term infants. We found that: (*1*) Ti is more prolonged with resistive than with elastic loads; (*2*) T tends to increase with loading, more so with resistive loads; and (*3*) peak nasal pressure is greater with resistive than with elastic loads. These findings suggest that: (*1*) control of Ti is flow dependent; (*2*) instantaneous frequency tends to decrease with loading; and (*3*) if peak nasal pressure reflects tension developed by the respiratory muscles, the latter does not represent the inhibitory information needed to terminate inspiration.

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26. Requests for reprints should be addressed to: H. Rigatto, M.D., Department of Paediatrics, Women's Centre, 700 William Ave., Winnipeg, Manitoba R3E 0Z3 (Canada).
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Hypoglycemia	pyruvate carboxylase
liver	pyruvate dehydrogenase
mitochondria	Reye's syndrome

Deficient Activity of Hepatic Pyruvate Dehydrogenase and Pyruvate Carboxylase in Reye's Syndrome

BRIAN H. ROBINSON,[25] D. GRANT GALL, AND ERNEST CUTZ

Departments of Paediatrics, Biochemistry, and Pathology, University of Toronto; Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada

Summary

The activity of certain hepatic enzymes involved in carbohydrate metabolism was measured in postmortem samples from six cases of Reye's symdrome. The activities of the two exclusively extramitochondrial enzymes, glucose-6-phosphatase and fructose-1,6-diphosphatase, were all within the normal range. Activities of pyruvate carboxylase and pryuvate dehydrogenase, both of which are exclusively mitochondrial enzymes, were below levels shown by control tissue in every case, the average being 21.7% of the lowest control value for pyruvate carboxylase and 11.6% of that for pyruvate dehydrogenase. Impaired pyruvate metabolism appears to be another feature in Reye's syndrome.

Speculation

The hypoglycemia and lactic acidemia frequently observed in Reye's syndrome are due to decreased activity in the mitochondria of pyruvate carboxylase and pyruvate dehydrogenase, respectively.

Reye's syndrome exhibits a number of features which are indicative of gross hepatic damage including elevated blood ammonia (1, 10) and serum glutamic oxalic transaminase (2), hypoglycemia (15), and clotting abnormalities (5). At the same time, liver tissue from patients with Reye's syndrome demon-

strates diffuse small droplet fatty change of hepatocytes without necrosis or associated inflammatory lesions and ultrastructural changes of mitochondria (11). These findings correlate with observations of decreased activity in two urea cycle enzymes present in liver mitochondria, ornithine transcarbamylase (EC. 2.1.3.3) (7, 18, 20, 22) and carbamyl phosphate synthetase (EC. 2.7.2.5) (7, 18). Histochemical observation of succinate dehydrogenase (EC. 1.3.99.1) and cytochrome oxidase (EC. 1.9.3.1) in Reye's syndrome has shown that these mitochondrial enzymes are also deficient in activity (3). In this report we extend the biochemical observations to include the virtual absence of two further specific mitochondrial enzymes in this disease.

CASE MATERIAL

Informed parental consent was obtained in all cases in which postmortem tissues from six patients with Reye's syndrome and from seven control subjects were studied. The group with Reye's syndrome consisted of three females and three males. Their clinical and laboratory findings are summarized in Table 1. All five patients demonstrated the classic prodromal illness followed by confusion, drowsiness, and rapid onset of encephalopathy as described by Reye et al. (15). In addition, they all showed elevated serum transaminases and blood ammonia. Low blood glucose was evident (<60 mg/100 ml) in four of the cases. In all six patients the diagnosis of Reye's syndrome was confirmed by the demonstration of characteristic diffuse small droplet fatty infiltration of liver, swollen mitochondria with loss of cristae, and absence of centrilobular necrosis (9).

