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Effects of vasopressin on the synthesis of phosphatidylethanolamines and phosphatidylcholines by isolated rat hepatocytes

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The effect of vasopressin on the biosynthesis of phosphatidylcholines and phosphatidylethanolamines was investigated in freshly isolated rat hepatocytes in suspension. Treatment of hepatocytes with vasopressin inhibits the incorporation of [*Me*-^{14}C]choline into phosphatidylcholines in a dose-dependent manner. The hormone does not affect the uptake, phosphorylation or oxidation of choline. Pulse-chase studies indicate that CTP:cholinephosphate cytidylyltransferase might be subject to hormonal regulation by vasopressin. In contrast with the inhibitory effect of vasopressin on the synthesis of phosphatidylcholines, this hormone stimulates the incorporation of [1,2-^{14}C]ethanolamine into phosphatidylethanolamines in a dose-dependent manner. Pulse and pulse-chase studies with labelled ethanolamine show that the conversion of ethanolaminephosphate to CDPethanolamine as well as the formation of phosphatidylethanolamines from CDPethanolamine and diacylglycerol are enhanced. Determination of the effect of vasopressin on the activity of the enzymes of the synthesis de novo of phosphatidylethanolamines demonstrates an increase of the activity of ethanolaminephosphotransferase, probably as a result of the increased amount of diacylglycerol in vasopressin-treated cells.

Introduction

There have been several studies in the past few years on the hormonal regulation of hepatic phosphatidylcholine synthesis. Geelen et al. [1,2] reported that exposure of isolated rat hepatocytes to glucagon, a cAMP-dependent hormone, exerted an inhibitory effect on the incorporation of [^{14}C]glucose, [^{14}C]acetate and [^{32}P]phosphate into phosphatidylcholines. Vance and coworkers [3] also examined the effects of glucagon on phosphatidylcholine synthesis. Incubation of rat hepatocytes in the presence of glucagon resulted in a diminished incorporation of choline into phosphatidylcholines, but dit not affect the activity of CTP:cholinephosphate cytidylyltransferase (EC 2.7.7.15), the rate-determining enzyme of the CDPcholine pathway [4]. However, short-term treatment of hepatocytes with cAMP analogues [4] did result in an inhibition of phosphatidylcholine synthesis, which was attended by a decreased activity of CTP:cholinephoshate cytidylyltransferase in the microsomes.

There are several indications that the synthesis of hepatic phosphatidylcholines is also regulated by Ca^{2+}-dependent hormones. Alemany et al. [5] reported a transient inhibition of the phosphatidylcholine synthesis by vasopressin and recently Haagsman et al. [6] showed that the biosynthesis

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of phosphatidylcholines is subject to an inhibition by α-adrenergic hormones.

Much less information is available with regard to the hormonal regulation of the synthesis of phosphatidylethanolamines via the CDPethanolamine pathway. Incubation of hepatocytes in the presence of glucagon resulted in a stimulation of the incorporation of [1,2-^{14}C]ethanolamine and of [^{32}P]phosphate into phosphatidylethanolamines [1] and Haagsman et al. [6] demonstrated that norepinephrine, while inhibiting phosphatidylcholine synthesis, stimulated the synthesis of phosphatidylethanolamines from labelled ethanolamine.

In the present study we present evidence that vasopressin also shows opposite effects on the synthesis de novo of phosphatidylcholines and phosphatidylethanolamines by isolated rat hepatocytes. The results of this study endorse the earlier suggestion from studies on the effects of hormones [6] as well as from dietary studies [7] that phosphatidylethanolamine and phosphatidylcholine synthesis are under independent hormonal control at one or more steps beyond the common diacylglycerol branchpoint.

Materials and Methods

Materials

[1,2-^{14}C]Ethanolamine and [*Me*-^{14}C]choline were obtained from New England Nuclear (Dreieichenhain, F.R.G.). CDP[1,2-^{14}C]ethanolamine was purchased from Amersham International (Amersham U.K.). [1,2-^{14}C]Ethanolaminephosphate was prepared from [1,2-^{14}C]ethanolamine according to Sundler [8]. Ham's F-12 medium was obtained from Flow Laboratories (Irvine, UK) and [arginine]vasopressin from Boehringer (Mannheim, F.R.G.). Bovine serum albumin (fraction V), collagenase (type I), glycerol kinase and glycerol-3-phospate dehydrogenase were purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of analytical grade and supplied by Baker Chemical Co. (Deventer, The Netherlands).

