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Hormonal control of fructose 2,6-bisphosphate concentration and of phosphofructokinase 2 in the rat liver during development

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In fetal rat liver the concentration of fructose 2,6-bisphosphate is decreased by administration of glucagon. The glucagon effect, i.e., the phosphorylation state of phosphofructokinase 2, dominates over the substrate supply. Insulin was found to increase fructose 2,6-bisphosphate only when exogenous glucose is supplied simultaneously. The total activity of phosphofructokinase 2 exhibits remarkable developmental changes. It is high at term, moderate in the fetal as well as in the mature organ, and low during suckling. The level of the enzyme during development is controlled by pancreatic and adrenal hormones.

INTRODUCTION

Fructose 2,6-bisphosphate (Fru 2,6-P_2) is of great significance to the coordination of hepatic glycolysis and gluconeogenesis. It is formed and hydrolyzed by the bifunctional enzyme 6-phosphofructo 2-kinase/fructose 2,6-bisphosphatase (PFK 2, ATP: D-fructose 6-phosphate 2-phosphotransferase, EC 2.7.7.–) (1–3). The activity of this enzyme is controlled by cAMP-dependent phosphorylation (4–7). Physiological concentrations of glucagon and other hormones giving rise to an increase of cAMP cause a decrease in the hepatic Fru 2,6-P_2 level. Insulin exhibits the opposite effect (8).

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In addition to the hormonal environment, the Fru 2,6-P_2 concentration is also influenced by the availability of its precursors glucose 6-phosphate and fructose 6-phosphate (9). Glucose was found to increase Fru 2,6-P_2 in hepatocytes from starved rats, but is without effect in parenchymal liver cells from diabetic animals (8).

In a previous communication, a polyphasic behaviour of the Fru 2,6-P_2 concentration in the developing rat liver was described (10). The present paper deals with the influences of glucagon, insulin and glucose on the Fru 2,6-P_2 concentration in fetal liver. In addition, evidence is presented that hepatic PFK 2 is an adaptive enzyme, the concentration of which was found to be controlled by pancreatic and adrenal hormones.

MATERIALS AND METHODS

Chemicals

The substrates and auxiliary enzymes were obtained from Boehringer GmbH, Mannheim (FRG). NADH was purchased from VEB Arzneimittelwerk, Dresden (GDR). Glucagon was from Novo Industrie GmbH, Mainz (FRG), dexamethasone from KRKA, Novo Mesto (Yugoslavia) and phentolamine from Ciba-Geigy AG, Basel (Switzerland). All other chemicals were of the highest purity commercially available.

Animals

Rats of a Wistar albino strain bred from this institute were used. In the prenatal experiments, the dams were laparotomized under ether anaesthesia and the fetuses intraperitoneally injected through the uterine wall with the respective hormone. The fetuses in the opposite uterus horn serving as controls received the same volume of 0.9 % (w/v) saline. The maternal abdomen was then closed. At the times indicated the fetuses were removed from the uterus by Caesarean section and decapitated. The livers were rapidly excised, freeze-clamped and immediately homogenized as described in (11).

Postnatal rats received intraperitoneal injections of either 0.1 mg dexamethasone, 0.5 mg glucagon, 10 units insulin, 5.0 mg testosterone, 5.0 mg progesterone or 5.0 mg estradiol, respectively (referred to 100 g of body weight). Phentolamine (5.0 mg per 100 g of body weight) was injected 15 minutes prior, and 4 hours after, the administration of glucagon. The animals were killed 24 hours after by decapitation. The livers were quickly removed and stored at $-20°C$ until the assay of total PFK 2 activity. Bilateral adrenalectomy was performed via the dorsolateral route five days before the experiment. Unless otherwise stated, mineralocorticoids were substituted by daily application of 15 μg deoxycorticosterone (Ciba-Geiby) per 100 g of body weight.

Assays

Fru 2,6-P_2 was estimated according to (11). For the measurement of PFK 2 the method described in (12) was modified as follows: The livers were homogenized in

three volumes of 10mM HEPES-buffer pH 7.4, 100mM KCl, 0.2mM EDTA and 2mM dithioerythritol. The homogenates were centrifuged for 40 minutes at 20,000 *g* (4°C) and the supernatants desalted on Sephadex G-25. The desalted samples were incubated at 37°C in a mixture of 20mM TRIS-HCl buffer pH 7.5, 0.2mM EDTA, 2mM dithioerythritol, 3mM inorganic phosphate, 100mM KCl, 10mM $MgCl_2$, 1mM fructose 6-phosphate, 3.5mM glucose 6-phosphate and 10mM ATP. Aliquots were withdrawn in intervals up to 20 minutes and assayed for Fru 2,6-P_2. In this assay the estimated PFK 2 activity is nearly independent of the phosphorylation state of the enzyme and, therefore, a measure of the enzyme amount (12). The PFK 2 activity is expressed as nmoles of Fru 2,6-P_2 formed per gram of liver wet weight per minute at 37°C under the described conditions.

