Studies on the mechanism of inhibition of hepatic cAMP accumulation by vasopressin

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Vasopressin elicited a dose-dependent inhibition of glucagon-induced cAMP accumulation in isolated hepatocytes. This response was not diminished by incubation of cells with the calmodulin antagonists trifluoperazine or chlorpromazine and was only slightly reduced in Ca^{2+}-depleted hepatocytes. Half-maximal inhibition of cAMP accumulation occurred at 8×10^{-11} M vasopressin, a dose which does not increase cytosolic Ca^{2+} in hepatocytes. Direct activation of adenylate cyclase by forskolin was significantly inhibited by vasopressin in Ca^{2+}-depleted cells. It is concluded that inhibition of hormone-induced cAMP accumulation by vasopressin in liver is not dependent on cellular Ca^{2+} mobilisation but may involve direct inhibition of adenylate cyclase.

Hepatocyte *cAMP* *Vasopressin* *Calcium* *Adenylate cyclase* *Glucagon*

1. INTRODUCTION

Recent studies have revealed that the vasoactive peptide vasopressin can elicit a number of responses in rat liver, including Ca^{2+} mobilization, phospholipid turnover and inhibition of hormonally induced cAMP accumulation [1–5]. There is much evidence that the metabolic responses to vasopressin in liver directly result from cellular Ca^{2+} mobilisation and elevation of cytosolic Ca^{2+} [6]. Since inhibition of glucagon-induced cAMP formation has also been demonstrated in rat hepatocytes incubated in the presence of the Ca^{2+} ionophore A23187 [5,7], it is possible that increases in cytosolic Ca^{2+} may play a primary role in cAMP inhibition by vasopressin, perhaps as a result of activation of Ca^{2+}-calmodulin-dependent cAMP phosphodiesterase [8]. Alternatively, direct inhibition of adenylate cyclase by a Ca^{2+}-independent mechanism could also mediate the response, as in the case of α_2-adrenergic agonists and angiotensin II [4,9]. We here investigated the role of Ca^{2+} in vasopressin-induced cAMP inhibition and demonstrate that this response is not secondary to Ca^{2+} mobilisation, but occurs by an independent mechanism.

2. EXPERIMENTAL

Parenchymal cells from the livers of fed male Sprague-Dawley rats (180–220 g body wt) were isolated and incubated as described [10]. Ca^{2+}-depleted hepatocytes were prepared as in [5] and, when appropriate, Ca^{2+}-repletion was achieved by re-addition of 4 mM Ca^{2+} to Ca^{2+}-depleted cells, 20 min prior to hormone addition. Hepatocyte cAMP levels were measured by radioimmunoassay of perchloric acid extracts as in [11].

Arginine-vasopressin, angiotensin II, trifluoperazine and chlorpromazine were from Sigma. Glucagon was a gift from Lilly (IN). A23187 and forskolin were from Calbiochem.

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3. RESULTS

The data presented in fig.1 demonstrate that, in isolated hepatocytes treated with 10^{-8} M glucagon, vasopressin (10^{-8} M) markedly reduced the extent of cAMP accumulation. In contrast, vasopressin did not alter basal cAMP levels in hepatocytes (table 1, [4,5]). The ability of vasopressin to lower glucagon-induced cAMP accumulation was most evident in cells incubated in medium containing Ca^{2+}, where up to 35% inhibition could be achieved at 10^{-8} M vasopressin (fig.1). The response was significantly ($p < 0.025$) attenuated when Ca^{2+}-depleted cells were employed (fig.1), although under these conditions vasopressin still caused significant inhibition of glucagon-induced cAMP accumulation ($p < 0.025$, fig.1). These data suggest that cAMP inhibition was not solely the consequence of cellular Ca^{2+}-mobilisation. In support of this, vasopressin was also observed to inhibit phosphorylase activation in response to low doses of glucagon under conditions of Ca^{2+}-depletion (not shown).