Isolation and incubation of hepatocytes

Hepatocytes were obtained from male Wistar rats (200–250 g), which were meal fed a stock-pelleted diet and had free access to water. Hepatocytes were isolated essentially according to Seglen [9], as described earlier [6]. The cells were suspended in Ham's F-12 medium supplemented with 2% defatted [10] and dialyzed [11] bovine serum albumin and buffered with 14.5 mM sodium bicarbonate, 12.5 mM 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulphonic acid (Tes) and 12.5 mM 4-morphelineethanesulphonic acid (Mes) at pH 7.4. The medium contained 0.1 mM choline and was routinely supplemented with 0.05 mM ethanolamine [6]. Incubations were carried out at 37°C for 60 min, unless indicated otherwise, in 25-ml Erlenmeyer flasks containing 5–7 mg cellular protein per ml. During incubation the flasks were continuously gassed with 95% oxygen/5% carbon dioxyde.

Extraction and analysis of phospholipids and intermediates

Incubations of hepatocytes were terminated by the addition of 6.5 volumes of chloroform/methanol (1:1, v/v). Lipids were extracted as described, by Sundler et al. [12]. Phospholipids were separated by thin-layer chromatography on silica gel G using chloroform/methanol/water (65:35:4, v/v) as developing solvent. Neutral lipids were separated on silica gel G using petroleum ether (b.p. 40–60°C)/ether/acetic acid (80:20:2, v/v) as the eluent. For the determination of radiolabel incorporated into water-soluble metabolites another procedure was used. After the incubation the cells were washed twice, by centrifugation and resuspension, with 6 ml ice-cold Krebs-Ringer buffer containing 0.1 mM choline and 0.05 mM ethanolamine. After addition of chloroform/methanol (1:2, v/v) [13], samples were separated in a water-soluble and a lipid-soluble fraction. The chloroform phase was washed twice with 4 ml methanol/water (5:4, v/v). The combined upper phases were analyzed by thin-layer chromatography as described by Pritchard and Vance [14], using either choline (3 μmol), cholinephosphate (3 μmol), and CDPcholine (2 μmol) or ethanolamine (0.4 μmol), ethanolaminephosphate (1 μmol) and CDPethanolamine (0.3 μmol) as carriers. Spots of choline intermediates were made visible with iodine vapour and ethanolamine intermediates were detected with ninhydrin.

For determination of the pool sizes of ethanolaminephosphate and CDPethanolamine, the water-soluble intermediates were extracted as described above and, subsequently, separated by high-performance liquid chromatography (HPLC) using an LKB TSK DEAE-3SW column (7.5 × 150 mm). The mobile phase was 0.01 M sodiumphosphate buffer (pH 5.6). Ethanolaminephospate was determined as described by Sundler and Åkesson [15]. The CDPethanolamine-containing fractions were subjected to a second HPLC separation using an LKB Lichrosorb reversed phase C18 column (4 × 250 mm) that was eluted with 0.1% sodium phosphate buffer (pH 2.5). The CDPethanolamine was determined spectrophotometrically at a wavelength of 280 nm. Full details of this method will be published elsewhere (Tijburg, L.B.M., Geelen, M.J.H., Van Golde, L.M.G., unpublished results).

Subcellular fractionation

At the end of the incubation period, the cells were washed twice, by centrifugation and resuspension, with ice-cold homogenization buffer, containing 0.145 M NaCl/10 mM Tris-chloride (pH 7.8)/1 mM EDTA/10 mM NaF [16]. The cells were homogenized with 50 strokes of a tight fitting Dounce homogenizer. The homogenate was centrifuged for 15 min at 12 000 × *g*, followed by centrifugation of the supernatant for 60 min at 105 000 × *g*. The microsomal pellet was resuspended in a buffer containing 0.25 M sucrose/10 mM Tris-chloride (pH 7.4)/1 mM EDTA/10 mM NaF at a concentration of 5 mg microsomal protein per ml.

