Mechanisms for the Effects of Ethanol on Hepatic Phosphatidate Phosphohydrolase

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1. The effects of the intramuscular administration of glycerol and dihydroxyacetone (40mmol per kg body wt.), sorbitol and glucose (20mmol per kg body wt.) or NaCl (1.5mmol per kg body wt. in 10ml of water per kg body wt.) were investigated on soluble phosphatidate phosphohydrolase and certain metabolites in rat liver. 2. The effects of ethanol and glycerol on phosphatidate phosphohydrolase were also studied in isolated perfused livers. 3. The administration of glycerol, sorbitol and dihydroxyacetone *in vivo* increased hepatic phosphatidate phosphohydrolase activity by 137, 63 and 32% respectively in 4h. 4. A significant positive correlation was found between the hepatic *sn*-glycerol 3-phosphate concentration and phosphatidate phosphohydrolase after the administration of various substrates *in vivo*. 5. The soluble phosphatidate phosphohydrolase activity tended to increase during perfusions of isolated rat livers without added substrates, and neither ethanol nor glycerol produced additional effects. 6. The activity of soluble phosphatidate phosphohydrolase was 2.5 times higher in the livers of hyperthyroid rats than in normal rats. This activity was not influenced by intragastric ethanol or glycerol administration, nor was the concentration of *sn*-glycerol 3-phosphate changed by these compounds. 7. It is concluded that the ethanol-induced increase in hepatic phosphatidate phosphohydrolase may at least in part be mediated by the hepatic concentration of metabolites, probably by the concentration of *sn*-glycerol 3-phosphate.

The ethanol-induced increase in hepatic triacylglycerol synthesis is usually attributed to either the redox change or the increase in the *sn*-glycerol 3-phosphate concentration in the liver (Nikkilä & Ojala, 1963; Ylikahri, 1970; Fellenius *et al.*, 1973). Some experiments with propan-2-ol and pyrazole argue against this hypothesis, however. Propan-2-ol is capable of producing fatty liver (Mallov & Bloch, 1956; Gaillard & Derache, 1965), although it causes only a slight change in the hepatic *sn*-glycerol 3-phosphate concentration (Forsander, 1967; Nordmann *et al.*, 1973). Results from experiments with pyrazole(s) are more contradictory. Bustos *et al.* (1970) and Estler (1974) report that pyrazole prevents the ethanol-induced increase in the hepatic *sn*-glycerol 3-phosphate concentration, but not the hepatic fat accumulation caused by ethanol, but opposite results have also been obtained in similar experiments (Morgan & DiLuzio, 1970; Blomstrand & Forsell, 1971).

It has also been suggested that ethanol affects hepatic triacylglycerol synthesis by other means. Abrams & Cooper (1976) show that it increases the uptake of fatty acids into the liver, and Scheig & Isselbacher (1965) observe that *in vivo* it increases the capacity of the triacylglycerol-synthesizing pathway, so that an increased rate of esterification is also

observed in liver homogenates or microsomal fractions from ethanol-treated rats *in vitro* in the absence of ethanol.

The ethanol-induced stimulation of triacylglycerol synthesis may at least in part be attributed to an increase in the activity of hepatic cytoplasmic phosphatidate phosphohydrolase (EC 3.1.3.4) which has been shown to precede the ethanol-induced accumulation of triacylglycerols in the liver (Savolainen, 1977). Of the enzymes of triacylglycerol synthesis studied so far, it is only the activity of this enzyme that is stimulated by a single dose of ethanol (Pritchard *et al.*, 1977). Increased activity of phosphatidate phosphohydrolase is also observed after prolonged ethanol ingestion (Lamb & Fallon, 1977). It has been suggested that phosphatidate phosphohydrolase controls the rate of triacylglycerol synthesis in the liver (Lamb & Fallon, 1974a; Fallon *et al.*, 1977). The regulatory role of phosphatidate phosphohydrolase is also supported by the pattern of glycerol incorporation into various lipid classes after ethanol administration (Pritchard & Brindley, 1977; Savolainen, 1977). The activity of hepatic phosphatidate phosphohydrolase is also reported to be elevated after subtotal hepatectomy (Mangiapanne *et al.*, 1973), starvation (Vavrečka *et al.*, 1969; Mangiapanne *et al.*, 1973; Kinnula *et al.*, 1978) or a

carbohydrate-rich diet (Lamb & Fallon, 1974b), but no information is available on the mechanism by which these treatments stimulate the enzyme activity.