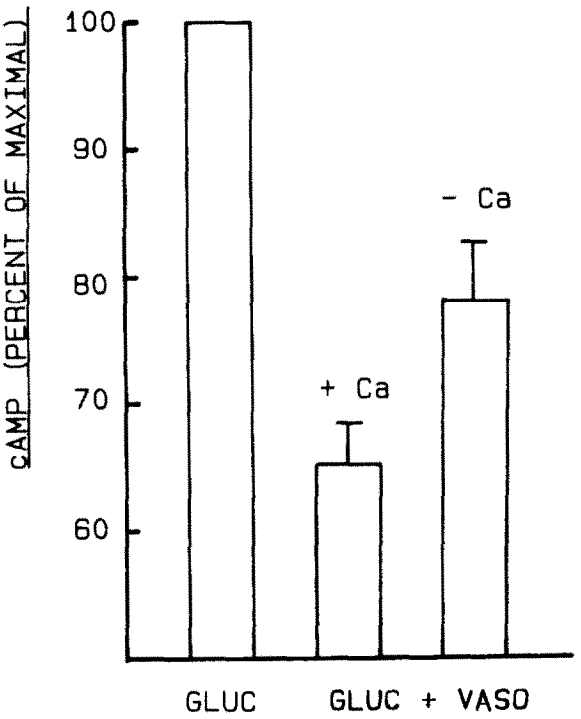

Fig.1. Effect of calcium depletion on the inhibition of cAMP accumulation by vasopressin. Ca^{2+}-depleted hepatocytes were prepared as described in section 2, and Ca^{2+} was added back to a proportion of the cells. Glucagon (10^{-8} M) and vasopressin (10^{-8} M) were added and samples removed for cAMP measurements 2 min later. Results are expressed as percentages of the maximal cAMP level in response to glucagon alone, and represent mean values ± SEM from 4 separate cell preparations each incubated in triplicate. Basal cAMP levels were not altered in Ca^{2+}-depleted *vs* Ca^{2+}-replete cells (0.36 ± 0.06 pmol/mg cells) and under these conditions glucagon increased cAMP to 4.63 ± 1.3 pmol/mg cells and 3.14 ± 0.76 pmol/mg cells, respectively (mean values from 4 experiments).

Pre-incubation of hepatocytes with the calmodulin inhibitors trifluoperazine and chlorpromazine at concentrations up to 100 μM, failed to diminish vasopressin's ability to inhibit glucagon-induced cAMP accumulation (fig.2).

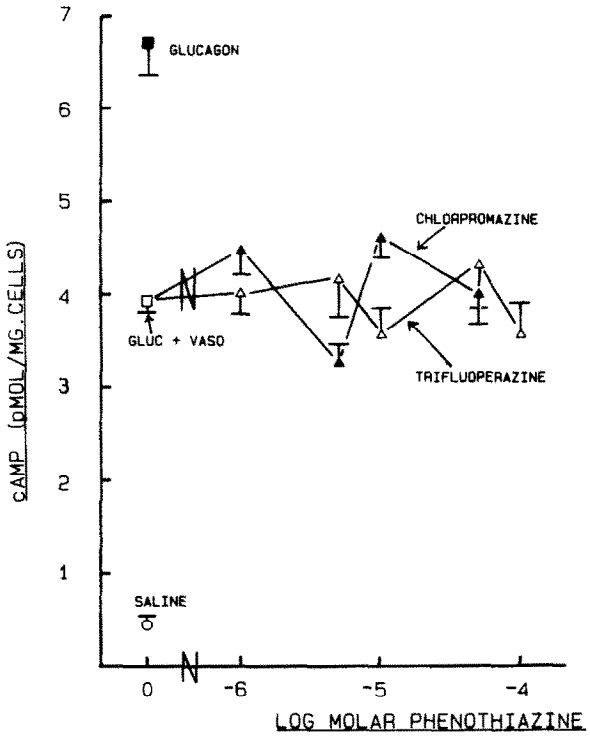

Fig.2. Effect of calmodulin antagonists on vasopressin-mediated cAMP inhibition. Isolated hepatocytes were preincubated for 15 min with different concentrations of either trifluoperazine or chlorpromazine as shown. After this time glucagon (10^{-8} M) and vasopressin (10^{-8} M) were added and incubation continued for a further 2 min, when samples were withdrawn for determination of cAMP content. Data are expressed as mean values ± SEM for quadruplicate incubations from an experiment representative of two.

