Effects of vasopressin and corticosterone on fatty acid metabolism and on the activities of glycerol phosphate acyltransferase and phosphatidate phosphohydrolase in rat hepatocytes

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(Received 4 July 1983/Accepted 19 September 1983)

1. The effects of vasopressin on the short-term control of fatty acid metabolism were studied in isolated rat hepatocytes. 2. Vasopressin increased the oxidation of oleate to CO_2 and decreased the formation of ketones in hepatocytes from Wistar rats, but not from Brattleboro rats. 3. Incubation with vasopressin for 30 min increased the conversion of oleate into triacylglycerol by 17% and 32% in hepatocytes from Wistar and Brattleboro rats respectively. The corresponding increases for the phospholipid fraction were 19% and 42%. When Wistar-rat hepatocytes were incubated with corticosterone for 6 h there was a 19% increase in triacylglycerol synthesis, and a 52% increase if vasopressin was added 30 min before the end of the incubation. 4. Glycerol phosphate acyltransferase activity was not significantly increased by vasopressin. 5. Incubation for 5–60 min with vasopressin increased the V_{max} of phosphatidate phosphohydrolase by 48% and 32% respectively in hepatocytes from Wistar and Brattleboro rats. These increases were antagonized if EGTA was added to the medium used for incubating the hepatocytes. The replacement of vasopressin by $5 \mu\text{M}$ -ionophore A23187 produced a significant increase of 13% in the phosphohydrolase activity. It is therefore likely that the effects of vasopressin on the phosphohydrolase are mediated by Ca^{2+} . 6. These results are discussed in relation to the possible function of phosphatidate phosphohydrolase in controlling the turnover of phosphoinositides, the synthesis of phosphatidylethanolamine, phosphatidylcholine and triacylglycerol, and the secretion of very-low-density lipoproteins.

The vasopressor and antidiuretic effects of vasopressin have been known for many years, but more recently it has become apparent that vasopressin is able to modify hepatic metabolism. Changes in carbohydrate metabolism that occur in response to vasopressin treatment include stimulation of glycogenolysis (Hems & Whitton, 1973), activation of phosphorylase (Hems *et al.*, 1975; Keppens & de Wulf, 1975) and stimulation of gluconeogenesis (Hems & Whitton, 1973; Hutson *et al.*, 1976). Vasopressin is also reported to affect the metabolism of oleate in isolated hepatocytes, increasing the incorporation of the fatty acid into lipids and its oxidation to CO_2 and decreasing incorporation into ketones (Williamson *et al.*, 1980).

In the present study investigations were carried

Abbreviation used: PAP, phosphatidate phosphohydrolase (EC 3.1.3.4).

out to determine whether homogenates prepared from hepatocytes incubated with vasopressin show an increased ability to synthesize glycerolipids. The activities of PAP and glycerol phosphate acyltransferase (EC 2.3.1.15), which may play an important role in regulating glycerolipid synthesis *in vivo*, were also determined (see Brindley & Sturton, 1982, for review). These experiments were performed by using hepatocytes prepared from both Wistar rats and Brattleboro rats. Brattleboro rats are not able to synthesize vasopressin (Valtin *et al.*, 1965). They also have other differences in hormone concentrations compared with the Long-Evans strain from which they are derived; these changes may include decreased corticotropin, corticosterone and aldosterone concentrations, and increased oxytocin concentrations (see Sokol & Zimmerman, 1982, for review). However, the

complete lack of vasopressin makes them an interesting experimental model.

The interaction of vasopressin with corticosterone was also measured in the present study. It was already known that glucocorticoids could increase the activity of PAP in perfused liver (Lehtonen *et al.*, 1979), and in isolated hepatocytes (Jennings *et al.*, 1981; Lawson *et al.*, 1982a,b). The esterification of glycerol phosphate by homogenates obtained from hepatocytes was also increased after incubation with corticosterone (Lawson *et al.*, 1982b). These changes could help to maintain or increase the rate of glycerolipid synthesis in times of stress particularly when the supply of fatty acid to the liver is high (Lawson *et al.*, 1982a,b). Vasopressin is also released in response to stress such as haemorrhagic shock (Hems, 1979), and it might therefore augment the action of glucocorticoids.