MATERIALS AND METHODS

Liver tissue was obtained at autopsy within 2 hr after death, frozen in liquid nitrogen, and stored at −100° until used for determinations. Tissue stored in this way showed no loss of enzyme activity over an 18-month period. For the enzyme assays to be listed below, small pieces of tissue were thawed, homogenized in 10 volumes of ice-cold 0.25 M sucrose, 5 mM Tris-HCl buffer, pH 7.4, and centrifuged at 500 × g for 10 min to remove

cell debris. Glucose-6-phosphatase was measured by the method of Swanson (20), fructose-1,6-diphosphatase by the method of Pontremoli (14), phosphoenolpyruvate carboxykinase by the method of Roobol and Alleyne (17), and pyruvate carboxylase by the method of Crabtree et al. (8).

The assay of whole tissue pyruvate dehydrogenase activity was carried out as described by Taylor et al. (21) using 300 to 400 mg freshly thawed tissue, homogenized in 10 volumes of ice-cold buffer, containing 10 mM potassium phosphate, 1 mM EDTA, 1 mM dithiothreitol, and 1% fatty acid free bovine serum albumin, pH 7.4. Aliquots of 0.1 ml were then added to assay buffer (11 mM potassium phosphate, 1.1 mM EDTA, 2.8 mM $MgCl_2$, 1.6% bovine serum albumin (fatty acid free), 1.2 mM dithiothreitol, 0.3 mM pyruvate labeled with 0.12 μCi [1-^{14}C]pyruvate/ml at pH 7.4 and 37°). The flasks were fitted with plastic inserts holding strips of filter paper (1 × 3 cm) saturated with 0.2 ml hyamine and closed with a rubber stopper. The reaction was stopped after a given time by injecting 0.8 ml 0.08 M citric acid, 0.04 M Na_2HPO_4 buffer (pH 3.0). After 30 min of further incubation the filter strips were removed with forceps and placed in 10 ml toluene-ethanol (9:1 v/v) scintillation fluid and the ^{14}C activity measured in a Beckman liquid scintillation spectrometer.

RESULTS

The activities of the key gluconeogenic enzymes in postmortem liver plus that of pyruvate dehydrogenase are shown in Table 2. The acitvities of the two exclusively extramitochondrial enzymes, glucose-6-phosphatase and fructose-1,6-diphosphatase, were all within the normal range except for the diphosphatase activity of case 2, which was just outside the range of our controls, 0.59 μmol/min/g liver, compared with the lowest value in control tissue of 0.72. Activities of phosphoenolpyruvate carboxykinase, which has a bimodal distribution in man with approximately equal activities in both the mitochondrial and cytosolic compartments (4), were also within the range shown by controls except that case 2 was again slightly below the lowest value exhibited in control tissue. Activities of pyruvate carboxylase and pyruvate dehydrogenase, both of which are exclusively

Table 1. Clinical summary[1]

Patient	Age	Sex	Serum NH_3, μg/dl	SGOT, units/ml	Blood glucose, mg/dl	Days from onset of encephalopathy to death
1	14 yr	F	448	305	116	4
2	6 yr	M	587	1,512	48	3
3	10 mo	F	790	1,110	36	3
4	11 yr	F	199	2,500	108	4
5	3 yr	M	638	1,080	55	4
6	18 mo	M	410	175	25	4
Normal subjects			<100	<60		

[1] Values given are for the lowest blood glucose and the highest NH_3 and serum glutamic oxalacetic transaminase (SGOT) recorded during the admission.

Table 2. Enzyme activities in postmortem liver (micromoles per g per min)[1]

Enzyme	Patient						Controls[2] (mean + SEM)	Range
	1	2	3	4	5	6		
G6Pase	1.98	1.62	3.40	3.67	2.10	4.10	2.55 ± 0.52	(1.19–4.80)
F16Dpase	1.95	0.59	2.25	2.44	1.46	0.62	1.40 ± 0.34	(0.72–3.20)
PEPCK	0.94	0.59	1.70	2.43	1.79	0.91	1.89 ± 0.44	(0.69–3.21)
Pyruvate carboxylase	0.097	0.004	0.230	0.240	0.092	0.218	0.91 ± 0.16	(0.61–1.66)
Pyruvate dehydrogenase	0.011	0.052	0.041	0.048	0.233	0.055	0.77 ± 0.10	(0.645–1.256)

[1] G6Pase: glucose-6-phosphatase; F16Dpase: fructose-1,6-diphosphatase; PEPCK: phosphoenolpyruvate carboxylase.