Since these agents did not alter the cAMP response to glucagon alone (not shown), the results indicate that calmodulin-dependent processes play no major role in the inhibitory action of vasopressin.

The data presented in fig.3 demonstrate that inhibition of glucagon-induced cAMP accumulation by vasopressin was dose-dependent with the half-maximal response being observed at a hormone concentration as low as 8×10^{-11} M.

The diterpene forskolin is a potent activator of adenylate cyclase in a variety of tissues [12] and caused a dose-dependent increase in cAMP levels in isolated hepatocytes (table 1). In Ca^{2+}-depleted cells, which were employed to eliminate any Ca^{2+}-mediated inhibition of cAMP accumulation, increases in cAMP in response to forskolin were markedly inhibited by vasopressin (table 1). The extent of this inhibition was similar to that induced by angiotensin II (table 1) which can directly inhibit adenylate cyclase in liver plasma membranes [4,9].

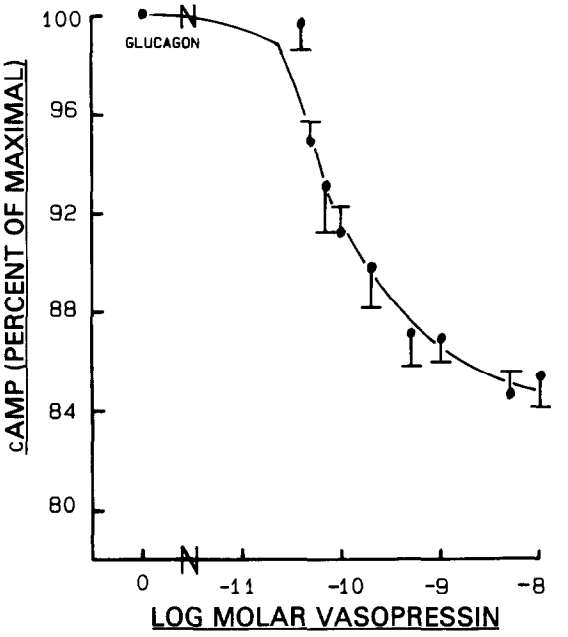

Fig.3. Dose–response curve for inhibition of glucagon-induced cAMP accumulation in hepatocytes, by vasopressin. Isolated hepatocytes were incubated with 10^{-8} M glucagon in the presence of increasing doses of vasopressin. Samples were withdrawn 2 min after hormone addition for determination of cell cAMP content. Results are expressed as percentages of the maximal cAMP response in the absence of vasopressin, and represent mean values ± SEM obtained from 3 separate cell preparations each replicated 6-times.

Table 1

Dose–response relationship for hepatocyte cAMP accumulation in response to forskolin

[Forskolin] (M)	Cell cAMP content (pmol/mg cells) Additions to medium		
	Saline	Vasopressin (10^{-8} M)	Angiotensin II (10^{-8} M)
0	1.94	2.04	1.95
5×10^{-7}	1.77		
10^{-6}	2.06		
5×10^{-6}	3.02		
10^{-5}	7.08		
2×10^{-5}	9.81 ± 0.95	7.31 ± 0.51[a]	
5×10^{-5}	12.75 ± 1.37	8.14 ± 0.95[a]	6.53 ± 0.82[b]

Ca^{2+}-depleted hepatocytes were incubated with increasing concentrations of forskolin in the absence or presence of vasopressin or angiotensin II for 1 min, after which time samples were withdrawn into 0.6 N perchloric acid for measurement of cAMP content. Data are mean values obtained from triplicate incubations in an experiment representative of two. [a] $p < 0.05$, [b] $p < 0.01$ *vs* forskolin alone

