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INVOLVEMENT OF A PLASMA MEMBRANE PHOSPHODIESTERASE IN THE NEGATIVE CONTROL OF CYCLIC AMP LEVELS BY VASOPRESSIN IN RAT HEPATOCYTES

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Abstract—Vasopressin has been shown previously to lower the glucagon-induced increase of cyclic AMP levels in isolated rat hepatocytes by way of an enhanced phosphodiesterase (EC 3.1.4.17) activity. Five phosphodiesterase inhibitors were tested for their ability to prevent vasopressin from lowering cyclic AMP levels in intact hepatocytes and for their inhibitory effect *in vitro* on soluble and particulate phosphodiesterase activities partially purified from hepatocytes.

Three soluble activities have been separated by DEAE-cellulose chromatography: a phosphodiesterase hydrolyzing both cyclic AMP and cyclic GMP, a form stimulated by cyclic GMP and a cyclic AMP-specific activity. The absence of any statistically significant correlation between the *in vivo* (in intact cells) and the *in vitro* (on isolated phosphodiesterases) potencies of the inhibitors does not support a role for the cytosolic phosphodiesterases in mediating the vasopressin-induced decrease in cyclic AMP levels. No statistically significant correlation was observed between the inhibition of the vasopressin effect on cyclic AMP accumulation and the inhibition of phosphodiesterase activity either associated with the native plasma membranes or solubilized from these membranes with 0.4 M NaCl. In contrast, a statistically significant correlation was observed between the degree of inhibition of the vasopressin effect in the intact cells and the degree of inhibition of the intrinsic phosphodiesterase still associated with the plasma membranes after high-salt treatment. These data indicate that a phosphodiesterase activity integral to the plasma membrane is very likely involved in the negative control of cyclic AMP levels by vasopressin.

Vasopressin exerts a powerful glycogenolytic effect in the liver by increasing the cytosolic concentration of calcium ions (for a recent review see [1]). The peptide has also been shown to produce an inhibition of glucagon-stimulated cyclic AMP accumulation in isolated hepatocytes [2–4]. The physiological significance of this effect is not readily apparent but can be related to the inhibition provoked by the same hormone on cyclic AMP accumulation in smooth muscle cells exposed to beta adrenergic agonists [5].

Recent results obtained in the liver point to a stimulation of the phosphodiesterase activity rather than to an inhibition of the adenylate cyclase activity: (i) no effect of vasopressin is observed on liver adenylate cyclase activity [6, 7], (ii) vasopressin is able to partially counteract the glycogenolytic effect of exogenous cyclic AMP [4]; (iii) the inhibitory effect of vasopressin on cyclic AMP levels is no longer observed when hepatocytes are incubated in the presence of several phosphodiesterase inhibitors, in particular 1-methyl-3-isobutylxanthine (MIX)§ [4].

The effects of vasopressin in rat hepatocytes present some analogies with the effects of carbamoylcholine in dog thyroid. Indeed, like vasopressin, carbamoylcholine enhances the intracellular concentration of Ca^{2+} [8] and inhibits the accumulation of cyclic AMP induced by thyrotropin. This effect of carbamoylcholine results from an increase in cyclic AMP phosphodiesterase activity [9].

Phosphodiesterase inhibitors are useful tools in cyclic nucleotide research. They have been used in several studies with smooth, skeletal and heart muscles to demonstrate that data obtained *in vitro* can be used to accurately predict their relative abilities to alter cyclic AMP or cyclic GMP levels in intact cells [10, 11]. Using several relatively specific phosphodiesterase inhibitors, tested on acellular enzymatic preparations and on cyclic AMP accumulation in thyroid slices, we have been able to demonstrate that the negative control of cyclic AMP levels exerted by carbamoylcholine is mediated by the soluble calmodulin-sensitive phosphodiesterase secondary to an increase of intracellular Ca^{2+} levels [12, 13]. The same strategy has been followed in this study.