Measurement of enzyme activities

Ethanolamine kinase (EC 2.7.1.82) activity was determined as described by Weinhold and Rethy [17]. Labelled ethanolaminephosphate was separated from ethanolamine by paper chromatography (Whatman, No. 3 MM), using ethanol/2-propanol/NH_3 (65 : 20 : 35, v/v) as developing solvent. CTP : ethanolaminephosphate cytidylyltransferase (EC 2.7.7.14) was determined in the cytosolic fraction, essentially as described by Sundler [8], except for some minor adaptations. Briefly, the final incubation volume was 150 μl, containing 20 mM Tris-chloride (pH 7.8)/10 mM $MgCl_2$/2 mM CTP/1 mM [1,2-^{14}C]ethanolaminephosphate/5 mM dithioerythritol/150–250 μg cytosolic protein. The formation of CDP[1,2-^{14}C]ethanolamine was linear with the protein concentration from 100 to 300 μg for at least 30 min. Incubations were carried out at 37 °C for 10 min. The reaction was terminated by boiling for 2 min and CDPethanolamine was separated from ethanolaminephosphate by thin-layer chromatography as described above. The standard assay mixture for the ethanolaminephosphotransferase assay (EC 2.7.8.1) contained in a final volume of 200 μl: 20 mM Tris-chloride (pH 7.4)/10 mM $MgCl_2$/0.2 mM CDPethanolamine/4 mM dithioerythritol/150–250 μg microsomal protein. The endogenous diacylglycerol served as the second substrate for the ethanolaminephosphotransferase reaction. The incubations were carried out for 8 min and the formation of radioactive phosphatidylethanolamines was determined by the filter disk method according to Goldfine [18].

Determination of diacylglycerol

After extraction and separation of the neutral lipids as described above, diacylglycerols were extracted from the silica with 4 × 2 ml diethyl ether. The diethyl ether was evaporated and the diacylglycerols were hydrolyzed with 0.5 M ethanolic KOH at 70 °C for 30 min. The samples were neutralized with perchloric acid and glycerol was, subsequently, determined by a modification of the procedure of Wieland [19]. Briefly, the reaction mixture contained in a total volume of 1 ml: 0.14 M glycine (pH 9.8)/0.14 M hydrazine/0.5 mM ATP/0.2 mM NAD^+/3.7 U/ml glycerol-3-phosphate dehydrogenase/0.5 U/ml glycerol kinase. The amount of NADH formed was followed spectrophotometrically at a wavelength of 340 nm. In our hands the reaction was linear with the glycerol concentration from 0 to 40 μM.

Other analytical procedures

Protein concentration was determined by the method of Lowry et al. [20], using bovine serum albumin as a standard. To quantitate radioactivity from samples analyzed by thin-layer chromatography, silica was scraped off the plates into counting vials.

Statistical analysis

All results are presented as mean ± S.D., except

where indicated otherwise. Student's t-test was used for determination of significance. The values shown in the figures are the mean of duplicate incubations of one representative cell preparation. Each experiment was carried out at least twice.

Results

Pulse studies with [1,2-^{14}C]ethanolamine and [Me-^{14}C]choline

The addition of vasopressin to rat hepatocytes stimulated the incorporation of [1,2-^{14}C]ethanolamine into phosphatidylethanolamines in a dose-dependent way (Fig. 1A). Maximal stimulation was reached at a concentration of 100 nM. Upon exposures of hepatocytes to this concentration of vasopressin the incorporation of [1,2-^{14}C]ethanolamine into phosphatidylethanolamines increased from 4.11 ± 0.60 nmol/mg cellular protein per h for control cells, to 6.01 ± 0.64 nmol/mg cellular protein per h for hormone-treated cells ($n = 5$, $P < 0.01$). It has been reported by Alemany et al. [21] that vasopressin induced a stimulation of phosphatidylethanolamine-N-methyltransferase in rat hepatocytes. In the present study the incorporation of [1,2-^{14}C]ethanolamine into phosphatidylcholines was indeed lower in control incubations (0.27 ± 0.05 nmol/mg per h) than in vasopressin-treated hepatocytes (0.36 ± 0.05 nmol/mg per h ($n = 5$, $P < 0.05$). Whether this effect was due to an increased specific activity of phosphatidylethanolamines or to an activation of the methyltransferase, as suggested by Alemany et al. [21] cannot be concluded from these experiments.