Materials and methods

Materials

Most of the materials used have been described previously (Jennings *et al.*, 1981). Oleic acid, [arginine]vasopressin (Synthetic; grade VI) and Hyamine hydroxide were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Newborn-calf serum was supplied by Gibco Bio-Cult Ltd., Hounslow, Middx., U.K., and treated as described by Jennings *et al.* (1981). Aluminium oxide (Brockman type II, pH 9.3–9.7) and Fisofluor were obtained from Fisons, Loughborough, Leics., U.K. Ionophore A23187 was obtained from Boehringer Corp. (London), Lewes, E. Sussex, U.K., and [^{14}C]oleic acid was purchased from Amersham International, Amersham, Bucks., U.K.

Animals

Male Wistar rats (250–300 g) were obtained and treated as described previously (Jennings *et al.*, 1981). Male Brattleboro rats (280–360 g) were supplied by Dr. S. Gardiner, Department of Physiology and Pharmacology, University of Nottingham. They were homozygous for diabetes insipidus and were derived from a Long–Evans parent strain (Laycock, 1977).

Hepatocytes were prepared from Wistar rats as described by Jennings *et al.* (1981). The Brattleboro rats differed in that they were anaesthetized with an increased dose of sodium pentobarbitone (80 mg/kg body wt.). The technique for perfusing the liver was also modified in the Brattleboro rats. In our hands this improved the yield and viability of the cells, and the method was easier to perform. The vena cava was clamped above the right kidney and then the diaphragm was punctured. The thorax was cut up each side and lifted back to expose the heart. The right atrium was cut and a

cannula was inserted and secured. The hepatic portal vein was cut and the blood allowed to run to waste. A volume of 200 ml of perfusion buffer was allowed to pass through the liver while it was being removed from the rat. The liver was placed on a filter suspended above the reservoir, and the perfusate draining from the hepatic portal vein was collected and recirculated. The amount of collagenase added was decreased to 50 mg and CaCl_2 to 1 mM. With this method Trypan Blue exclusion was greater than 90%, and less than 10% of the total lactate dehydrogenase activity was found in the supernatant after sedimenting the cells.

Incubation of hepatocytes

The incubation procedure was similar to that described by Jennings *et al.* (1981), except that newborn-calf serum replaced the foetal-calf serum and CaCl_2 was increased to 2.5 mM. In experiments with hepatocytes from Wistar rats, 12.5–15 mg wet wt. of cells was added per incubation. This was increased to 20–25 mg wet wt. for Brattleboro rats. The cells were preincubated for at least 90 min at 37°C with an atmosphere of O_2/CO_2 (19:1) before the start of the experiment.

Addition of hormones, EGTA, CaCl_2 and A23187

Corticosterone was added at the start of the experiment as described by Jennings *et al.* (1981). Vasopressin, EGTA (adjusted to pH 7.4 with KOH) and CaCl_2 stock solutions were diluted with 0.15 M-NaCl such that injection of 50 μl samples into the flasks gave the required final concentration. Control flasks were injected with 50 μl of 0.15 M-NaCl alone. The calcium ionophore A23187 was added in 20 μl of methanol. Control incubations for these experiments also contained 20 μl of methanol. In fact, the addition of methanol had no significant effect on the activity of phosphatidate phosphohydrolase. In the experiments in which the incorporation of oleate was measured, the vasopressin was added 30 s before the fatty acid.

Determination of fatty acid incorporation into glycerolipids and oxidation products

Hepatocytes from Wistar rats were preincubated as described above in 3.7 ml of medium. To this was added, in a volume of 0.3 ml: 10 μl of 1 M-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] adjusted to pH 7.4 with KOH, and 0.18 ml of newborn-calf serum, and 55 μl of 65.5 mM-[^{14}C]oleic acid (0.2 Ci/mol; containing a 20% molar excess of KOH). The volume was made up to 0.3 ml with modified incubation medium (Jennings *et al.*, 1981; and see above).

Hepatocytes from Brattleboro rats were preincubated as described above in 3.8 ml of medium. To this was added, in a final volume of 0.2 ml, 10 μl of

1 M-Hepes adjusted to pH 7.4 with KOH, 0.1 ml of newborn-calf serum, and 30 μ l of 66.7 mM-[1- 14 C]-oleic acid (0.2 Ci/mol; containing a 20% molar excess of KOH). The volume was made up to 0.2 ml with modified incubation medium.

