

Effects of glucose on phosphorylase and glycogen synthase in hepatocytes from diabetic rats

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The effects of glucose on phosphorylase and glycogen synthase were investigated in hepatocytes isolated from acutely (40 h) and chronically (90 h) alloxan-diabetic rats. The glucose-induced inactivation of phosphorylase proceeded normally in all conditions. The ensuing activation of glycogen synthase was slightly blunted in acute diabetes, but became virtually absent in 72 h diabetes of similar severity. In hepatocytes from rats with various degrees of chronic diabetes, the maximal activation of glycogen synthase (at 60 mM-glucose) was inversely correlated with the plasma glucose concentration.

In normal animals a glucose load causes first the inactivation of phosphorylase in the liver, and then an activation of glycogen synthase (Stalmans *et al.*, 1974). Addition of glucose reproduces these changes in the isolated perfused liver and in isolated hepatocytes (Hue *et al.*, 1975). The current explanation is that glucose binds to phosphorylase *a* and that the resulting complex is a superior substrate for phosphorylase phosphatase, whence the inactivation of phosphorylase and the inhibition of glycogenolysis. The removal of phosphorylase *a*, which is a strong inhibitor of glycogen synthase phosphatase, allows the latter enzyme to convert synthase *b* into the *a* form, which in turn determines the rate of glycogen synthesis (Stalmans, 1976; Curnow & Larner, 1979; Hers, 1981).

Diabetic animals maintain a low amount of synthase *a* in the liver in spite of an elevated glucose concentration, and they do not respond to a glucose load (Steiner, 1966; Kreutner & Goldberg, 1967; Hornbrook, 1970). The exact lesion in diabetes has, however, not been established. Our purpose was to study the effects of duration and severity of the diabetes on the glycogenic response of isolated hepatocytes to glucose.

Experimental

Biochemicals

Collagenase (type I) and alloxan monohydrate were obtained from Sigma Chemical Co.; streptozotocin was a gift from the Upjohn Co. The source of other relevant products has been described (Doperé *et al.*, 1980).

Animals

We used male Wistar rats weighing about 250 g and fed *ad libitum*. Unless otherwise stated, diabetes was induced under light diethyl ether anaesthesia by an intravenous injection of alloxan (50 mg/kg body wt.) at 17 h. The diabetic rats were used either 40 h ('acute') or 90 h ('chronic') later. In the experiments shown in Fig. 4 the amount of injected alloxan was varied between 35 and 50 mg/kg, and the rats were used 90 h later; animals treated with streptozotocin (50 mg/kg, intravenously) were used after 1–2 weeks.

Hepatocytes

These were isolated as described by Hue *et al.* (1975), with a few modifications. Heparin was not used. The liver was removed from the rat, preperfused without recirculation with 150 ml of Krebs–Henseleit (1932) bicarbonate buffer without Ca^{2+} and Mg^{2+} , and then perfused with 100 ml of recirculating medium. Collagenase (50 mg) was added after 10 min and the perfusion terminated about 20 min later. After filtration through nylon tissue (100 mesh), the cells were washed three times by sedimentation for 30 s at 50 g from a 10-fold excess of unmodified Krebs–Henseleit bicarbonate buffer, pH 7.4. Samples (10^7 cells in 2 ml) were incubated in closed plastic vials (20 ml) with linear shaking (120 strokes/min) under an atmosphere of O_2/CO_2 (19:1). The hepatocytes were always preincubated for 15 min with 2 mM-glucose before an additional amount of glucose was added. In the regular incubations, the extracellular medium contained 144 mM- Na^+ and 6 mM- K^+ (Krebs & Hen-

seleit, 1932); the 'K⁺-rich medium' contained 89 mM-Na⁺ and 60 mM-K⁺.

