Endocrine control of lysosomal alterations in rat liver during the perinatal period

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Summary. Within hours of birth, some physical properties of liver lysosomes are modified. These alterations, which may be related to the autophagic vacuoles formation known to occur during this period, were inhibited by insulin administration. Glucagon, a potent inducer of autophagy in adult rat liver, did not anticipate this process in fetal liver. Our results suggest that the decrease of plasma insulin immediately after birth is an important factor in the development of hepatic autophagy.

Glucagon-induced autophagy in adult rat liver has been the subject of numerous investigations[1-3]. The sequestration of cytoplasmic constituents within autophagic vacuoles after glucagon treatment is associated with an increased osmotic sensitivity and fragility of hydrolases-bearing particles[4,5]. It seems from these studies that preexisting lysosomes are the source of hydrolases found in autophagic vacuoles.

In the first postnatal hours, conspicuous lysosomal changes occur in rat hepatocytes[6,7]. The appearance of numerous autophagic vacuoles is associated with changes in sedimentation rates of hydrolases-bearing particles[8]. These lysosomal alterations are preceded by changes in the levels of the circulating pancreatic hormones: plasma insulin falls and plasma glucagon increases immediately after birth[9]. This new hormonal balance, in which glucagon is high in relation to insulin, is known to favour accelerated catabolism.

In the present investigation, we have studied the role of these hormones in the postnatal development of hepatic autophagy. We have determined the sedimentation rates and the osmotic sensitivity of liver lysosomes after hormonal administration to fetuses or newborn rats. Acid phosphatase and N-acetyl-β-D-glucosaminidase (NAGase) were chosen as representative for the group of the lysosomal hydrolases.

Materials and methods. Wistar rats were used. Some fetuses were delivered by cesarean section after 21.5 days of gestation after the sacrifice of the mother, and were injected s.c. with 1 U of insulin (Rapitard Novo) or saline. They were maintained in an incubator at 37 °C and killed 2.5 h later. Other fetuses, after pentobarbital anesthesia of the mother, were injected s.c. through the uterus with 100 µg of zinc glucagon (Novo) or saline. They were maintained in utero and killed 2.5 h later. The doses used here are clearly unphysiological, but were adopted because they were known to be effective on the development of autophagy in adult rat liver.

Osmotic fragility of hepatic lysosomes was studied on homogenates exposed to 0.15 M sucrose for 30 min at 0 °C, then brought back to 0.25 M sucrose before assay, and analysed for 'free' and total activities of lysosomal hydrolases according to Deter and de Duve[2]. For the determination of the subcellular localization of NAGase, rat liver was homogenized and fractionated according to a modified method of differential centrifugation as previously described[8]. N-Acetyl-β-D-glucosaminidase (EC.3.2.1.30) activity was determined according to Findlay et al.[10] with p-nitrophenyl 2-acetamido 2-deoxy β-D-glucopyranoside as substrate. Acid phosphatase (EC.3.1.3.2) activity was measured according to Gianetto and de Duve[11] with β-glycerophosphate as substrate. Total activity was determined in the presence of 0.1% (w/v) Triton X-100. Protein determination was made by the method of Lowry et al.[12].

Results and discussion. As shown on table 1, in the fetal rat liver, the activity of NAGase is mainly associated with the F_3 fraction. Already 2.5 h after birth, enzyme activity dropped in this fraction, while it increased to about 50% in the F_2 fraction. This shift of NAGase activity, which suggests an increase of the sedimentation velocity of particles containing this enzyme, is correlated with the development of cytological alterations: the distributional patterns of NAGase and of acid phosphatase come back to normal when signs of enhanced autophagy have disappeared[8]. The increase of the 'free' activity of lysosomal enzymes after birth (table 2) is probably a reflection of an increased lysosomal volume. It is likely that a part of lysosomal enzymes is transferred to particles of larger size which become more sensitive to osmotic shock. Our results show that the prevention of postnatal hypoinsulinemia by insulin administration at birth inhibited the development of these

Table 1. Effect of insulin and glucagon on intracellular distribution of N-acetyl-β-D-glucosaminidase in the rat liver during the perinatal period

	Total activity (U/g)	Percentage values			
		F_1	F_2	F_3	F_4
Newborn, at delivery	2.15 ± 0.12	10.6 ± 0.48	19.2 ± 0.79	67.0 ± 1.30	3.1 ± 0.04
	n = 7	(0.50)	(1.08)	(3.15)	(0.07)
Newborn, 2.5 h old					
Saline	2.50 ± 0.09	11.6 ± 0.48	28.9 ± 0.70	56.5 ± 0.91	3.0 ± 0.04
	n = 8	(0.58)	(1.86)	(2.79)	(0.06)
Insulin	2.38 ± 0.06	11.0 ± 0.64	22.5 ± 0.47	64.0 ± 1.43	2.5 ± 0.02
	n = 8	(0.55)	(1.42)	(2.99)	(0.06)
Fetus of 21.5 days					
Saline	2.06 ± 0.16	9.5 ± 0.45	18.6 ± 0.69	68.5 ± 1.14	3.3 ± 0.03
	n = 5	(0.47)	(1.05)	(2.87)	(0.09)
Glucagon	2.14 ± 0.07	9.5 ± 0.62	21.1 ± 0.85	66.4 ± 1.17	3.0 ± 0.02
	n = 6	(0.44)	(1.26)	(2.71)	(0.09)

