Activation of rat liver plasma-membrane diacylglycerol kinase by vasopressin and phenylephrine

Mark H. RIDER and Arnaud BAQUET

Hormone and Metabolic Research Unit,

Institute of Cellular and Molecular Pathology (ICP) and Louvain University Medical School, Avenue Hippocrate 75, B-1200 Brussels, Belgium

Plasma-membrane fractions were prepared from the livers of rats injected with 0.15 M-NaCl (controls) or vasopressin (1 nmol/kg body wt.). When assayed in the presence of deoxycholate, vasopressin increased the $V_{\max}$ of plasma-membrane diacylglycerol kinase 2–4-fold, and the apparent K_m of the enzyme for 1,2-dioleoyl-*sn*-glycerol was doubled. The effect of vasopressin on the $V_{\max}$ of plasma-membrane diacylglycerol kinase was twice as great between pH 7 and 8.5 than at pH 6 or 6.5. Vasopressin doubled the activity of diacylglycerol kinase in the plasma-membrane fraction when the enzyme was assayed with phosphatidylserine rather than deoxycholate as stimulator, and when either 1-stearoyl-2-arachidonoyl-*sn*-glycerol or 1,2-dioleoyl-*sn*-glycerol was the substrate. In perfused livers vasopressin (10 nM) increased the $V_{\max}$ of plasma-membrane diacylglycerol kinase 2-fold, and phenylephrine (3 μ M) gave a similar effect. Vasopressin doubled diacylglycerol kinase activity in hepatocytes that had been preincubated for 55 min, but not in cells that had only been preincubated for 15 min.

INTRODUCTION

Vasopressin and other Ca^{2+} -mobilizing hormones stimulate phosphatidylinositol turnover in the liver. The hormone-stimulated breakdown of phosphatidylinositol 4,5-bisphosphate involves the G-protein-mediated activation of a membrane-associated phospholipase C [1,2]. This in turn leads to the generation of two potential second messengers, namely inositol 1,4,5-trisphosphate, which mobilizes Ca^{2+} from stores in the endoplasmic reticulum, and diacylglycerol (DAG), which activates protein kinase C [3–5].

DAG activates protein kinase C by increasing its affinity for Ca^{2+} and promoting the translocation of the enzyme from the cytosol to the plasma membranes [6]. DAG kinase (EC 2.7.1.107) catalyses the formation of phosphatidic acid from DAG and ATP, and could be an important factor in controlling the concentration of DAG in the membranes and hence protein kinase C activation. Indeed, in platelets, DAG analogues, which inhibit DAG kinase activity *in vitro*, lead to a longer-lasting DAG signal in cells stimulated by thrombin [7], and another DAG kinase inhibitor, R59022, potentiates protein kinase C activation by thrombin in these cells [8].

DAG kinase belongs to the so-called ‘ambiquitous’ family of enzymes. Its activity is distributed between soluble and particulate fractions, and in rat liver approx. 50% of the activity recovered from the initial homogenate is found in the soluble fraction and 30% in microsomal fractions (microsomes) [9]. Both soluble and microsomal DAG kinase have similar specific activities and K_m values for DAG and ATP [9]. Translocation of the enzyme from cytosol to membranes has been observed in rat brain and rat liver homogenates incubated with phospholipase C or DAG [10] and in neutrophils treated with chemotactic peptide or phorbol ester [11]. Recently

DAG kinase has been purified to homogeneity from pig [12] and rat [10] brain cytosol, and polyclonal antibody raised against the pig brain soluble enzyme precipitated the soluble, microsomal and synaptosomal activities [13], suggesting that one form of enzyme is present in all three fractions.

A detailed study of plasma-membrane DAG kinase has not been undertaken to our knowledge. Since the pool of DAG in the plasma membrane is of prime importance for protein kinase C activation, we have studied some properties of DAG kinase in plasma membranes isolated from rat liver. We report that vasopressin activates rat liver plasma-membrane DAG kinase *in vivo*, in the perfused liver and in hepatocytes incubated *in vitro*. In addition, phenylephrine was found to activate plasma-membrane DAG kinase in perfused livers.

