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Metabolic responses to intermittent hepatic dearterialization in the rat

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Hepatic dearterialization is a palliative treatment for irresectable liver tumours. In the current study, the metabolic consequences of hepatic dearterialization were examined in the rat. Liver glycogen content was reduced to an average of 84% following 60 min dearterialization and was further reduced to an average of 16% following 60 min reperfusion. Plasma concentration of β-hydroxybutyric acid was elevated by an average of 65% following 60 min hepatic dearterialization. In contrast, hepatic dearterialization did not alter cholesterol and triglyceride plasma levels. In addition, the hepatic activity of hepatic lipase was reduced by 29% after 60 min of hepatic dearterialization, a reduction which remained after 60 min of reperfusion. Clearance of intravenously administered antipyrine, which reflects the activity of liver microsomal enzymes, was reduced by 37% after 60 min of hepatic dearterialization. In conclusion hepatic dearterialization is accompanied by marked activity in the processes related to carbohydrate, lipid and xenobiotic metabolism. These effects should be taken into account when treating patients with hepatic dearterialization.

Irresectable liver malignancies can be palliatively treated with intermittent hepatic dearterialization (1–3). This mode of treatment has been increasingly used since an implantable occluder was developed allowing repeated ischaemia over long periods (4). This treatment has been promising for tumour size reduction and tumour calcification (3,5). However, the metabolic consequences are far from established. Recently, a small vascular occluder was developed for experimental studies of intermittent hepatic dearterialization in the rat (6). Daily short-term dearterialization periods were found to reduce the growth rate of an inoculated implantable tumour in the rat liver (7). In the present study, we used the vascular occluder to study the metabolic consequences of hepatic dearterialization in the healthy rat. We examined the influence of 60 min of hepatic dearterialization followed by 60 min of reperfusion in the rat. The following processes were considered: liver glycogen content; liver oxidative capacity as indirectly determined by plasma β-hydroxybutyric acid levels (8); serum cholesterol and triglyceride levels; the activity of hepatic lipase in the liver and post-heparin plasma (9); and the activity of liver microsomal enzymes as assessed by plasma antipyrine clearance (10,11).

Materials and Methods

Animals

Male inbred Wistar-Furth rats (Anticimex, Stockholm, Sweden), weighing 250–350 g, were used. They were kept on a standard laboratory diet (Astra-Ewos, Södertälje, Sweden) and tap water ad libitum.

Anesthesia

Laparatomy for the dearterialization procedure, removal of liver tissue and vessel cannulation for blood sam-

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pling were all performed during anesthesia induced by intraperitoneal injection of chloral hydrate (0.25 g/kg; Apoteksbolaget, Stockholm, Sweden).

Dearterialization procedure

In the anaesthesized rats, the falciform ligament and the peritoneal attachments were divided. The gastroduodenal artery was ligated. Thereafter, a vascular occluder was placed around the proper hepatic artery. The occluder is a short ring-shaped Silastic cuff with a Prolene 4-0 suture placed through its wall in a purse-string fashion. The Prolene suture is threaded through a polyethylene tube (PE 100) which is tunnelled subcutaneously to the tail of the rat. Vascular occlusion is achieved by traction of the Prolene suture. Previous angiographic study has shown that this hepatic ischaemia technique is reversible (6). Control rats underwent a sham operation, where a laparatomy was performed but the vascular occluder was not applied. Vascular occlusion was performed 1 day after surgery.

Vessel cannulation

For blood sampling and for intravenous injections, a cannula (Portex®, Hythe, Kent, U.K.) was inserted in a femoral vein and artery, respectively.