The cells were then incubated in a Braun WTR-1 Orbital Shaker at 90 rev./min and 37°C for normally 30 min. The formation of 14 CO₂ was determined essentially as described by Whitelaw & Williamson (1977) in duplicate incubations. The reaction was stopped by injection of 0.4 ml of 20% (v/v) HClO₄ through the Subaseal of the incubation flasks. Hyamine hydroxide (0.5 ml) was injected into a small tube suspended in the flask, and 14 CO₂ was collected by shaking for 1 h at 37°C. A sample of the Hyamine hydroxide was removed for scintillation counting. The contents of the flask were cooled and centrifuged in a bench centrifuge, and a sample of the supernatant was taken for scintillation counting in Fiso fluor. The acid-soluble radioactivity has been reported to consist primarily of ketone bodies (Christiansen, 1977). Flasks that had not been incubated were used to obtain blank values.

The incorporations of [1- 14 C]oleate into glycerolipids were measured in triplicate. After 30 min the samples were cooled rapidly on ice and centrifuged at 50 g for 4 min. The supernatant was aspirated and the lipids were extracted (Hajra, 1968) and analysed by t.l.c. (Brindley & Bowley, 1975).

Determination of enzyme activities and ATP concentrations

The cells were harvested and homogenates were prepared as described by Jennings *et al.* (1981). Three concentrations of protein were used for each enzyme assay, and the mean specific activities were calculated by linear-regression analysis. The techniques for measuring the incorporation of glycerol phosphate into lipid with a palmitoyl-CoA-generating system, lactate dehydrogenase (to account for cell breakage and recovery) and ATP concentrations have been described by Lawson *et al.* (1982b). The activity of PAP was determined as described by Jennings *et al.* (1981), except that the extraction procedure was modified as follows (R. Hopewell & S. C. Butterwith, unpublished work). The reaction was stopped with 1.88 ml of chloroform/methanol (1:2, v/v). To this was added 0.625 ml of chloroform containing 0.4% of olive oil, and 0.875 ml of water. After centrifugation a 0.9 ml sample of the bottom phase was added to 2 ml of chloroform/methanol (19:1, v/v) and 1 g of aluminium oxide. After vigorous shaking and centrifugation in a bench centrifuge, a sample of the solvent was taken and dried down for liquid-scintillation counting. This procedure gave a complete recovery of diacylglycerol while removing almost all of the [3 H]phosphatidate.

Statistical analysis

The results were analysed by a paired *t* test.

Experimental and results

Effects of vasopressin and corticosterone on the metabolism of oleate

Under the conditions used in these experiments, most of the metabolized oleate was converted into glycerolipid by the hepatocytes of Wistar and Brattleboro rats (Table 1). The distribution of oleate between different lipid classes in Wistar rats was 74 $\pm$ 12% triacylglycerol, 9 $\pm$ 4% diacylglycerol, 2 $\pm$ 2% monoacylglycerol, 1 $\pm$ 1% phosphatidic acid, 2 $\pm$ 1% phosphatidylethanolamine and 13 $\pm$ 8% phosphatidylcholine (means $\pm$ S.D. for five separate experiments). The distribution was similar in hepatocytes from Brattleboro rats, with 67 $\pm$ 13% triacylglycerol, 12 $\pm$ 10% diacylglycerol and 21 $\pm$ 5% phospholipid (means $\pm$ S.D. for three separate experiments). Addition of hormones did not significantly affect the distribution of oleate between different lipid classes in either Brattleboro- or Wistar-rat hepatocytes (results not shown).

The presence of 100 nM-vasopressin significantly increased the incorporation of [14 C]oleate into total glycerolipid, triacylglycerol, and phospholipid and its conversion into 14 CO₂ by hepatocytes of Wistar rats. The incorporation of 14 C into acid-soluble products was significantly decreased. By contrast, vasopressin had no significant effect on the conversion of oleate into 14 CO₂ and acid-soluble products with the hepatocytes of the Brattleboro rats. However, there was a significant stimulation in the incorporation of oleate into total lipids and triacylglycerols and into the total phospholipid fraction.