4. DISCUSSION

It has been surmised for several years that treatment of isolated rat hepatocytes with vasopressin leads to an increase in the cytosolic Ca^{2+} concentration which then mediates the subsequent metabolic events associated with hormone stimulation [6]. Recently, direct evidence in support of this concept has been provided [1]. Vasopressin has also been shown to inhibit the cAMP response to glucagon in liver cells [4,5], and since ionophore A23187 also inhibits this response [5,7] under conditions where Ca^{2+}-influx into the cell is enhanced [13], it seems possible that this action of vasopressin could also be the result of cellular Ca^{2+}-mobilisation. One mechanism which might account for this effect, would be that Ca^{2+} mobilisation in response to the hormone causes activation of calmodulin-dependent cAMP phosphodiesterase, leading to enhanced degradation of cAMP [8]. Such a mechanism has been implicated in part, in the ability of muscarinic

cholinergic agonists to antagonise hormone-stimulated cAMP accumulation in rat prostate gland [14] and in cultured fibroblasts [15]. Pre-incubation of isolated hepatocytes with the calmodulin antagonists trifluoperazine and chlorpromazine, at concentrations sufficient to allow penetration into liver cells [16] and which inhibit calmodulin-dependent processes in other tissues (e.g., [17]), failed, however, to attenuate vasopressin-induced inhibition of cAMP accumulation (fig.2). Furthermore, total depletion of the hormone-sensitive Ca^{2+} pool in hepatocytes (by treatment with EGTA, see [11,18]) also failed to prevent cAMP inhibition by vasopressin (fig.1). It should be noted however, that under these conditions the extent of inhibition was reduced compared to that in controls to which Ca^{2+} had been added back (fig.1). These data suggest, therefore, that cellular Ca^{2+} mobilisation in response to vasopressin plays only a relatively minor role in the mediation of cAMP inhibition.

Further support for this suggestion derives from a comparison of the sensitivity of Ca^{2+}-mobilisation and cAMP inhibition to the hormone. As shown in fig.3, the half-maximal concentration of vasopressin required for inhibition of glucagon-induced cAMP formation was about 8×10^{-11} M. In contrast, Ca^{2+}-mobilisation and phosphorylase activation (which results from the increased cytosolic Ca^{2+} level [6,13]) require a 5-fold greater concentration of vasopressin for half-maximal effects [13,18]. Indeed, cytosolic Ca^{2+} is not significantly increased by the dose of vasopressin which mediates half-maximal effects on cAMP accumulation (fig.1, [18]).

An alternative mechanism by which vasopressin could antagonize hepatic cAMP accumulation is through inhibition of adenylate cyclase. In this respect, angiotensin II (a vasopressor hormone which, like vasopressin, causes both Ca^{2+}-mobilisation and cAMP inhibition in liver; see [5]) has been demonstrated to directly inhibit adenylate cyclase activity in liver plasma membranes [4,9]. It is possible, therefore, that, by analogy with angiotensin II, vasopressin may also cause a direct inhibition of hepatic adenylate cyclase. The data in table 1 are in accord with this contention. Forskolin is a generalised activator of adenylate cyclase [12] whose action can be inhibited by a variety of hormones which exert a negative control over adenylate cyclase [19]. Vasopressin was here observed to lower forskolin-mediated cAMP increases in Ca^{2+}-depleted hepatocytes, consistent with a direct inhibition of adenylate cyclase (table 1). In addition, angiotensin II exerted a similar influence.

These data are consistent with a direct effect of vasopressin to inhibit liver cell adenylate cyclase. In this respect it has been observed that the binding of hormones which inactivate adenylate cyclase to their receptors is modified by guanine nucleotides [20]. This has been demonstrated in liver for both angiotensin II and α_2-adrenergic agonists [4,20]. Furthermore, the binding of [^{3}H]vasopressin to liver membranes is also decreased by GTP [21] consistent with a possible negative coupling at the level of adenylate cyclase. In one study [9], however, direct inhibition of liver plasma membrane adenylate cyclase activity by vasopressin was not observed under conditions where angiotensin II was inhibitory. It should be emphasized, however, that the conditions required to obtain effects in that study were very stringent [9] and may have been inappropriate for the vasopressin response. In view of the differential sensitivity of cellular Ca^{2+}-mobilisation and hormone-induced cAMP inhibition to vasopressin (fig.3, [18]) it is possible that different vasopressin receptors mediate these respective effects, as is the case for the α-adrenergic receptors.

In conclusion, our data suggest that the ability of vasopressin to lower hepatic cAMP levels is largely independent of cellular Ca^{2+}-mobilisation and results from interaction with a receptor of higher affinity than that which induces Ca^{2+}-mobilisation. This high affinity receptor may be directly linked to inhibition of adenylate cyclase.

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