Experimental protocols and chemical determinations

Liver glycogen. The liver was removed, rapidly frozen to −20 °C and stored until analysis. The liver was removed in controls ($n = 11$), after 60 min hepatic dearterialization without reestablishing an arterial flow ($n = 6$), or after 60 min of reperfusion following the 60 min dearterialization ($n = 5$). Liver specimens (100 mg) were boiled in 30% KOH (2 ml) for 30 min, and 3 ml ethanol (96%) were added. Pellets from 10 000 × g centrifugation were reconstituted in distilled water for glucose measurement by the glucose oxidase technique (12,13). The liver glycogen content was expressed as mg released glucose per 100 mg liver tissue (wet weight).

Plasma levels of β-hydroxybutyric acid, cholesterol and triglycerides. Seven rats underwent hepatic dearterialization for 60 min. Blood was taken immediately before occlusion, immediately before the end of occlusion, and 60 min after the end of hepatic dearterialization. Blood was sampled in preheparinized tubes, immediately centrifuged and plasma removed and stored at −20 °C until analysis. Plasma levels of D-(−)-β-hydroxybutyric acid were determined spectrophotometrically with the use of β-hydroxybutyric dehydrogenase (Sigma Chemicals, St. Louis, MO, U.S.A.) which catalyzes the reduction of nicotinamide adenine dinucleotide (NAD) by β-hydroxybutyric acid (14). Plasma levels of cholesterol and triglycerides were analysed with commercial kits from Boehringer-Mannheim, F.R.G.

Hepatic lipase. Both the hepatic and post-heparin plasma activities of hepatic lipase were determined. The blood sampling was performed, in controls ($n = 6$), after 60 min of hepatic dearterialization ($n = 6$) and in a group subjected to 60 min dearterialization and 60 min perfusion ($n = 6$). Blood was sampled 10 min after an intravenous injection of heparin (10 IU/100 g body weight) to release the enzyme from the capillaries (15). The sample was immediately centrifuged and plasma removed and stored at −20 °C until analysis. Two days later, liver tissue was taken from the same animals without any preceding dearterialization ($n = 6$) and after 60 min hepatic dearterialization ($n = 6$) or from rats subjected to 60 min hepatic dearterialization followed by 60 min reperfusion ($n = 6$). Approximately 500 mg of tissue was taken and frozen at −20 °C until analysis. A 2 day span between blood sampling and liver tissue removal was chosen since the enzyme is replenished to hepatic capillaries within 24 h (16). Five hundred mg of liver tissue were homogenized in 5 ml cold Krebs-Ringer-Hepes-buffer (pH 7.4). One ml of the suspension was centrifuged for 5 min at 6 °C and the fatty supernatant was discarded. One ml cold 0.1 M Tris buffer (pH 9.0), containing 15 IU heparin was added to the retained microsomal fraction and, after incubation for 15 min in an ice-bath, the suspension was centrifuged as above and the supernatant removed for assay (17). Assay of hepatic lipase activity was performed by the method of Nilsson-Ehle and Ekman (9) using dioleoyl-phosphatidylcholine (final concentration 0.067 mg/ml) as substrate emulsifier. The result is related to the tissue protein content, as determined by the method of Lowry et al. (18).

Antipyrine metabolism. Antipyrine clearance was studied by the one-sample method in 19 rats (11). Catheters were placed in the femoral vein and the hepatic arterial occluder under chloral hydrate anesthesia as described above. One group of eight sham operated rats served as controls. A second group of 11 rats underwent 60 min of hepatic artery occlusion. Antipyrine (4 mg; Sigma Chemicals, St. Louis, MO, U.S.A.) was injected in the venous catheter immediately preceding the arterial occlusion procedure. Five hours after administration, 500 μl tail blood was sampled and plasma was separated. Using metabolic cages, urine was collected on ice for 24 h subsequent to antipyrine administration. Plasma and urine were stored at −20 °C for later analysis by HPLC (14). The clearance of antipyrine (CL) was calculated as $CL = [\ln(D/V) - \ln(c)] \times V/t$, where D is the dose, V is the volume of distribution and c is the plasma concentration at time t. V is estimated as 0.66-times the body weight. The

clearance of antipyrine through each metabolic pathway was calculated as the fraction of the dose excreted as the particular metabolite multiplied by CL.

