The activation of glycogen synthase in hepatocytes from rats with a glycogen storage disorder (*gsd/gsd*)

Colin Watts, Jane R. Redshaw and Kevin R. Gain

Department of Clinical Biochemistry, Medical School, University of Otago, P.O.Box 913, Dunedin, New Zealand

Received 14 June 1982

1. INTRODUCTION

Rats from the NZR/Mh strain have a genetically-determined deficiency of phosphorylase *b* kinase activity in the liver which in homozygotes (*gsd/gsd*) produces a poor activation of glycogen phosphorylase and very high liver glycogen concentrations [1]. They also have low active glycogen synthase activities [1] and previous studies on liver homogenates have shown an inhibition of glycogen synthase–phosphatase by high glycogen concentrations [2]. This appears to be the major control of glycogen synthesis in liver of *gsd/gsd* and fed normal animals [2] rather than the control of glycogen synthase–phosphatase by active glycogen phosphorylase [3]. It is of interest to know if, and to what extent, glycogen synthase in *gsd/gsd* liver can be activated. Here, we describe the effects of glucose on the active forms of glycogen synthase and phosphorylase in hepatocytes from *gsd/gsd* and normal livers. In *gsd/gsd* cells with high glycogen levels, the activation of glycogen synthase in response to 80 mM glucose was blunted despite the very low active phosphorylase activities and was inversely correlated with the glycogen content of the cells. The findings do not support the concept of phosphorylase inactivation being a prerequisite for activation of glycogen synthase [3].

2. MATERIALS AND METHODS

Hepatocytes were prepared [4] from male rats of a Wistar-derived normal strain and the NZR/Mh strain. Livers were perfused with a calcium-free Krebs-Henseleit–Hepes buffer (pH 7.4) containing 7.3 mM glucose, 3.3 mM pyruvate, 2.3 mM glutamate with 0.3 mg/ml collagenase (Sigma Chemical Co., St Louis MO). After 30 min, the liver was minced and incubated for 15 min at 37°C in a medium containing 2.5 mM calcium, 25 mM glucose and 1.5% gelatin in the above buffer. The dispersed cells were then filtered through gauze and washed 3 times with glucose-free incubation medium.

Cell suspensions were diluted 1:10 with incubation medium and preincubated at 37°C for 15 min with constant oxygenation (100% O_2) before incubation with glucose. Incubations were started by adding cell suspension to flasks containing glucose at 10–80 mM final conc. Following incubation, 0.5 ml aliquots for assay of enzymes and glycogen were added to 0.5 ml 200 mM Tris–HCl (pH 7.8) containing 20 mM EDTA, 200 mM NaF and 20 mM cysteine and frozen in an alcohol/solid CO_2 bath. Aliquots from each flask were also spotted on millipore filter discs under suction and dried to constant weight for determination of a dry cell weight.

Enzyme activities and glycogen content were measured on samples after thawing. Sonication of the samples did not improve the yield of enzymes or glycogen. One freeze/thaw treatment gave identical yields of active forms of the enzymes compared with homogenization of the cell suspensions in a Dounce hand homogenizer. In comparison, 75–85% of the inactive enzymes were recovered. Glycogen synthase was measured directly on the thawed cell homogenate as in [5]. Phosphorylase was measured on a 1:5 dilution of the cell homogenate in 200 mM glycerophosphate buffer, pH 6.1 containing 5 mM EDTA, 200 mM NaF and 400

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

mM sucrose. Active phosphorylase was measured at 37°C as in [1]. Inactive phosphorylase was measured under the same incubation conditions but without caffeine and in the presence of 5 mM 5′-AMP and 10% 1:2-dimethoxyethane. The solvent activation of inactive phosphorylase [6] gave the same results as activation by phosphorylase kinase. For all enzymes, 1 unit of activity was that amount which catalyzed the incorporation of 1 μmol substrate into glycogen/min. Results are expressed as units/g dry wt of cells. Glycogen was measured on an aliquot of the cell homogenate by an amyloglucosidase method [1], and the values expressed as glucose equivalents.