The present study investigates the mechanism by which ethanol administration stimulates cytoplasmic phosphatidate phosphohydrolase activity. In a search for possible regulators of phosphatidate phosphohydrolase activity, compounds other than ethanol that are capable of causing cellular NAD^+ reduction (glycerol, sorbitol) or an increase in the concentration of *sn*-glycerol 3-phosphate (dihydroxyacetone) were injected into starved rats *in vivo*. Measurements were made of the activity of cytoplasmic phosphatidate phosphohydrolase and the hepatic concentrations of *sn*-glycerol 3-phosphate, dihydroxyacetone phosphate, lactate and pyruvate. In hyperthyroid rats, administration of glycerol (Williamson *et al.*, 1969) or ethanol (Ylikahri, 1970) has no effect on the hepatic *sn*-glycerol 3-phosphate concentration. In order to investigate further the role of *sn*-glycerol 3-phosphate as a regulator of soluble phosphatidate phosphohydrolase, the activity of the latter was also measured after the administration of ethanol or glycerol to thyroxine-treated rats.

In order to differentiate between direct and indirect effects on the liver, the response of isolated perfused livers to ethanol and glycerol was also investigated.

Materials and Methods

Reagents

Phosphatidic acid (disodium salt) from egg phosphatidylcholine was obtained from Koch-Light Laboratories, Colnbrook, Bucks., U.K. Enzymes, phosphatidylcholine, dihydroxyacetone, streptomycin sulphate and penicillin G were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Glycerol was from E. Merck A.G., Darmstadt, Germany, and ethanol (94%, w/v) from the State Alcohol Monopoly (ALKO), Helsinki, Finland. Coenzymes were obtained from Boehringer G.m.b.H., Mannheim, Germany. Eagle's minimum essential medium for tissue culture and glutamine were purchased from the Orion Pharmaceutical Co., Espoo, Finland. L-Thyroxine, sodium salt, was obtained from Neofarma Pharmaceutical Co., Seinäjoki, Finland.

Animals

Male Sprague-Dawley rats weighing 210–320 g were used. These were starved for 48 h before use in the experiments *in vivo* (injection of glycerol, dihydroxyacetone, sorbitol or glucose), while the rats used in thyroxine experiments and the donors of both liver and blood in the experiments with isolated perfused livers were fed *ad libitum* until they were killed. Sodium L-thyroxine (1 mg/day per kg body weight)

was administered subcutaneously for 7 successive days.

Experiments *in vivo*

Food-deprived rats were injected intramuscularly with 4M-glycerol, 4M-dihydroxyacetone, 2M-sorbitol, 2M-glucose or 0.15M-NaCl (10 ml/kg body wt.), and then anaesthetized 4 h or 8 h later with diethyl ether. The feeding and thyroxine-treated rats were given ethanol (65 mmol/kg body wt.), glycerol (40 mmol/kg body wt.) or an equal volume of water (25 ml/kg body wt.) by gastric intubation, and then anaesthetized 4 h later with diethyl ether. Liver samples for the metabolite determinations were taken by the freeze-clamp technique (Wollenberger *et al.*, 1960), and immediately afterwards a piece of the fresh liver was placed into an ice-chilled beaker for enzyme determination.

Perfusion experiments

Each rat was anaesthetized with intraperitoneally administered pentobarbital (Nembutal, 60 mg/kg body wt.), and the bile duct cannulated. Immediately afterwards the portal vein and inferior vena cava were cannulated. Perfusion *in situ* was started immediately with Krebs-Ringer bicarbonate solution in equilibrium with O_2/CO_2 (19:1). The liver was removed and placed in a perfusion chamber equipped with a thermostat. It was perfused in a closed system with fresh rat blood diluted to a haematocrit of 30% with Eagle's minimum essential medium supplemented with 10 mg of glutamine, 2500 units of penicillin G and 2.5 mg of streptomycin sulphate per 50 ml (Ohisalo *et al.*, 1974). After a stabilization period of 15 min, an initial sample was taken from the liver for enzyme assay by ligating and excising the three smallest lobes. After 6 h (Table 4) or 4 h (Table 5), samples for metabolite determination were taken from the liver by the freeze-clamp technique and a piece of fresh liver was removed for enzyme assay.

