Vasopressin inhibits cyclic AMP accumulation and adenylate cyclase activity in cerebral preparations

Michael E. Newman

Research Department, Jerusalem Mental Health Center, POB 140, Jerusalem, Israel

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Arginine-vasopressin (AVP) and lysine vasopressin (LVP) elicited a dose-dependent inhibition of noradrenaline-sensitive cyclic AMP accumulation in rat cerebral cortical slices, and of forskolin-stimulated adenylate cyclase activity in a rat cerebral cortical membrane preparation. In both cases LVP was more potent than AVP, and exerted half-maximal effects at concentrations similar to those found effective in binding studies on rat hippocampal membranes. In hippocampal slices, AVP did not affect cyclic AMP accumulation at low concentrations but potentiated the effect of noradrenaline at higher concentrations. In caudate membranes, AVP inhibited dopamine-stimulated adenylate cyclase with a similar dose-dependency to that seen for forskolin activation in cortex membranes.

Vasopressin *cAMP* *Adenylate cyclase* *Brain*

1. INTRODUCTION

The biochemical actions of vasopressin include stimulation of adenylate cyclase in the kidney [1] and mobilisation of the phospholipid phosphatidylinositol by a Ca-dependent mechanism in liver and aorta [2], these actions providing the bases for the antidiuretic and vasopressor effects of the hormone, respectively. Binding sites for vasopressin which are linked to these activities have been found in these tissues, and have been designated V_2 and V_1 receptors, respectively [3,4]. In brain, vasopressin has been shown to affect processes of learning and memory, possibly by modulation of the activity of the noradrenergic system [5,6]. Binding sites for vasopressin with nanomolar affinities have recently been demonstrated in cerebral, particularly hippocampal, tissue [7–9] but the relationship of these sites to the physiological processes mediated by vasopressin in the brain, and the biochemical mechanisms involved in these processes, remain unclear. We have demonstrated [10] that AVP and its derivative DDAVP, which is an extremely potent antidiuretic agonist and also has marked effects on learning as measured by extinction of the conditioned avoidance response [11], inhibit noradrenalin-sensitive cyclic AMP accumulation in slices derived from cortex, caudate and hippocampus of rats both in vivo and in vitro. An inhibitory effect of vasopressin on adenylate cyclase has recently been shown for basal activity in human platelets [12] and for forskolin-stimulated activity in hepatocytes [13], although the relationship of the inhibition to V_1 or V_2 receptor activity is not known. In striatal homogenates [14] vasopressin at high (micromolar) concentrations was shown to potentiate the stimulatory effect of dopamine on adenylate cyclase. Here, the effects of various concentrations of AVP and LVP on cyclic AMP accumulation induced by noradrenaline and forskolin in rat brain slices, and on adenylate cyclase activity in membrane preparations derived from cortex and caudate, have been examined.

Abbreviations: AVP, arginine-8-vasopressin; DDAVP, 1-deamino-[8-D-arginine]-vasopressin; IBMX, 3-isobutyl-1-methylxanthine

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2. MATERIALS AND METHODS

Male rats of the Sabra strain (150–200 g) were used for all experiments. For preparation of slices, brain areas were dissected on a cooled dish and sliced using a McIlwain tissue chopper set at 0.35 mm. All slices from each area were preincubated for 30 min at 37°C in Krebs-Ringer's bicarbonate buffer containing 1.29 mM $CaCl_2$, gassed with 95% O_2:5% CO_2. The slices were then collected on a Buchner funnel and distributed among vials containing 5 ml Krebs-Ringer's with additions for 20 min incubation. After this period the slices were transferred to test-tubes, centrifuged, the medium decanted, and the pellets homogenised in 2 ml (for cortex) or 1 ml (for caudate or hippocampus) of 95% ethanol. Aliquots of the supernatants were evaporated to dryness under N_2 and cyclic AMP determined by displacement of [^{3}H]cAMP (Radiochemical Centre, Amersham) using a protein binding method based on that of Brown et al. [15].

For preparation of membranes, brain areas were homogenised in 10 vols of 50 mM Tris–HCl (pH 7.4), 2 mM EGTA, using a Polytron homogeniser, and centrifuged at 1000 × *g* for 10 min. The resultant supernatant was centrifuged at 20000 × *g* for 20 min and the pellet produced washed in the original volume of homogenising buffer. It was then resuspended to a protein concentration of about 1 mg/ml, and 3-ml aliquots stored at −70°C.