3. RESULTS

The changes in the active forms of glycogen synthase and phosphorylase in response to various glucose concentrations are shown in table 1. Values for the total (active + inactive) activities of each enzyme are not shown as they remained constant for each group. In the absence of glucose, active glycogen synthase represented 2% of the total in normal and 0.8% in *gsd/gsd* hepatocytes, while active phosphorylase represented 34% in normal cells and 5% in *gsd/gsd* cells. In normal cells, basal active phosphorylase was high, but a significant decrease in activity was seen at 20 mM glucose ($P < 0.001$ compared with zero glucose) and was reduced by 80% at 60 mM glucose. Active phosphorylase was very low in hepatocytes from *gsd/gsd* animals but the residual activity was still inhibited 80% by glucose. The activation of glycogen synthase was not so

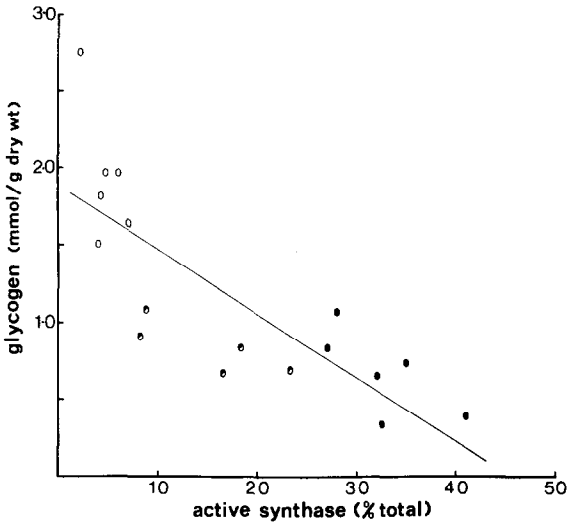

Fig.1. Relationship between activation of glycogen synthase by 80 mM glucose and the glycogen content of hepatocytes from *gsd/gsd* (○), fed normal (●) and 24 h starved/24 h refed (◐) rats. Cells were incubated with glucose for 10 min.

Table 1

Response of hepatocytes from fed normal and *gsd/gsd* rats to glucose

	Glucose (mM)					
	0	10	20	40	60	80
No.	6	4	5	6	6	6
Active synthase						
Normal	0.13 ±0.01	0.17 ±0.01	0.23 ±0.03	0.67 ±0.05	1,36 ±0.09	2.06 ±0.20
gsd/gsd	0.06 ±0.01	0.08 ±0.003	0.14 ±0.02	0.18 ±0.03	0,25 ±0.03	0.30 ±0.02
Active phosphorylase						
Normal	36.2 ±2.2	28.4 ±6.0	16.2 ±2.4	9.8 ±1.6	6,8 ±0.3	6.9 ±1.8
gsd/gsd	5.4 ±0.6	4.3 ±0.7	2.5 ±0.4	1.8 ±0.4	1,0 ±0.1	1.0 ±0.3

Cells incubated with glucose for 15 min; values are mean ± SEM and units/g dry wt cells