In the perfusions with ethanol an initial concentration of 80 mM was added to the perfusion medium. Thereafter 0.625 M-ethanol was infused at a rate of 1.6 ml/h in order to compensate for its metabolism and evaporation and to maintain the concentration near its initial value throughout the experiment. The pH of the perfusion medium was monitored continuously and kept constant by infusing NaHCO_3 at a suitable rate. In the first series of perfusions with glycerol (Table 4) the initial concentration was 10 mM and the perfusion was carried out without any further additions of glycerol. In the second series (Table 5) the initial concentration of glycerol was 2 mM. To prevent glycerol-induced depletion of phosphate (Woods & Krebs, 1973), an equimolar amount of sodium phosphate, pH 7.4, was added to the per-

fusion medium simultaneously with the initial dose of glycerol. During the perfusion, 0.5M-glycerol was infused at a rate of 0.81 ml/h. This resulted in an almost constant concentration of glycerol in the perfusion medium throughout. In the experiments with fatty acid (Table 5), the initial concentration of oleic acid in the perfusion medium was 1 mM and thereafter 25 mM-oleic acid in 12.5% (w/v) bovine serum albumin was infused at a rate of 2.7 ml/h in order to compensate for its metabolism and to maintain the concentration near its initial value throughout the experiment.

The viability of the liver was monitored by checking its appearance and the colour difference between the affluent and effluent medium (O_2 consumption) and by measuring bile production, which was 0.8–1.8 ml/6 h. This agrees with previously reported values (Brauer *et al.*, 1951).

Treatment of tissue samples

The liver samples for metabolite determination were pulverized in a mortar under liquid N_2 and extracted with ice-cold aq. 6% (v/v) $HClO_4$. The extraction was repeated and the filtrate neutralized to pH 6.5 with 3.75M- K_2CO_3 containing 0.5M-triethanolamine hydrochloride for the lactate, *sn*-glycerol 3-phosphate and dihydroxyacetone phosphate determinations and with 2M- K_2HPO_4 for the pyruvate determinations. The $HClO_4$ -insoluble fraction of the tissue was washed with 2% (v/v) $HClO_4$, and the precipitate stored at $-70^\circ C$.

The fresh liver sample was homogenized in 4 vol. of 0.225M-sucrose containing 1 mM-EDTA and 50 mM-Tris/HCl, pH 7.5, in a glass homogenizer fitted with a Teflon pestle (all procedures at 2–4°C). The homogenate was then centrifuged at 15000g for 20 min, and the supernatant further centrifuged at 105000g for 60 min. Then 0.23 g of $(NH_4)_2SO_4$ /ml was added to the supernatant. After standing for 15 min and centrifugation at 10000g for 20 min, the pellet surface was washed gently three times with 1 ml of 0.25M-sucrose containing 0.5 mM-dithiothreitol and 20 mM-Tris/HCl, pH 7.4. It was then dissolved in this buffer; $83 \pm 4\%$ of the enzyme activity (mean $\pm$ S.D. for six experiments) was recovered in the pellet in the $(NH_4)_2SO_4$ precipitation. Phosphatidate phosphohydrolase was either assayed immediately or stored at $-20^\circ C$ until assayed.

Assay of phosphatidate phosphohydrolase

The aqueous dispersion of phosphatidic acid used as substrate was obtained by sonication in 0.25M-sucrose, adjusted to pH 9–10 with NaOH, for 1 min at 20 kHz in an MSE Ultrasonic Disintegrator, the pH then being adjusted to 7.0 with HCl. Phospha-

tidylcholine was sonicated in 0.25M-sucrose for 1 min in a separate tube. The incubation medium contained 20 μ mol of Tris/HCl, pH 7.5, 1 μ mol of $MgCl_2$, 0.8 μ mol of phosphatidylcholine, 0.5 μ mol of dithiothreitol, 0.2 μ mol of phosphatidic acid and a suitable amount of the sample [the 105000g supernatant or $(NH_4)_2SO_4$ fraction] in a total volume of 0.5 ml. After a 10 min incubation at $37^\circ C$, 1 ml of 10% (w/v) trichloroacetic acid was added, and after centrifugation the protein-free supernatant was extracted with water-saturated diethyl ether. P_i in the water phase was determined as described by Ames & Dubin (1960). Under the conditions of the assay the enzyme activity was linearly proportional to the amount of tissue extract added, and showed zero-order kinetics for at least 20 min.

The activity of the microsomal phosphatidate phosphohydrolase was determined by the method described by Lamb & Fallon (1974a), which uses an aqueous dispersion of phosphatidate as the substrate.