§ Abbreviations used: MIX, 1-methyl-3-isobutylxanthine; 7-benzyl MIX, 1-methyl-3-iso-butyl-7-benzylxanthine; IIX, 1-isoamyl-3-isobutylxanthine; 8-MeOMe MIX, 1-methyl-3-isobutyl-8-methoxymethylxanthine; Ro 20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; PMSF, phenylmethylsulfonyl fluoride; cyclic AMP, adenosine 3′,5′-monophosphate.

As in other mammalian tissues, liver phosphodiesterase activity consists of multiple soluble and particulate-associated enzymes [14]. The same inhibitors as those used in dog thyroid, four xanthine derivatives and Ro 20-1724, have been compared in this work. We have attempted to determine which type of phosphodiesterase is involved in the decrease of cyclic AMP concentration provoked by vasopressin in rat hepatocytes challenged with glucagon.

MATERIALS AND METHODS

Studies with cells. Liver cells from male Wistar rats (200–250 g body weight) were prepared and incubated in Krebs–Henseleit bicarbonate buffer equilibrated with CO_2/O_2 (19:1) as previously described [15]. Cells (5×10^6 cells/ml) suspended in this medium were incubated for 20–30 min in the presence of 10 mM glucose and 14 mM bacitracin at 37° in closed plastic 20 ml vials with continuous shaking. Further experimental details are described in the legend of Table 1. Aliquots of the cell suspension were frozen in liquid nitrogen for later cyclic AMP determinations. Proteins were precipitated with a final concentration of 10% trichloroacetic acid which was subsequently removed by washing with 5 ml diethylether. Cyclic AMP concentration was measured in the supernatant fraction by a competitive protein-binding technique [16].

Phosphodiesterase assay. Phosphodiesterase activity was measured with 1 or 3 μM [^{3}H]cyclic AMP or 3 μM [^{3}H]cyclic GMP as previously described [17]. Incubation was performed at 30° for 10–30 min to obtain about 20% hydrolysis of the substrate in the absence of inhibitor. This reaction was followed by complete conversion of [^{3}H]AMP to tritiated dephosphorylated products by incubation with an excess of 5′-nucleotidase (EC 3.1.3.5). The dephosphorylated nucleotides were separated from the cyclic nucleotides by chromatography on QAE-Sephadex A-25 columns. Phosphodiesterase activity was expressed as pmol cyclic AMP hydrolyzed/min/mg protein. Concentration of protein was determined by the procedure of Bradford [18].

Separation of the soluble phosphodiesterases from rat hepatocytes. A hepatocyte suspension (20 ml of 5×10^6 cells/ml) was incubated with glucose and bacitracin for 15 min at 37°, as described above. The incubation was stopped by addition of 60 ml ice-cold buffer containing 10 mM Tris–HCl (pH 7.4), 2 mM EDTA, 0.25 M sucrose. The diluted cell suspension was centrifuged at 50 *g* and the pellet was resuspended in 10 ml ice-cold buffer A (20 mM Tris–HCl (pH 7.4), 2 mM $MgCl_2$, 0.4 mM PMSF, 2 mM benzamidine, 5 μM leupeptin, soybean trypsin inhibitor (25 mg/l), 5 mM 2-mercapthoethanol and 0.25 M sucrose). The cells were homogenized at 4° with a Ultra-Turax homogenizer (3 × 5 sec) at full speed. The homogenate was centrifuged at 250,000 *g* for 30 min at 4°. About 50% of the total phosphodiesterase activity was recovered in the supernatant fraction with 1 μM cyclic AMP as substrate (not shown). The crude supernatant fraction (about 30 mg protein) was applied to DEAE-cellulose (DE-52) column (0.9 × 13 cm). The column was washed with 50 ml buffer A without sucrose. Phosphodiesterase activity was eluted by a gradient of 0–0.5 M $(NH_4)_2SO_4$ (400 ml:400 ml) prepared in buffer A without sucrose [12]. Peak fractions were concentrated with an Amicon PM10 ultrafiltration cell and stored in aliquots in liquid nitrogen in the presence of bovine serum albumin (1 mg/ml).