Adenylate cyclase was measured in a total assay volume of 0.2 ml in a medium containing 25 mM Tris–HCl, 0.5 mM ATP, 2 mM $MgCl_2$, 1 mM EGTA, 1.5 mg/ml creatine phosphate, 0.2 mg/ml creatine phosphokinase, and 1 mM IBMX. Experiments involving dopamine stimulation of cyclase in caudate membranes were conducted in the additional presence of 1 μM GTP. Incubations were started by addition of protein and terminated after 10 min at 30°C by addition of 0.8 ml absolute ethanol. The tubes were then centrifuged and cyclic AMP in aliquots of the supernatants determined as above.

3. RESULTS

Incubation of cerebral cortical slices with 50 μM noradrenaline led to a 3-fold increase in cyclic

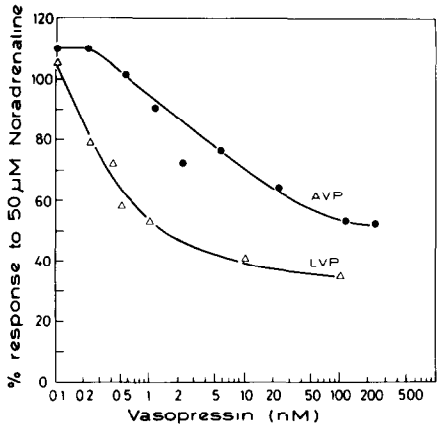

Fig.1. Dose-response curve for inhibition of noradrenaline-sensitive cyclic AMP accumulation in rat cortical slices by AVP and LVP. Basal cyclic AMP levels were 17.2 ± 2.7 pmol/mg protein and levels in the presence of 50 μM noradrenaline 49.5 ± 4.3 pmol/mg protein. Each point represents the mean of 4 separate determinations.

Table 1

Effects of AVP on hormonal stimulation of cyclic AMP formation in hippocampal and caudate preparations

[AVP] (nM)	Preparation	
	Hippocampal slices [stimulating agent: noradrenaline (50 μM)]	Caudate membranes [stimulating agent: dopamine (50 μM)]
0.7	94 ± 20	83 ± 8
14	117 ± 23	70 ± 9
55	99 ± 17	65 ± 5
110	139 ± 16	66 ± 6
220	165 ± 35	63 ± 7
550	169 ± 13	45 ± 8

Results are expressed as percentages of values obtained in the presence of noradrenaline or of dopamine alone, and are mean ± SE of 6 observations in each case. Basal cyclic AMP levels in hippocampal slices were 10.2 ± 2.2 pmol/mg protein, and levels in the presence of 50 μM noradrenaline 38.1 ± 2.5 pmol/mg protein. Adenylate cyclase activity in caudate membranes in the presence of 1 μM GTP was 35 ± 2 pmol/min per mg protein, and in the additional presence of 50 μM dopamine 67 ± 5 pmol/min per mg protein

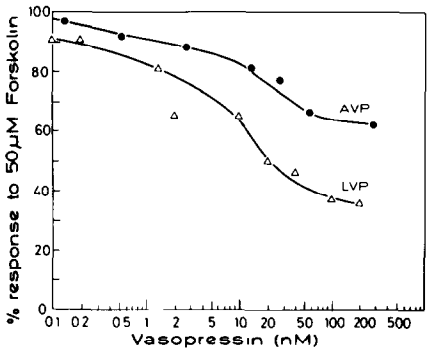

Fig.2. Dose-response curve for inhibition of forskolin-stimulated adenylate cyclase activity in rat cortical membranes by AVP and LVP. Basal adenylate cyclase in these membranes was 58 ± 14 pmol/min per mg protein and activity in the presence of 50 μM forskolin 380 ± 58 pmol/min per mg protein. Each point represents the mean of 4 separate determinations.

AMP content. Addition of increasing concentrations of AVP to the preparation resulted in a dose-dependent inhibition of the response (fig.1). LVP in cortical slices was more effective than AVP, and produced dose-dependent inhibition of the response to noradrenaline with a half-maximally effective concentration of 0.3 nM (fig.1). In hippocampal slices, noradrenaline alone produced a 4-fold increase in cyclic AMP levels. AVP at low concentrations had no effect on this response (table 1), but at concentrations above 0.2 μM appeared to potentiate the noradrenaline stimulation.