[2] Control tissue was from seven individuals all less than 10 years of age taken at autopsy within 2 hr of death and showing no signs of liver disease.

mitochondrial enzymes, were below the levels shown by control tissue in every case, the average being 21.7% of the lowest control value for pyruvate carboxylase and 11.6% of that for pyruvate dehydrogenase. Preincubation of tissue extracts with Ca^{++} and Mg^{++} as described by Robinson and Sherwood (16) to elicit maximal pyruvate dehydrogenase activity resulted in only slight (<10%) increase in activity in tissue from both patients and control subjects.

DISCUSSION

Defective urea cycle function in a damaged liver leading to hyperammonemia has been suggested as central to the pathogenesis of encephalopathy in Reye's syndrome (18, 19, 22), although it has now been shown that the activities of hepatic ornithine transcarbamylase and carbamyl phosphate synthetase slowly return to normal after the acute phase of the disease (7, 18). This is so whether or not the progression of the disease leads to fatality (7). However, ultrastructural changes and reduction of succinic oxidase and cytochrome oxidase activities suggest that a more general demise in mitochondrial function may be one of the early events in this disease (3, 13). Hypoglycemia exhibited in Reye's syndrome (15) thought to be related to depletion of hepatic glycogen reserves, may be due, at least in part, to impaired gluconeogenesis (11). Failure to convert 3-carbon substrates such as pyruvate, alanine, and lactate into glucose leads to an accumulation of those metabolites (12). Thus, impaired pyruvate metabolism appears to be another feature in Reye's syndrome.

The results presented demonstrate abnormalities of two further mitochondrial enzyme pathways. First, although the extramitochondrial enzymes of gluconeogenesis such as glucose-6-phosphatase and fructose-1,6-diphosphatase show normal activity in Reye's syndrome, the activity of pyruvate carboxylase, which is exclusively mitochondrial, is defective in activity. Such an impairment must mean that the gluconeogenic pathway is not operating at anywhere near its normal capacity. Second, pyruvate dehydrogenase, which allows 3-carbon substrates to be oxidized by the Krebs' cycle, is also defective in activity. This defect may be because pyruvate dehydrogenase is in the inactive form since fatty acids are the major incoming hepatic energy substrate in this disease (11). However, preincubation with Ca^{++} and Mg^{++} to cause reactivation did not increase the activity to a normal level.

The observed deficiencies of ornithine transcarbamylase and carbamyl phosphate synthetase are reported to be temporary in those subjects with Reye's syndrome in which they have been measured (7, 18). Although it was not possible for us to make serial measurements, it is obvious from our data that in these cases which are all fatal instances, no such recovery of either pyruvate dehydrogenase or pyruvate carboxylase to normal levels was evident. We suggest that the disturbance of mitochondrial integrity by an agent or agents unknown (23) leads to a loss of enzyme activity in many metabolic pathways in which the mitochondria are involved, including the urea cycle, the Krebs' cycle, and gluconeogenesis.

CONCLUSION

The activities of two mitochondrial enzymes, pyruvate dehydrogenase and pyruvate carboxylase, were shown to be deficient in six fatal cases of Reye's syndrome. This adds credence to the postulate that nonselective deletion of mitochondrial enzymes

due to disruption of mitochondrial integrity (19) is a causative factor in the pathogenesis of Reye's syndrome.

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25. Requests for reprints should be addressed to: B. H. Robinson, Ph.D., Research Institute, The Hospital for Sick Children, 555 University Ave., Toronto, Ont. M5G 1X8 (Canada).
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