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Glycogenolysis in liver of phosphorylase kinase-deficient rats during liver perfusion and ischaemia

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Liver glycogen degradation and phosphorylase activity were measured in normal and phosphorylase kinase-deficient (*gsd/gsd*) rats. During perfusion or ischaemia, *gsd/gsd*-rat livers showed a brisk glycogenolysis. There was also a small (1.9-fold) but significant transient increase in their phosphorylase *a* activity during ischaemia, despite their phosphorylase *b* kinase deficiency; it seems unlikely, however, that this was the main determinant of the glycogenolysis.

In recent years an inbred strain of rats (MZR/Mh) with a glycogen-storage disease (*gsd/gsd*) has been reported (Malthus & Clark, 1977; Malthus *et al.*, 1980). They are deficient in hepatic phosphorylase *b* kinase, the enzyme that activates glycogen phosphorylase, and consequently have great difficulty in degrading their liver glycogen.

Liver glycogen acts as a reservoir of glucosyl units, which can be mobilized rapidly to maintain blood glucose homoeostasis. Glycogen phosphorylase, the main glycogenolytic enzyme, is normally present in livers in an inactive *b* form and is activated into the *a* form during hypoxia, ischaemia or administration of hormones (Hems & Whitton, 1980; Sharma *et al.*, 1980).

Clark *et al.* (1982) have shown glycogenolysis in ischaemic livers of neonatal *gsd/gsd* rats. In the present studies we have examined glycogen degradation and glucose output in *gsd/gsd* and normal livers during perfusion and total ischaemia.

Materials and methods

Rats (200–250g) homozygous for phosphorylase *b* kinase deficiency (*gsd/gsd*) were obtained from the Department of Clinical Biochemistry, Medical School, University of Otago, Dunedin, New Zealand, and bred in the Animal House at St. George's Hospital Medical School. Wistar rats (200–250g) were purchased from Bantin and Kingman, Hull, U.K., and fed on Oxoid 41B diet in our Animal House for 5–10 days before the experiment. Livers were either obtained from freshly killed rats (see Hems & Brosnan, 1970) or perfused with bicarbonate-buffered saline containing bovine serum albumin and washed rat erythrocytes but no glucose (Hems *et al.*, 1966). The zero-time point in perfusion experiments was the moment when the liver colour changed, showing that a uniform perfusion had occurred. It was 2–3min after the start of perfusion. In experiments with ischaemic livers, the rats were killed by cervical dislocation and the livers rapidly removed. A portion was immediately freeze-clamped (10–15s after death) and the livers were kept at 37°C. All results are expressed per g wet weight.

All reagents were purchased from British Drug Houses (Poole, Dorset, U.K.), except for the following: [U-^{14}C]glucose 1-phosphate was purchased from The Radiochemical Centre, Amersham, Bucks., U.K.; enzymes were purchased from either Boehringer Corp., London W.5, U.K., or Sigma Chemical Co., Poole, Dorset, U.K. Diazyme (1,4-amyloglucosidase) enzyme was kindly given by Professor B. E. Ryman of Charing Cross Hospital Medical School, London.

Statistical significance was tested by Student's *t* test.

Assay of glycogen and glucose

Freeze-clamped liver samples (50mg) (Sharma *et al.*, 1980) were rapidly weighed and digested in 1ml of boiling 30% (w/v) KOH. The glycogen was precipitated with 2ml of ethanol and 50μl of 10% (w/v) Na_2SO_4, washed with 4 × 3ml of ethanol and determined by a modification of the method of Huijing (1970). The assay mixture (1ml) contained 40mM-sodium acetate, pH4.8, 500μg of Diazyme and extract containing less than 200μg of glycogen. The mixture was incubated at 37°C overnight and glucose produced was analysed by Trinder's method, in which the peroxide formed by glucose

oxidase is assayed with 4-aminophenazone and phenol (see Kaplan & Szabo, 1979).

The perfusates (100μl) were deproteinized with 6% (v/v) $HClO_4$ (900μl) and centrifuged at 8500 *g* for 1 min. The glucose in the supernatant was assayed as detailed in the glycogen assay above.