MATERIALS AND METHODS

Materials

Fed male Wistar rats weighing 180–200 g were used throughout. Percoll was from Pharmacia, Uppsala, Sweden. 1,2-Dioleoyl-*sn*-glycerol, 1-stearoyl-2-arachidonoyl-*sn*-glycerol, vasopressin, phosphatidylserine and phosphatidate (from egg-yolk lecithin) were from Sigma, St. Louis, MO, U.S.A. Silica-gel 60 F-254 plates were from Merck, Darmstadt, Germany. Sodium deoxycholate was from BDH, Poole, U.K. [γ - ^{32}P]ATP and [2- ^3H]AMP were from Amersham International, Amersham, Bucks., U.K. All other biochemicals were from Boehringer, Mannheim, Germany.

Treatment of animals

Nembutal-anaesthetized rats (60 mg/kg body wt., intraperitoneally) were injected intravenously with 0.15 M-NaCl (controls) or with vasopressin (1 nmol/kg

body wt.). After 5 min, a portion of liver (about 3 g) was rapidly excised, minced and homogenized in 8 vol. (v/w) of 0.25 M-sucrose/20 mM-KF/2 mM-EGTA/5 mM-EDTA/1 mM-dithioerythritol/10 mM-Tris/HCl, pH 7.4 (sucrose buffer), at 0 °C with a Potter-Elvehjem homogenizer. Plasma membranes were then prepared as described below.

Rat livers were perfused *in situ* as previously described [14] with Krebs-Henseleit buffer containing 10 mM-glucose. After 10 min, 0.15 M-NaCl (controls), vasopressin (10 nM) or phenylephrine (3 μ M) was added to the perfusion medium and perfusion was continued for 10 min. Approx. 3 g of liver was then excised and homogenized as described above.

Rat hepatocytes were prepared [15] and incubated in 10 ml volumes of Krebs-Henseleit buffer (approx. 1 g wet wt. of cells per incubation flask) with 10 mM-glucose for 15 or 55 min. Then 0.15 M-NaCl or vasopressin (10 nM) was added and the incubation was continued for 5 min. Next 20 ml of ice-cold sucrose buffer was added to the incubation mixture before harvest of the cells by centrifugation at 1000 $g_{\max}$ for 20 s. The cell pellet was homogenized in 8 vol. (v/w) of sucrose buffer with a Dounce homogenizer (30 strokes). Plasma membranes were isolated as described below.

Preparation of Percoll gradients

Stock Percoll was made 90 % (v/v) in 0.25 M-sucrose before use. The suspension was then diluted in sucrose buffer to give a Percoll concentration of 22.5 % (v/v). The gradient was prepared by pipetting 30 ml of this solution into a 50 ml centrifuge tube and layering 2 ml of sucrose buffer on top.

Isolation of plasma membranes

Homogenates prepared from livers or hepatocytes were centrifuged (500 $g_{\max}$ for 2.5 min) to remove nuclei and cell debris. The supernatant was decanted, and the pellet was extracted once with sucrose buffer (4 ml/g wet wt. of tissue) and the combined supernatants (referred to in the text as the homogenate) were centrifuged (27 500 $g_{\max}$ for 15 min). The pellet was resuspended in

sucrose buffer (4 ml/g wet wt. of tissue), and 5 ml of this suspension was layered on top of a 32 ml Percoll gradient (see above) which was then centrifuged (27 000 $g_{\max}$ for 15 min) in a Sorvall RC-2B centrifuge fitted with a SS-34 rotor. The first 5 ml on top of the gradient, which contained contaminating soluble enzymes, was removed and discarded. The underlying 10 ml, containing the plasma-membrane fraction, was diluted in 5 vol. (v/v) of 0.15 M-NaCl/20 mM-KF/2 mM-EGTA/5 mM-EDTA/1 mM-dithioerythritol/10 mM-Tris/HCl, pH 7.4 at 0 °C (saline buffer). The plasma membranes were collected by centrifugation (27 500 $g_{\max}$ for 15 min) and washed once in 10 ml of saline buffer. The washing removed Percoll from the plasma-membrane fraction. Finally the plasma membranes were collected by centrifugation and resuspended (Teflon/glass hand-powered homogenizer at a protein concentration of 2–3 mg/ml) in sucrose buffer (which prevented the aggregation of membranes observed in saline buffer). For phosphorylase assay a sample of 27 500 g supernatant was taken, and in some experiments the whole supernatant was centrifuged at 105 000 g for 1 h to prepare microsomal and soluble fractions.