Preparation of plasma membranes from rat hepatocytes. A hepatocyte suspension obtained as in the preparation of soluble phosphodiesterases was centrifuged at 50 *g*. The cell pellet was resuspended in a buffer B (5 mM Tris–HCl (pH 7.4), 0.4 mM PMSF, 2 mM benzamidine, 5 μM leupeptin, soybean trypsin inhibitor (25 mg/l) and 0.25 M sucrose) so as to reach a concentration of 10^7 cells/ml. The homogenization was performed with a Dounce

Table 1. Effect of phosphodiesterase inhibitors on the ability of vasopressin to inhibit glucagon-dependent cyclic AMP accumulation

Inhibitor	Cyclic AMP (pmol/mg protein)			Relief of vasopressin effect (%)
	Control	Glucagon	Glucagon + Vasopressin	Inhibition by vasopressin (%)
Experiment 1				
Dimethylsulfoxide	3.8 ± 0.8 (3)	25.9 ± 1.4 (3)	18.8 ± 1.3 (3)	32.1 ± 3.4[a]	
MIX	7.8 ± 1.0 (3)	138.3 ± 5.8 (3)	135.2 ± 4.8 (3)	2.4 ± 1.6[c]	92.5 ± 10.0
IIX*	6.7 ± 1.0 (3)	137.6 ± 9.1 (3)	144.3 ± 21.6 (3)	−5.1 ± 2.0[c]	115.6 ± 8.0
Ro 20-1724	10.5 ± 0.7 (3)	141.8 ± 3.8 (3)	126.5 ± 4.8 (3)	11.6 ± 1.5[b]	63.9 ± 1.7
Experiment 2				
Dimethylsulfoxide	3.7 ± 0.5 (7)	38.8 ± 1.9 (7)	25.0 ± 1.9 (7)	39.3 ± 3.9[a]	
8-MeOMeMIX	9.0 ± 1.6 (4)	118.4 ± 10.8 (4)	78.0 ± 5.8 (4)	36.7 ± 8.9[a]	6.6 ± 0.3
7-benzyl MIX	5.3 ± 1.1 (3)	92.5 ± 6.4 (3)	58.7 ± 6.8 (3)	38.8 ± 10.5[a]	1.3 ± 0.3

Hepatocytes were incubated for 5 min in the presence of 0.1 mM of a phosphodiesterase inhibitor dissolved in dimethylsulfoxide at a final concentration of 1%; in control experiments 1% dimethylsulfoxide was added to the medium. A subsequent 2 min incubation was performed in the presence of 5 nM glucagon or 5 nM glucagon and 25 nM vasopressin. The values of cyclic AMP concentration are means ± SEM (N experiments). The statistical significance of the vasopressin effect was estimated by the paired *t*-test: [a]$P < 0.02$; [b]$P < 0.05$; [c]$P > 0.1$. In order to compare the two sets of experiments, the percent relief of the vasopressin effect by each inhibitor is listed in the last column.

*IIX concentration: 0.05 mM due to its low solubility.

homogenizer using three series of 25 strokes each. After each series the preparation was centrifuged at 250 *g* for 10 min. The three supernatant fractions were pooled and centrifuged at 1500 *g* for 10 min. The final pellet was resuspended in buffer B without sucrose. Five and one half volumes of 70.7% sucrose in buffer B without sucrose were then added. Thirty-two ml of the suspension were overlayed with 16 ml 48.2% and 8 ml 42.5% sucrose respectively. The preparation was centrifuged for 1 hr at 66,000 *g*. The plasma membranes (monitored with 5′-nucleotidase activity) were recovered at the 42.5–48.2% sucrose interface. They were washed twice with 4 vol. of buffer B without sucrose and sedimented at 12,000 *g* for 10 min. The membranes were resuspended in buffer B without sucrose at a concentration of 1–2 mg of protein per ml. Phosphodiesterase activity was measured in preparations which were either fresh or had been stored in liquid nitrogen; no differences were noted between fresh and frozen preparations.

Treatment of plasma membranes with 0.4 *M*-NaCl. The plasma membrane preparation (in buffer B without sucrose) was diluted to 0.5–1 mg of protein per ml in a solution of 40 mM Tris–HCl (pH 7.5), 0.4 M NaCl, 20 mM 2-mercaptoethanol (final concentrations). High-salt solubilization of phosphodiesterase activity was performed as described by Marchmont and Houslay [19]. Both the high-salt-solubilized proteins and the high-salt-treated membranes were used immediately for phosphodiesterase assay.

