Vasopressin inhibits the adenylate cyclase activity of human platelet particulate fraction through V_1-receptors

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Arg^8-vasopressin inhibited the adenylate cyclase activity of human platelet particulate fraction up to a maximum of 27% (IC_{50} = 1.2 nM). This inhibition required the presence of 10 μM GTP and was optimal with 100 mM NaCl. Orn^8-vasopressin had similar effects. 1-Deamino-Val^4, D-Arg^8-vasopressin did not by itself affect adenylate cyclase activity but competitively inhibited the action of Arg^8-vasopressin (pA_2 = 7.74). Arg^8-vasopressin did not inhibit adenylate cyclase in intact platelets but instead caused platelet aggregation, an effect that was also competitively inhibited by 1-deamino-Val^4, D-Arg^8-vasopressin (pA_2 = 7.82). Thus, platelets possess vasopressin receptors of the V_1 type that, under appropriate conditions, can mediate either inhibition of platelet adenylate cyclase or platelet aggregation.

Vasopressin *Adenylate cyclase* *Receptor* *Platelet aggregation*

1. INTRODUCTION

Vasopressin receptors can be classified into two principal types on the basis of structure-activity and binding studies with vasopressin analogues [1,2]. These receptor types mediate the classical pressor and antidiuretic actions of AVP. Authors in [3] have termed the former, V_1-receptors; these also appear to mediate the glycogenolytic action of vasopressin on hepatocytes and to act by stimulating phosphoinositide breakdown and Ca^{2+} mobilization. Antidiuretic receptors, which activate adenylate cyclase, were termed V_2-receptors. Relatively specific agonists and antagonists acting at these receptors have been synthesized and it is of particular interest that some potent antidiuretic agonists (e.g., dVDAVP) are potent pressor antagonists [1]. AVP is also an inducer of platelet aggregation [4]. Initial studies with analogues suggested that this effect of AVP resembled its pressor action [4], a finding that has recently been confirmed using a specific antagonist [5].

In contrast to agonists such as epinephrine, acetylcholine and angiotensin II [6,7], which can also mobilize Ca^{2+} ions, AVP has not been observed to inhibit adenylate cyclase in broken cell preparations from any tissue so far examined [2,7]. Moreover, experiments with platelets have shown that AVP is unable to block increases in cyclic AMP caused by activators of adenylate cyclase, although several other aggregating agents, such as ADP and epinephrine, readily do so [8]. More recently, AVP was also found to have no effect on cyclic AMP accumulation in cultured aortic smooth muscle cells [9]. In one apparently contrary report [10], AVP decreased cyclic AMP accumulation in the presence of glucagon in rat hepatocytes, though the mechanism was not determined. We have recently observed that platelet-activating factor can inhibit the adenylate cyclase activity of human platelet particulate fraction [11], although in intact platelets from this species no direct effect of this compound on cyclic AMP formation can be detected [12]. The latter apparently conflicting observations have prompted us to study

Abbreviations: AVP, Arg^8-vasopressin; OVP, Orn^8-vasopressin; dVDAVP, 1-deamino-Val^4, D-Arg^8-vasopressin; EGTA, ethylene glycol bis (β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid

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the effects of AVP and analogues on human platelet adenylate cyclase, using similar particulate fractions. We have found that AVP, like platelet-activating factor, possesses a latent ability to inhibit human platelet adenylate cyclase.

2. MATERIALS AND METHODS

2.1. *Materials*

Tris–ATP, disodium ATP (prepared by phosphorylation of adenosine and therefore essentially GTP-free), and Tris–phosphocreatine were obtained from Sigma (St. Louis, MO). dVDAVP was a generous gift from Dr M. Manning, Medical College of Ohio (Toledo, OH). [2,8-^{3}H]ATP (29 Ci/mmol) was obtained from ICN (Irvine, CA). When the actions of GTP and NaCl were studied, the [^{3}H]ATP was diluted with GTP-free disodium ATP to a specific activity of 20 mCi/mmol and then eluted with water from a small column containing Dowex 50 resin (H^+ form). The [^{3}H]ATP obtained was adjusted to pH 7.4 with Tris. All other materials were obtained from sources listed in [4] or [11].