Determination of metabolite concentrations

The methods employed for the measurement of *sn*-glycerol 3-phosphate (Hohorst, 1970), dihydroxyacetone phosphate (Bücher & Hohorst, 1970), lactate (Gawehn & Bergmeyer, 1970), pyruvate (Czok & Lamprecht, 1970), DNA (Hubbard *et al.*, 1970) and long-chain fatty acyl-CoA esters after extraction and hydrolysis (Williamson & Corkey, 1969), in the form of CoA, by the 2-oxoglutarate dehydrogenase (EC 1.2.4.2) method (Garland, 1964) were as described by the authors cited. Glycerol in the perfusion medium was determined as described by Eggstein & Kuhlmann (1970) and ethanol by g.l.c. on a Porapak Q column (Blackmore, 1969), with propan-2-ol as internal standard.

Results

Phosphatidate phosphohydrolase in vivo

Of the compounds tested in the experiments *in vivo*, glycerol had the greatest effect on phosphatidate phosphohydrolase, increasing its activity in the livers of the starved rats by 2.4-fold in 4 h (Table 1), and dihydroxyacetone and sorbitol gave increases of 32 and 63% respectively. The activity of phosphatidate phosphohydrolase in the livers of the feeding rats given ethanol or glycerol was 85 and 60% higher respectively than that in the livers of the control rats (see Table 3). Thyroxine treatment abolished the effects of ethanol and glycerol on phosphatidate phosphohydrolase (see Table 3), but also increased its activity by 2.5-fold ($P < 0.001$) in the livers of the control rats.

Table 1. Activity of soluble phosphatidate phosphohydrolase and concentrations of metabolites in rat liver 4 h after the injection of glycerol, dihydroxyacetone, sorbitol, glucose or NaCl

The rats were starved for 48 h and then injected intramuscularly with 4 M-glycerol, 4 M-dihydroxyacetone, 2 M-sorbitol, 2 M-glucose or 0.15 M-NaCl (10 ml/kg body wt.). Liver samples were taken 4 h after the injection. The results are means $\pm$ S.E.M. for six to eight animals in each group. *, ** and *** Statistical significance at levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively for the difference between the control (saline) group and the experimental groups.

	NaCl	Glucose	Glycerol	Dihydroxyacetone	Sorbitol
Soluble phosphatidate phosphohydrolase (nmol/s per g wet wt.)	2.62 $\pm$ 0.24	2.15 $\pm$ 0.19	6.20 $\pm$ 0.46***	3.45 $\pm$ 0.16**	4.28 $\pm$ 0.43**
sn-Glycerol 3-phosphate (nmol/g wet wt.)	214 $\pm$ 25	273 $\pm$ 31	2619 $\pm$ 558***	226 $\pm$ 14	423 $\pm$ 40***
Dihydroxyacetone phosphate (nmol/g wet wt.)	20 $\pm$ 2	31 $\pm$ 4***	44 $\pm$ 4***	25 $\pm$ 4*	40 $\pm$ 5***
Glycerophosphate/dihydroxyacetone phosphate	10.0 $\pm$ 0.8	10.1 $\pm$ 1.7	61.9 $\pm$ 12.0***	9.1 $\pm$ 0.8	11.7 $\pm$ 1.1
Lactate (nmol/g wet wt.)	286 $\pm$ 40	368 $\pm$ 42	642 $\pm$ 59***	436 $\pm$ 91	610 $\pm$ 69**
Pyruvate (nmol/g wet wt.)	30 $\pm$ 6	49 $\pm$ 6*	39 $\pm$ 5	47 $\pm$ 6*	91 $\pm$ 12***
Lactate/pyruvate	11.1 $\pm$ 2.0	7.8 $\pm$ 0.8	19.3 $\pm$ 3.0*	8.8 $\pm$ 2.2**	7.5 $\pm$ 1.0**
Long-chain fatty acyl-CoA (nmol/g wet wt.)	59 $\pm$ 3	47 $\pm$ 2**	35 $\pm$ 3**	40 $\pm$ 4	43 $\pm$ 5

Table 2. Activity of soluble phosphatidate phosphohydrolase and concentrations of metabolites in rat liver 8 h after the injection of glycerol, dihydroxyacetone, sorbitol, glucose or NaCl

Experimental conditions were as in Table 1, except that the liver samples were taken 8 h after the injection. The results are means $\pm$ S.E.M. for four to six animals in each group. Symbols are as in Table 1.

