Phosphatidylethanolamine metabolism in rat liver after partial hepatectomy

Control of biosynthesis of phosphatidylethanolamine by the availability of ethanolamine

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The effect of partial (70 %) hepatectomy on phosphatidylethanolamine (PE) synthesis was studied in rat liver during the first 4 post-operative days. Between 4 and 96 h after partial hepatectomy, the mass of PE increased from 30 % to 80 % of sham-operation values. In line with the increase in PE mass, the rate of PE synthesis *in vivo* from [¹⁴C]ethanolamine was stimulated 1.6- and 1.3-fold at 22 and 48 h after partial hepatectomy respectively. Surprisingly, the activity of CTP:phosphoethanolamine cytidyltransferase (EC 2.7.7.14) was virtually unchanged after partial hepatectomy. In addition, neither ethanolamine kinase (EC 2.7.1.82) nor ethanolaminephosphotransferase (EC 2.7.8.1) showed any changes in activity over the time period studied. Hepatic levels of ethanolamine and phosphoethanolamine were drastically increased after partial hepatectomy, as compared with sham operation, whereas levels of CDP-ethanolamine and microsomal diacylglycerol were not affected. Interestingly, partial hepatectomy caused the concentration of free ethanolamine in serum to increase from 29 μ M to approx. 50 μ M during the first day after surgery. In hepatocytes isolated from non-operated animals, incorporation of [³H]ethanolamine into PE was stimulated by increasing the ethanolamine concentration from 10 up to 50 μ M, whereas the radioactivity associated with phosphoethanolamine only increased at ethanolamine concentrations higher than 30 μ M. Taken together, our results indicate that the observed increase in serum ethanolamine concentration after partial hepatectomy is probably responsible for both the increase in PE biosynthesis and the accumulation of ethanolamine and phosphoethanolamine in regenerating liver.

INTRODUCTION

The rapid restoration of liver mass (compensatory hyperplasia or 'liver regeneration') following partial (70 %) hepatectomy offers a good tool to study growth-related events in a physiologically controlled situation *in vivo*. Although this model system has been extensively used to study regulation of cellular proliferation [1–3], far less information is available on the metabolism of neutral lipids [4–6] and phospholipids [7,8] in the regenerating liver.

We recently showed [8] that, after partial hepatectomy, there is an increased synthesis of phosphatidylcholine (PC), caused by enhanced activity of CTP:phosphocholine cytidyltransferase (EC 2.7.7.15). It is generally believed that translocation of this enzyme from cytosol to endoplasmic reticulum is instrumental in the stimulation of its activity [9,10]. In regenerating liver, however, increased activity of this cytidyltransferase is mainly caused by an increase in the amount of enzyme [8].

It is obvious that increased membrane biogenesis after partial hepatectomy also implicates enhanced biosynthesis of phospholipids other than PC. This prompted us to investigate the effect of partial hepatectomy on phosphatidylethanolamine (PE) biosynthesis. PE can be synthesized by three different routes. In addition to synthesis via CDP-ethanolamine, PE can also be formed by a Ca²⁺-dependent base-exchange reaction or by decarboxylation of phosphatidylserine (PS). Although there are reports that decarboxylation of PS is the sole route for PE biosynthesis in cultured cells [11,12], recent studies indicate that several tissues, including liver, derive a substantial part of their PE from the CDP-ethanolamine pathway [13,14].

Previous reports showed that CTP:phosphoethanolamine cytidyltransferase (EC 2.7.7.14) can be considered as a possible

regulatory site of PE synthesis [15–17]. In addition, the availability of diacylglycerols [15,16,18] and ethanolamine [15] may also play an important role in the regulation of PE biosynthesis. In the present paper, we conclude that synthesis of PE in the regenerating liver is primarily governed by the supply of ethanolamine to the liver. Increased substrate pressure, owing to elevated serum levels of ethanolamine, leads to enhanced PE synthesis and to a concomitant accumulation of ethanolamine and phosphoethanolamine in the liver remnant.