In a membrane preparation from cerebral cortex, both AVP and LVP inhibited the stimulation of adenylate cyclase activity due to addition of 50 μM forskolin (fig.2). LVP was again more potent than AVP and produced half-maximal inhibition of the response at a concentration of 7 nM. Membranes from caudate nucleus showed a doubling of activity when incubated with 50 μM dopamine. The dopamine stimulation was also dose-dependently inhibited by AVP (table 1). In hippocampal membranes, AVP did not affect either basal or serotonin-stimulated adenylate cyclase activity over the entire concentration range tested.

4. DISCUSSION

The present results show that AVP and LVP at concentrations comparable to those used in binding studies [3,4,7–9] are capable of inhibiting both hormone and forskolin-induced cyclic AMP accumulation in preparations of both slices and membranes from rat cortex and caudate. Indeed, the concentration of AVP giving half-maximal inhibition of noradrenaline-induced cyclic AMP accumulation in cortical slices, 3.2 nM, was very similar to the K_d determined by Barberis [7] for binding of this compound to hippocampal slices, 2.8 nM. For LVP identical values of 7 nM were obtained for half-maximal inhibition of forskolin-stimulated adenylate cyclase in cortex membranes in the present work, and for binding in hippocampal membranes. The concentrations found effective in this study are greater than the in vivo levels of vasopressin in the brain as found by several authors [16–18]. Hamburger-Bar et al. [16] found levels of 10–50 pg/mg protein, corresponding to a concentration of 1–50 pM, in hippocampus and caudate, while the levels in hypothalamus were in general 1000-fold higher. Modulation of the in vivo level of vasopressin will therefore result in changes in both receptor occupation and the degree of inhibition of adenylate cyclase, so that both these activities are likely to be involved in the physiological effects of the hormone.

The present results confirm our earlier findings [10] of inhibition by vasopressin of the noradrenaline-induced accumulation of cyclic AMP in rat cerebral cortex and caudate slices. In hippocampal slices, previous results obtained with a single concentration of vasopressin [10] indicated no effect in vitro or after acute treatment of animals, but inhibition of the noradrenaline response after chronic treatment. Church [19] obtained potentiation of the noradrenaline response in mouse hippocampal slices by 1 μM LVP, and the present results indicate that a similar phenomenon occurs with rat hippocampal slices and AVP. In caudate membranes, Courtney and Raskind [14] observed potentiation of the response to dopamine at AVP concentrations of 1 μM and above. The present results show that at lower, physiological concentrations, AVP inhibits the dopamine response, with a concentration dependence similar to that seen for inhibition of the response to noradrenaline in the cortex slice preparation. Inhibition by vasopressin of forskolin stimulation of adenylate cyclase has not previously

been demonstrated in cerebral preparations, although this effect was observed in rat hepatocytes [13].

These results do not permit any definitive conclusions as to whether the effects observed are related to V_1 or V_2 receptor function, or whether they bear any relation to the actions of vasopressin on memory and learning. With regard to the former question, values obtained for half-maximal inhibition of adenylate cyclase in the present experiments are similar to those obtained by Vanderwel et al. [12], who concluded the effect to be mediated by the V_1 receptor/Ca^{2+} mobilisation mechanism, in platelets. However, Morgan et al. [13] observed inhibition of forskolin-stimulated adenylate cyclase in hepatocytes with a half-maximal response in the same range at 0.1 nM even in Ca^{2+}-depleted cells, and concluded that the effect does not involve Ca^{2+} mobilisation. The observation that LVP was more potent than AVP in these experiments may indeed imply that the effect described here is mediated by a third class of vasopressin receptors, as has also been proposed for the action of vasopressin in stimulating ACTH release from the anterior pituitary gland [20]. Increases in cyclic AMP have been proposed to be associated with improved performances of animals in tests of memory and learning [21], while the effects of vasopressin derivatives on learning have been considered to be localised to the hippocampus [6]. The differential effects of AVP observed in the present study in inducing a rise in cyclic AMP accumulation in hippocampus, and a fall in the other brain areas tested, may therefore be relevant to molecular processes underlying learning and memory phenomena. Further work to resolve these questions is in progress.

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