Assays

Plasma glucose was measured by the glucose oxidase method (Dahlqvist, 1961). For the enzyme

assays 50 μ l of cell suspension was added to 10 μ l of a solution containing 60 mM-glycylglycine, 3% (w/v) glycogen, 0.6 M-KF and 0.12 M-EDTA, pH 7.4, and frozen in liquid N₂. After thawing, glycogen synthase *a* and *a*+*b* were measured as described by Doperé *et al.* (1980), except for the use of 20 μ l of

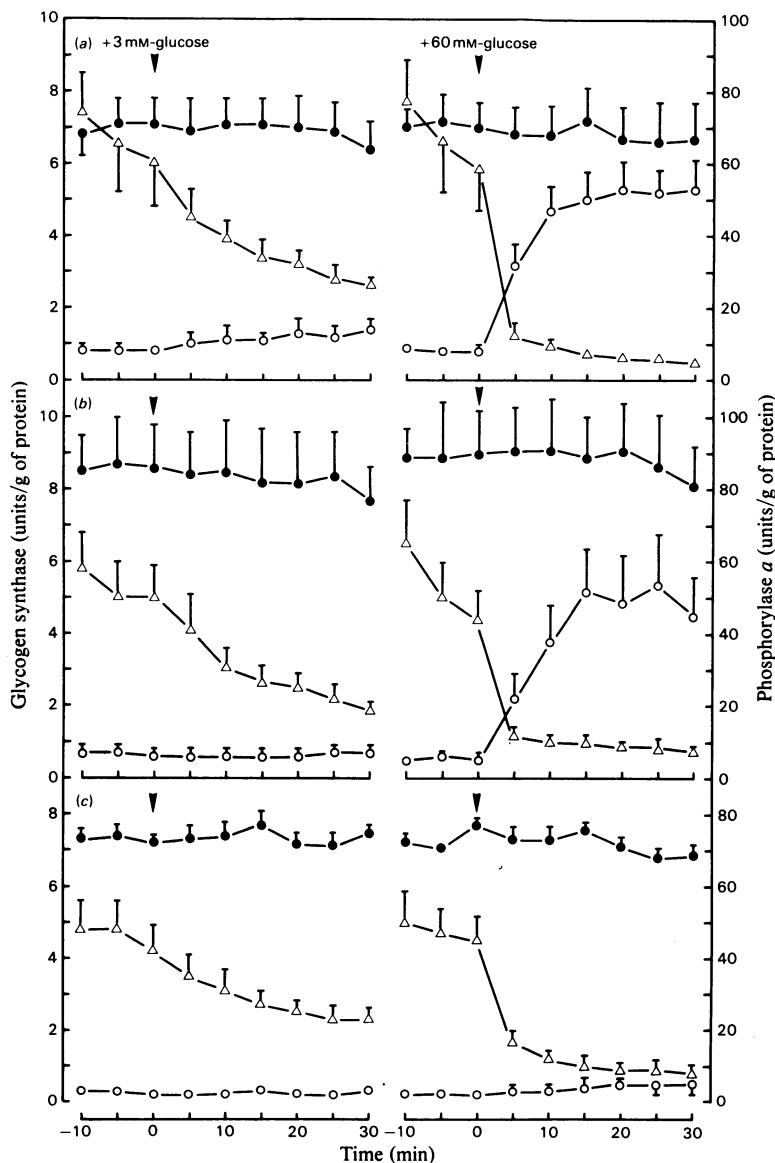


Fig. 1. Effects of two glucose concentrations on the inactivation of phosphorylase and the activation of glycogen synthase in hepatocytes from normal and diabetic rats

Cells were preincubated for 15 min with 2 mM-glucose before addition (arrow) of 3 mM-glucose (left panel) or 60 mM-glucose (right panel). (a) Intact rats (plasma glucose = 10.6 ± 0.6 mM; $n = 5$); (b) acutely alloxan-diabetic rats (plasma glucose = 48.9 ± 3.9 mM; $n = 4$); (c) chronically alloxan-diabetic rats (plasma glucose = 50.6 ± 11.7 mM; $n = 4$). Δ , Phosphorylase *a*; \circ , synthase *a*; \bullet , synthase *a*+*b*. Control cell preparations incubated with 50 nM-glucagon for 10 min contained 103 ± 12 units of phosphorylase *a*/g of protein ($n = 4$).

sample and incubation for 30 min. Phosphorylase *a* was measured as described by Hue *et al.* (1975), but at 25°C.