EXPERIMENTAL

Materials

L-[³H]Serine, 1,2-dioleoyl-L-3-phosphatidyl-L-[3-¹⁴C]serine, [1,3-³H]glycerol and [³H]ethanolamine were purchased from Amersham International (Amersham, Bucks., U.K.). [1,2-¹⁴C]ethanolamine was obtained from New England Nuclear (Dreieichenhain, Germany) and CDP-[1,2-¹⁴C]ethanolamine was supplied by ICN Radiochemicals (Irvine, U.K.). Phospho[1,2-¹⁴C]ethanolamine was prepared from [1,2-¹⁴C]ethanolamine as described [18].

Phospholipase C from *Clostridium welchii* and *Bacillus cereus*, BSA and Triton X-100, purified for membrane research, were purchased from Boehringer (Mannheim, Germany), whereas dansyl chloride was obtained from Janssen Chimica (Beerse, Belgium). All other chemicals were of analytical grade, obtained from Baker Chemical Co. (Deventer, The Netherlands).

Animals and surgery

Partial (70 %) hepatectomies [19] were routinely performed between 09:00 and 10:00 h on male Wistar rats (150–200 g) under light ether anaesthesia. Sham operations were performed

Abbreviations used: DMEM, Dulbecco's modified Eagle's medium; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

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as a control for surgical stress. All animals had been kept under an inverted light/dark cycle (dark between 06:00 and 18:00 h) for at least 2 weeks before surgery, and they were allowed free access to water and pelleted standard diet [18] before and after surgical procedures.

Studies *in vivo* on synthesis of PE and PS

To estimate the rates of PE and PS synthesis *in vivo*, rats were injected intraperitoneally with 0.5 ml of physiological saline containing 1 μ Ci of [14 C]ethanolamine (4.0 mCi/mmol), or intraportally with 0.25 ml of physiological saline containing either 75 μ Ci of [1,3- 3 H]glycerol (0.5 Ci/mmol) or 50 μ Ci of [3 H]serine (37.8 Ci/mmol) respectively. At the time points indicated in the Figure and Table legends, rats were killed. Livers were removed and subsequently homogenized in a Teflon/glass homogenizer with 4 vol. of ice-cold buffer containing 145 mM-NaCl, 10 mM-Tris/HCl (pH 7.4), 1 mM-EDTA and 10 mM-NaF. Lipids were extracted from homogenates as described by Sundler *et al.* [20]. Phospholipids were separated by t.l.c. on silica gel G with chloroform/methanol/ NH_3 (13:7:1, by vol.) as developing agent. Label incorporation into the PE or PS headgroup was quantified by liquid-scintillation counting as described [13].

Enzyme assays

Homogenates, prepared as described above (see 'Studies *in vivo*'), were centrifuged twice for 5 min at 600 *g*, followed by centrifugation of the combined supernatants at 12000 *g* for 15 min. The pellet (mitochondrial fraction) was resuspended in a buffer containing 250 mM-sucrose, 10 mM-Hepes (pH 7.5) and 1 mM-EDTA, at a final concentration of 1.5 mg of protein/ml. The supernatant was centrifuged once more (60 min at 105000 *g*). The final supernatant was employed as the cytosolic fraction, whereas the pellet (microsomal fraction) was resuspended in a buffer containing 250 mM-sucrose, 10 mM-Tris/HCl (pH 7.4), 1 mM-EDTA and 10 mM-NaF to a final concentration of 5 mg of protein/ml.

Ethanolamine kinase (EC 2.7.1.82) activity was determined in the cytosolic fraction as described by Weinhold & Rethy [21]. Labelled phosphoethanolamine was separated from ethanolamine by paper chromatography (Whatman 3 MM), with ethanol/propanol/ NH_3 (13:4:7, by vol.) as developing solvent. CTP:phosphoethanolamine cytidyltransferase activity was measured in the cytosolic fraction, as described in ref. [22]. We were not able to detect any CTP:phosphoethanolamine cytidyltransferase activity in the microsomal fraction. The activity of ethanolaminephosphotransferase (EC 2.7.8.1) was measured in the microsomal fraction with endogenous diacylglycerol as substrate [23]. Incubations were carried out for 8 min and the formation of radioactive PE from [14 C]CDP-ethanolamine was quantified by the filter-disc method, as described by Goldfine [24].