2.2. *Preparation of platelet particulate fractions and assay of adenylate cyclase*

Human platelets were isolated and washed as in [4], except that they were finally resuspended at 25 mg wet wt/ml in buffer (pH 7.4) containing 150 mM Tris–HCl and 5 mM Tris–EGTA. After freezing and thawing this suspension, the particulate fraction was isolated and resuspended for assay of adenylate cyclase as in [11], except that when the action of added GTP was studied, endogenous guanine nucleotides were removed by homogenization of the pellet in buffer containing 10 mM Tris–HCl and 5 mM Tris–EGTA, pH 7.4. After centrifugation, this extraction procedure was repeated once more. In addition to the compounds specified in section 3, adenylate cyclase assay mixtures (250 μl) all contained 75 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 0.4 mM Tris–[^{3}H]ATP, 4 mM Tris–phosphocreatine, 20 units of creatine phosphokinase/ml, 1 mM cyclic AMP, 1 mM 3-isobutyl-1-methylxanthine, 1 mg of crystallized bovine serum albumin/ml, 0.4 mM dithiothreitol and 0.4 mM Tris–EGTA. After incubation for 10 min at 30°C, assays were terminated and cyclic [^{3}H]AMP isolated and quantitated [11]. The platelet protein present in assays was measured as in [11].

2.3. *Measurement of changes in platelet cyclic [^{3}H]AMP*

Human heparinized platelet-rich plasma prepared as in [4] was labelled by incubation with 2 μM [^{3}H]adenine for 90 min at 37°C. Samples (final vol. 0.5 ml containing 1.5–2.0 $\times$ 10^8 platelets) were incubated at 37°C with appropriate additions. Incubations were terminated after 0.5 min by addition of 1 ml of 15% (w/v) trichloroacetic acid. Cyclic [^{3}H]AMP was isolated and quantitated as in [11].

2.4. *Measurement of platelet aggregation*

Aggregation of human platelets in heparinized platelet-rich plasma, prepared as in [4], was measured turbidimetrically using a Payton Aggregometer (Scarborough, Ont.). The change in light transmittance (ΔT) 0.5 min after addition of AVP or analogues was determined. When antagonism of the action of AVP by dVDAVP was studied, platelets were incubated with dVDAVP for 20 s before addition of AVP.

2.5. *Analysis of dose–response curves*

To obtain best estimates of the concentrations of AVP causing half-maximal inhibition of adenylate cyclase (IC_{50} values) or half-maximal platelet aggregation (EC_{50} values), Hill plots of the results were constructed by linear regression. To avoid bias, only data points falling between 10 and 90% of the maximum effect were used. The inhibitory effects of dVDAVP on the actions of AVP were analyzed as in [13] to give pA_2 values for dVDAVP. IC_{50} or EC_{50} values, determined as above, were used to calculate the dose-ratio of AVP required to overcome the inhibitory effect of each concentration of dVDAVP studied.

3. RESULTS

When human platelet particulate fractions were washed to remove guanine nucleotides, addition of GTP alone had a biphasic effect on adenylate cyclase activity, tending to stimulate at low concentrations (0.1 μM) and to inhibit at high concentrations (10 μM) (table 1). Enzyme activity was enhanced by 100 mM NaCl, particularly in the

Table 1

Inhibition of the adenylate cyclase activity of human platelet particulate fraction by AVP; effects of NaCl and GTP

Additions		Adenylate cyclase activity (pmol·min^{-1}·mg protein^{-1})		Inhibition of adenylate cyclase by AVP (%)
NaCl (mM)	GTP (μM)	Without AVP	With AVP	
0	0	57.3 ± 0.7	55.8 ± 0.8	2 ± 2
	0.1	66.6 ± 0.7	58.3 ± 1.0	12 ± 2[a]
	10	41.8 ± 0.8	35.5 ± 0.8	15 ± 3[a]
100	0	69.1 ± 1.3	64.8 ± 0.3	6 ± 2
	0.1	83.4 ± 1.3	76.3 ± 1.8	9 ± 3
	10	74.3 ± 0.9	55.1 ± 0.9	26 ± 2[b]