Results are expressed as means \pm S.E.M. for observations on the indicated numbers (*n*) of animals or cell preparations.

Results

Fig. 1 shows effects of two extreme glucose concentrations on the key enzymes of glycogen metabolism in isolated hepatocytes from normal and diabetic rats. In all conditions phosphorylase was partially inactivated in the presence of 5 mM-glucose, and near-completely at a very high glucose concentration (60 mM). Activation of glycogen synthase in normal hepatocytes was pronounced at the high glucose concentration (Fig. 1*a*). The latter response was somewhat slower, and its extent slightly blunted, in hepatocytes from acutely diabetic rats (Fig. 1*b*). In contrast, almost no activation of synthase occurred in diabetes of longer standing but otherwise similar severity, as judged by the extent of hyperglycaemia (Fig. 1*c*).

The effect of intermediate glucose concentrations is illustrated in Fig. 2. The inactivation of phos-

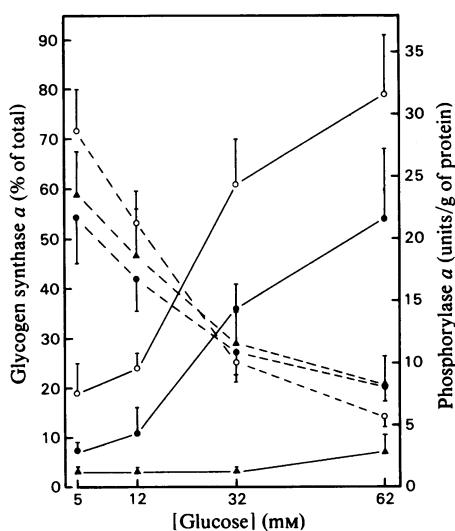


Fig. 2. Final activities of phosphorylase *a* and synthase *a* in hepatocytes incubated with various glucose concentrations

The same hepatocyte preparations were used as in Fig. 1. O, Normal rats; ●, acutely alloxan-diabetic rats; ▲, chronically alloxan-diabetic rats. —, Concentration of phosphorylase *a*, 20–30 min after incubation with the indicated amount of glucose; —, percentage of synthase in the *a* form during the same time period.

phorylase was similarly sensitive to glucose in all conditions. The activation of synthase in hepatocytes from control and acutely diabetic rats appeared also similarly sensitive to glucose, although in the latter case the maximal response at 60 mM-glucose was somewhat decreased (see also Fig. 1). In severe chronic diabetes there was virtually no response of synthase to glucose throughout.

When the concentration of extracellular K^+ is increased at the expense of Na^+ , the inactivation of phosphorylase, and accordingly the activation of synthase, are facilitated in normal hepatocytes (Hue *et al.*, 1975). When hepatocytes from chronic severely diabetic rats were incubated in a K^+ -rich medium, the activity of phosphorylase *a* reached after 30 min of incubation at glucose concentrations between 5 and 60 mM was 20–30% lower than the values in Fig. 2 (results not shown). However, the activation of glycogen synthase was not improved.

Fig. 3 shows the maximal activation of glycogen synthase (at 60 mM-glucose) in hepatocytes isolated at various intervals after administration of alloxan (50 mg/kg). Beyond 40 h the capacity for activation of synthase decreased rapidly, and became negligible after 3 days. The glycaemia was uniformly high from 40 h onwards.

In contrast, further work on chronically diabetic rats indicated that the defect in the conversion of synthase *b* into *a* is related to the severity of the diabetic state (Fig. 4): there is a significant inverse correlation between the maximal extent of synthase

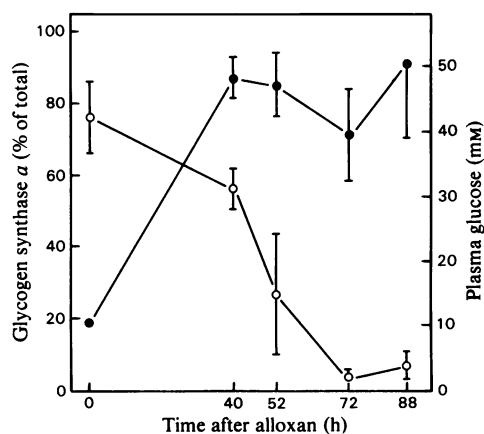


Fig. 3. Maximal degree of synthase activation in hepatocytes isolated at various times after injection of alloxan. At the indicated times after injection hepatocytes were isolated ($n=3-5$) and incubated in the presence of 60 mM-glucose. O, Percentage of synthase in the *a* form 20–30 min after addition of glucose. ●, Plasma glucose concentration at the time of death. Control rats (0 h) were not injected.