PS decarboxylase (EC 4.1.1.65) activity was measured in the mitochondrial fraction as the formation of [14 C]PE from [14 C]PS. The reaction mixture (final volume 0.4 ml) consisted of 100 mM- KH_2PO_4 (pH 6.8), 10 mM-EDTA, 50 μ M-phosphatidyl-[3- 14 C]serine (3.5×10^6 d.p.m./ μ mol), 0.5 mg of purified Triton X-100/ml, and either enzyme (100–200 μ g of mitochondrial protein) or appropriate buffer for background determination [25]. Assays were carried out for 30 min at 37 °C and were terminated by adding 3.4 ml of chloroform/methanol/water (15:29:6, by vol.), followed by lipid extraction [26]. Radioactive PE was isolated by t.l.c. as described above (see 'Studies *in vivo*'), and was quantified by liquid-scintillation counting.

Isolation and incubation of hepatocytes

Hepatocytes were isolated from male Wistar rats (150–200 g)

essentially as in ref. [27], as described by Haagsman *et al.* [28]. To study the rate of PE synthesis as a function of ethanolamine concentration, hepatocytes were isolated from non-operated animals. The hepatocytes were suspended in Krebs–Ringer bicarbonate buffer (pH 7.4), supplemented with 10 mM-glucose, 1% (w/v) defatted and dialysed BSA, and various concentrations of [3 H]ethanolamine. Incubations (final volume 2.0 ml; cell concn. 4–6 mg of protein/ml) were carried out at 37 °C in a metabolic shaker (82 oscillations/min) in 25 ml Erlenmeyer flasks under an atmosphere of O_2/CO_2 (19:1). Radioactivity associated with PE or phosphoethanolamine was quantified exactly as described in ref. [16].

PE catabolism was studied in hepatocytes, isolated from sham-operated or partially hepatectomized rats, at 22 h after surgery. At 1 h before cell isolation, rats had been injected intraperitoneally with 0.25 ml of physiological saline containing 75 μ Ci of [3 H]ethanolamine (29.5 Ci/mmol), to accomplish pre-labelling of PE *in vivo*. Freshly isolated hepatocytes were suspended in Dulbecco's modified Eagle's medium (DMEM), supplemented with 1% BSA. The cells were plated in 3 ml of medium on collagen-coated culture dishes (60 mm diam.) at a density of 2.5×10^6 hepatocytes per dish. Cells were allowed to attach to the dishes for 2 h, washed once with DMEM, and were further incubated for up to 6 h with 2 ml of DMEM containing 1% BSA and 0.25 mM-ethanolamine. At the end of the incubation period, cells were harvested as in ref. [29], and lipids and water-soluble metabolites were analysed [16].

Chemical analyses

Unless indicated otherwise, lipids were extracted and phospholipids separated as described above (see 'Studies *in vivo*'). The mass of hepatic PE was determined by phosphorus analysis as described by Bartlett [30]. Diacylglycerols were purified by t.l.c. on silica gel G, with light petroleum (b.p. 40–60 °C)/diethyl ether/acetic acid (35:15:1, by vol.) as eluent. Butylated hydroxytoluene was added as an anti-oxidant. Diacylglycerols were then extracted from the silica, hydrolysed, and determined by a modification [22] of the procedure of Wieland [31]. Samples of diacylglycerol were directly converted into the corresponding methyl esters, to quantify the fatty acid composition by g.l.c. [32].

The water-soluble PE precursors, ethanolamine, phosphoethanolamine and CDP-ethanolamine, were extracted and subsequently separated by h.p.l.c., followed by a sensitive chemical analysis, exactly as described by Tijburg *et al.* [33]. Serum ethanolamine levels were determined exactly as reported by Lipton *et al.* [34], and serum choline levels essentially as in ref. [33]. Protein was measured as described by Lowry *et al.* [35], with BSA as a standard.