Assay of phosphorylase a

Freeze-clamped liver samples were rapidly weighed, pulverized and homogenized with 3.5 vol. of ice-cold buffer, pH 7.0, containing 50 mM-glycylglycine and 100 mM-NaF. Phosphorylase activity in the whole homogenate (20μl) was immediately assayed by measuring the incorporation of [U-^{14}C]glucose 1-phosphate into glycogen at 30°C. The incubation mixture (220μl) contained 1% glycogen, 50 mM-glucose 1-phosphate, 0.1 M-NaF, 0.5 mM-EDTA, 0.5 mM-caffeine and 5 mM-glycylglycine, pH 6.5. These conditions minimized the activity of phosphorylase *b* (see Hems *et al.*, 1976).

Results

During the first 10 min perfusion, glucose output from normal livers (Fig. 1) was 6.6 ± 0.6 μmol/min per g; in this period the output from *gsd/gsd* livers was significantly less (2.5 ± 0.2 μmol/min per g; $P = 0.008$). In the period 10–65 min of perfusion, normal livers produced only 0.28 ± 0.01 μmol of glucose/min per g, whereas the rate in *gsd/gsd* livers was 0.94 ± 0.04 μmol/min per g. The difference was significant ($P = 0.006$). Over the whole perfusion (65 min) the total glucose outputs in normal and *gsd/gsd* livers (83.9 ± 6.6 and 75.2 ± 2.4 μmol/g) were not significantly different. Thus the phosphorylase kinase-deficient livers released glucose in a slower but more sustained manner.

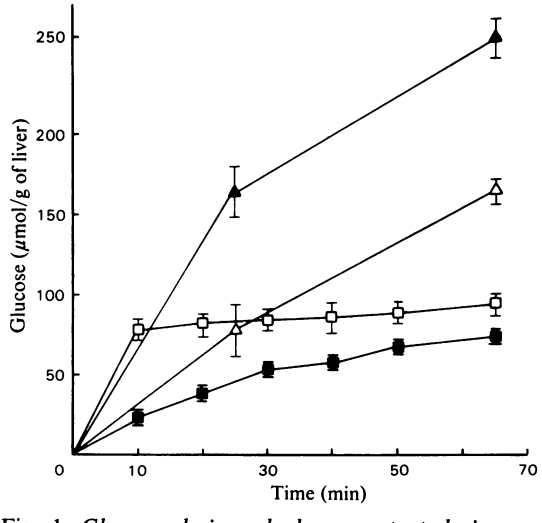

Fig. 1. *Glycogenolysis and glucose output during perfusion of normal and gsd/gsd-rat livers*
Livers were perfused, under the conditions stated in the text, with 60 ml of medium. Glucose output (□, ■) and decrease in glycogen content (△, ▲) were measured in normal (□, △) and *gsd/gsd* (■, ▲) rat livers. Results are expressed as means ± S.E.M. for four or five observations.