Incubating hepatocytes from Wistar rats with corticosterone had no significant effect on the incorporation of oleate to glycerolipids in the three experiments. However, the addition of vasopressin to cells that had been pretreated with corticosterone resulted in a significant increase in the incorporation of oleate into glycerolipids. Although not statistically significant, this appeared to be particularly into triacylglycerols rather than into phospholipids (Table 1). It was therefore decided to investigate whether these differences in glycerolipid synthesis could be explained by changes in the activities of glycerol phosphate acyltransferase or PAP.

Effect of vasopressin and corticosterone on the activities of enzymes involved in glycerolipid synthesis

Vasopressin had no significant effect on the total activity of glycerol phosphate acyltransferase as measured directly with exogenous palmitoyl-CoA

Table 1. *Effects of vasopressin and corticosterone on the metabolism of oleate by rat hepatocytes*

Conversion of [$1-^{14}C$] oleate into:	Wistar rats				Brattleboro rats	
	Relative activity (%) in the presence of:				(V) Control value (nmol converted/ min per mg dry wt. of cells)	(IV) Relative activity (%) in the presence of 100nM-vasopressin
	(I) Control value (nmol converted/ min per mg dry wt. of cells)	(II) 100nM- vasopressin	(III) 10 μ M- corticosterone	(IV) 100nM vasopressin + 10 μ M- corticosterone		
(A) $^{14}CO_2$	0.19 $\pm$ 0.08 (8)	123 $\pm$ 11 (8) (I) versus (II)†† 85 $\pm$ 9 (6)	Not measured	Not measured	0.060 $\pm$ 0.012 (4)	111 $\pm$ 13 (4)
(B) Acid-soluble products	0.41 $\pm$ 0.09 (6)	(I) versus (II)*** 118 $\pm$ 4 (5)	Not measured	Not measured	0.319 $\pm$ 0.025 (3)	93 $\pm$ 30 (3)
(C) Total glycerolipid	0.80 $\pm$ 0.11 (5)	(I) versus (II)†† 117 $\pm$ 8 (4)	121 $\pm$ 26 (4) (III) versus (IV)*	145 $\pm$ 24 (4) (I) versus (IV)*	0.902 $\pm$ 0.429 (3)	130 $\pm$ 9 (3) (V) versus (VI)*
(D) Triacylglycerol	0.58 $\pm$ 0.10 (4)	(I) versus (II)* 119 $\pm$ 3 (4)	119 $\pm$ 38 (3)	152 $\pm$ 27 (3)	0.569 $\pm$ 0.159 (3)	132 $\pm$ 7 (3) (V) versus (VI)**
(E) Total phospholipid	0.13 $\pm$ 0.10 (4)	(I) versus (II)†	119 $\pm$ 38 (3)	119 $\pm$ 39 (3)	0.189 $\pm$ 0.045 (3)	142 $\pm$ 5 (3) (V) versus (VI)†

Table 2. *Effects of vasopressin and corticosterone on the activities of enzymes involved in glycerolipid synthesis*

Hepatocytes were incubated with vasopressin for 30 min as indicated. When corticosterone was used, the cells were preincubated with this hormone for 330 min before addition of vasopressin. Homogenates were prepared from the cells as described by Jennings *et al.* (1981). In rows (A)–(C) palmitoyl-CoA was generated in the incubation from palmitate, whereas in row (D) it was added directly. The results are means $\pm$ s.d. for the numbers of independent experiments shown in parentheses. The level of significance is shown as described in Table 1.