Expression of results

Results are presented as means $\pm$ s.d. of the numbers of experiments indicated in the legends. Significance was determined by Student's *t* test.

RESULTS

PE synthesis *in vivo* after partial hepatectomy

After 70% hepatectomy, a large portion of hepatocytes in the liver remnant enters the cell cycle. This results in a synchronized wave of DNA synthesis at 24 h [36] and subsequent mitosis [37] at 30 h after surgery. Fig. 1(a) shows the level of PE, the second most abundant phospholipid in rat liver, as a function of time after surgery. The mass of PE, expressed in μ mol/liver, increased after partial hepatectomy in a manner analogous to the res-

Fig. 1. Effect of partial hepatectomy on level and rate of synthesis of hepatic PE at different times after surgery

(a) Level: PE was isolated from livers of sham-operated (○) or partially hepatectomized (●) rats. Data are expressed as function of total liver weight after surgery. Each time point represents means $\pm$ S.D. of four different rats. (b) Synthesis: incorporation *in vivo* of [14 C]ethanolamine into PE was determined in livers of sham-operated (○) and partially hepatectomized (●) rats, 10 min after intraperitoneal injection of [14 C]ethanolamine. Each time point represents means $\pm$ S.D. of three different rats. Data are expressed as nmol of [14 C]ethanolamine incorporated, by using the concentration of serum ethanolamine, as shown in Fig. 3, to calculate the specific radioactivity. Significantly different from sham-operated rats: * $P < 0.02$; ** $P < 0.05$.

toration of total liver mass (results not shown), whereas sham operation did not cause any significant changes in PE level.

We then investigated whether or not the mass recovery of PE was exclusively caused by an increased synthesis of PE via the CDP-ethanolamine pathway. The formation of PE *in vivo* from [14 C]ethanolamine, expressed as nmol of ethanolamine incorporated, was stimulated 1.6- and 1.3-fold at 22 h and 48 h after surgery respectively (Fig. 1b). In addition, the incorporation of [3 H]glycerol into PE was also significantly enhanced at 22 h after surgery (hepatectomized liver 140710 ± 23160 , sham-operated liver 85840 ± 39650 d.p.m./g of liver; $P < 0.05$; from serum glycerol concentrations of sham-operated and partially hepatectomized rats [29], this can be calculated to be 1.21 ± 0.40 and 0.40 ± 0.19 nmol/g of liver respectively). Since newly synthesized PE is rapidly degraded in hepatocytes [38], the increased radioactivity associated with PE might be caused by inhibition of PE catabolism rather than by stimulation of PE synthesis. To check this possibility, PE catabolism was determined in pulse-chase experiments with [3 H]ethanolamine, using hepatocytes isolated

Fig. 2. Effect of partial hepatectomy on the rate of PE breakdown in hepatocytes isolated 22 h after surgery

Hepatocytes were isolated from partially hepatectomized (●) or sham-operated (○) rats, which had been injected with [3 H]ethanolamine to pre-label PE *in vivo* (see the Experimental section). The disappearance of [3 H]-labelled PE was monitored during a chase period of 6 h in the presence of 0.25 mM unlabelled ethanolamine. The results shown are means of duplicate incubations and were reproducible in two independent experiments.

from partially hepatectomized or sham-operated rats. It appeared, however, that the rate of PE degradation in cells from hepatectomized rats was similar to that in cells from sham-operated animals (Fig. 2). This indicated that the elevated incorporation of ethanolamine into PE, induced by partial hepatectomy, is due to a net increase in hepatic PE synthesis.

