HORMONAL REGULATION OF HEPATIC SOLUBLE PHOSPHATIDATE PHOSPHOHYDROLASE

Induction by cortisol in vivo and in isolated perfused rat liver

Mervi A. LEHTONEN, Markku J. SAVOLAINEN and Ilmo E. HASSINEN
Department of Medical Biochemistry, University of Oulu, Kajaanintie 52 A, SF-90220 Oulo 22, Finland

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1. Introduction

The development of acute ethanol-induced fatty liver is associated with a marked increase in the activity of the cytoplasmic phosphatidate phosphohydrolase (EC 3.1.3.4) in the liver [1,2], but the mechanism by which ethanol administration stimulates the activity of this enzyme is not known.

It is well established that an intact pituitary gland and adrenal cortex are necessary for the production of acute ethanol-induced fatty liver [3–5], but the hormones involved in this process have not been identified, although ethanol is known to influence the plasma concentrations of several hormones [6–9]. The present study investigates the effects on hepatic soluble phosphatidate phosphohydrolase of selected hormones which could be anticipated to be involved in the development of ethanol-induced fatty liver. The activity of phosphatidate phosphohydrolase in rat liver increased significantly after in vivo administration of somatotropin (growth hormone), corticotropin-(1–24)-tetracosapeptide and cortisol. A 3-fold increase in the enzyme activity in perfused liver indicated that cortisol acts directly on the liver cells, probably by increasing the synthesis of enzyme protein.

2. Materials and methods

2.1. *Hormones and reagents*

Corticotropin-(1–24)-tetracosapeptide (Synacthen, a synthetic polypeptide possessing adrenocorticotropic activity) was purchased from Ciba-Geigy (Basel), insulin (Actrapid) from Novo Industri (Copenhagen), and other hormones from Sigma Chemical Co. (St Louis, MO). L-Thyroxine was dissolved in 0.1 M NaOH and adjusted to pH 10 with HCl. Glucagon (1 mg/ml) was dissolved in 10 mM HCl and diluted to 0.5 mg/ml with 0.25 M sodium phosphate (pH 7.4) just before injection. Somatotropin (growth hormone) was dissolved in 0.04 M acetic acid and other hormones in 0.15 M NaCl.

Phosphatidic acid (disodium salt, prepared from egg phosphatidylcholine) was obtained from Koch-Light Labs (Colnbrook, Bucks), and Eagle's modified minimum essential medium (Auto-Pow 11-110 with Earle's salts) from Flow Labs (Irvine).

2.2. *In vivo experiments*

Fed male Sprague-Dawley rats (240–400 g) were used. One group of rats was injected with each of the hormones shown in table 1. The control rats received an equal amount of the appropriate vehicle (1 ml/kg body wt). After 4 h the rats were anaesthetized with pentobarbital (60 mg/kg body wt) and a piece of liver was taken, rinsed with saline, blotted and prepared for the determination of soluble phosphatidate phosphohydrolase activity as in [1].

2.3. *Perfusion experiments*

Each rat was anaesthetized with pentobarbital and the portal vein and the inferior vena cava cannulated. Perfusion was started immediately with Krebs-Ringer

Address correspondence to: Dr M. Savolainen

bicarbonate solution in equilibrium with O_2/CO_2 (19:1). The liver was removed and perfusion continued at 35°C in a recirculating system incorporating a membrane oxygenator [10] constructed from 5 m Dow Corning silastic tubing (type 602–235), 1.47 mm i.d. and 0.24 mm wall thickness. The perfusate flow was 3 ml . min^{-1} . g $liver^{-1}$, and the oxygenator was able to maintain the arterial oxygen at 0.81 mM as measured with an oxygen electrode during the perfusion. The oxygen consumption was 2.06 μmol . min^{-1} . g $liver^{-1}$, which is in accordance with data on perfusions with hemoglobin-free [11] and erythrocyte-containing [12] media. Eagle's minimum essential medium (70 ml) was used as the perfusion fluid, supplemented with 1.8 mmol $NaHCO_3$, 0.14 mmol glutamine, 3500 units penicillin G and 3.5 mg streptomycin sulphate. Five mg cortisol-21-succinate (sodium salt), 70 μg actinomycin D or 70 mU insulin were added where indicated. In the perfusions with insulin, further insulin was infused into the perfusion medium at a rate of 0.5 mU . min^{-1}.