In contrast to the effect of vasopressin on the synthesis of phospatidylethanolamines, the incorporation of [Me-^{14}C]choline into phosphatidylcholines was inhibited, as is shown in Fig. 1B. At a concentration of 100 nM vasopressin, the choline incorporation was 75% of that measured in control cells (0.88 ± 0.16 nmol/mg per h for

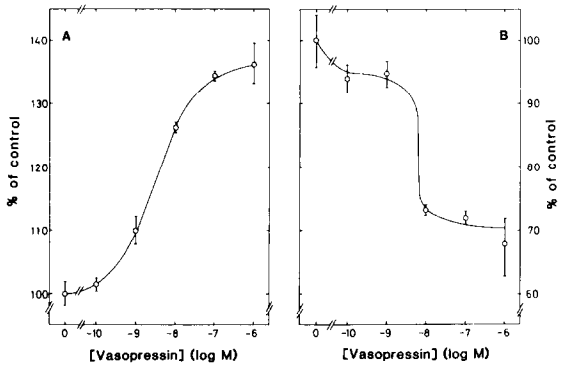

Fig. 1. The effects of vasopressin on the incorporation of [1,2-^{14}C]ethanolamine into phosphatidylethanolamines (A) and on the incorporation of [Me-^{14}C]choline into phosphatidylcholines (B). Freshly isolated rat hepatocytes were incubated with different concentrations of vasopressin for 60 min. Values are the mean of three separate incubations of one cell preparation. The incorporation of [1,2-^{14}C]ethanolamine in control cells was 5.10 ± 0.10 nmol/mg per h; the incorporation of [Me-^{14}C]choline into phosphatidylcholines was 2.04 ± 0.09 nmol/mg per h.

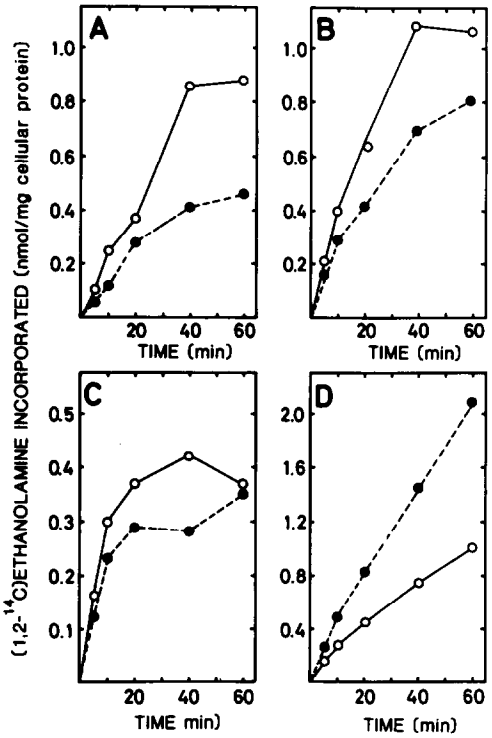

Fig. 2. The effect of vasopressin on the incorporation of [1,2-^{14}C]ethanolamine into phosphatidylethanolamines and ethanolamine intermediates. Hepatocytes were incubated with [1,2-^{14}C]ethanolamine in the presence (●) or absence (○) of 100 nM vasopressin. At various times up to 1 h, cells were washed twice and the radioactivity incorporated into cellular ethanolamine metabolites was determined. Values are the mean of two separate incubations of one representative hepatocyte preparation. A, ethanolamine; B, ethanolaminephosphate; C, CDPethanolamine; D, phosphatidylethanolamines.

control cells; 0.65 ± 0.12 nmol/mg per h for hormone-treated cells, $n = 5$, $P < 0.05$). Incubations of hepatocytes with angiotensin (10^{-6} M) resulted in effects which were comparable with those of vasopressin, i.e., a stimulatory effect on phosphatidylethanolamine synthesis and an inhibitory effect on phosphatidylcholine synthesis (results not shown).

Incubation of cells with ethanolamine in the presence of vasopressin resulted within 5 min in a stimulation of the synthesis of phosphatidylethanolamines (Fig. 2, panel D). Concomitantly, accumulation of label in ethanolamine and the intermediates ethanolaminephosphate (panel B) and CDPethanolamine (panel C) was much lower in vasopressin-treated cells than in control cells.