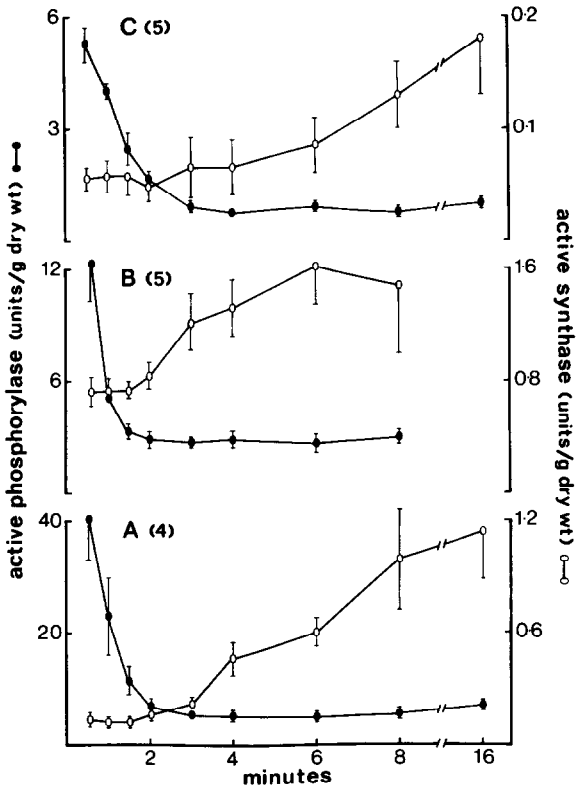

Fig.2. Active glycogen synthase (○) and phosphorylase (●) during incubation of cells with 40 mM glucose from (A) fed normal, (B) 24 h starved normal and (C) fed *gsd/gsd* rats. Number of animals are shown in parentheses. Results plotted as mean with bars representing SEM.

responsive to low glucose concentrations. Although a significant increase was seen with both types of cells at 20 mM glucose ($P < 0.02$ compared with zero glucose), the response at higher glucose concentrations was far less in the *gsd/gsd* hepatocytes with only a 5-fold increase at 80 mM glucose compared with a 20-fold increase in normal cells.

The activation of glycogen synthase by 80 mM glucose was inversely correlated ($r = -0.82$, $P < 0.001$) with the initial glycogen content of *gsd/gsd* and normal hepatocytes (fig.1). There was no measurable change in glycogen concentration in any hepatocyte preparation after exposure to glucose. The relationship between the activation of glycogen synthase and inactivation of phosphorylase was studied during exposure of the cells to 40 mM glucose. In hepatocytes from fed normal rats (fig.2A), active phosphorylase fell rapidly to a minimum at 2 min whereas active glycogen synthase did not rise significantly until 3 min ($P < 0.02$ compared with initial values). In hepatocytes from starved normal rats (fig.2B) initial active phosphorylase activities were < ⅓rd and active glycogen synthase activities 4-times higher than those in cells from fed animals. However, the response to glucose was identical to that of the cells from fed animals with a rapid inactivation of phosphorylase and a delayed activation of glycogen synthase. The starved cells did not tolerate exposure to 40 mM glucose for > 10 min as they showed loss of cell contents by a reduction in the dry cell wt/0.5 ml aliquot and a marked activation of phosphorylase. In *gsd/gsd* hepatocytes (fig.2C), the initial activities of active phosphorylase were the same as the maximally-depressed ones in the fed cells. However, the residual activity was still reduced rapidly by glucose to almost zero by 3 min but despite the very low active phosphorylase activities, activation of glycogen synthase showed a prolonged lag phase after glucose and did not rise significantly until 8 min ($P < 0.05$ compared with initial values). Again, no significant change in hepatocyte glycogen concentration was detected after the 16 min incubation.

4. DISCUSSION

The glucose effects on fed normal hepatocytes reported here support the concept of glucose directly stimulating liver glycogen synthesis by activation of glycogen synthase [7,8]. The mechanism whereby glucose achieves these effects, however, is debatable. Glucose is said to act by binding to active phosphorylase, producing a conformational change which renders the enzyme more susceptible to inactivation by phosphorylase phosphatase [9]. Active phosphorylase is considered to be a potent inhibitor of glycogen synthase–phosphatase from studies in vivo using fed animals [10,11] and using isolated hepatocytes [12,13]. Therefore, an inactivation of this enzyme is said to be required before stimulation of glycogen synthase–phosphatase and activation of glycogen synthase can occur [3]. However, there are situations where this relationship does not hold, e.g., in livers of starved animals [12,14], hepatocytes from starved animals [8,15] and in vitro [16,17]. Our findings with hepatocytes from *gsd/gsd* animals do not indicate that the activation

of glycogen synthase is controlled by active phosphorylase. The *gsd/gsd* cells have a normal phosphorylase, but the active form remains very low because of the phosphorylase kinase deficiency. Despite this, initial active glycogen synthase activity was also very low and there was still an obvious lag phase in the activation of the enzyme by glucose (fig.2C). In addition, the lag phase in the activation of glycogen synthase in response to glucose in hepatocytes from starved animals with low active phosphorylase levels (fig.2B) is not consistent with phosphorylase control of glycogen synthase activation.