2.4. *Assay of phosphatidate phosphohydrolase activity*

The activity of the cytoplasmic phosphatidate phosphohydrolase was determined using an aqueous dispersion of phosphatidate as the substrate [1], except that 0.2 mM EGTA [ethyleneglycol-bis-(β-aminoethyl ether)*N,N'*-tetracetic acid] was included in the incubation medium [13]. Protein was determined by the biuret method [14].

3. Results and discussion

A single intraperitoneal injection of a saline pituitary homogenate equivalent to a whole rat pituitary gland [3] increased the activity of the hepatic phosphatidate phosphohydrolase in preliminary experiments by more than 2-fold (data not shown). Since this injection is also capable of causing fat accumulation in the liver [3], the effects of those pituitary hormones, the secretion of which is influenced by the administration of ethanol were investigated in vivo (table 1). Somatotropin and corticotropin increased the activity of hepatic phosphatidate phosphohydrolase by 66% and 43%, respectively, whereas vasopressin was without effect. A dose of 10 U vasopressin/kg body wt was needed to cause a significant increase in the phosphatidate phosphohydrolase, but the physical signs observed in the rat indicated that this was probably secondary to some toxic effect.

Since the effect of corticotropin could be expected to be due to the release of adrenal corticosteroids, the effects of cortisol were also ascertained. Cortisol

Table 1
Activity of soluble phosphatidate phosphohydrolase in rat liver 4 h after an intraperitoneal injection of hormone

Hormone	Dose (kg body wt^{-1})	Soluble phosphatidate phosphohydrolase activity (nmol. s^{-1} . g wet wt^{-1})		Change
		Hormone-treated rats	Control rats	
Somatotropin	2 U	4.52 ± 0.27 (6)	2.73 ± 0.31 (6)	66%[b]
Corticotropin[a]	25 U	4.69 ± 0.40 (6)	3.53 ± 0.32 (6)	43%[b]
Vasopressin	2.5 U	3.78 ± 0.33 (6)	3.28 ± 0.35 (6)	n.s.
Cortisol	5 mg	5.56 ± 0.30 (7)	2.95 ± 0.41 (7)	88%[c]
Thyroxine	5 mg	3.42 ± 0.52 (4)	3.59 ± 0.45 (4)	n.s.
Glucagon	0.5 mg	4.26 ± 0.63 (5)	3.28 ± 0.22 (5)	n.s.

[a] Synthetic corticotropin-(1–24)-tetracosapeptide
[b] $P < 0.01$ and
[c] $P < 0.001$ between hormone-treated and control rats

Hormones were injected intraperitoneally (glucagon subcutaneously) into fed rats and liver samples taken 4 h later. Results are means ± SEM from 4–7 experiments in each group (no. expts in parenthesis). n.s., not statistically significant

Table 2
Effects of hormones on the activity of soluble phosphatidate phosphohydrolase in isolated perfused rat liver

	Perfusions	Soluble phosphatidate phosphohydrolase activity (nmol. s^{-1} . g $protein^{-1}$)		
		At 0 h	At 4 h	Change
Control	5	5.65 ± 0.59	6.31 ± 1.04	n.s.
Cortisol	5	5.42 ± 0.39	14.85 ± 1.65	274%[a]
Actinomycin D	4	6.40 ± 0.97	4.94 ± 0.41	n.s.
Actinomycin D + cortisol	4	5.12 ± 0.44	4.74 ± 0.56	n.s.
Insulin	5	5.92 ± 0.93	7.18 ± 0.80	n.s.