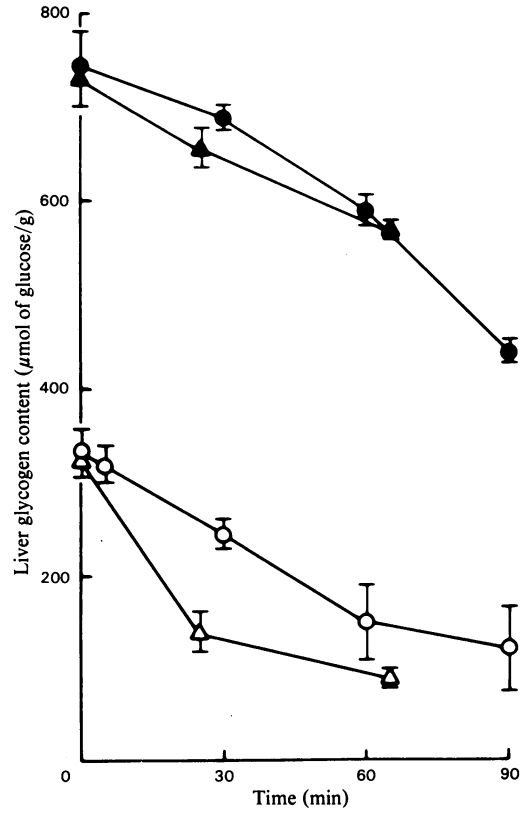

Fig. 2. *Glycogen content of normal and gsd/gsd-rat livers during perfusion and ischaemia*
Glycogen content was measured during perfusion (△, ▲) or total ischaemia (○, ●) in normal (△, ○) or *gsd/gsd* (▲, ●) rat livers. Other details were as in Fig. 1.

The breakdown of glycogen in normal livers between 25 and 65 min perfusion (Fig. 2) was significantly greater than that in *gsd/gsd* livers (3.6 ± 0.4 versus 2.5 ± 0.5 μmol/min per g; $P = 0.003$). These data have been recalculated in terms of glucosyl units made available and are shown in Fig. 1. Evidently much of the degraded glycogen was not released as glucose.

During total ischaemia (Fig. 2) the rates of glycogenolysis over 60 min in the normal and *gsd/gsd* livers were not significantly different. In the period 60–90 min of total ischaemia, in the *gsd/gsd*

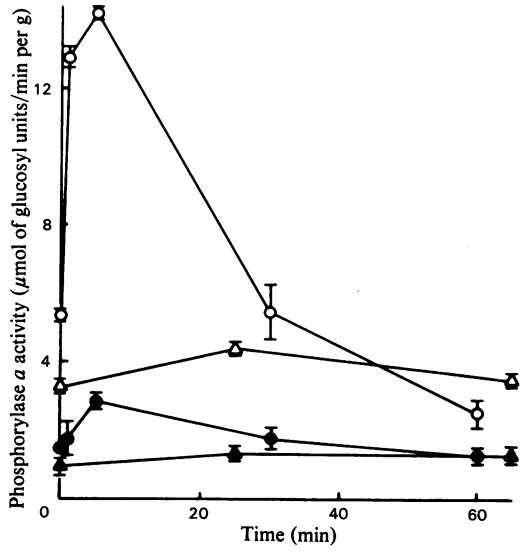

Fig. 3. *Phosphorylase a activity in normal and gsd/gsd-rat livers during perfusion and ischaemia*
Phosphorylase *a* activity in normal (△, ○) and *gsd/gsd* (▲, ●) rat liver was determined during perfusion (△, ▲) and total ischaemia (○, ●) by the methods stated in the text. Other details were as in Fig. 1.

liver glycogenolysis was more rapid (3.4 ± 0.7 versus 5.1 ± 0.3 μmol/min per g of liver; $P = 0.007$), perhaps because glycogen was becoming severely depleted in normal livers (Fig. 2).

Contrary to our expectations, there was a significant increase (1.9-fold) in phosphorylase *a* activity in the first 5 min of ischaemia in *gsd/gsd*-rat livers (see Fig. 3; $P = 0.003$). In the later stages of ischaemia the phosphorylase *a* content of *gsd/gsd* livers was not significantly different from that in normoxic livers. In normal livers there was a transient 2.7-fold increase in phosphorylase *a* activity, similar to that reported by Sharma *et al.* (1980).

In normal rat livers there was a significant increase in liver phosphorylase *a* during the first 25 min perfusion (from 3.3 ± 0.3 to 4.4 ± 0.4 μmol of glucosyl units/g; $P = 0.002$), but after 65 min perfusion the activity was not significantly different from normal. In perfused *gsd/gsd*-rat livers no significant changes occurred (Fig. 3).

Discussion

The main purpose of the present experiments was to study the role of phosphorylase *b* in liver glycogenolysis. Since *gsd/gsd* rats are deficient in liver phosphorylase kinase, and none of their glycogen phosphorylase is considered to be in the *a* form, any glycogenolysis that they show would be presumed to be phosphorylase-*b*-mediated. The results show a marked glycogenolysis in response to total ischaemia or during liver perfusion. How far can this be attributed to phosphorylase *b*?

