDAVP produce severe pressor effects. Because of the general availability of dDAVP nowadays, it would be recommendable to use this peptide as the antidiuretic standard when the duration of antidiuretic effects is to be estimated.

Carba substitution of the disulfide bridge in oxytocin[11,12] and vasopressin series[5,13] has proved successful in terms of higher affinity of carba-analogues to some receptors and of higher protractiveness of some biological responses[14,15]. In the rat kidney receptor assay the 6-carba analogue of dDAVP has somewhat lower ability to activate adenylate cyclase than has dDAVP (ref.[16]). Nevertheless its metabolic stability and most probably the altered compartementalisation together surpass the effect of the decreased affinity. These properties make the 6-carba analogue of dDAVP the most potent antidiuretic analogue we have tested in the series of deamino vasopressin having D-arginine in position 8. Undoubtedly new metabolically stable dDAVP analogues will be prepared with the object of increasing renal receptor affinity. Whether such analogues represent major steps forward in the therapeutic field remains to be seen.

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