	NaCl	Glucose	Glycerol	Dihydroxyacetone	Sorbitol
Soluble phosphatidate phosphohydrolase (nmol/s per g wet wt.)	2.17 $\pm$ 0.14	2.46 $\pm$ 0.20	4.15 $\pm$ 0.31***	2.73 $\pm$ 0.41	2.64 $\pm$ 0.32
sn-Glycerol 3-phosphate (nmol/g wet wt.)	217 $\pm$ 14	311 $\pm$ 9**	295 $\pm$ 43*	272 $\pm$ 24**	374 $\pm$ 22**
Dihydroxyacetone phosphate (nmol/g wet wt.)	19 $\pm$ 2	36 $\pm$ 2***	42 $\pm$ 2***	36 $\pm$ 3***	45 $\pm$ 2***
Glycerophosphate/dihydroxyacetone phosphate	11.9 $\pm$ 1.2	10.1 $\pm$ 1.2	7.1 $\pm$ 1.0**	7.8 $\pm$ 0.8*	8.4 $\pm$ 0.7*

Table 3. Activity of soluble phosphatidate phosphohydrolase and concentrations of metabolites in the livers of hyperthyroid and euthyroid rats 4 h after the administration of ethanol or glycerol

Sodium L-thyroxine was given to one group of rats in subcutaneous injections (1 mg/kg body wt.) for 7 successive days before the experiment, the other group receiving an equal volume of 0.15 M-NaCl adjusted to pH 10 with NaOH. No food deprivation preceded the experiments. A single dose of ethanol (65 mmol/kg), glycerol (40 mmol/kg) or water (25 ml/kg) was given perorally to the rats, and liver samples were taken 4 h later. The results are means $\pm$ S.E.M. from five animals in each group. ††† Statistical significance at a level of $P < 0.001$ for the difference between the normal control rats and thyroxine-treated rats. Other symbols are as in Table 1.

	Control	Ethanol	Glycerol	Thyroxine	Thyroxine + ethanol	Thyroxine + glycerol
Soluble phosphatidate phosphohydrolase (nmol/s per g wet wt.)	2.15 $\pm$ 0.20	3.98 $\pm$ 0.31***	3.44 $\pm$ 0.51*	5.34 $\pm$ 0.14†††	5.20 $\pm$ 0.28	5.84 $\pm$ 0.20
sn-Glycerol 3-phosphate (nmol/g wet wt.)	224 $\pm$ 51	467 $\pm$ 58**	663 $\pm$ 242	136 $\pm$ 25	155 $\pm$ 17	118 $\pm$ 5
Dihydroxyacetone phosphate (nmol/g wet wt.)	39 $\pm$ 4	21 $\pm$ 1**	38 $\pm$ 6	42 $\pm$ 5	35 $\pm$ 2	32 $\pm$ 1
Glycerophosphate/dihydroxyacetone phosphate	5.5 $\pm$ 0.6	23.3 $\pm$ 4.3**	21.6 $\pm$ 7.5*	3.3 $\pm$ 0.5	4.4 $\pm$ 0.5	3.6 $\pm$ 0.1
Lactate (nmol/g wet wt.)	857 $\pm$ 153	802 $\pm$ 67	1097 $\pm$ 281	1160 $\pm$ 96	1003 $\pm$ 140	919 $\pm$ 97
Pyruvate (nmol/g wet wt.)	109 $\pm$ 19	35 $\pm$ 5*	131 $\pm$ 37	167 $\pm$ 15	136 $\pm$ 18	127 $\pm$ 12
Lactate/pyruvate	7.9 $\pm$ 0.4	23.7 $\pm$ 3.8**	9.1 $\pm$ 1.9	7.0 $\pm$ 0.3	7.6 $\pm$ 0.6	7.4 $\pm$ 0.8
Triacylglycerol (μ mol/g wet wt.)	4.7 $\pm$ 0.6	5.6 $\pm$ 0.6	5.7 $\pm$ 0.5	4.9 $\pm$ 0.1	5.1 $\pm$ 0.4	5.1 $\pm$ 0.1

Hepatic metabolite concentrations in vivo

The concentrations of the metabolites tested were almost equal after the injection of NaCl or glucose, so that these may be regarded as basal values. Glycerol had a striking effect on the hepatic concentration of *sn*-glycerol 3-phosphate, leading to a more than 12-fold increase in 4 h (Table 1). By 8 h after injection, the *sn*-glycerol 3-phosphate concentration was already on the decrease, being only about twice the basal value (Table 2). Sorbitol also raised the *sn*-glycerol 3-phosphate concentration, by a factor of 2 in 4 h, but the subsequent decline was somewhat slower (Tables 1 and 2).