DAG kinase assay

DAG kinase was assayed at 30 °C in 0.5 ml of 100 mM-Tris/HCl/20 mM-NaF/1 mM-dithioerythritol, pH 7.4, containing 5 mM-MgCl₂. For the study of the pH/activity profile of DAG kinase, the buffer consisted of 33 mM-Mes, 33 mM-Hepes, 33 mM-Tris/HCl, 20 mM-NaF, 1 mM-dithioerythritol and 5 mM-MgCl₂, adjusted to the pH indicated in the Figure legends with either KOH or HCl. The DAG suspension was prepared as follows: chloroform was evaporated from the DAG solution under N₂, and the residue was sonicated at 0 °C for 15 s (Branson sonicator, setting no. 3) in sodium deoxycholate (10 mg/ml)/20 mM-Tris/HCl, pH 7.4. The DAG suspension was included in the assay at the concentrations indicated in the Figure legends together with deoxycholate (1 mg/ml final concn.). In some experiments, DAG kinase was assayed with DAG sonicated in 20 mM-Tris/HCl, pH 7.4, and with concentrations of phosphatidylserine (sonicated

Fig. 1. Time course of ³²P incorporation into chloroform-soluble reaction products by plasma membranes from control and vasopressin-treated rats

Liver plasma membranes (40 μ g) from control (○, □) and vasopressin-treated rats (●, ■) were incubated with 1 mg of deoxycholate/ml (□, ■) or 0.3 mg of 1,2-dioleoyl-*sn*-glycerol/ml and 1 mg of deoxycholate/ml (○, ●) and 0.5 mM-[γ -³²P]ATP (10 000 c.p.m./nmol) at 30 °C. Reactions were stopped at the indicated times to determine ³²P incorporation into chloroform-soluble reaction products as described in the Materials and methods section.

in 20 mM-Tris/HCl, pH 7.4) as indicated. The assays, containing 25–75 μ g of plasma-membrane protein, were started with 0.5 mM- $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (sp. radioactivity 5000–10000 c.p.m./nmol). After 15 min the reactions were stopped with 3 ml of chloroform/methanol (1:1, v/v) containing 0.1 M-HCl, and the incorporation of ^{32}P into chloroform-soluble products was determined as in ref. [9].

The assays were performed in the presence and the absence of exogenously added DAG, and the DAG-independent rates of incorporation of radioactivity into chloroform-soluble reaction products were subtracted from the total rates of incorporation (with added DAG) to correct for the presence of endogenous DAG in the membranes and for the presence of other lipid kinases such as phosphatidylinositol kinases. The incorporation of ^{32}P into chloroform-soluble products was linear up to at least 20 min in the presence and the absence of added diolein, when 1 mg of deoxycholate/ml (Fig. 1) or 0.5 mg of phosphatidylserine/ml was included to stimulate DAG kinase (not shown). In plasma membranes from control rats, added DAG increased the incorporation of ^{32}P into chloroform-soluble products 2–3-fold when deoxycholate was present (Fig. 1). Vasopressin treatment did not affect the DAG-independent rate of incorporation of radioactivity into chloroform-soluble products, but caused a 2-fold increase in the total rate of incorporation of ^{32}P into chloroform-soluble products compared with the control (Fig. 1). When microsomes were prepared from the 27500 *g* supernatant as described above and assayed under identical conditions, added dioleoylglycerol caused an 8–10-fold stimulation of ^{32}P incorporation into lipid products compared with the rate observed in the presence of deoxycholate alone (results not shown). These findings are in agreement with the results of Kanoh & Åkesson [9], who observed a similar stimulation of the reaction rate by dioleoylglycerol.

To identify the reaction product, a sample of the chloroform layer was taken, evaporated to dryness under N_2 , and 50–100 μ g of carrier phosphatidate was added to the residue. Lipids were separated by t.l.c. on silica-gel 60 F-254 plates with chloroform/methanol/ NH_3 (12:6:1, by vol.) as solvent, and identified with I_2 vapour. The spot corresponding to authentic phosphatidate was cut out and its radioactivity was counted. These studies

showed that 80% and 34% of the chloroform-soluble ^{32}P was incorporated into phosphatidate ($R_F = 0.25$) in the presence and the absence of exogenous diolein respectively. Moreover, in plasma membranes from vasopressin-treated rats the incorporation of ^{32}P into the phosphatidate spot was increased 2-fold. Plasma-membrane DAG kinase activities were sometimes expressed as pmol of phosphatidate formed per munit of 5'-nucleotidase to correct for differences in homogenization and recovery.