Activity in homogenate	Wistar rats				Brattleboro rats	
	Relative activity (%) in the presence of:				(V) Control value (nmol converted/min per unit of lactate dehydrogenase)	(VI) Relative activity in the presence of 100nM-vasopressin (%)
	(I) Control value (nmol formed/min per unit of lactate dehydrogenase)	(II) 100nM- vasopressin	(III) 10 μ M- corticosterone	(IV) 100nM vasopressin + 10 μ M- corticosterone		
(A) Glycerol phosphate into total lipids	1.89 $\pm$ 1.01 (7)	118 $\pm$ 26 (7)	118 $\pm$ 16 (3)	117 $\pm$ 24 (3)	2.17 $\pm$ 0.99 (4)	99 $\pm$ 11 (4)
(B) Glycerol phosphate into phospholipids	1.54 $\pm$ 1.16 (7)	106 $\pm$ 27 (7)	102 $\pm$ 34 (3)	101 $\pm$ 11 (3)	1.99 $\pm$ 1.16 (4)	89 $\pm$ 24 (4)
(C) Glycerol phosphate into neutral lipids	0.38 $\pm$ 0.31 (7)	131 $\pm$ 26 (7) (I) versus (II)**	160 $\pm$ 42 (3)	174 $\pm$ 10 (3) (I) versus (II)***	0.364 $\pm$ 0.277 (4)	153 $\pm$ 37 (4) (V) versus (VI)*
(D) Glycerol phosphate acyltransferase	1.45 $\pm$ 0.55 (10)	110 $\pm$ 28 (5)	119 $\pm$ 26 (6)	124 (1)	0.887 $\pm$ 0.357 (3)	108 $\pm$ 7 (3)
(E) Phosphatidate phosphohydrolase	1.65 $\pm$ 0.82 (10)	148 $\pm$ 53 (10) (I) versus (II)**	151 $\pm$ 25 (5) (I) versus (III)**	180 $\pm$ 72 (5)	1.93 $\pm$ 1.01 (8)	132 $\pm$ 13 (8) (V) versus (VI)††

(Table 2, row D), or by generating palmitoyl-CoA from palmitate (row A) with hepatocytes from Wistar or Brattleboro rats. However, it did increase the proportion of neutral-lipid synthesis by preparations from Wistar rats (row C). Corticosterone also failed to stimulate the activity of glycerol phosphate acyltransferase (Table 2, row D), but there were indications of a slight increase in the total esterification of glycerol phosphate (row A). These effects of a 360 min incubation with corticosterone have been reported and discussed elsewhere (Lawson *et al.*, 1982b). The addition of 100 nM-vasopressin at 30 min before the end of this incubation failed to produce any obvious effect on the total esterification of glycerol phosphate when using cells from Wistar rats (Table 2, row A).

Vasopressin produced a significant stimulation in the activity of PAP with cells taken from Brattleboro or Wistar rats (Table 2, row E). This effect was maximum at 100 nM-vasopressin (Fig. 1), and it was observed at the shortest incubation time used, which was 5 min. Thereafter the activity remained approximately constant for at least 55 min (results not shown). The stimulation by vasopressin of PAP activity was most clearly seen when the activity was measured at high concentra-

tions of magnesium phosphatide (Fig. 2). The PAP activities given in Table 2 were measured at 0.6 mM-phosphatide, and they are therefore approximately $V_{\max}$ values. These changes in PAP activity are reflected in the proportion of the esterified glycerol phosphate that was isolated in the neutral-lipid fraction (Table 2, row C).

Incubation of hepatocytes with corticosterone for 360 min also increased the activity of PAP, as expected from previous work (Jennings *et al.*, 1981; Lawson *et al.*, 1982a,b). The addition of 100 nM-vasopressin for the last 30 min of this incubation produced a further stimulation of PAP activity in four of the five experiments performed (Table 2, row E), but this did not reach statistical significance in a paired *t* test.

Effects of Ca^{2+} , EGTA and A23187 on the stimulation of PAP

These experiments were performed to investigate whether the effect of vasopressin in stimulating the activity of PAP was dependent on the presence of Ca^{2+} in the medium. Since there does not

Fig. 1. Effect of the vasopressin concentration on the stimulation of phosphatide phosphohydrolase activity in isolated hepatocytes

The activities are expressed relative to that obtained when no hormone was added to the incubations. The results are given as means $\pm$ S.D. Two Brattleboro and two Wistar rats were used for this series of experiments.

Fig. 2. Effect of the phosphatide concentration on the stimulation of PAP by incubating hepatocytes with vasopressin

Hepatocytes were incubated in the absence (●) or presence (Δ) of 100 nM-vasopressin, and the activity of PAP was measured in the homogenate at different concentrations of phosphatide. The phosphatide/ Mg^{2+} molar ratio was maintained at 1:2 in all incubations. These results were obtained by using hepatocytes from a Wistar rat, and they were reproduced in an independent experiment.

appear to be any essential difference in the effects of vasopressin on this activity in hepatocytes derived from Brattleboro and Wistar rats, the results have been combined in Table 3.

The addition of 5 mM-EGTA to the incubations did not change the activity of PAP in the absence of vasopressin. However, the presence of EGTA decreased the effect of vasopressin so that there was no longer a significant increase in PAP activity (Table 3). The calcium ionophore A23187 had no significant effect on PAP activity at 1 μ M. At 5 μ M there was a significant stimulation, but this was not as large as was obtained with vasopressin.