Of the compounds tested, ethanol and glycerol increased the molar concentration ratios of lactate/pyruvate and of *sn*-glycerol 3-phosphate/dihydroxyacetone phosphate, which describe the redox state of the cytosolic NAD couple (Tables 1 and 3). These changes were absent in the case of the hyperthyroid rats (Table 3). The differential response of the *sn*-glycerol 3-phosphate/dihydroxyacetone phosphate and lactate/pyruvate ratios in the glycerol-treated rats (Tables 1 and 3) may be explained by a disequilibrium of the *sn*-glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) reaction during high rates of glycerol phosphorylation alone or together with an enhanced mitochondrial NADH oxidation during the ATP consumption of the glycerol kinase (EC 2.7.1.30) reaction. This discrepancy is also noted by Woods & Krebs (1973).

Experiments with isolated perfused livers

The changes in the activity of phosphatidate phosphohydrolase *in vivo* were rapid enough to warrant experiments with perfused livers. The intention was to test whether the effects observed *in vivo* with ethanol (Savolainen, 1977) or other compounds (this

work) were due to a direct effect upon the liver, or were indirect, mediated by hormonal mechanisms or other tissues, e.g. by changes in the supply of substrate to the liver. Thus only ethanol and glycerol were tested in perfused livers. The results are presented in Tables 4 and 5.

Ethanol had no statistically significant effect on phosphatidate phosphohydrolase activity as compared with the control perfusions. The activity during perfusion of the livers treated with a medium containing glycerol was twice that in the controls, but there was large experimental variation, and the change was not statistically significant. Ethanol caused a 92% increase in the hepatic concentration of *sn*-glycerol 3-phosphate and an 8-fold increase in the ratio of *sn*-glycerol 3-phosphate/dihydroxyacetone phosphate. On the other hand, the increase in the *sn*-glycerol 3-phosphate concentration caused by the glycerol was only 20% (Table 4). The limited effects of glycerol on metabolite concentrations at the termination of the 6 h perfusion were probably due to its mode of administration. The reason for the increase in the activity of the soluble phosphatidate phosphohydrolase in relation to the initial values in all the experiments cannot be ascertained on the basis of the present results. The addition of oleic acid to the perfusion medium, in the presence or absence of glycerol, did not affect the enzyme activity (Table 5). The activity of the microsomal phosphatidate phosphohydrolase increased as compared with the initial values in the livers perfused with oleic acid and glycerol ($P < 0.01$), but did not change during the other perfusions (Table 5). This indicates that the observed increase in soluble phosphatidate phosphohydrolase activity was not due to detachment of the enzyme from the membranes of the endoplasmic reticulum. However, when considering these changes it must be borne in mind that the method used probably overestimates the activity of the micro-

Table 4. *Effect of ethanol and glycerol on the activity of soluble phosphatidate phosphohydrolase and concentrations of metabolites in isolated rat livers perfused for 6 h*

Experimental conditions were as described in the Materials and Methods section. Liver samples for the measurement of DNA content and the activity of phosphatidate phosphohydrolase were taken at the beginning (0h) and end (6h) of the perfusions. Samples for the determination of metabolite concentrations were taken only at the end of the perfusions. Symbols are as in Table 1.

	Control	Ethanol	Glycerol
Soluble phosphatidate phosphohydrolase (nmol/s per mg of DNA)			
at 0h	0.78 ± 0.11	0.92 ± 0.05	0.94 ± 0.14
at 6h	1.07 ± 0.13	1.23 ± 0.09	1.94 ± 0.44
<i>sn</i> -Glycerol 3-phosphate (nmol/mg of DNA)	231 ± 43	592 ± 31**	278 ± 40
Dihydroxyacetone phosphate (nmol/mg of DNA)	31 ± 1	10 ± 2***	34 ± 2
Glycerophosphate/dihydroxyacetone phosphate	7.6 ± 1.7	64.1 ± 8.6***	8.1 ± 0.8
Lactate (nmol/mg of DNA)	1022 ± 65	1147 ± 354	1103 ± 81
Pyruvate (nmol/mg of DNA)	131 ± 10	12 ± 4***	206 ± 66
Lactate/pyruvate	7.7 ± 0.6	108 ± 40**	6.7 ± 1.4
Number of experiments	4	3	4

Table 5. *Effect of glycerol on the activity of soluble phosphatidate phosphohydrolase and concentrations of metabolites in isolated rat livers perfused with or without fatty acid for 4 h*