Other assays

Glutamate dehydrogenase [16], lactate dehydrogenase [17], NADP^+ -cytochrome *c* reductase [18], phosphorylase *a* [14] and 5'-nucleotidase [19] activities were measured as described in the references. One unit of enzyme activity is the amount of enzyme which catalyses the formation of 1 μ mol of product/min under the stated conditions. Protein was measured as described by Bradford [20], with bovine serum albumin as standard.

RESULTS AND DISCUSSION

Effect of vasopressin on plasma-membrane DAG kinase activity *in vivo*

Table 1 shows a comparison of the specific activities of DAG kinase and marker enzymes in liver homogenates and plasma-membrane fractions from control and vasopressin-treated rats. The enzyme markers were glutamate dehydrogenase (mitochondria), lactate dehydrogenase (cytosol), NADP^+ -cytochrome *c* reductase (microsomes) and 5'-nucleotidase (plasma membranes). Vasopressin treatment did not affect the specific activities of the enzyme markers or their recoveries in the plasma-membrane fraction. In addition, vasopressin had no effect on the specific activity of DAG kinase in the homogenate, but doubled the specific activity of DAG kinase in the plasma-membrane fraction and increased the recovery of the enzyme in this fraction (Table 1). The specific activities of 5'-nucleotidase show that plasma membranes were purified 13-fold from the homogenate and that the recovery was 25%. There was some enrichment in specific activity of the microsomal marker NADP^+ -cytochrome *c* reductase in the plasma-membrane fraction. One could argue that the presence of

Table 1. Specific activities of marker enzymes and DAG kinase in homogenate and plasma-membrane fractions

Homogenates and plasma-membrane fractions were prepared from the livers of control and vasopressin-treated rats as described in the Materials and methods section and assayed for marker enzymes and DAG kinase. DAG kinase activity was measured in the presence of 0.3 mg of 1,2-dioleoyl-*sn*-glycerol/ml and 1 mg of deoxycholate/ml. The results are means $\pm$ S.E.M. for four separate experiments: * indicates a significant effect of vasopressin ($P < 0.05$, unpaired *t* test).

Enzyme activity	Specific activity (munits/mg of protein)				Activity recovered in plasma membranes (%)	
	Homogenate		Plasma membranes		Control	Vasopressin
	Control	Vasopressin	Control	Vasopressin		
Lactate dehydrogenase	1970 $\pm$ 210	2070 $\pm$ 260	150 $\pm$ 120	170 $\pm$ 140	0.11 $\pm$ 0.12	0.13 $\pm$ 0.13
Glutamate dehydrogenase	633 $\pm$ 130	573 $\pm$ 210	220 $\pm$ 50	180 $\pm$ 30	0.61 $\pm$ 0.17	0.60 $\pm$ 0.22
NADP^+ -cytochrome <i>c</i> reductase	38 $\pm$ 15	36 $\pm$ 14	106 $\pm$ 50	100 $\pm$ 52	4.70 $\pm$ 1.50	4.60 $\pm$ 1.60
5'-Nucleotidase	4.5 $\pm$ 1.3	5.2 $\pm$ 1.3	60 $\pm$ 24	67 $\pm$ 18	24 $\pm$ 6	17 $\pm$ 5
DAG kinase	0.25 $\pm$ 0.03	0.25 $\pm$ 0.03	0.30 $\pm$ 0.04	0.66 $\pm$ 0.19*	1.8 $\pm$ 0.3	3.8 $\pm$ 0.7*

DAG kinase in the plasma-membrane fraction was due to contamination by microsomes, which contain DAG kinase of specific activity 0.3 munit/mg of protein, a value similar to that found in plasma membranes.

Fig. 2. Saturation curve for 1,2-dioleoyl-*sn*-glycerol of DAG kinase from control (○) and vasopressin-treated (●) rats

Plasma membranes from control (○) and vasopressin-treated (●) rats were isolated as described in the Materials and methods section and assayed for DAG kinase with the indicated concentration of 1,2-dioleoyl-*sn*-glycerol at pH 7.4. DAG kinase activities in plasma membranes from vasopressin-treated rats were significantly different from the controls at all concentrations of 1,2-dioleoyl-*sn*-glycerol tested ($P < 0.05$, unpaired t test). The results are means $\pm$ S.E.M. for five separate experiments.