An experiment was performed to assess the effect of EGTA and A23187 on cellular ATP concentrations and on the percentage of the lactate dehydrogenase in the cell pellet (results not shown). These values provide information about cell damage. Incubation for 15 min with up to 10 mM-EGTA had no effect on ATP concentrations in hepatocytes from either Wistar or Brattleboro rats. The percentage of the total lactate dehydrogenase in the cell pellet did not alter. However, the presence of A23187 resulted in a time- and concentration-dependent decrease in ATP concentrations for both Brattleboro and Wistar rats. For example, a 10 min incubation in the presence of 5 μ M-A23187 resulted in an 80% fall in ATP concentrations in cells from Wistar rats. Under these conditions the percentage of lactate dehydrogenase found in the cell pellet fell from $89 \pm 4\%$ to $76 \pm 4\%$ (mean $\pm$ S.D. for quadruplicate incubations). This indicates that the A23187 was damaging the cells. An incubation time of 5 min with 1 μ M-A23187 was chosen in Table 3 for investigating the effects on PAP activity, since this produced no significant change in ATP concentration or lactate dehydrogenase activity. This incubation time also minimized the cell damage at 5 μ M-A23187.

Discussion

The present study confirms the observations of Williamson *et al.* (1980) that vasopressin can stimulate the production of CO₂ and triacylglycerol from oleate, whereas it decreases the formation of ketones in hepatocytes from Wistar rats (Table 1). With hepatocytes from Brattleboro rats the increase in triacylglycerol synthesis was observed, but the production of CO₂ and ketones was not significantly altered. A similar result to that for Brattleboro rats has been reported with hepatocytes from starved Wistar rats (Sugden & Watts, 1983). These authors demonstrated that the lack of effect on the production of CO₂ and ketones is caused by the limitation of oxaloacetate supply resulting from the need to maintain gluconeogenesis rather than the tricarboxylic acid cycle. This situation would also exist for the Brattleboro rats which have diabetes insipidus. Vasopressin has also been reported to have no effect on CO₂ evolution or ketogenesis in hepatocytes from obese mice (Edwards *et al.*, 1981).

The mechanism for the increase in the synthesis of triacylglycerols that was reported in Table 1 was examined further. Previous work had shown that vasopressin treatment increased the activity of glycerol phosphate acyltransferase in hepatocytes of female rats (Sugden *et al.*, 1980a). The microsomal activity showed the greatest increase, about 30%. In our experiments with hepatocytes from male rats there was no significant increase in the total glycerol phosphate acyltransferase activity that was measured directly with palmitoyl-CoA, or with a palmitoyl-CoA-generating system (Table 2). The microsomal activity in this system would have contributed about 30% of the total glycerol phosphate acyltransferase (Lawson *et al.*, 1982b). Any change in the microsomal glycerol phosphate acyltransferase activity would therefore have been

Table 3. Effects of Ca²⁺, EGTA and A23187 on the stimulation of phosphatidate phosphohydrolase activity by vasopressin

In section (A), hepatocytes were incubated at 37°C where shown for 10 min with 5 mM-EGTA and then treated with or without 100 nM-vasopressin for a further 15 min. Section (B) shows the effects of incubating hepatocytes with A23187 for 5 min. Results are given as means $\pm$ S.D. relative to the activity obtained in the absence of additions. The numbers of independent experiments are shown in parentheses by giving the numbers of Brattleboro and Wistar rats respectively.

Additions		Relative activity (%)	Significance of difference from control
(A)	(I) None (control)	100 (3+3)	
	(II) Vasopressin	137 $\pm$ 14 (3+3)	$P < 0.005$
	(III) EGTA	109 $\pm$ 16 (2+2)	Not significant
	(IV) EGTA + vasopressin	114 $\pm$ 15 (3+3)	Not significant
(B)	(I) None (control)	100 (5+3)	
	(II) A23187 (1 μ M)	101 $\pm$ 26 (3+1)	Not significant
	(III) A23187 (5 μ M)	113 $\pm$ 9 (4+3)	$P < 0.01$

relatively small and at the limits of detection, taking into account the variation between experiments.