Experimental conditions were as described in the Materials and Methods section. Liver samples for the measurement of the concentration of DNA and triacylglycerol and the activity of soluble and microsomal phosphatidate phosphohydrolase were taken at the beginning (0h) and end (4h) of the perfusions. Samples for the determination of metabolite concentrations were taken only at the end of the perfusions. * and ** Statistical significance at levels of $P < 0.05$ and $P < 0.01$ respectively for the difference between the control perfusions and the perfusions with glycerol. † and †† Statistical significance at levels of $P < 0.05$ and $P < 0.001$ respectively for the difference between the control perfusions with fatty acid and the perfusions with fatty acid and glycerol. ‡ Statistical significance at a level of $P < 0.01$ for the difference between the samples taken at 0 and 4 h.

	Control	Glycerol	Control+oleate	Glycerol+oleate
Soluble phosphatidate phosphohydrolase (nmol/s per mg of DNA)				
at 0h	1.09 ± 0.08	0.90 ± 0.06	0.94 ± 0.10	1.18 ± 0.21
at 4h	1.45 ± 0.14	1.43 ± 0.31	1.15 ± 0.28	1.47 ± 0.08
Microsomal phosphatidate phosphohydrolase (nmol/s per g of microsomal protein)				
at 0h	99 ± 6	110 ± 8	111 ± 9	119 ± 4
at 4h	97 ± 5	121 ± 9	118 ± 14	147 ± 1‡
Hepatic triacylglycerol (μmol/mg of DNA)				
at 0h	3.38 ± 0.13	2.88 ± 0.14	3.25 ± 0.45	2.78 ± 0.23
at 4h	2.77 ± 0.14	2.75 ± 0.15	3.02 ± 0.51	3.37 ± 0.20
sn-Glycerol 3-phosphate (nmol/mg of DNA)	116 ± 33	366 ± 45**	88 ± 15	205 ± 28†
Dihydroxyacetone phosphate (nmol/mg of DNA)	32 ± 4	33 ± 3	25 ± 3	24 ± 3
Glycerophosphate/dihydroxyacetone phosphate	3.4 ± 0.6	11.3 ± 1.9*	3.5 ± 0.2	8.4 ± 0.3††
Lactate (nmol/mg of DNA)	705 ± 136	865 ± 185	485 ± 147	660 ± 135
Pyruvate (nmol/mg of DNA)	115 ± 6	63 ± 22	71 ± 24	69 ± 19
Lactate/pyruvate	6.3 ± 1.5	28.1 ± 20.1	7.1 ± 0.5	10.0 ± 0.8†
Number of experiments	3	3	3	3

somal phosphatidate phosphohydrolase by 20–30% because of phospholipase A-type activities and sn-glycerol 3-phosphate-dephosphorylating enzymes present (Sturton & Brindley, 1978).

The changes in the hepatic triacylglycerol concentration were similar to but smaller than those observed by Fellenius *et al.* (1973). There was a statistically significant ($P < 0.01$) difference between the changes in triacylglycerol concentration in the livers perfused with oleic acid and glycerol (an increase of 0.59 ± 0.25 μmol/mg of DNA during the 4 h perfusion) and those in the control perfusions (a decrease of 0.60 ± 0.24 μmol/mg of DNA).

Discussion

The previously reported increase in hepatic phosphatidate phosphohydrolase activity after ethanol loading follows a time course suggestive of a close causal relationship between the activity of this enzyme and the accumulation of triacylglycerol (Savolainen, 1977). On the other hand, it has been repeatedly shown that a correlation exists between hepatic sn-glycerol 3-phosphate concentration and triacylglycerol accumulation both *in vivo* (Nikkilä & Ojala, 1963; Ylikahri, 1970) and in isolated livers perfused with fatty acid (Fellenius *et al.*, 1973).

In the present case, a positive correlation was found between phosphatidate phosphohydrolase activity and sn-glycerol 3-phosphate concentration in the liver of normal rats given various oxidizable substrates, and the best fit was obtained by a semi-logarithmic plot of the experimental data (Fig. 1). This correlation cannot, however, be used as an argument for a causal relationship between the variables.

It is significant that, although thyroxine treatment increases the activity of phosphatidate phosphohydrolase, this is not accompanied by an increase in the hepatic sn-glycerol 3-phosphate concentration (Table 3). The data could be interpreted as indicating that thyroxine determines the basal activity of phosphatidate phosphohydrolase by an unknown mechanism, and a metabolite-mediated control is superimposed upon the hormonal control. The experiments with ethanol and glycerol in thyroxine-treated animals (Table 3) also support this interpretation.