[a] $p < 0.05$
[b] $p < 0.01$

The particulate fraction was washed twice with hypotonic buffer prior to resuspension for assay (see section 2). When present, AVP was 100 nM. Values for adenylate cyclase activity are means ± SEM from 3 or 4 determinations. The significance of inhibition by AVP was determined using Student's t-test

presence of 10 μM GTP. Addition of AVP caused an inhibition of adenylate cyclase activity that was completely GTP-dependent and was potentiated by NaCl (table 1). Optimal inhibition was obtained in the presence of 10 μM GTP, when 100 nM AVP decreased adenylate cyclase activity by 17 ± 2% without NaCl (mean ± SE, 6 expts) and by 27 ± 2% with 100 mM NaCl (mean ± SE, 8 expts). Under these conditions, the AVP concentration required for half-maximal inhibition was 1.2 ± 0.4 nM (7 expts). OVP caused the same maximal inhibition and was only slightly less potent (IC_{50} = 2.7 ± 0.9 nM, 3 expts), but dVDAVP alone had no significant effect on platelet adenylate cyclase activity (fig.1A). However, dVDAVP was found to be a competitive inhibitor of the action of AVP on platelet adenylate cyclase (fig.2). Calculation gave a pA_2 for dVDAVP of 7.74 ± 0.04 (4 expts), which corresponds to a K_i of 18 ± 2 nM.

We were able to confirm our previous observation that AVP has no effect on cyclic AMP formation in intact platelets [8], using a wider range of AVP concentrations (10 nM–1 μM). These experiments were carried out with heparinized platelet-rich plasma containing platelets labelled by preincubation with [^{3}H]adenine. Samples of this material were incubated with indomethacin (10 μM), phosphocreatine (2 mM) and creatine phosphokinase (100 units/ml) for 5 min before ad-

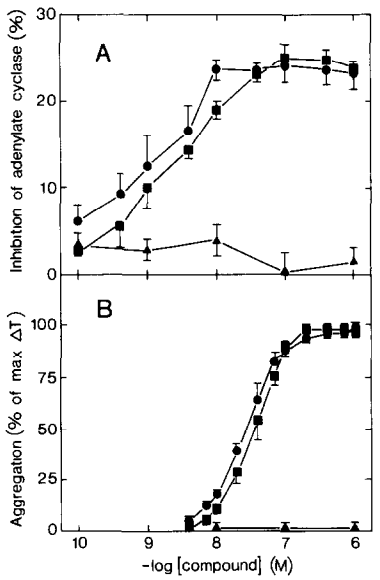

Fig.1. Dose–response curves for: (A) the inhibition of platelet adenylate cyclase; (B) the induction of platelet aggregation by AVP (●), OVP (■) and dVDAVP (▲). Values are means ± SEM from 3 separate experiments.

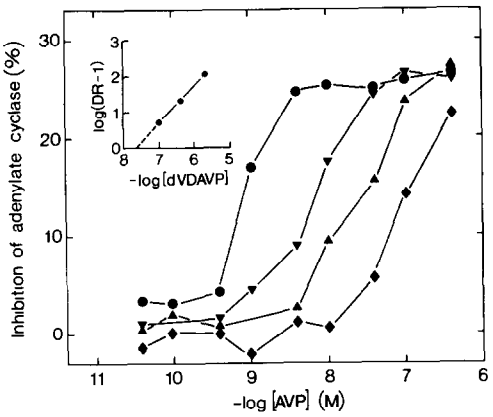

Fig.2. Effects of different concentrations of dVDAVP on the dose–response curve for inhibition of adenylate cyclase by AVP. The dVDAVP concentrations used were: none (●); 100 nM (▼); 400 nM (▲); 2000 nM (♦). Values are means of 3 determinations. Inset: Schild plot of the effect of dVDAVP; dose-ratios (DR) of AVP causing half-maximal inhibition of adenylate cyclase at different dVDAVP concentrations were calculated as noted in section 2.

dition of 0.2 μM prostaglandin E_1 with or without AVP, to prevent any indirect inhibition of adenylate cyclase as a result of thromboxane A_2 formation or the release of ADP. In these experiments, prostaglandin E_1 caused a 10-fold increase in platelet cyclic [^{3}H]AMP but no inhibitory effect on this of any of the AVP concentrations tested was detected.