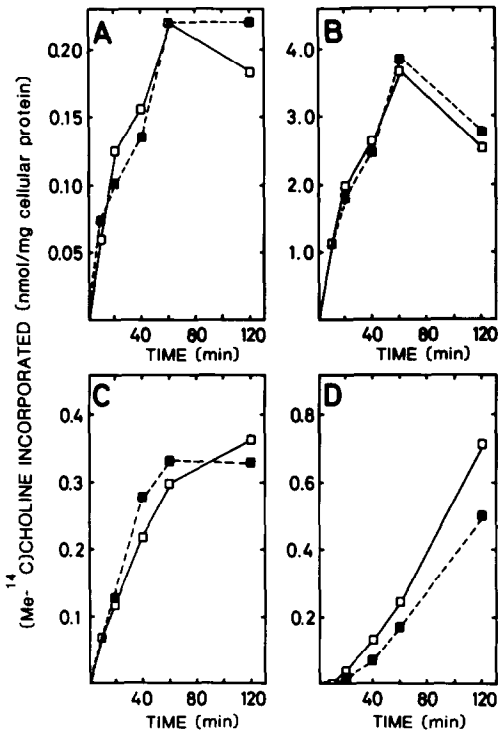

Fig. 3. The effect of vasopressin on the incorporation of [*Me*-^{14}C]choline into phosphatidylcholines and choline metabolites. Hepatocytes were incubated with [*Me*-^{14}C]choline in the presence (■) or absence (□) of 100 nM vasopressin. At various times up to 2 h, cells were washed twice and the radioactivity incorporated into cellular choline metabolites was determined. Values are the mean of two separate incubations of one representative cell preparation. A, choline; B, betaine; C, cholinephosphate; D, phosphatidylcholines.

This observation suggested a stimulating effect of vasopressin on the last step of the synthesis, the formation of phosphatidylethanolamines from CDPethanolamine and diacylglycerol, a reaction which is catalyzed by ethanolaminephosphotransferase.

Fig. 3 shows that the inhibitory effect of vasopressin on the incorporation of choline into phosphatidylcholines is not due to a decreased uptake (Fig. 3A) or phosphorylation (Fig. 3C) of choline, nor to an increased oxidation to betaine (Fig. 3B). Also the accumulation of label in CDPcholine was not affected by exposure of the cells to vasopressin (not shown). The inhibition of phosphatidylcholine synthesis is a persistent effect for a period of at least 2 h.

Pulse-chase studies with [1,2-^{14}C]ethanolamine and [Me-^{14}C]choline

When hepatocytes were pulsed with [1,2-^{14}C]ethanolamine and, subsequently, chased with unlabelled ethanolamine, the disappearance of label from the intermediates was very fast (Fig. 4), indicating a high rate of biosynthesis and degradation of phosphatidylethanolamines. Therefore, the

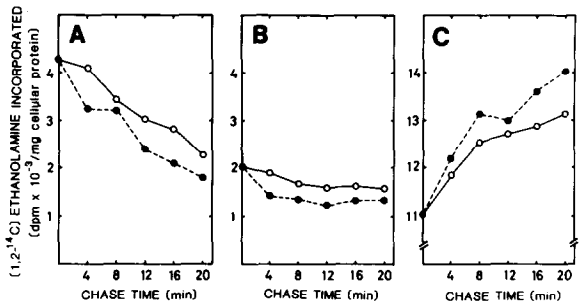

Fig. 4. Pulse-chase study of the metabolism of [1,2-^{14}C]ethanolamine by rat hepatocytes. The isolated cells (375 mg cell protein in a total volume of 50 ml Ham's F-12 medium with 0.1 mM choline and 0.05 mM ethanolamine) were pulsed with [1,2-^{14}C]ethanolamine (6300 dpm/nmol) for 30 min. The cells were washed and chased in Ham's F-12 supplemented with 0.05 mM unlabelled ethanolamine, in the absence (○) or presence (●) of 100 nM vasopressin. At various times up to 20 min, samples were washed and radioactivity in ethanolamine metabolites was determined. A, ethanolaminephosphate, B, CDPethanolamine; C, phosphatidylethanolamines plus phosphatidylcholines. The values are the mean of duplicate determinations of one representative cell preparation. This experiment was repeated twice with similar results.

chase period had to be extremely short for cells pulsed with ethanolamine compared to cell pulsed with labelled choline. Pulse-chase studies showed that the enhanced incorporation of [1,2-^{14}C]ethanolamine into phospholipids (Fig. 4C) is accompanied by an accelerated diappearance of label from ethanolaminephosphate (Fig. 4A), suggesting that the effect of vasopressin might be exerted on the activity of the putative rate-limiting enzyme of the pathway, CTP:ethanolaminephosphate cytidylyltransferase [15]. However, the disappearance of label from CDPethanolamine (Fig. 4B) is also slightly enhanced in vasopressin-treated cells, indicating that the formation of phosphatidylethanolamines from CDPethanolamine and diacylglycerol may also be stimulated. Although these experiments were carried out three times with similar results, the magnitude of the effects was not very impressive. The conclusions from the pulse-chase study are, however, strongly corroborated by preliminary measurements of the pool sizes of ethanolaminephosphate and CDPethanolamine in the presence and absence of vasopressin. The pool size of ethanolaminephosphate indeed decreased from 3.66 ± 0.37 nmol/mg protein in control cells to 2.16 ± 0.17 nmol/mg protein in vasopressin-treated cells, while that of CDPethanolamine diminished from 249 ± 32 pmol/mg in control cells to 152 ± 8 pmol/mg in hormone-treated cells. The values found for the control cells are in good agreement with those reported earlier by Sundler and Åkesson [15].