Activities of the enzymes in the CDP-ethanolamine pathway and PS decarboxylase

In order to understand better the mechanism responsible for the stimulation of PE synthesis *de novo*, we determined the activities of enzymes in the CDP-ethanolamine pathway. Table 1 clearly shows that the activity of CTP:phosphoethanolamine cytidyltransferase, the putative key regulatory enzyme of this pathway [15], was the same in liver cytosol from partially hepatectomized and sham-operated rats, at 4, 22 and 96 h after surgery. At 48 h after partial hepatectomy its activity was even slightly decreased relative to sham-operated controls (Table 1). This indicates that alterations in the activity of CTP:phosphoethanolamine cytidyltransferase cannot account for the

Table 1. Effect of partial hepatectomy on enzyme activities in the CDP-ethanolamine pathway at different times after surgery

At the indicated times after partial hepatectomy (PH) or sham operation (SO), subcellular fractions of rat liver were prepared and the activities (nmol/min per mg of protein) of ethanolamine kinase, CTP:phosphoethanolamine cytidyltransferase (CT) and ethanolaminephosphotransferase determined. Ethanolamine kinase and CT activities were measured in the cytosolic fraction, whereas ethanolaminephosphotransferase activity was assayed in the microsomal fraction. Control values (at $t = 0$ h) of the three enzyme activities were: 0.66 ± 0.08 , 1.81 ± 0.05 and 0.27 ± 0.03 nmol/min per mg of protein respectively. Values are expressed as means $\pm$ S.D. for n rats. Significantly different from sham-operated rats: * $P < 0.001$.

Treatment	Time after surgery (h) ...	Activity (nmol/min per mg of protein)			
		4	22	48	96
Ethanolamine kinase ($n = 3$)	SO	0.56 ± 0.04	0.64 ± 0.06	0.80 ± 0.08	0.72 ± 0.10
	PH	0.54 ± 0.04	0.74 ± 0.12	0.96 ± 0.14	0.82 ± 0.02
CT ($n = 5$)	SO	1.97 ± 0.24	2.09 ± 0.17	2.22 ± 0.17	2.02 ± 0.11
	PH	1.85 ± 0.16	1.96 ± 0.17	1.70 ± 0.12^a	1.89 ± 0.12
Ethanolaminephosphotransferase ($n = 3$)	SO	0.23 ± 0.01	0.28 ± 0.03	0.23 ± 0.02	0.22 ± 0.03
	PH	0.20 ± 0.04	0.23 ± 0.01	0.23 ± 0.01	0.25 ± 0.04

Table 2. Effect of partial hepatectomy on the fatty acid composition of microsomal diacylglycerols 22 h after surgery

Diacylglycerols were extracted from the microsomal fraction of partially hepatectomized (PH) or sham-operated (SO) rats 22 h after surgery. The fatty acid composition of the diacylglycerols was determined by g.l.c. as described in the Experimental section. Significantly different from sham-operated rats: ^a*P* < 0.001; ^b*P* < 0.01.

Treatment	Fatty acid composition (%)					
	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:4}
SO	36.6 ± 0.8	4.4 ± 0.7	15.3 ± 1.8	22.1 ± 0.6	16.4 ± 2.0	5.0 ± 1.2
PH	26.8 ± 0.8 ^a	4.1 ± 1.1	11.1 ± 1.6	27.7 ± 0.5 ^b	24.4 ± 1.6 ^b	4.9 ± 0.5

Table 3. Effect of partial hepatectomy on the amounts of precursors of PE at different times after surgery

Ethanolamine and its metabolites were separated and quantified as described in the Experimental section. Each value represents means ± s.d. for three different rats. Amounts of precursors in control (non-operated) rats were 0.29 ± 0.02 (ethanolamine), 1.17 ± 0.23 (phosphoethanolamine) and 0.09 ± 0.01 (CDP-ethanolamine) µmol/g of liver. Significantly different from sham-operated rats: ^a*P* < 0.01; ^b*P* < 0.02.