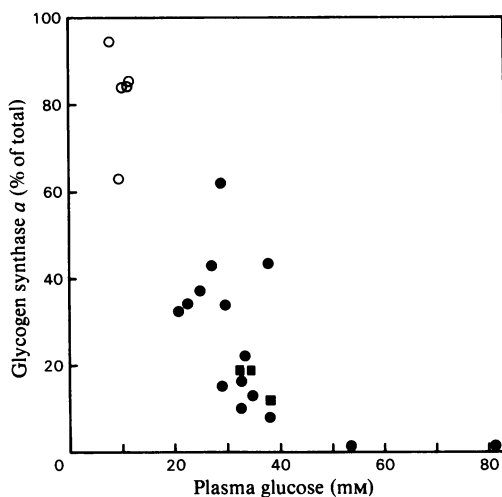


Fig. 4. Relationship between the glycaemia and the maximal degree of synthase activation in isolated hepatocytes from rats with chronic diabetes of variable severity

Hepatocytes were incubated with 60 mM-glucose; synthase *a* (as % of total) is the mean value recorded after 20–30 min. ○, Normal rats; ●, 90 h after injection of various amounts of alloxan; ■, chronically streptozotocin-diabetic rats. Linear regression coefficient (up to a glycaemia of 40 mM): $r = 0.85$; $P < 0.001$.

activation in isolated hepatocytes, and the plasma glucose concentration (up to 40 mM); above the latter value the activation of synthase was totally impaired.

Discussion

Miller and co-workers have reported that the control of phosphorylase and synthase activities by glucose is lost in livers isolated from rats made diabetic for 2–6 days (Miller *et al.*, 1973; Miller, 1978). More recently, however, they reported that glucose did induce some inactivation of phosphorylase, and speculated that the degree of diabetes could be a possible explanation (Miller, 1979). Our results do not substantiate the latter proposal: the glucose-promoted inactivation of phosphorylase occurred normally, irrespective of either the duration or the severity of the diabetic state. We presume that in earlier work changes in phosphorylase activity have been obscured by an assay that was not specific for the *a* form of phosphorylase.

We observe further that glucose can still produce an important activation of glycogen synthase in hepatocytes from recently (40h) diabetic rats. Paradoxically, glycogen synthase remains inactive in the liver of the acutely diabetic animal in spite of the

high prevailing glucose concentration (see the Introduction). This means that there is no significant defect in the liver of the acutely diabetic animal, and that the absence of hepatic glycogen synthesis *in vivo* is largely explained in terms of extrahepatic changes. Consonant with this conclusion are the observations that acutely diabetic animals respond to insulin with activation of hepatic synthase within 5–15 min (Gold, 1970; Bishop *et al.*, 1971; Nichols & Goldberg, 1972; Miller & Larner, 1973; Hers *et al.*, 1974).

In chronically diabetic animals there is an intrahepatic defect as well. It is limited to the activation of glycogen synthase, and its importance correlates with the severity of the diabetic state. Restoration of this defect requires hours rather than minutes of insulin treatment before activation of glycogen synthase or glycogen synthesis occurs (Steiner, 1966; Bishop *et al.*, 1971; Miller, 1978, 1979). The exact nature of the defect has not been established. Current proposals include a decreased synthase phosphatase activity (Tan & Nuttall, 1976; Miller *et al.*, 1981), as well as an alteration in glycogen synthase *b*, which would then be a poor substrate for synthase phosphatase (Tan & Nuttall, 1976; Akatsuka *et al.*, 1982). Evidence against the latter proposal has been presented by Bahnak & Gold (1982).

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