RESULTS AND DISCUSSION

In the fetal liver exogenous glucagon injection was found to lower the Fru 2,6-P_2 concentrations increased under these conditions (Table 1). An increase of the hexose monophosphates, which in the fetal liver probably results from the low glucose 6-phosphatase activity, is known to augment the Fru 2,6-P_2 concentration in the liver of the adult animal (9). The fall of the Fru 2,6-P_2 concentration after intrauterine glucagon administration to the fetuses shows that the phosphorylation state of the PFK 2 is apparently more important for the regulation of the Fru 2,6-P_2 concentration than the availability of the precursors. Glucose is capable of diminishing the glucagon effect (Fig. 1), probably by enhancement of insulin secretion.

Simultaneous application of insulin and glucose leads to an up to tenfold rise of Fru 2,6-P_2 concentration, while insulin alone decreases the Fru 2,6-P_2 concentration (Fig. 1). We suggest that insulin channels glucose 6-phosphate mainly to glycogen, hence, when the hormone is injected without simultaneous glucose administration, the

Table 1. Influence of insulin and glucagon on the concentrations of fructose 2,6-bisphosphate, glucose 6-phosphate and fructose 6-phosphate in the fetal rat liver at the 21st day of gestation

	Treatment		
Metabolite	Control	Glucagon	Insulin
Fructose 2,6-bisphosphate [nmoles per g of wet weight]	0.56 ± 0.17	0.01 ± 0.01*	0.32 ± 0.13**
Glucose 6-phoshate [μmoles per g of wet weight]	1.25 ± 0.28	1.90 ± 0.21*	0.85 ± 0.27**
Fructose 6-phosphate [μmoles per g of weight]	0.24 ± 0.04	0.38 ± 0.09*	0.16 ± 0.05**

The hormones (0.32 units of insulin or 50 μg of glucagon per fetus) were administered as described in "Materials and Methods". The animals were killed 60 minutes later and the livers of similarly treated littermates pooled. Results are given as mean of 4–5 experiments ± S.E.M. Asterisks indicate the statistical comparison between each group of hormone-treated animals and the control group: *$p < 0.05$, **$p < 0.10$.

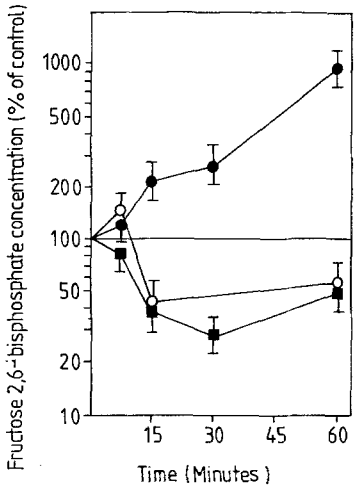

Fig. 1 Time dependence of the glucagon and insulin effects on the hepatic fructose 2,6-bisphosphate concentration in fetal rats at the 21st day of gestation. Results are given as per cent of the control which was found to contain 0.56 ± 0.17 nmoles fructose 2,6-bisphosphate per g wet weight. Each value represents the mean of 6–8 animals. The bars indicate the S.E.M. Conditions: ●, 2 units of insulin + 4.32 mg glucose, ○, 2 units of insulin, ■, 0.05 mg of glucagon + 4.32 mg glucose. All dosages refer to the whole animal.

hexose monophosphates are preferentially converted to glycogen and consequently the Fru 2,6-P_2 concentration is lowered. When insulin is administered together with exogenous glucose the Fru 2,6-P_2 synthesis is elevated. This interpretation is in line with the observation that insulin alone gives rise to a temporary increase of the Fru 2,6-P_2 concentration. In this transient phase the hepatic hexose monophosphate concentration is high enough to meet the requirements for both the synthesis of glycogen and of Fru 2,6-P_2. The late insulin effect (decrease of Fru 2,6-P_2) reflects the deficiency of the substrate rather than an inactivation of PFK 2.

These results show that the regulation of the Fru 2,6-P_2 concentration in the fetal liver occurs principally by the same mechanisms as in the adult organ. The developmental changes of the Fru 2,6-P_2 concentration can qualitatively be interpreted in terms of the cAMP-dependent reversible phosphorylation of PFK 2. In fetal liver the raised Fru 2,6-P_2 concentration reflects the high insulin/glucagon ratio. The diminution of this ratio at term leads to a steep decrease of Fru 2,6-P_2, which stays at this very low level as long as the glucagon concentration is high (throughout the suckling period). Around weaning, when the circulating glucagon concentration is decreasing, the hepatic Fru 2,6-P_2 concentration increases again (10).

In the adult liver, Fru 2,6-P_2 is many fold higher than in the fetal organ, although the insulin concentration in the adult animal is known to be lower than in the fetus. This raises the question as to whether the amount of PFK 2 is also hormonally controlled.