Treatment	Time after surgery (h) ...	Amount (µmol/g of liver)			
		4	22	48	96
Ethanolamine	SO	0.30 ± 0.03	0.44 ± 0.08	0.32 ± 0.02	0.26 ± 0.04
	PH	0.68 ± 0.04 ^a	1.10 ± 0.20 ^b	1.47 ± 0.16 ^a	0.62 ± 0.02 ^a
Phosphoethanolamine	SO	1.25 ± 0.12	1.14 ± 0.09	1.20 ± 0.11	1.18 ± 0.05
	PH	1.98 ± 0.12 ^a	3.23 ± 0.23 ^a	3.66 ± 0.39 ^a	1.94 ± 0.18 ^b
CDP-ethanolamine	SO	0.09 ± 0.01	0.08 ± 0.02	0.11 ± 0.01	0.08 ± 0.01
	PH	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.10 ± 0.01

observed increase in PE biosynthesis. It is noteworthy that, in contrast with CTP:phosphocholine cytidyltransferase [8,9], CTP:phosphoethanolamine cytidyltransferase is exclusively located in the cytosolic fraction. The activities of ethanolamine kinase and ethanolaminephosphotransferase were not affected by partial hepatectomy (Table 1). The activity of PS decarboxylase, the enzyme which converts PS into PE, was not changed 22 h after surgery (sham-operated 0.76 ± 0.06, partially hepatectomized 0.69 ± 0.05 nmol/min per mg of protein).

Effect of partial hepatectomy on the amounts of precursors of PE at different times after surgery

Since the enzyme activities of the CDP-ethanolamine pathway had been measured *in vitro* under optimal (*V*_{max}) conditions which do not necessarily reflect the situation in the liver *in situ*, we also measured pool sizes of intermediates of PE synthesis in the CDP-ethanolamine pathway. It has been reported previously [15,16,18,20] that the activity of ethanolaminephosphotransferase, an enzyme which is tightly bound to the endoplasmic reticulum [39], is influenced by the availability and fatty acid composition of diacylglycerols. There were no significant changes in the levels of microsomal diacylglycerols over the time period studied. At 22 h after surgery the amount of diacylglycerol was 2.84 ± 0.22 and 2.46 ± 0.12 nmol/mg of microsomal protein for partially hepatectomized and sham-operated rats respectively. Moreover, in agreement with data reported by Slater *et al.* [40] for total microsomal lipid, we observed an increase in the unsaturated fatty acid/saturated fatty acid ratio in diacylglycerol after partial hepatectomy (Table 2). We therefore conclude that the availability of appropriate diacylglycerol species [41] for PE biosynthesis is not changed by liver resection.

Partial hepatectomy also led to a dramatic and very reproducible rise in the hepatic pool sizes of ethanolamine and phosphoethanolamine (Table 3). At 48 h after surgery, the

amounts of ethanolamine and phosphoethanolamine in livers from partially hepatectomized rats were increased 4.6- and 3-fold respectively. Interestingly, these pool sizes were already significantly expanded at an early time point (4 h) after partial hepatectomy (Table 3). On the other hand, the pool size of CDP-ethanolamine did not change significantly during the time period studied (Table 3).

Serum level of ethanolamine

These results posed the conceptual problem that enzyme activities in the CDP-ethanolamine pathway remained nearly unchanged after partial hepatectomy (Table 1), whereas at the same time PE mass was increased (Fig. 1a) and the levels of ethanolamine and, particularly, phosphoethanolamine were elevated (Table 3). In an attempt to clarify this point, we measured the concentration of ethanolamine in serum of rats that had been subjected to either partial hepatectomy or sham operation. The ethanolamine concentration found in serum from non-operated rats is in good agreement with data reported by Sundler & Åkesson [15]. Fig. 3 shows that, already at 4 h after partial hepatectomy, the level of ethanolamine in serum was increased. At 22 h after surgery the serum ethanolamine concentration was elevated up to 50.9 µM, as compared with 28.1 µM in sham-operated rats, thereby substantially increasing the supply of ethanolamine to the regenerating liver. It may be noted that, at 22 h after surgery, the concentration of choline in serum from sham-operated animals (19.9 ± 2.8 µM; *n* = 3) agreed well with literature data [42], and that this value was not significantly different from the choline concentration determined in serum from partially hepatectomized animals (20.4 ± 1.6 µM; *n* = 3).