We were, however, able to demonstrate a consistent increase, of about 50% and 30% respectively, in the activity of PAP in the hepatocytes of Wistar and Brattleboro rats when they were treated with vasopressin (Table 2 and Fig. 1). This effect was seen within 5 min of exposure of the cells to vasopressin, and it resulted from a change in the $V_{\max}$ of the phosphohydrolase (Fig. 2 and Table 2). A more detailed kinetic analysis has not been presented, since the phosphatidate is added as an aggregate and its interaction with PAP is complex.

Many of the effects of vasopressin are known to be associated with changes in the distribution and flux of Ca^{2+} in cells (Exton, 1980; Kirk *et al.*, 1981; Williamson *et al.*, 1981; Blackmore *et al.*, 1982). In agreement with this, the addition of EGTA to the incubations decreased the effect of vasopressin on PAP activity so that no significant stimulation was obtained (Table 3). Furthermore, the addition of $5\mu\text{M}$ -A23187 significantly stimulated PAP activity. However, it did not produce as large a stimulation as did vasopressin, even when the conditions were controlled so that little, if any, obvious cell damage occurred. This was assessed by measuring the concentration of ATP in the cells and by the release of lactate dehydrogenase. It has previously been shown that A23187 can decrease ATP concentrations in pancreatic islets (Tamarit-Rodriguez *et al.*, 1978). The failure of A23187 to mimic completely the vasopressin-induced stimulation of PAP activity might be explained by the results of Garrison *et al.* (1979). They reported that the ionophore did not produce the same phosphorylation pattern of cytosolic proteins in incubated hepatocytes as was found after stimulation of the α -receptor, which produces a similar labelling pattern to that of vasopressin (Garrison & Wagner, 1982). There were phosphorylated proteins that appeared after stimulation with the α -type agonists that were not seen with A23187. Therefore this ionophore may not be able to mimic all of the effects of vasopressin. More work is needed to elucidate how vasopressin stimulates PAP activity, and how Ca^{2+} may be involved in this process.

The present work, however, demonstrates that the activity of PAP could be regulated in two different ways. There is the acute control that is illustrated with vasopressin (Table 2). A longer-term increase in the synthesis of PAP is promoted by glucocorticoids (Lehtonen *et al.*, 1979; Jennings *et al.*, 1981). This glucocorticoid effect is opposed by insulin (Lawson *et al.*, 1982a,b). As expected, the effects of vasopressin and corticosterone on PAP activity appear to be additive (Table 2).

There is now a large body of evidence to suggest

that the long-term control of hepatic PAP activity by glucocorticoids operates *in vivo* and that it is important in the regulation of glycerolipid synthesis (Brindley & Sturton, 1982; Lawson *et al.*, 1982a,b). It is too early to be certain whether the stimulation of PAP activity by vasopressin has a physiological significance, since there are a number of problems to be considered. First, the concentrations of vasopressin used (100 nM) are far in excess of those reported normally *in vivo* (Hems, 1979). However, concentrations of up to 1 nM may occur after haemorrhagic shock (Hems, 1979). One experiment has been reported which investigated whether vasopressin could alter the hepatic activity of PAP 4 h after its injection into rats (Lehtonen *et al.*, 1979). PAP activity was not significantly changed. However, it is unlikely that an acute effect would persist for that length of time, since vasopressin has a half-life of only a few minutes *in vivo* (Robertson, 1977).

The response of PAP to the presence of vasopressin is not as sensitive as that of glycogen phosphorylase, where the stimulation approaches a maximum at 1 nM (Blackmore *et al.*, 1978; Thomas *et al.*, 1983; Creba *et al.*, 1983). The PAP response is more similar to that seen in the stimulated breakdown of phosphatidylinositol 4,5-bisphosphate, the depletion of phosphatidylinositol, the increase in phosphatidate labelling, and the binding of vasopressin to the hepatocytes (Cantau *et al.*, 1980; Kirk *et al.*, 1981; Thomas *et al.*, 1983; Creba *et al.*, 1983). A high degree of receptor occupancy is therefore required for the stimulation of PAP. A further similarity between the response of PAP and that of the breakdown of phosphatidylinositol 4,5-bisphosphate is that neither appears to be stimulated by A23187 to the extent seen with vasopressin. However, both of the responses are diminished when the medium and cells are depleted of Ca^{2+} (Table 3; Prpic *et al.*, 1982; Thomas *et al.*, 1983; Creba *et al.*, 1983). Several other differences between the effect of vasopressin and A23187 on phospholipid turnover in hepatocytes have been documented by Takenawa *et al.* (1982).