The smaller and slower response of glycogen laden *gsd/gsd* hepatocytes to glucose activation of glycogen synthase (table 1) and the correlation of glycogen synthase activation with glycogen concentration in these and normal cells (fig.1) strongly supports the concept of glycogen having a major role in the control of its own rate of synthesis as suggested previously for liver [2,18] and other tissues [19,20].

Finally, it is of interest to note that, although the *gsd/gsd* hepatocytes have very low active phosphorylase, the enzyme still responds rapidly to glucose. Preliminary results (not shown) indicate that this decrease can be reversed by glucagon, suggesting that the *gsd/gsd* rat may not have a complete absence of liver phosphorylase *b* kinase.

ACKNOWLEDGEMENTS

This work is supported by a grant from the Medical Research Council of New Zealand. We thank Mr R. Malthus for breeding the *gsd/gsd* rats.

REFERENCES

[1] Malthus, R., Clark, D.G., Watts, C. and Sneyd, J.G.T. (1980) Biochem. J. 188, 99–106.
[2] Watts, C. and Malthus, R. (1980) Eur. J. Biochem. 108, 73–77.
[3] Hers, H.-G. (1976) Annu. Rev. Biochem. 45, 167–189.
[4] Loten, E.G., Francis, S.H. and Corbin, J.D. (1980) J. Biol. Chem. 255, 7838–7844.
[5] Watts, C. and Gain, K.R. (1976) Biochem. J. 160, 263–270.
[6] Uhing, R.J., Janski, A.M. and Graves, D.J. (1979) J. Biol. Chem. 254, 3166–3169.
[7] Mulmed, L.N., Gannon, M.C., Gilboe, D.P., Tan, A.W.H. and Nuttall, F.Q. (1979) Diabetes 28, 231–236.
[8] Katz, J., Golden, S. and Wals, P.A. (1979) Biochem. J. 180, 389–402.
[9] Madsen, N.B., Kasvinsky, P.J. and Fletterick, R.J. (1978) J. Biol. Chem. 253, 9097–9101.
[10] Stalmans, W., De Wulf, H. and Hers, H.-G. (1974) Eur. J. Biochem. 41, 127–134.
[11] Van de Werve, G., Stalmans, W. and Hers, H.-G. (1977) Biochem. J. 162, 143–146.
[12] Hue, L., Bontemps, F. and Hers, H.-G. (1975) Biochem. J. 152, 105–114.
[13] Witters, L.A. and Avruch, J. (1978) Biochemistry 17, 406–410.
[14] Curnow, R.T. and Nuttall, F.Q. (1972) J. Biol. Chem. 247, 1892–1898.
[15] Cuidal, C.J., Massague, J. and Guinovart, J.J. (1979) FEBS Lett. 99, 321–324.
[16] Gilboe, D.P. and Nuttall, F.Q. (1978) J. Biol. Chem. 253, 4078–4081.
[17] Van de Werve, G. (1981) Biochem. Biophys. Res. Commun. 102, 1323–1329.
[18] Wititsuwannakul, D. and Kim, K.-H. (1979) J. Biol. Chem. 254, 3562–3569.
[19] Danforth, W.H. (1965) J. Biol. Chem. 240, 588–593.
[20] Saheki, R. and Tsuiki, S. (1968) Biochem. Biophys. Res. Commun. 31, 32–36.