[a] $P < 0.001$ between the values at 0 h and 4 h

Livers were perfused with hemoglobin-free perfusion medium (Eagle's minimum essential medium) for 4 h, samples being taken at the beginning and end. Results are means ± SEM from 4–5 perfusions in each group. n.s., not statistically significant

administration in vivo (5 mg/kg body wt) increased the activity of the soluble phosphatidate phosphohydrolase by 88% ($P < 0.01$) (table 1).

The effect of cortisol was also tested in perfused liver (table 2), the intention being to verify whether the in vivo results had been due to a direct effect upon the liver. Cortisol increased the activity of hepatic phosphatidate phosphohydrolase by 274% ($P < 0.001$), but insulin had no statistically significant effect as compared with the control perfusions. Addition of actinomycin D to the perfusion medium prevented the cortisol-induced increase in phosphatidate phosphohydrolase activity. These results suggest that the increased activity was due to an enhanced synthesis of enzyme protein.

Only recently, other evidence has been obtained on the hormonal regulation of phosphatidate phosphohydrolase. It was demonstrated [15] that noradrenaline rapidly reduces the activity of the Mg^{2+}-dependent phosphatidate phosphohydrolase in adipocytes and insulin rapidly restores this activity [16]. The results presented in table 2, however, demonstrate that the hormonal regulation of triacylglycerol synthesis may differ in principle in the liver, as it is not affected by insulin. In addition to this tissue-specific difference in the hormone-mediated regulation, the enzyme also shows developmental changes in its regulatory properties, viz. under conditions in which foetal lung phosphatidate phosphohydrolase activity is increased by maternal corticosteroid administration, the hepatic activity of this enzyme decreases [17].

Thyroxine does not change the hepatic phosphatidate phosphohydrolase within 4 h after administration (table 1), although a 2.5-fold increase is observed when the injections are continued for 1 week [18]. This indicates that the effects of thyroxine on phosphatidate phosphohydrolase follow the pattern set by other effects of the thyroid hormones on metabolism, which usually take several days [19]. This effect is interesting, in that it does not result in triacylglycerol accumulation [18], although hepatic neutral glycerolipid synthesis does increase in vitro [20].

It has been demonstrated that dibutyryl cyclic AMP stimulates the activity of the microsomal phosphatidate phosphohydrolase [21]. In the present study glucagon did not affect the activity of the soluble phosphatidate phosphohydrolase in vivo, although it is known to increase the hepatic concentration of cyclic AMP.

Increased hepatic phosphatidate phosphohydrolase activity has been observed after sub-total hepatectomy [22], laparotomy [22], fasting [23,24], a carbohydrate-rich diet [25], induction of diabetes by streptozotocin [26] administration of ethanol [1,2,27,28], glycerol [18,27], sorbitol [18,27], fructose [27] or dihydroxyacetone [18]. Partial hepatectomy [29], laparotomy [29] and ethanol loading [7] are reported to result in

increased plasma corticosteroid concentrations, and the increase in the phosphatidate phosphohydrolase activity after the partial hepatectomy can be blocked by actinomycin D [22].

Glucocorticoids stimulate the synthesis of triacylglycerol in liver [30,31], but the mechanism is not known. Phosphatidate phosphohydrolase appears to have an important role in the regulation of neutral glycerolipid synthesis in liver [32,33]. It responds rapidly and dramatically to changes in the physiological and pharmacological status of animals [1,2,18,22–28] and parallels the capacity of the liver to synthesize triacylglycerols [1,34]. The present results demonstrate that cortisol has a direct increasing effect on the activity of hepatic phosphatidate phosphohydrolase, a phenomenon also observable in an isolated perfused liver. The results thus lend support to the hypothesis that under most of the above-mentioned conditions adrenocortical steroids may be the mediators of the effects on phosphatidate phosphohydrolase. This may have some bearing on the enhanced synthesis of hepatic triacylglycerols in certain stress states (e.g., ethanol loading, partial hepatectomy, laparotomy) and presumably also in obesity, at least in genetically obese mice, in which there is hyperfunction of the adrenal cortex [35] and increased activity of phosphatidate phosphohydrolase [32].

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