Statistics. Means ± S.E. are demonstrated. Student's paired and unpaired *t*-tests were used for statistical determinations. The rate of antipyrine metabolism was compared between the groups by means of one-way analysis of variance and the least significant difference was used for comparison of means.

Results

Liver glycogen content

After 60 min hepatic dearterialization, the liver glycogen content had decreased to 26.0 ± 1.5 mg/g liver ($n = 6$) compared to 31.0 ± 1.0 mg/g liver in controls ($n = 11$; $p < 0.01$). After 60 min reperfusion, the liver glycogen content was reduced further to 4.8 ± 0.6 mg/g liver ($p < 0.001$).

Plasma β-hydroxybutyric acid

Plasma levels of β-hydroxybutyric acid were elevated from 4.0 ± 0.5 to 6.6 ± 1.3 mg/l at the end of 60 min of hepatic dearterialization ($n = 7$; $p < 0.05$). After 60 min reperfusion, the plasma β-hydroxybutyric acid levels remained elevated at 6.4 ± 0.7 mg/l.

Plasma cholesterol and triglycerides

Before dearterialization, plasma levels of cholesterol were 1.02 ± 0.10 mmol/l and those of triglycerides were 0.67 ± 0.09 mmol/l ($n = 7$). These values were not significantly altered by hepatic dearterialization.

Hepatic lipase

The activity of hepatic lipase in liver tissue was reduced to 4.9 ± 0.6 mU/mg liver ($n = 6$) after 60 min dearterialization compared to 7.0 ± 0.5 mU/mg liver in controls ($n = 7$; $p < 0.01$). It remained low after 60 min reperfusion at 4.2 ± 1.3 mU/mg liver ($n = 6$). Enzyme activities in post-heparin plasma were, however, not altered during hepatic dearterialization or reperfusion. Thus, the post-heparin plasma levels of hepatic lipase were 186 ± 9 mU/ml in control rats ($n = 7$), 196 ± 29 mU/ml in rats at the end of 60 min hepatic dearterialization ($n = 6$), and 174 ± 17 mU/ml in rats undergoing 60 min hepatic dearterialization followed by 60 min reperfusion ($n = 6$).

Antipyrine metabolism

Hepatic dearterialization for 60 min reduced plasma clearance of antipyrine to on the average 63% ($n = 8$) compared to the controls ($n = 11$; $p < 0.05$). The hepatic dearterialization procedure decreased the calculated renal clearance of unchanged antipyrine and the formation of 3-hydroxymethyl-antipyrine more than that representing formation of 4-hydroxy-antipyrine (Table 1). The ratio between the excreted amount of the 3-hydroxymethyl and 4-hydroxymethyl metabolites was significantly lower in the group subjected to the 60 min dearterialization than in the control group. The 24 h urinary excretion of antipyrine as unchanged compound and metabolites was significantly lower in the group subjected to 60 min hepatic dearterialization than in the control group (Table 1).

TABLE 1

Antipyrine metabolism in control rats ($n = 8$) and in rats subjected to hepatic dearterialization for 60 min ($n = 11$)

	Controls	60 min
Plasma antipyrine CL (ml/min/kg)	3.76 ± 0.24	2.36 ± 0.25[a]
Clearance to HMAP (μl/min/kg)	1274 ± 192	333 ± 49[a]
Clearance to NORAP (μl/min/kg)	173 ± 31	61 ± 15[a]
Clearance to OHAP (μl/min/kg)	354 ± 55	195 ± 30[a]
Renal antipyrine CL (μl/min/kg)	142 ± 38	17 ± 4[a]
Ratio HMAP/OHAP	3.9 ± 0.5	1.7 ± 0.1[a]
24 h urinary excretion of antipyrine + metabolites (% of dose)	52 ± 7	26 ± 3[a]

[a] Denotes $p < 0.05$ vs. the control group. CL, clearance; HMAP, 3-hydroxymethyl-antipyrine; NORAP, norantipyrine; OHAP, 4-hydroxy-antipyrine.