Accumulation of phosphoethanolamine in isolated hepatocytes

In view of these findings, we hypothesized that *in vivo* the supply of ethanolamine could well be rate-limiting for PE

Fig. 3. Effect of partial hepatectomy on the level of free ethanolamine in serum at different times after surgery

Ethanolamine levels were measured in serum from sham-operated (○) or partially hepatectomized (●) rats. Each time point represents the mean $\pm$ S.D. of three different rats. Significantly different from sham-operated rats: * $P < 0.001$; ** $P < 0.01$.

Fig. 4. Effect of exogenous ethanolamine concentration on the rate of [³H]ethanolamine incorporation into PE and phosphoethanolamine by isolated rat hepatocytes

Freshly isolated hepatocytes were incubated for 45 min in the presence of different ethanolamine concentrations. Incorporation of [³H]ethanolamine into PE (●) and phosphoethanolamine (○) was determined. Values are means of duplicate incubations of a representative experiment.

synthesis *de novo* via the CDP-ethanolamine pathway. To test this hypothesis, hepatocytes isolated from non-operated rats were incubated with different concentrations of [³H]ethanolamine. Next, the radioactivity incorporated into PE and phosphoethanolamine was determined (Fig. 4). It is clear from Fig. 4 that, at low ethanolamine concentrations, a linear relationship exists between the incorporation of ethanolamine into PE and the concentration of ethanolamine applied. In agreement with previous observations in our laboratory [16], maximal incorporation into PE was reached in the concentration range of 0.05–0.1 mM-ethanolamine. Only at ethanolamine concentrations higher than 30 μ M, phosphoethanolamine accumulated. These data suggest that, at ethanolamine concentrations below 30 μ M, ethanolamine supply rather than CTP:phosphoethanolamine cytidyltransferase activity limited the rate of PE synthesis.

DISCUSSION

In the present study we examined the regulation of PE biosynthesis in regenerating rat liver. As expected, the total amount of hepatic PE increased after partial hepatectomy (Fig. 1a). This was accompanied by an enhanced rate of incorporation of [¹⁴C]ethanolamine into PE (Fig. 1b), whereby the specific radioactivity of ethanolamine in serum was used to calculate the rate of PE biosynthesis. Increased formation of labelled PE may be due to: (i) decreased catabolism of PE; (ii) stimulation of the Ca²⁺-dependent base-exchange reaction; and (iii) activation of the CDP-ethanolamine pathway. Pulse-chase experiments with radioactive ethanolamine demonstrated that the degradation of PE in hepatocytes, isolated from partially hepatectomized rats, was similar to that in cells from sham-operated animals (Fig. 2). Moreover, Sundler *et al.* [43] reported that, at physiological ethanolamine concentrations, only approx. 9% of hepatic ethanolamine incorporation into PE could be attributed to Ca²⁺-dependent base-exchange. Although this portion increases significantly at higher ethanolamine concentrations, it is unlikely that the base-exchange reaction could account for the observed increased formation of PE (Fig. 1b). As in addition the route via decarboxylation of PS appears to be unchanged, we consider that in regenerating liver PE biosynthesis via the CDP-ethanolamine pathway is stimulated.

Recently we reported that in regenerating liver the biosynthesis of PC was elevated as a result of increased activity and amount of CTP:phosphocholine cytidyltransferase [8], the key regulatory enzyme of the CDP-choline pathway. Measurement of the corresponding enzyme of the CDP-ethanolamine route, CTP:phosphoethanolamine cytidyltransferase, showed no difference in its activity in livers from partially hepatectomized or sham-operated rats (Table 1). This indicates that alterations in the activity of CTP:phosphoethanolamine cytidyltransferase cannot account for the observed increase in PE biosynthesis. These data show that in the regenerating liver PE biosynthesis is regulated differently from the biosynthesis of PC via the CDP-choline route, as the enhanced formation of PC after partial hepatectomy was shown to be caused by an increased activity and amount of CTP:phosphocholine cytidyltransferase [8].