The increase in hepatic phosphatidate phosphohydrolase activity found after subtotal hepatectomy or sham operation (Mangiapanne *et al.*, 1973), ethanol administration (Savolainen, 1977) or hypoxia (Kinula *et al.*, 1978) could be similarly attributed to an increase in the hepatic sn-glycerol 3-phosphate concentration. The sn-glycerol 3-phosphate concentra-

Fig. 1. Correlation between *sn*-glycerol 3-phosphate concentration and phosphatidate phosphohydrolase activity in rat liver *in vivo* after the administration of various oxidizable substrates

Experimental conditions were as in Table 1. ●, Glycerol; ▲, dihydroxyacetone; ■, glucose; ○, sorbitol; △, saline. The line was fitted by least-squares regression analysis of the values for enzyme activity and *sn*-glycerol 3-phosphate concentration. For the semi-logarithmic plot, $r = 0.80$; $P < 0.001$.

tion in regenerating liver has not been measured, but in the sham-operated animals a hypoxic period during the surgical operation could also explain the increase in phosphatidate phosphohydrolase activity.

The involvement of certain ethanol-induced changes in metabolite concentrations in the regulation of hepatic phosphatidate phosphohydrolase activity is supported by the finding that thyroxine treatment abolished the effects of ethanol and glycerol on this enzyme (Table 3). Regulation by metabolites is also suggested by the results of Wood & Lamb (1978), who found that ethanol increases phosphatidate phosphohydrolase activity in hepatocytes in monolayer cultures, i.e. in the absence of hormones. The effect of ethanol is probably indirect and attributable to the metabolic changes caused by ethanol oxidation in the hepatocytes, since it could be prevented by the addition of pyrazole to the culture medium.

Other regulators, e.g. hormones, cannot be excluded on the basis of the available literature and the present results, however. Partial hepatectomy, sham operation, ethanol loading and hypoxia are associated with stress, and the contribution of those hormones known to be released in a stress situation (corticosteroids, catecholamines) remains unclear.

The experiments on isolated perfused livers similarly indicate that metabolites are not the only

regulators of hepatic phosphatidate phosphohydrolase. Although ethanol and glycerol tended to increase the phosphatidate phosphohydrolase activity, the increase was smaller than *in vivo*, and the interpretation of the results is hampered by the increase in the enzyme activity even in livers perfused without the addition of substrates. It has been suggested that plasma non-esterified fatty acids may regulate hepatic phosphatidate phosphohydrolase activity (Mangiapanne *et al.*, 1973), but the addition of fatty acid to the perfusate in this instance had no effect on the enzyme.

Hepatic phosphatidate phosphohydrolase activity also increases after starvation (Vavrečka *et al.*, 1969; Kinnula *et al.*, 1978) and after a carbohydrate-rich diet (Lamb & Fallon, 1974b). Since the hepatic *sn*-glycerol 3-phosphate concentration does not fluctuate markedly under various nutritional conditions (Guynn *et al.*, 1972), the changes in the enzyme activity cannot be related to the change in *sn*-glycerol 3-phosphate concentration, but rather to hormonal factors, although at present no specific hormone can be identified as being responsible for this.

There are reports suggesting hormonal regulation of phosphatidate phosphohydrolase activity in the lung (Brehier *et al.*, 1977) and in adipose tissue (Cheng & Saggerson, 1978), but none concerning its regulation in the liver. The present results (Table 3) serve to confirm that hormones also regulate the activity of the phosphatidate phosphohydrolase in the cytoplasmic fraction of the liver. Whether it is a case of the activation of the enzyme or of an increase in the amount of enzyme protein cannot be determined from the present results.

The increased activity of hepatic phosphatidate phosphohydrolase in the hyperthyroid rats (Table 3) is in good agreement with the results of Roncari & Murthy (1975), which show a significant enhancement of diacylglycerol and triacylglycerol synthesis in liver homogenates from thyroxine-treated rats. The increased hepatic triacylglycerol synthesis in the hyperthyroid state is probably related to the enhanced synthesis of very-low-density lipoproteins, which occurs in the presence of a thyroid hormone excess (Nikkilä & Kekki, 1972), and this is probably the reason why no triacylglycerol accumulates in the livers of hyperthyroid rats (Table 3). Lipoprotein synthesis is also affected, of course, by the nutritional status and food intake of the animal, which are in turn influenced by thyroxine administration (Keyes & Heimberg, 1978).

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