Neither of the above assumptions is strictly true: some phosphorylase kinase activity and some phosphorylase *a* activity are found in *gsd/gsd* liver homogenates.

Thus the first question to be answered is the relevance of these low enzyme activities. Malthus *et al.* (1980) found a low phosphorylase kinase activity (0.5 ± 0.39 unit of phosphorylase *a* converted/min per g) in extracts from *gsd/gsd* livers whereas Watts *et al.* (1982) found low (5% of total) but significant phosphorylase *a* activities in *gsd/gsd* hepatocytes. When Watts *et al.* (1982) incubated their hepatocytes in glucose, there was a 5-fold decrease in phosphorylase *a* activity. These observations argue for a physiological role for the phosphorylase kinase activity measured by Malthus *et al.* (1980). However, very little glycogen turnover occurs in these livers (Clark *et al.*, 1982).

A more certain indication of true phosphorylase kinase activity would be a rise in phosphorylase *a* activity following some stimulus. Malthus *et al.* (1980) found no rise in phosphorylase *a* activity after glucagon administration, and Blackmore & Exton (1981) failed to find a rise after administration of phenylephrine, vasopressin, glucagon or the calcium ionophore A23187. The present report of a significant increase in phosphorylase *a* activity during ischaemia in *gsd/gsd* livers is, to our knowledge, the first evidence for significant phosphorylase kinase activity in the intact livers of these animals. The physiological relevance of this observation is less clear. The highest phosphorylase *a* activity in ischaemic *gsd/gsd* livers was 2-fold lower than the activity in normoxic normal livers and was 4.9-fold lower than the activity in ischaemic normal livers. Thus it seems unlikely that this caused the same rate of glycogenolysis as was observed in ischaemic normal livers. Indeed the rate of glycogenolysis in normal livers from 30 to 60 min of ischaemia was identical with that in the same livers in the first 30 min, although the phosphorylase *a* content of these livers was elevated at 5 and 10 min and normal or even subnormal between 30 and 60 min. This suggests that phosphorylase *a* content was not the main determinant of glycogenolysis in ischaemia, even in normal livers.

The situation is more clear-cut in the perfused livers, where a brisk glycogenolysis occurred (Figs. 1 and 2), with no additional phosphorylase *a* formation, in either normal or *gsd/gsd*-rat livers.

How could phosphorylase-*b*-mediated glycogenolysis occur in these livers? AMP or IMP (Stalmans & Gevers, 1981) will activate liver

phosphorylase *b in vitro*, and both these nucleotides rise in concentration during liver ischaemia. Such a mechanism has been proposed for phosphorylase kinase-deficient muscle (Rahim *et al.*, 1976, 1980). Stalmans & Gevers (1981) considered its relevance in liver, but found that liver phosphorylase *b* was almost inactive *in vitro* at the concentrations of substrates and inhibitors present in ischaemic-liver extracts. There must be some uncertainty about the concentrations of small molecules in the cellular compartment occupied by the enzyme, however, and the properties of glycogen phosphorylase *in vivo* may differ from those of the purified enzyme. Muscle phosphorylase, for instance, behaves differently when isolated in crude 'glycogen particle' extracts (reviewed by Griffiths, 1981), and the same may be true of the liver enzyme.

Alternatively one could postulate the presence of an amylase or glucosidase able to degrade glycogen by an alternative pathway (Devos & Hers, 1980). One would also need to postulate a mechanism whereby this pathway could be activated both in ischaemia (when, for instance, lysosomal enzymes might become available) and during liver perfusion. Clark *et al.* (1982) found that glycogenolysis of double-labelled glycogen in livers of *gsd/gsd* rats *post mortem* followed a random pattern, suggesting a hydrolytic mechanism.

In summary, we conclude that the glycogenolysis in ischaemic or perfused *gsd/gsd*-rat livers is probably due to AMP- or IMP-activated phosphorylase *b* and that a similar mechanism may be important in normal livers under similar circumstances.

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