Pulse-chase studies with labelled choline showed that the inhibition of phosphatidylcholine synthesis, although rather small in this particular experiment (Fig. 5), was attended by a statistically significant delayed disappearance of label from cholinephosphate. At 40 min the amount of label in cholinephosphate in vasopressin-treated cells was $132 \pm 15\%$ of that in control cells ($n = 4$, $P < 0.05$). and at 60 min $128 \pm 15\%$ ($n = 4$, $P < 0.05$). On the other hand, the amount of labelled CDPcholine remained constant during the chase period, except for the small, transient increase that was observed both in control cells and in vasopressin-treated cels in the first 10 min. These observations suggest that the activity of cholinephosphate cytidylyltransferase is subject to hormonal regulation by vasopressin.

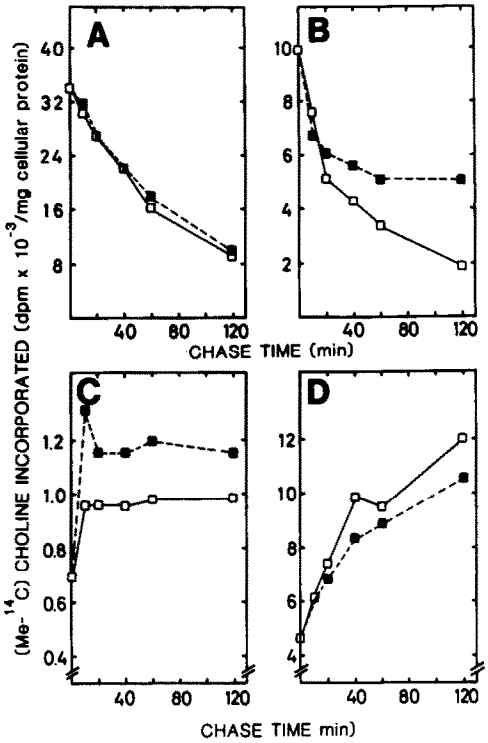

Fig. 5. Pulse-chase study of the metabolism of [*Me*-^{14}C]choline by rat hepatocytes. The cells (460 mg cell protein in a total volume of 50 ml Ham's F-12 medium with 0.1 mM choline and 0.05 mM ethanolamine) were pulsed for 30 min with [*Me*-^{14}C]choline (15 900 dpm/nmol). The hepatocytes were washed twice and subsequently chased in Ham's F-12, containing 0.1 mM unlabelled choline, in the absence (□) or presence (■) of 100 nM vasopressin. At various times up to 2 h, samples were taken. The cells were washed and the radioactivity in choline metabolites was determined. A, betaine; B, cholinephosphate; C, CDPcholine; D, phosphatidylcholines. The values are the mean of duplicate determinations of one representative cell preparation. This experiment was repeated twice with similar results.

Determination of the enzyme activities

From the pulse-label and pulse-chase studies described above it became clear that vasopressin stimulates phosphatidylethanolamine synthesis and inhibits the synthesis of phosphatidylcholines. The latter effect seems to be comparable with the inhibition of the phosphatidylcholine synthesis by norepinephrine as described by Haagsman et al. [6] and by glucagon or cAMP analogues [1–4]. Inhibition of the phosphatidylcholine synthesis by Ca^{2+}-dependent [6] as well as cAMP-dependent hormones [3,4] seems to be exerted on the level of

cholinephosphate cytidylyltransferase.

In the present study we focused our attention on the mechanism of the stimulatory effect of vasopressin on the synthesis of phosphatidylethanolamines. Therefore, we decided to determine the effect of vasopressin on the activities of ethanolamine kinase, ethanolaminephosphate cytidylyltransferase and ethanolaminephosphotransferase. Table I shows that the activity of ethanolaminephosphate cytidylyltransferase was high compared to the activity of ethanolamine kinase. This was also reported by Groener et al. [22] and by Schneider and Vance [23]. On the other hand, it was shown that the activity of cholinephosphate cytidylyltransferase in rat liver is much lower than the activity of choline kinase [3,4,22].