Materials. [Arg^8]vasopressin (grade VI), PMSF, 5′-nucleotidase (*Crotalus atrox* venom), soybean trypsin inhibitor, benzamidine, MIX, and leupeptin were purchased from Sigma Chemical Company (St Louis, MO). Glucagon was from Novo Laboratories (Copenhagen, DK). Ro 20-1724 was kindly provided by Hoffman-La Roche Company (Nutley, NJ); IIX, 7-benzyl MIX and 8-MeOMe MIX were prepared according to published procedures [20–22]. Tritiated cyclic AMP and cyclic AMP assay kit were obtained from the Radiochemical Centre (Amersham, U.K.) Cyclic AMP was from Boehringer (Mannheim, F.R.G.). DE-52 was purchased from Whatman (Maidstone, U.K.). QAE Sephadex A-25 was provided by Pharmacia (Uppsala, Sweden). Bovine brain calmodulin was purified to homogeneity as described earlier [23].

RESULTS AND DISCUSSION

Effect of phosphodiesterase inhibitors on cyclic AMP accumulation in rat hepatocytes

In a previous study we have shown that vasopressin, in a time- and dose-dependent way, activates the glycogen phosphorylase and prevents the rise of cyclic AMP levels provoked by glucagon [4]. According to these results, hepatocytes preincubated for 5 min with either dimethylsulfoxide (1%) or any of the phosphodiesterase inhibitors (dissolved in dimethylsulfoxide) were challenged with 5 nM glucagon in the absence or in the presence of 25 nM vasopressin for a period of 2 min. Cyclic AMP concentration was measured in the cell suspensions. The results of two sets of experiments are listed in Table 1.

Vasopressin had no effect by itself on cyclic AMP levels (not shown) but inhibited the glucagon-provoked increase of cyclic AMP by 32–39%. This inhibition was totally relieved by MIX or IIX. Only a 10% inhibition persisted in the presence of Ro 20-1724. 7-Benzyl MIX and 8-MeOMe MIX did not interfere with the vasopressin effect (Table 1).

The absence of vasopressin effect on basal levels of cyclic AMP can indicate that an increase of cyclic AMP levels or/and an interaction of glucagon with its receptor is an essential step in the mechanism of action of vasopressin. It can also be related to the well documented lack of effect of the hormone on adenylate cyclase activity [6, 7].

Inhibition of partially purified soluble and plasma membrane phosphodiesterases

The soluble phosphodiesterases present in a 250,000 *g* hepatocyte supernatant fraction were separated by DEAE-cellulose chromatography. Three major peaks of activity, assayed at 3 μM cyclic AMP as substrate were eluted from the column by a salt gradient (Fig. 1). The first peak of activity hydrolyzed both cyclic AMP (Fig. 1) and cyclic GMP (not shown); it was insensitive to 2 mM EGTA (Fig. 1) or to 1 μM calmodulin in the presence of 2 mM Ca^{2+} (not shown). The second peak hydrolyzed both cyclic AMP (Fig. 1) and cyclic GMP (not shown); cyclic AMP hydrolysis was stimulated up to seven-fold by 3 μM cyclic GMP (Fig. 1). The third enzyme specifically hydrolyzed cyclic AMP and was insensitive to cyclic GMP and EGTA (Fig. 1).

The inhibition of phosphodiesterase activities by the five inhibitors used in this study was systematically measured with a range of inhibitor concentration from 1 to 50 or 100 μM. The rank order of potency of the inhibitors was the same at the different concentrations tested; therefore, only the inhibitions at 10 μM are presented.