Vasopressin treatment did not affect the specific activity of microsomal DAG kinase (result not shown), and the recovery of DAG kinase in microsomes from liver homogenates from control rats was $24.5 \pm 2.5\%$, which was identical with the value of $24.5 \pm 3.5\%$ (mean $\pm$ range of two experiments) for the recovery of DAG kinase in microsomes prepared from liver homogenates of vasopressin-treated rats. However, the hormone doubled the specific activity of plasma-membrane DAG kinase without affecting the specific activity or recovery of NADP^+ -cytochrome *c* reductase in the plasma-membrane fraction (Table 1). Comparison of the specific activities of DAG kinase in the homogenate and plasma-membrane fractions of control rats shows that there was almost no purification of DAG kinase in the plasma membrane fraction. This is probably because DAG kinase is distributed between cytosol, microsomes and plasma membranes, with the plasma-membrane enzyme representing about 2% of the total activity (Table 1).

Fig. 2 shows the effect of vasopressin on the saturation curves of plasma-membrane DAG kinase for 1,2-dioleoyl-*sn*-glycerol. The $V_{\max}$ values calculated by computer fitting [21] of the data in Fig. 2 were approximately doubled, and in addition the calculated apparent K_m for 1,2-dioleoyl-*sn*-glycerol was increased from 0.02 mg/ml for the controls to 0.04 mg/ml for the DAG kinase activity of plasma membranes from rats treated with vasopressin. Therefore, since the K_m is doubled together with a doubling of $V_{\max}$, no change in actual rate would result if the substrate concentration remained unchanged in the K_m range. However, the hormone-stimulated breakdown of phosphatidylinositol phosphates probably increases the plasma-membrane concentration of DAG and the main effect of vasopressin on DAG kinase is likely to be a doubling of $V_{\max}$. The inclusion of proteinase inhibitors in the homogenization medium did not affect the results, i.e. a doubling of the $V_{\max}$ of DAG kinase (result not shown). In some experiments, such as those shown in Fig. 3(a), vasopressin increased the $V_{\max}$ of DAG kinase by 4-fold. DAG

Fig. 3. pH/activity profile (a) and sensitivity to phosphatidylserine (b) of plasma-membrane DAG kinase from control (○) and vasopressin-treated (●) rats

Plasma membranes from control and vasopressin-treated rats were assayed for DAG kinase with 0.3 mg of 1,2-dioleoyl-*sn*-glycerol/ml. The diolein was sonicated with 1 mg of deoxycholate/ml in Fig. 3(a), but in Fig. 3(b) the diolein was sonicated in 20 mM-Tris/HCl, pH 7.4. Phosphatidylserine was sonicated separately in 20 mM-Tris/HCl, pH 7.4, and added at the concentrations indicated. The results are means $\pm$ ranges of two separate experiments.

kinase activity in liver plasma membranes from control-treated rats did not change over the pH range 6–8.5. However in plasma membranes from vasopressin-treated rats, a doubling of DAG kinase activity was observed when the pH was increased from 6.5 to 7, and the effect of vasopressin was twice as great between pH 7 and 8.5 than at pH 6 or 6.5.

Previous workers have assayed DAG kinase with phospholipid rather than deoxycholate [7,12]. Fig. 3(b) shows that plasma-membrane DAG kinase was stimulated 4–6-fold by 0.5 mg of phosphatidylserine/ml. Moreover, vasopressin doubled DAG kinase activity over the range of phosphatidylserine concentrations used, but the hormone did not affect the sensitivity of the enzyme to phosphatidylserine stimulation (Fig. 3b). The DAG kinase activities of plasma-membrane fractions from control and vasopressin-treated rats measured with 0.5 mg of phosphatidylserine/ml (Fig. 3b) were similar to those measured with 1 mg of deoxycholate/ml (Fig. 2), which represents the optimal concentration of detergent required to stimulate plasma-membrane DAG kinase (result not shown). In addition, when phosphatidylserine was used to stimulate DAG kinase and 1-stearoyl-2-arachidonoyl-*sn*-glycerol was the substrate, control plasma-membrane DAG kinase activity was 0.17 ± 0.04 munit/mg of protein, which was increased to 0.30 ± 0.04 munit/mg of protein by vasopressin ($P < 0.05$, $n = 4$).