The stimulated breakdown of phosphatidylinositol and phosphatidylinositol 4,5-bisphosphate that is caused by the Ca^{2+} -mobilizing hormones leads to the production of diacylglycerol (Takenawa *et al.*, 1982; Thomas *et al.*, 1983). This diacylglycerol may have a number of physiological implications, including the stimulation of a protein kinase (Kuo *et al.*, 1980; Kishimoto *et al.*, 1980; Kiss & Mhina, 1982; Michell, 1983). Subsequently the diacylglycerol can be converted into phosphatidate and cycled back into the inositol-containing lipids. An increased labelling of phosphatidate is observed during stimulation by the Ca^{2+} -mobiliz-

ing hormones (Thomas *et al.*, 1983). Our results show that this phenomenon is accompanied by an increase in the activity of PAP, which could provide a significant factor in controlling the flux of intermediates during the accelerated lipid turnover. The enhanced PAP activity could increase the net rate of diacylglycerol production and accumulation, and restrict the resynthesis of the inositol-containing lipids.

The other function of the increased diacylglycerol production could be to facilitate the synthesis of phosphatidylcholine, phosphatidylethanolamine and triacylglycerol. Vasopressin and angiotensin are thought to alter the balance between the synthesis of phosphatidylethanolamine and phosphatidylcholine, since these hormones stimulate phospholipid methyltransferase (Alemany *et al.*, 1981). They also inhibit the CDP-choline pathway of phosphatidylcholine synthesis, possibly at the level of the choline phosphotransferase (Alemany *et al.*, 1982). These aspects were not investigated in the present work, but a significant increase in the synthesis of total phospholipids (mainly phosphatidylcholine) was observed with Brattleboro- and Wistar-rat hepatocytes after vasopressin treatment. The increase in PAP activity that was produced by vasopressin (Table 2) was also accompanied by increases in the synthesis of triacylglycerols in hepatocytes of both Brattleboro and Wistar rats (Table 1). It is difficult to be certain that these effects resulted directly from the changes in PAP activity, since it has been reported that the effect of vasopressin in stimulating the synthesis of triacylglycerols was not dependent on Ca^{2+} (Sugden *et al.*, 1980b). However, in our experimental model the depletion of Ca^{2+} by EGTA resulted in a 75% inhibition of fatty acid esterification. This approach was therefore not pursued, since glycerolipid synthesis seems to require the presence of bivalent cations in the incubation medium. Thomas *et al.* (1983) also reported that Ca^{2+} was needed for normal phospholipid turnover in hepatocytes. Studying the turnover of triacylglycerols in whole cells involves the interaction of a variety of metabolic pathways, and it is difficult to predict the changes in flux from the activities of an individual enzyme. Similar difficulties exist in understanding the effects of vasopressin on glucose release, and the production of CO_2 from fatty acids.

The stimulation of PAP activity by vasopressin does, however, fit into a pattern. It has been demonstrated that the long-term control of the PAP activity in the liver is analogous to that of a gluconeogenic enzyme (Brindley & Lawson, 1983), namely that the activity is increased by glucocorticoids, and this effect is antagonized by insulin (Lawson *et al.*, 1982a,b). Evidence is now presented that vasopressin stimulates PAP activity

acutely. This is also reminiscent of the control of gluconeogenesis, although the mechanism of vasopressin action may not be identical. In conditions of metabolic stress, including starvation and diabetes, the liver is providing the brain with glucose and ketones, erythrocytes with glucose, and muscle tissue with ketones and triacylglycerols as a form of energy (Brindley, 1981; Brindley & Lawson, 1983). The increased transport of triacylglycerols to muscle is particularly evident when the supply of fatty acids to the liver is high, e.g. in severe ketotic diabetes (Pelech *et al.*, 1983). This may explain the basis for a co-ordinated control for the hepatic synthesis of glucose and triacylglycerol in stress conditions.

The work was supported by an S.E.R.C. studentship to A. D. P. and subsequently by an M.R.C. project grant. We thank Dr. N. Lawson for his assistance in preparation of hepatocytes for initial experiments.

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