The first peak of phosphodiesterase activity eluted from the DEAE-cellulose chromatography (Fig. 1) differed from the calmodulin-sensitive phosphodiesterase of dog thyroid and other tissues (the first phosphodiesterase activity to be eluted by using the same experimental conditions [25–27]). Indeed, the hepatocyte phosphodiesterase was insensitive to 1 μM calmodulin in the presence of 2 mM Ca^{2+} or to 2 mM EGTA. It was also virtually insensitive to all the inhibitors tested (Table 2); a similar resistance to methylxanthines and papaverine has been described for a soluble phosphodiesterase isolated from canine ureter [28]. The resistance to the inhibitors that are effective on cyclic AMP accumulation (Table 1) leads us to exclude a role for this enzyme in the vasopressin action. Furthermore, the apparent absence of a calmodulin-sensitive phosphodiesterase reinforces the suggestion [29] that the vasopressin effect on cyclic AMP accumulation is not mediated by an increase in intracellular Ca^{2+} concentration. Indeed, (i) the inhibition provoked by vasopressin on cyclic AMP accumulation is still observed in Ca^{2+}-depleted cells [29], and (ii) although the increase in cytosolic Ca^{2+} concentration provoked by vasopressin is essentially over after 4 min [30], the attenu-

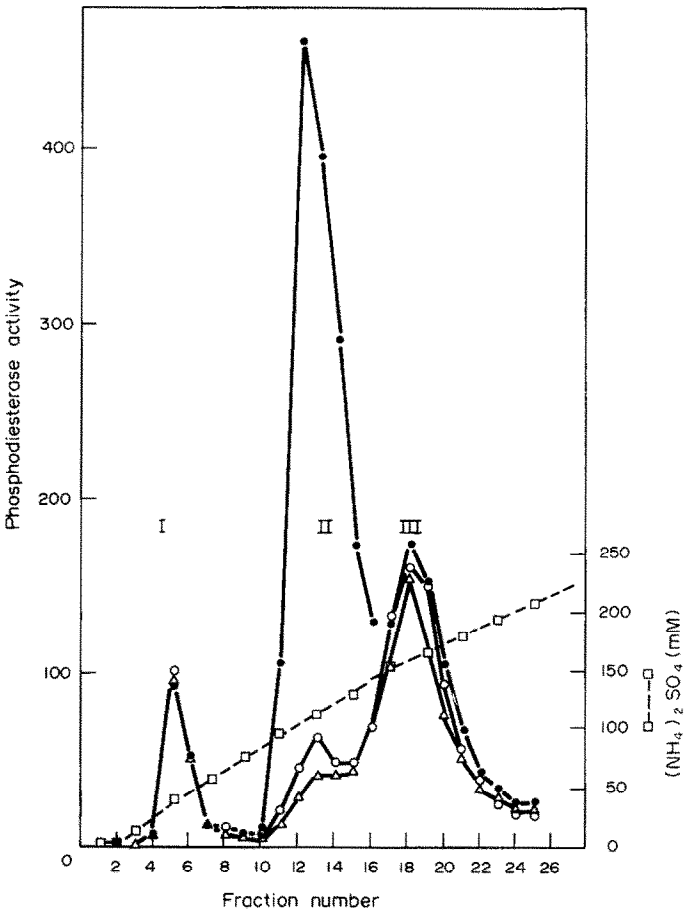

Fig. 1. DEAE-cellulose chromatography of cyclic AMP phosphodiesterase activity from a rat hepatocyte supernatant fraction. A crude supernatant fraction (about 30 mg protein) was applied to a DEAE-cellulose (DE-52) column 0.9 × 13 cm). The column was washed and phosphodiesterase activity was eluted by a salt gradient as described in Materials and Methods. Phosphodiesterase activity was determined with 3 μM cyclic AMP as substrate (○—○) in the additional presence of 2 mM EGTA (△—△) or 3 μM cyclic GMP (●—●). The activity is expressed as pmol cyclic AMP hydrolyzed/30 min/40 μl of each fraction.

ation of the increase in cyclic AMP levels persists [4]. Our data indicate that the mechanism of activation of cyclic AMP catabolism by vasopressin in hepatocytes is quite different from the mechanism of reduction of cyclic AMP accumulation by stimulation of muscarinic cholinergic receptors in dog thyroid [12] or 1321N1 human astrocytoma cells [31]; in these two systems, the reduction of cyclic AMP concentration occurs through the activation of a soluble Ca^{2+}/calmodulin-sensitive phosphodiesterase.