Vasopressin did not affect the activity of ethanolamine kinase, nor the activity of ethanolaminephosphate cytidylyltransferase in the $105\,000 \times g$ supernatant. The latter result was not in line with the results of the pulse-chase studies (Fig. 4), which indicated that ethanolaminephosphate cytidylyltransferase might be subject to regulation by vasopressin. However, the activity of ethanolaminephosphotransferase was increased in vasopressin-stimulated cells compared to control cells. Although this stimulation was not statistically significant, the enzyme activity was enhanced in each of the four experiments carried out. The stimulation of ethanolaminephosphotransferase in hormone-treated cells might be explained by the increase of the diacylglycerol content of the cells (Table II). After an incubation period of 10 min the diacylglycerol content in vasopressin-treated cells was enhanced to $125 \pm 5\%$ of the content of control cells. A much greater enhancement ($193 \pm 7\%$) was observed after an incubation period of 60 min.

TABLE I

EFFECTS OF VASOPRESSIN ON THE ACTIVITIES OF THE ENZYMES OF THE DE NOVO PHOSPHATIDYLETHANOLAMINE SYNTHESIS

Hepatocytes were incubated in the absence or presence of vasopressin (10^{-7} M) for 60 min. Subcellular fractionation and determination of the enzyme activities were performed as described in Materials and Methods. Values are mean ± S.D.. The number of cell preparations is indicated in parentheses.

Enzyme	Specific activity (nmol/mg protein per min)	
	Control	Vasopressin
Ethanolamine kinase	0.69 ± 0.06 (3)	0.64 ± 0.05 (3)
Ethanolaminephosphate cytidylyltransferase	2.20 ± 0.35 (4)	2.17 ± 0.29 (4)
Ethanolamine-phosphotransferase	0.23 ± 0.06 (4)	0.30 ± 0.08 (4)

TABLE II

EFFECT OF VASOPRESSIN ON THE DIACYLGLYCEROL CONTENT OF HEPATOCYTES

Cells were incubated in the absence or presence of vasopressin (10^{-7} M). At different time points incubations were terminated by the addition of chloroform/methanol (1:1, v/v). Determination of diacylglycerol content was carried out as described in Materials and Methods. The absolute amount of diacylglycerol was 0.73 ± 0.21 nmol/mg cell protein at the start of the incubation period and 0.40 ± 0.06 nmol/mg cell protein after 60 min of incubation. Results are means ± ranges for two different cell preparations, each determined in duplicate.

Incubation time (min)	Diacylglycerol content (% of control)
0	100%
10	125 ± 5%
30	129 ± 15%
60	193 ± 7%

Discussion

The present study demonstrates that vasopressin, a Ca^{2+}-dependent hormone, inhibits the biosynthesis of phosphatidylcholines from choline and stimulates the incorporation of ethanolamine into phosphatidylethanolamines in isolated hepatocytes. This emphasises the fact that phosphatidylcholine and phosphatidylethanolamine synthesis are under independent hormonal control at one or more steps beyond the diacylglycerol branchpoint.

The mechanism of the inhibition of the phosphatidylcholine synthesis seems to be very similar to the inhibition of biosynthesis of phosphatidylcholines by norepinephrine [6] and glucagon or cAMP analogues [3,4]. Neither vasopressin, nor norepinephrine affects the uptake, phosphorylation or oxidation of choline. However, pulse-chase studies with either of these hormones indi-

cate that the inhibition of the synthesis of phosphatidylcholines might be due to an inhibition of CTP:cholinephosphate cytidylyltransferase (Fig. 5). Alemany et al. [5] also reported an inhibition of choline incorporation into phosphatidylcholines by vasopressin. However, this effect was transient and the rate of phosphatidylcholine synthesis already reached control levels after 4 min. The authors suggested that the inhibitory effect of vasopressin was secondary to an increase in intracellular calcium, leading to an inhibition of cholinephosphotransferase. Although inhibition of this enzyme cannot be excluded, a direct decrease of the activity of cholinephosphotransferase by vasopressin was not demonstrated by these authors.