Effect of vasopressin on plasma-membrane DAG kinase activity in perfused livers and hepatocytes

Vasopressin doubled plasma-membrane DAG kinase activity in perfused livers as well as *in vivo* (Table 2). In livers perfused with phenylephrine, plasma-membrane DAG kinase was activated to an extent similar to that observed with vasopressin.

The activation of plasma-membrane DAG kinase by vasopressin was also obtained in hepatocytes, but only when the cells had been incubated for 55 min before addition of the hormone (Table 2). The DAG kinase activities in plasma membranes of control hepatocytes

preincubated for 15 min were about twice the activities in plasma membranes from the control livers *in vivo* or control perfused livers, and no effect of vasopressin was seen. However, in cells preincubated for 55 min, control plasma-membrane DAG kinase activity returned to basal and vasopressin doubled DAG kinase activity (Table 2). Vasopressin increased the activity of phosphorylase both in hepatocytes that had been preincubated for 15 min and in cells preincubated for 55 min (Table 2). Therefore the activation of DAG kinase by vasopressin cannot be related to the effect of the hormone on phosphorylase activation.

The activation of plasma-membrane DAG kinase in control hepatocytes preincubated for 15 min might have been the result of translocation of the enzyme from the cytosol to the plasma membranes during collagenase digestion. Phospholipase C, a possible contaminant of commercial preparations of collagenase, has been reported to cause the translocation of DAG kinase from the cytosol to the membranes in liver homogenates [10]. However in control perfused livers and in livers perfused with 4 units of phospholipase C (phosphatidylcholine-specific enzyme from *Bacillus cereus*)/ml, plasma-membrane DAG kinase was only increased from 4.6 ± 1.7 ($n = 8$) to 5.1 ± 1.3 ($n = 4$) pmol of phosphatidate formed/min per munit of 5'-nucleotidase ($P > 0.05$) respectively.

Our results show that vasopressin and phenylephrine activate plasma-membrane DAG kinase. Vasopressin and phenylephrine could have activated plasma-membrane DAG kinase by promoting the translocation of the enzyme from the cytosol to the plasma membranes or by causing a stable change in enzyme activity, such as covalent modification. It is difficult to make any conclusions as to whether vasopressin promotes translocation of DAG kinase from cytosol to the plasma membranes. An argument against a translocation mechanism is the fact that different kinetic properties of DAG kinase were observed in plasma membranes from vasopressin-treated rats (Fig. 3a). Although the amount of DAG kinase recovered in the plasma membranes is

Table 2. Effect of vasopressin and phenylephrine on plasma-membrane DAG kinase and phosphorylase activities of perfused rat liver and hepatocytes

Rat livers were perfused *in situ* without and with vasopressin (10 nM) or phenylephrine (3 μ M) as described in the Materials and methods section. Hepatocytes were preincubated (preinc.) for 15 min and 55 min before vasopressin (10 nM) was added. Plasma membranes were prepared and DAG kinase was assayed as described in the Materials and methods section in the presence of 0.3 mg of 1,2-dioleoyl-*sn*-glycerol/ml and 1 mg of deoxycholate/ml. Phosphorylase activities were measured [14] in post-27500 *g* supernatants, and are expressed as nmol of phosphate released/min per unit of lactate dehydrogenase to correct for differences in homogenization and recovery. The results are means $\pm$ S.E.M. for the numbers of experiments shown in parentheses. * and † indicate significant ($P < 0.05$) effects of hormone compared with the controls for paired *t* test and unpaired *t* test respectively; NM, not measured.