Table 2. Inhibition (%) of phosphodiesterase activities from the rat hepatocyte supernatant fraction

Inhibitor 10 μM	I cyclic AMP-cyclic GMP phosphodiesterase activity	II cyclic GMP-stimulated phosphodiesterase activity	III cyclic AMP-specific phosphodiesterase activity
MIX	4.6 ± 2.3[c]	53.6 ± 1.4[a]	60.5 ± 5.4[a]
IIX	−4.3 ± 3.3[c]	16.3 ± 5.3[d]	87.3 ± 1.0[a]
Ro 20-1724	−3.7 ± 4.3[c]	13.3 ± 3.3[d]	80.7 ± 0.6[a]
8-MeOMeMIX	6.0 ± 1.6[c]	34.7 ± 1.2[a]	21.8 ± 1.3[a]
7-benzyl MIX	6.3 ± 3.8[c]	16.7 ± 5.4[d]	52.3 ± 7.5[b]

Pooled fractions of the three peaks of activity resolved on DEAE-cellulose (Fig. 1) were assayed for cyclic AMP phosphodiesterase activity (1 μM for peak I and III and 3 μM in the presence of 3 μM cyclic GMP as effector for peak II). The phosphodiesterase inhibitors were dissolved in dimethylsulfoxide so that its final concentration was 0.5%.

Results are expressed as percent inhibition ±SEM (N = 3). The statistical significance of the degree of inhibition was estimated by the paired *t*-test: [a]$P < 0.002$; [b]$P < 0.01$; [c]$P < 0.05$; [d]$P < 0.1$; [e]$P > 0.1$.

No correlation was observed between the potencies of the phosphodiesterase inhibitors *in vivo* and their potencies to inhibit the cyclic GMP-stimulated phosphodiesterase. IIX and Ro 20-1724, two of the most potent inhibitors *in vivo* (Table 1), did not affect this enzymatic activity *in vitro* (Table 2). Moreover, vasopressin did not increase the cyclic GMP concentration in hepatocytes [32] so that the basal concentration of cyclic GMP (0.01 μM) would be entirely insufficient to activate the cyclic GMP-stimulated phosphodiesterase (a two-fold stimulation requires 0.1 μM cyclic GMP at 3 μM cyclic AMP substrate concentration [33]). Similar reasons tend to exclude a role for the cyclic GMP-stimulated phosphodiesterase present in liver plasma membranes: the K_a value for cyclic GMP activation amounts to 0.23 μM [34] at 0.25 μM cyclic AMP as substrate, in agreement with unpublished data from our laboratory.

IIX and Ro 20-1724 were the most potent inhibitors of the soluble cyclic AMP-specific phosphodiesterase. MIX and 7-benzyl MIX were fairly potent inhibitors whereas 8-MeOMe MIX was a rather weak inhibitor of this enzymatic form (Table 2). We analyzed the correlation between the potencies of four inhibitors (MIX, 7-benzyl MIX, 8-MeOMe MIX and Ro 20-1724) to relieve at 100 μM the vasopressin effect *in vivo* (Table 1) and to inhibit at 10 μM the cyclic AMP-specific phosphodiesterase *in vitro* (Table 2); there was a positive ($r = 0.65$) however not statistically significant ($P > 0.01$) correlation. Including IIX, the most potent inhibitor *in vivo* (tested however at 50 μM because of its low solubility) and *in vitro*, did not result in a statistically significant correlation ($r = 0.78$, $P > 0.1$). This rather weak correlation does not suggest a crucial role for the cyclic AMP-specific phosphodiesterase in the effect of vasopressin in intact hepatocytes, although we can not exclude a contribution of this enzyme in the overall disposal of cyclic AMP since after all, it is the predominant form in the cytosol. The inhibition of this form (and/or of the cyclic GMP-stimulated activity) can explain increased values of the basal cyclic AMP concentration in the presence of the inhibitors (Table 1).