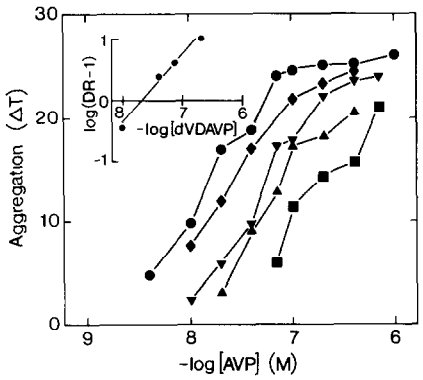

Fig.3. Effects of different concentrations of dVDAVP on the dose–response curve for induction of platelet aggregation by AVP. The dVDAVP concentrations used were: none (●); 10 nM (♦); 40 nM (▼); 70 nM (▲); 200 nM (■). Inset: Schild plot of the effect of dVDAVP; dose-ratios (DR) of AVP causing half-maximal platelet aggregation at different dVDAVP concentrations were calculated as noted in section 2.

Aggregation of platelets in heparinized platelet-rich plasma required much higher concentrations of AVP (EC_{50} = 27 ± 4 nM, 4 expts) and OVP (EC_{50} = 41 ± 8 nM, 3 expts) than were needed for inhibition of adenylate cyclase, but the relative activities of these two compounds were unaltered (fig.1B). Addition of dVDAVP alone had no effect on human platelets (fig.1B) but this compound was a potent competitive inhibitor of the aggregating action of AVP with a pA_2 of 7.82 ± 0.17 (6 expts) (fig.3). This pA_2 value for dVDAVP corresponds to a K_i of 16 ± 2 nM and does not differ significantly from that obtained when the compound was used to antagonize the inhibition of platelet adenylate cyclase by AVP.

4. DISCUSSION

The inhibition of platelet particulate fraction adenylate cyclase by AVP resembled the effects of other hormonal inhibitors of this enzyme in its dependence on a relatively high GTP concentration [6,14]. Potentiation of the inhibitory effects of hormones on adenylate cyclase by NaCl is also a well-documented effect in many biological systems [6], though in the platelet this has only been observed when precautions have been taken to prevent endogenous Ca^{2+}-dependent proteolysis [15]. Inhibition of adenylate cyclase with these characteristics is considered to be a receptor effect mediated by the N_i GTP-binding protein [6,14]. This is the first description of such an effect of AVP.

Our results indicate that the inhibition of adenylate cyclase by AVP is mediated by V_1-receptors. Thus, OVP has an enhanced ratio of vasopressor to antidiuretic activity (i.e., shows selectivity for V_1 receptors), whereas dVDAVP is a potent antidiuretic (V_2) agonist and vasopressor (V_1) antagonist [1]. The induction of platelet aggregation by AVP showed the same structural specificity as observed for the inhibition of adenylate cyclase. The identical pA_2 values for inhibition of both actions of AVP by dVDAVP are particularly significant and imply that very similar or identical receptors mediate both effects. Our

results show that a report claiming that platelet AVP receptors are of the renal V_2 type [16] is incorrect and support the conclusion previously drawn by ourselves [4] and another group [5] regarding the specificity of these receptors.

Although it is clear that platelet AVP receptors are of the V_1 type, our results do not exclude the possibility that different receptor subtypes are linked to the phosphoinositide breakdown associated with platelet aggregation induced by AVP [17] and to the inhibition of adenylate cyclase by this compound. More extensive analysis of the structure-activity relationships of a range of antagonists could reveal such a difference. However, it is also possible that different populations of identical AVP receptors are associated with the two different effector systems or that a single population links with one or the other system depending on the experimental conditions. The fact that no inhibition of adenylate cyclase by AVP could be detected in intact platelets favours the latter hypothesis, and also suggests that unidentified factors may exist that regulate the effector system with which the AVP receptors interact. Such a possibility has been envisioned previously [6]. The same phenomenon, that is a latent capacity to inhibit adenylate cyclase seen only in broken cell preparations, was also observed with respect to the effects of platelet-activating factor on human but not rabbit platelets [11,12].

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