Discussion

In the present experimental study in the rat, hepatic dearterialization lowered the liver glycogen content. Physiologically, the liver glycogen content is determined by a balance between glycogen formation, through gluconeogenesis and glucose uptake, and glycogen breakdown (19). Approximately 40% of the glycogen stored in the liver is formed through de novo gluconeogenesis, whereas 60% is synthesized through glucose taken up from the blood stream (20). In the rat, hepatic glucose production is not affected by hepatic dearterialization (21). This suggests that the reduction of glycogen content is not simply due to exaggerated glycogenolysis. Although the food intake in the rats was not measured, we consider it unlikely that reductions in liver glycogen were due to decreasing eating, since rats were not restrained and had free access to food during the dearterialization procedure. The re-

duction of liver glycogen is more likely due to reduced glycogen synthesis, either through reduced liver glucose uptake and/or inhibition of glycogen synthesizing enzymes. This in turn may be initiated by impaired insulin secretion, which has been shown to worsen during hepatic dearterialization in the rat (22). Also important, the reduction in glycogen stores seems to be long-term, since after 60 min of reperfusion, the liver glycogen content was reduced even further. This suggests an effect by hepatic dearterialization on glycogen stores, which is not readily reversible.

It is known that elevated plasma β-hydroxybutyric acid levels correlate to hepatic oxidative capacity, i.e., to mitochondrial function (8). Therefore, the elevated plasma concentration after 60 min dearterialization suggests that the mitochondria were affected by the procedure. This could be a mechanism underlying the inhibition of tumour growth seen after hepatic dearterialization, since the availability of energy might be restricted. In contrast, hepatic dearterialization did not change the cholesterol or triglyceride plasma level.

Hepatic activity of hepatic lipase was reduced by the dearterialization. This parameter reflects recently synthesized enzyme within hepatocytes, secreted enzyme in transit, and lipase bound to glycosaminoglycans in capillaries. It is only the latter pool which is released into post-heparin plasma (23). Since this plasma activity was not different between groups, our data indicate an inhibition of de novo enzyme synthesis during dearterialization. The lack of difference between the groups also suggests a low pre-formed lipase turnover.

The clearance of antipyrine is assumed to reflect the enzyme activities in liver microsomes, mainly the cytochrome P_{450} system (10,11,24). Hepatic dearterialization for 60 min decreased the plasma antipyrine clearance. Hepatic dearterialization thus may inhibit microsomal enzyme activities. Anesthesia and surgery per se may affect the hepatic metabolism of antipyrine (25). However, in the present study, the control group and the groups subjected to dearterialization were comparable in this respect. Therefore, the effects of hepatic dearterialization on antipyrine metabolism seem to be due to the dearterialization procedure. The 60 min hepatic dearterialization was found to have a differential effect on the individual elimination pathways of antipyrine. Thus, 3-methylhydroxylation was more depressed than 4-hydroxylation. The formation of each antipyrine metabolite is dependent on different isoenzymes of the cytochrome P_{450} system (26). This suggests that hepatic dearterialization inhibits the activity of the isoenzymes by different degree.

In conclusion, the current experimental study in the rat has demonstrated that hepatic dearterialization is accompanied by reduced liver glycogen content, elevation of plasma β-hydroxybutyric acid levels, reduction of hepatic activity of hepatic lipase and inhibition of the antipyrine clearance. Hence, hepatic dearterialization affects several different hepatic processes related to carbohydrate, lipid and xenobiotic metabolism. These findings could have clinical implications when hepatic dearterialization is performed in patients with diabetes or lipid disorders, and in patients under ongoing treatment with drugs metabolized by the hepatic microsomal enzymes.

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