Results are mean values ± SEM. Total activities are units of enzyme activity/g wet weight of tissue (1 unit is the amount of enzyme releasing 1 µmole of p-nitrophenol per min). The percentage distribution among fractions, is computed with respect to the sum of the values recovered in all the fractions: F_1 (5 min × 400 g); F_2 (10 min × 1500 g); F_3 (20 min × 22,500 g); F_4 supernatant fluid. The relative specific activities (percentage of enzyme activity/percentage of protein) are given in parentheses.

lysosomal alterations. Insulin probably acts by decreasing the fragility of the membrane of hydrolases-bearing particles and this effect is most likely due to a decreased formation of autophagic vacuoles. Such an inhibitory effect on the development of autophagy has been reported in adult rat liver: insulin inhibits autophagic-vacuoles formation in cultured hepatocytes[13,14] and in hepatocytes from perfused rat livers[15]. Hepatic autophagy in uncontrolled experimental diabetes may be corrected by insulin therapy[16].

Prenatal injection of glucagon does not lead to lysosomal alterations (tables 1 and 2), although glucagon under these conditions has a glycogenolytic effect (results not shown). It is suggested that the high fetal insulinemia[17] prevents the effect of exogenous glucagon. In adult rat liver, glucagon induces rapidly lysosomal alterations in animals fasted overnight, but not in fed animals[18]; possibly the decrease of plasma insulin level after short starvation period makes the hepatic lysosomes sensitive to the action of exogenous glucagon.

Our results indicate that a high level of plasma insulin during the perinatal period, whatever the glucagon level, prevents lysosomal alterations; and suggest that insulin lack by itself, which is the result of the fall of plasma insulin after birth, may largely contribute to the development of hepatic autophagy.

Table 2. Effect of insulin and glucagon on osmotic fragility of hepatic lysosomes during the perinatal period

	'Free' NAGase (per cent of total activity ± SEM)	'Free' acid phosphatase (per cent of total activity ± SEM)
Newborn, at delivery	30.2 ± 1.05 (8)	37.4 ± 1.47 (5)
Newborn, 2.5 h old		
Saline	46.2 ± 2.97 (5)	66.5 ± 2.01 (5)
Insulin	34.9 ± 1.71 (5)	46.5 ± 1.72 (6)
Fetus of 21.5 days		
Saline	27.9 ± 0.98 (6)	40.3 ± 1.51 (5)
Glucagon	29.2 ± 1.09 (6)	37.9 ± 2.12 (5)

Total and 'free' activities of NAGase and of acid phosphatase were assayed as given in materials and methods section. Numbers of experiments are given in parentheses.

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Corticosteroidogenesis by isolated human adrenal cells: Effect of serotonin and serotonin antagonists

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Summary. The direct effect of serotonin and antiserotonin agents on adrenal steroid biosynthesis was studied in isolated adrenal cells derived from patients with Cushing's syndrome. The results indicate that serotonin increases corticosterone production, while the serotonin antagonists cyproheptadine and methysergide depress adrenal steroid - particularly cortisol and aldosterone - biosynthesis.

Earlier studies utilizing in vitro systems have shown that serotonin, by direct action, enhances adrenal steroid synthesis in different species[2-4]. In isolated zona glomerulosa cells of the rat, serotonin in a concentration as low as 10^{-9} moles/l is capable of increasing significantly corticosterone and aldosterone production[2]. Based on these studies, it is now generally accepted that serotonin acts specifically on the zona glomerulosa and is one of the most potent stimulators of aldosterone biosynthesis in vitro[2-4]. It is therefore not surprising that serotonin antagonists decrease the aldosterone production of rat adrenal quarters and, when given concomitantly with serotonin, block the aldosterone stimulating effect of the latter[3]. The direct effect on steroid biosynthesis of serotonin and its antagonists has not so far been investigated in the hyperfunctioning human adrenal derived from patients with Cushing's syndrome due to ACTH overproduction.

Materials and methods. The study was made on isolated human adrenal cells obtained by surgery. The 1st patient had medullary carcinoma of the thyroid which caused ectopic ACTH syndrome, and was therefore subjected to bilateral adrenalectomy; histology of the adrenals revealed bilateral micronodular hyperplasia. The 2nd patient showed Cushing's syndrome of pituitary origin; histology of the removed adrenals again showed bilateral micronodular hyperplasia. Adrenal cells were isolated[5] using Collagenase Type I and DNA-ase I (Sigma Chemical Co. Ltd) and by mechanical dispersion[6]; the cells were resuspended in Krebs-Ringer bicarbonate buffer supplemented with glucose and albumin (KRBGA: pH 7.4; glucose: 0.2%; bovine serum albumin: 0.5%; K^+: 5.9 mmoles/l). Aliquots of 3×10^5 cell counts were incubated at 37 °C, in an atmosphere of 95% O_2 and 5% CO_2 for 120 min. Each substance tested was added to the medium at the beginning of incubation; the following compounds were used: $ACTH_{1-24}$ (Synacthen, CIBA Ltd, Basel), serotonin creatinine sulphate, and cyproheptadine hydrochloride (Reanal Chemical Works Ltd, Budapest), methysergide hydrogen maleinate (Sandoz Ltd, Basel). Steroid production was measured in 3 parallel cell cultures for each concentration of the