In the present study we focused our attention primarily on the activation of the synthesis of phosphatidylethanolamines by vasopressin. Fig. 2 demonstrates that enhanced incorporation of ethanolamine into phosphatidylethanolamines is accompanied by a diminished labelling of the intermediates. An explanation for this phenomenon might be a stimulation of the formation of phosphatidylethanolamines from diacylglycerol and CDPethanolamine, the last step in the phosphatidylethanolamine synthesis. This will induce an accelerated flow through the whole pathway with a concomitant decrease in the amount of labelled intermediates. Pulse-chase studies demonstrate that the enhancement of the biosynthesis of phosphatidylethanolamines is attended by an increased rate of disappearance of labelled ethanolaminephosphate as well as CDPethanolamine (Fig. 4). This suggests that not only ethanolaminephosphate cytidylyltransferase might be a target enzyme for the action of vasopressin. The determination of the activities of the enzymes of the phosphatidylethanolamine biosynthesis (Table I) are not entirely in line with the results obtained in pulse-chase experiments and in pool size determinations. Although the activity of ethanolaminephosphotransferase indeed tends to increase, incubation of hepatocytes in the presence of vasopressin does not affect the activity of the cytidylyltransferase.

Binding of vasopressin to its receptor initiates a cascade of signals, leading to the formation of inositol 1,4,5-triphosphate on the one hand and diacylglycerol on the other [24]. The latter compound activates protein kinase C, which in turn phosphorylates specific proteins. 12-*O*-tetradecanoyl 13-acetate simulates the second messenger activity of diacylglycerol. In this respect it is interesting to mention that the incorporation of [1,2-^{14}C]ethanolamine into phosphatidylethanolamines is stimulated in hepatocytes exposed to phorbol ester. This stimulation is attended by an enhancement of the activity of ethanolaminephosphate cytidylyltransferase (Tijburg, L.B.M., Schuurmans, E.A.J.M., Geelen, M.J.H. and Van Golde, L.M.G., unpublished observations). Our results with vasopressin, which stimulates phosphatidylethanolamine synthesis, but does not affect cytidylyltransferase, and with phorbol ester, which stimulates phosphatidylethanolamine synthesis and does affect cytidylyltransferase, are comparable with results reported by Vance and coworkers concerning the effect of glucagon on the phosphatidylcholine synthesis [3,4]. These investigators could not correlate the inhibition of the phosphatidylcholine synthesis in cells treated with glucagon to any change in the activity of cholinephosphate cytidylyltransferase [3]. However, short-term treatment of hepatocytes with cAMP analogues resulted in an inhibition of phosphatidylcholine synthesis, accompanied by an inhibition of the activity by cholinephosphate cytidylyltransferase.

The stimulation of ethanolaminephosphotransferase might be attributed to the increased amount of diacylglycerol in hormone-treated cells compared to control cells (Table II). Bocckino et al. [25] demonstrated a marked stimulation of the accumulation of 1,2-diacylglycerol in hepatocytes treated with vasopressin for maximally 10 min. From our results it is clear that the accumulation of diacylglycerol continues to increase in the presence of vasopressin for at least 60 min, although the stimulation is much smaller than that reported by Bocckino et al. [25].

The accumulation of diacylglycerol in vasopressin-treated cells may be due to the breakdown of inositol phospholipids, alone or in combination with a breakdown of phosphatidylcholines or to a stimulation of phosphatidate phosphohydrolase [26], the enzyme that produces diacylglycerol from phosphatidate.

The diacylglycerol formed in the presence of vasopressin is enriched in stearate and arachidonate [25]. There are several differences in the utilization of diacylglycerol by choline- and ethanolaminephosphotransferase [27,28], the latter one showing a preference for highly unsaturated species. It is very well possible that the newly formed diacylglycerol is a better substrate for ethanolaminephosphotransferase, which would explain the increase in the incorporation of ethanolamine into phosphatidylethanolamines.

Vance and coworkers [29] suggested that the supply of diacylglycerol is saturating, even for a highly stimulated phosphatidylcholine synthesis. However, recently this group reported that biosynthesis of phosphatidylcholines can be limited by the supply of CDPcholine as well as by diacylglycerol [30]. Furthermore, Sundler and Åkesson [15] suggested that the availibility of diacylglycerol might play an important role in the rate of phosphatidylethanolamine synthesis. It will be of great interest to establish the relative importance of the supply of CDPethanolamine on the one hand and that of diacylglycerol on the other in controlling the rate of phosphatidylethanolamine synthesis.

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