Treatment	DAG kinase activity (pmol of phosphatidate formed/min per munit of 5'-nucleotidase)			Phosphorylase <i>a</i> activity (nmol of P _i released/min per unit of lactate dehydrogenase)		
	Perfused liver	Hepatocytes preinc. 15 min	Hepatocytes preinc. 55 min	Perfused liver	Hepatocytes preinc. 15 min	Hepatocytes preinc. 55 min
Control	4.6 ± 1.7 (8)	9.4 ± 3.8 (4)	4.8 ± 1.8 (4)	8.6 ± 4.9 (8)	15.7 ± 3.8 (4)	10.9 ± 2.2 (4)
Vasopressin	10.3 ± 2.7 (4)†	9.6 ± 3.6 (4)	9.1 ± 1.8 (4)*	16.1 ± 3.1 (4)†	26.4 ± 3.8 (4)*	22.6 ± 1.5 (4)*
Phenylephrine	7.7 ± 2.4 (6)†	NM	NM	25.1 ± 4.7 (6)†	NM	NM

low compared with the activity in microsomes and cytosol fractions, it probably plays an important role in synthesizing the plasma-membrane phosphatidate pool. The vasopressin- and phenylephrine-induced activation of plasma-membrane DAG kinase could eventually contribute to the replenishment of the plasma-membrane phosphoinositide pool.

The activation of DAG kinase by vasopressin and phenylephrine might antagonize protein kinase C activation, since both enzymes can compete for the same ligand, namely 1-stearoyl-2-arachidonoyl-*sn*-glycerol, which is liberated by hormone-stimulated phosphoinositide breakdown. Indeed, in hepatocytes incubated with phenylephrine, protein kinase C does not seem to become activated [22]. However, the results of Garrison and co-workers [23] suggest that protein kinase C becomes activated in hepatocytes incubated with vasopressin.

We thank Dr. Louis Hue for his advice and critical reading of the manuscript and Mrs. Thérèse Lambert for typing assistance. The work was supported by the Région Bruxelloise and the FRSM (Belgium). M.H.R. is a Fellow of the ICP.

REFERENCES

- Berridge, M. J. (1987) *Annu. Rev. Biochem.* **56**, 159–193
- Litosch, I. & Fain, J. N. (1986) *Life Sci.* **39**, 187–194
- Michell, R. H., Kirk, C. J., Jones, L. M., Downes, C. P. & Creba, J. A. (1981) *Philos. Trans. R. Soc. London Ser. B* **296**, 123–137
- Charest, R., Prpic, V., Exton, J. H. & Blackmore, P. F. (1985) *Biochem. J.* **227**, 79–90
- Fakehar, P. S. & Kirk, C. J. (1981) *Biochem. J.* **194**, 167–172
- Nishizuka, Y. (1986) *Science* **233**, 305–312
- Bishop, W. R., Ganong, B. R. & Bell, R. M. (1986) *J. Biol. Chem.* **261**, 6993–7000
- Chaffoy de Courcelles, D., Roevens, P. & Van Belle, H. (1985) *J. Biol. Chem.* **260**, 15763–15770
- Kanoh, H. & Åkesson, B. (1978) *Eur. J. Biochem.* **85**, 225–232
- Besterman, J. M., Pollenz, R. S., Booker, E. L. & Cuatrecasas, P. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9378–9382
- Ishitoya, J., Yamakawa, A. & Takenawa, T. (1987) *Biochem. Biophys. Res. Commun.* **144**, 1025–1030
- Kanoh, H., Kondoh, H. & Ono, T. (1983) *J. Biol. Chem.* **258**, 1767–1774
- Kanoh, H., Iwata, T., Ono, T. & Suzuki, T. (1986) *J. Biol. Chem.* **261**, 5597–5602
- Hue, L., Bontemps, F. & Hers, H. G. (1975) *Biochem. J.* **152**, 105–114
- Hue, L., Feliu, J. E. & Hers, H. G. (1978) *Biochem. J.* **176**, 791–797
- Martin, B. R. & Denton, R. M. (1970) *Biochem. J.* **117**, 861–877
- Saggerson, E. D. (1974) *Biochem. J.* **140**, 211–224
- Phillips, A. H. & Langdon, R. G. (1962) *J. Biol. Chem.* **237**, 2652–2660
- Belsham, G. J., Denton, R. M. & Tanner, M. J. A. (1980) *Biochem. J.* **192**, 457–467
- Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254
- Barlow, R. B. (1983) *Biodata Handling with Microcomputers*, pp. 116–121, Elsevier, Cambridge
- Williamson, J. R., Cooper, R. H., Joseph, S. K. & Thomas, A. P. (1985) *Am. J. Physiol.* **248**, C203–C216
- Garrison, J. C., Johnsen, D. E. & Campanille, C. P. (1984) *J. Biol. Chem.* **259**, 3283–3292

Received 3 March 1988/13 June 1988; accepted 20 June 1988