The pool sizes of both ethanolamine and phosphoethanolamine were significantly enhanced in the regenerating liver (Table 3). A similar increase in the pool size of phosphoethanolamine in regenerating liver was also demonstrated by ³¹P-n.m.r. analysis [44]. Ethanolamine could be continuously generated in the liver by an enzyme-catalysed cycle, requiring only trace amounts of PS and PE, i.e. by decarboxylation of PS, followed by base-exchange with serine, thereby causing release of free ethanolamine and re-synthesis of PS [45,46]. However, partial hepatectomy affected neither the incorporation *in vivo* of label from [³H]serine into PS or PE (results not shown), nor the activity of mitochondrial PS decarboxylase as measured *in vitro*. This suggests that the increase in hepatic ethanolamine levels originates from an extrahepatic source. Indeed, the concentration of ethanolamine in serum increased by more than 2-fold after partial hepatectomy (Fig. 3).

The intrahepatic ethanolamine level in control animals can be calculated to be below the K_m value of ethanolamine kinase. Using published conversion factors [47,48], one arrives at a cytosolic volume of approx. 0.54 ml/g wet wt of liver. From this number and the data of Table 3, it appears that, at 48 h after partial hepatectomy, the intracellular ethanolamine concentration amounts of 2.72 mM, as compared with 0.54 mM in liver of non-operated animals. Published values for the apparent K_m of purified ethanolamine kinase for ethanolamine range from 0.6 mM [21,49,50] to 1.2 mM [51], thereby indicating that partial

hepatectomy causes substrate saturation of the enzyme. For the cytidyltransferase (K_m for phosphoethanolamine of 0.065 mM [52]), the situation is less clear. In both the sham-operated and partially hepatectomized livers the concentration of phosphoethanolamine is far above the K_m of CTP:phosphoethanolamine cytidyltransferase. This means that a regulatory role for CTP:phosphoethanolamine cytidyltransferase, as claimed previously [15–17], probably implies compartmentation of phosphoethanolamine as suggested by Sundler [53].

Previous reports from our [16] and other [15] laboratories demonstrated that in isolated hepatocytes the rate of PE synthesis increased at increasing concentrations of exogenous ethanolamine. Since we observed no accumulation of radioactive phosphoethanolamine in isolated hepatocytes at exogenous ethanolamine concentrations below 30 μ M (Fig. 4), we propose that in this lower concentration range the supply of ethanolamine limits PE formation. At ethanolamine concentrations higher than 30 μ M, phosphoethanolamine does accumulate (Fig. 4) and the activity of CTP:phosphoethanolamine cytidyltransferase may become rate-limiting.

In summary, the results of Figs. 3 and 4 suggest that the increase in concentration of serum ethanolamine, from approx. 29 μ M to 50 μ M, leads to an increase in PE biosynthesis (Fig. 1b) as well as to intrahepatic accumulation of ethanolamine and phosphoethanolamine (Table 3).

The present work raises two intriguing questions that remain to be addressed. The first concerns the still unknown origin of extrahepatic ethanolamine. It has been shown [54,55] that serum levels of several amino acids are considerably, and progressively, increased during liver regeneration. However, the serum level of serine was only slightly increased after partial hepatectomy, and showed little or no variation over the time period studied after surgery [54,55]. It could well be then that under these circumstances, elsewhere in the mammalian organism, serine is continuously mobilized and converted into ethanolamine via the enzyme-catalysed cycle mentioned above.

The second question pertains to a possible regulatory role of increased intracellular levels of (phospho)ethanolamine in the regenerating liver. ^{31}P -n.m.r. studies have already indicated that the phosphomonoester peak, comprising phosphocholine and phosphoethanolamine, is increased not only in tumours of various human tissues ([44], and reviewed in ref. [56]), but also in developing liver [57] and brain [57,58] relative to adult tissue. According to Gyulai *et al.* [58], phosphoethanolamine is the major constituent of this monoester peak in neonatal dog brain. Therefore, developmental studies in liver and other mammalian tissues may provide an additional clue to the role of phosphoethanolamine in the regenerating liver. It may remain useful to search in future studies for possible effects of phosphoethanolamine on activities of growth-related enzymes in liver.

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