The amount of PFK 2, as measured under conditions where the maximum activity represents the enzyme concentration, shows remarkable changes in the course of growth and development (Table 2). It is maximum after birth and decreases in the suckling period. After weaning, an increase is again observed. The enzyme activity found in the liver of the adult animal is attained at around the 25th day of life. In the fetal liver a similar specific activity is found as in the adult.

Since, coincidentally, with these developmental alterations, the concentrations of the circulating glucocorticoid and pancreatic hormones are also changing remarkably, the influences of these hormones on the hepatic PFK 2 concentration were studied.

Table 2. Developmental changes of phosphofructokinase 2 in rat liver. Results are expressed as mean ± S.E.M. The number of animals are given in parentheses

Age	PFK 2 activity
Prenatal (21st day of gestation)	5.19 ± 0.78 (6)
Term	12.82 ± 0.86 (4)
Postnatal (days after birth)	
1	11.16 ± 0.44 (4)
4	3.67 ± 0.13 (4)
15	1.54 ± 0.10 (10)
16	2.33 ± 0.75 (7)
18	3.37 ± 1.27 (3)
25	5.84 ± 0.75 (3)
Adult (female)	5.18 ± 0.67 (5)

Bilateral adrenalectomy decreases the activity of the hepatic PFK 2 in rats of the suckling and postweaning stages (Table 3). Dexamethasone is capable of restoring the PFK 2 activity in adrenalectomized rats and also of increasing the enzyme amount in the intact animal prior to the age at which activity is naturally increased. Other steroid hormones such as deoxycorticosterone, estradiol and progesterone were found to be without effect, while testosterone diminishes the dexamethasone action (Table 3).

Table 3. Regulation by steroid hormones of the hepatic phosphofructokinase 2 in young rats

Age [days]	Treatment	PFK 2 Activity
15	Control	1.54 ± 0.10 (10)
	Dexamethasone	2.99 ± 0.22 (5)*
	Dexamethasone + testosterone	2.26 ± 0.26 (5)*,**
	Dexamethasone + progesterone	2.75 ± 0.39 (4)*
	Dexamethasone + estradiol	2.74 ± 0.39 (4)*
16	Control	2.33 ± 0.75 (7)
	Adrenalectomy	1.38 ± 0.20 (3)*
25	Control	5.84 ± 0.75 (3)
	Adrenalectomy	3.79 ± 0.29 (3)*
22	Adrenalectomy + orchidectomy (control)	1.50 ± 0.34 (3)
	Dexamethasone	3.07 ± 0.37 (3)*
	Estradiol	1.72 ± 0.06 (3)
	Deoxycorticosterone	1.62 ± 0.10 (3)

Results are given as mean ± S.E.M. The number of animals is indicated in parentheses. The ages given in the table are those at the end of each experiment. The 22 days old post-operative animals were not substituted with deoxycorticosterone but received physiological saline to drink at libitum. The control values are taken from Table 2 and refer to non-injected animals. *means significantly different ($p < 0.05$) in comparison with the respective control group; ** means significantly different ($p < 0.05$) in comparison with dexamethasone-treated group.

Glucagon was found to be ineffective in inducing PFK 2 in the liver of intact suckling animals. However, it enhances the enzyme activity if applied together with the alpha-adrenergic antagonist phentolamine (Table 4), while a simultaneous injection of glucagon and of the beta-antagonist propranolol is ineffective (results not shown). Phentolamine on its own gives rise to a small but statistically insignificant increase of

Table 4. Participation of the alpha-adrenergic system in the control of hepatic phosphofructokinase 2 activity by glucagon in suckling rats

Treatment	PFK 2 Activity
Control	1.54 ± 0.10 (10)
Glucagon	0.91 ± 0.32 (4)
Glucagon + phentolamine	3.21 ± 0.29 (4)*
Glucagon + phentolamine + insulin (0.1 unit/g body weight)	2.00 ± 0.30 (4)**
Phentolamine	2.03 ± 0.30 (5)

Hormone injections were performed at the 14th day after birth and the animals killed 24 hours later. The control value was taken from Table 2 and represents non-injected animals. Results are shown as mean ± S.E.M. with the number of animals in parenthesis. * means significantly different compared with the control group ($p<0.01$); ** means significantly different from the group treated with glucagon and phentolamine ($p<0.025$).

the PFK 2 activity (Table 4). Because rat liver is reported to contain much less $alpha_2$-receptors (13) than $alpha_1$-receptors (13). from the results of the action of phentolamine an antagonistic effect of the $alpha_1$-adrenergic system on the glucagon-mediated inducibility of PFK 2 becomes most likely. In this respect PFK 2 behaves similarly to serine dehydratase and tyrosine aminotransferase (unpublished results). Insulin was found to counteract the PFK 2 induction both by glucagon (Table 4) and by glucocorticoids (results not shown).

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