In the next step of our study we investigated the degree of inhibition of the total phosphodiesterase activity associated with the plasma membrane fraction obtained from isolated hepatocytes. Plasma membranes obtained from whole liver are known to possess a low K_m phosphodiesterase [14, 19, 24]. We have confirmed, as described by Marchmont and Houslay [19], that native plasma membranes from isolated hepatocytes contain a low K_m cyclic AMP phosphodiesterase activity.

The most potent inhibitors of the total plasma membrane phosphodiesterase activity were MIX, IIX and Ro 20-1724 (Table 3). As compared with the cyclic AMP-specific soluble phosphodiesterase, the degree of inhibition by the inhibitors correlated better with their ability to prevent the vasopressin effect on cyclic AMP accumulation in intact hepatocytes (-IIX: $r = 0.84$, $P > 0.1$; +IIX: $r = 0.82$, $P < 0.1$).

As described by Marchmont and Houslay [19], part of the plasma membrane phosphodiesterase activity can be solubilized by high-salt 0.4 M NaCl treatment. We estimated that 45% of the phosphodiesterase activity (at 1 μM cyclic AMP) was so released from hepatocyte plasma membranes. The solubilized enzyme had a high apparent affinity for cyclic AMP (apparent $K_m = 0.72$ μM) but, contrary to the results presented by Marchmont and Houslay [19], the phosphodiesterase activity still bound to the plasma membranes after 0.4 M NaCl treatment also displayed a high affinity for cyclic AMP (apparent $K_m = 1.08$ μM). Assayed at 1 μM cyclic AMP, this intrinsic phosphodiesterase displayed the same relative pattern of inhibition as the native plasma membrane activity (Table 3). For this intrinsic activity we observed a statistically significant correlation between the *in vitro* potency of MIX, Ro 20-1724, 7-benzyl MIX and 8-MeOMe MIX and their ability to prevent the vasopressin effect on cyclic AMP ($r = 0.96$, $P < 0.05$); with IIX, r mounted to 0·97, $P < 0.01$). This type of correlation was lost if comparison was made with the enzymatic activity removed from its native environment by high-salt treatment. As for the plasma membranes, we observe here again that the presence of membranes seems to be a favourable factor to obtain a better correlation; this factor could be of a physico-chemical nature but it is not sure that this would alter the rank order of potencies of the different inhibitors. Consequently, the intrinsic phosphodiesterases may represent the target enzyme for the negative control of cyclic AMP levels exerted by vasopressin in isolated hepatocytes. Extensive studies concerning a possible direct effect of vasopressin on purified membranes or the involvement of regulatory factors

Table 3. Inhibition (%) of hepatocyte plasma membrane phosphodiesterase activities

Inhibitor 10 μM	Total plasma membrane phosphodiesterase activity	Intrinsic phosphodiesterase activity	NaCl-solubilized phosphodiesterase activity
MIX	31.9 ± 4.3[a]	32.4 ± 4.1[b]	41.4 ± 3.3[a]
IIX	27.6 ± 3.8[a]	36.4 ± 2.5[a]	20.0 ± 1.4[a]
Ro 20-1724	23.4 ± 1.7[a]	31.1 ± 3.5[b]	14.4 ± 3.1[c]
8-MeOMeMIX	−5.3 ± 2.1[e]	14.0 ± 3.2[e]	−0.2 ± 2.4[e]
7-benzyl MIX	14.5 ± 3.2[c]	16.8 ± 2.2[b]	9.2 ± 4.5[e]

Total plasma membrane, intrinsic, and NaCl-solubilized fractions were assayed for cyclic AMP phosphodiesterase activity (1 μM). Phosphodiesterase inhibitors were dissolved as described in Table 2. Results are expressed as percent inhibition ±SEM (N = 4). The statistical significance of the degree of inhibition was estimated by the paired t-test: [a]$P < 0.002$; [b]$P < 0.01$; [c]$P < 0.05$; [d]$P < 0.1$; [e]$P > 0.1$.

(GTP, phorbol esters . . .) have up to now yielded inconclusive results.

In conclusion, our data suggest that the decrease of cyclic AMP accumulation caused by vasopressin in hepatocytes incubated in the presence of glucagon, results, at least in part, from the activation of a phosphodiesterase integral to the plasma membrane.

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