GLYCOGEN SYNTHESIS IN ISOLATED PARENCHYMAL RAT LIVER CELLS

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1. Introduction

The synthesis of liver glycogen and the activity of glycogen synthetase (EC 2.4.1.11) are strongly stimulated by glucose both *in vivo* [1, 2], in the perfused liver [3, 4] and in liver homogenates [5]. Glucose, besides being a substrate for glycogen synthesis, appears to activate glycogen synthetase phosphatase (activating enzyme) by an as yet incompletely characterized mechanism [6]. Insulin and glucocorticoid hormone also stimulate glycogen formation *in vivo*, but it is not clear to what extent these hormones interact and how their actions are related to the glucose effect [6, 7].

The availability of intact parenchymal rat liver cells in large quantities [8–10] offers an opportunity to study the regulation of glycogen synthesis in a well-defined *in vitro* system. These cells have previously been shown to respond to both glucocorticoid [11] and polypeptide hormone [12], and in the present communication their capacity for glucose-stimulated glycogen synthesis is demonstrated.

Abbreviations:
HEPES = *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; TES = *N*-Tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid; Tricine = *N*-Tris-(hydroxymethyl) methylglycine.

2. Materials and methods

Male Wistar rats (250–300 g) were maintained on a controlled feeding and illumination schedule [13] unless otherwise stated. Suspensions of rat liver cells were prepared by perfusion of the isolated liver with collagenase (hyaluronidase omitted) essentially as previously described [9, 10]. The parenchymal cells were purified by differential centrifugation [11] and suspended at a concentration of 8–10 × 10^6 cells/ml (80–100 mg/ml) in suspension buffer, pH 7.6 (37°) of the following composition: 4.0 g NaCl, 0.4 g KCl, 0.15 g KH_2PO_4, 0.1 g Na_2SO_4, 0.13 g $MgCl_2 \cdot 6\,H_2O$, 0.18 g $CaCl_2 \cdot 2\,H_2O$, 7.2 g HEPES, 6.9 g TES, 6.5 g Tricine, 2.1 g NaOH and H_2O *ad* 1000 ml. The final yield of purified cells was in the range 30–50%; more than 95% of the cells were parenchymal, and 80–90% of these were intact by the trypan blue exclusion test.

0.5 ml aliquots of cell suspension were pipetted into 15 ml centrifuge tubes and incubated at 37° in a gyrorotatory shaker at 215 rpm. All added components were in isotonic solution (280–290 mosM), and the final incubation volume never exceeded 0.65 ml. Reactions were stopped by the addition of 0.2 ml 10% perchloric acid (PCA), and after two washings of the precipitates with 0.5 ml 2% PCA at 4°, the combined PCA extracts were used for analysis of acetoacetate and β-hydroxybutyrate by enzymatic methods [14] and glucose and lactate as previously described [13]. Glycogen was also completely extracted by PCA, and could be precipitated by ethanol [15], hydrolyzed in 0.5 M H_2SO_4 and determined as glucose.

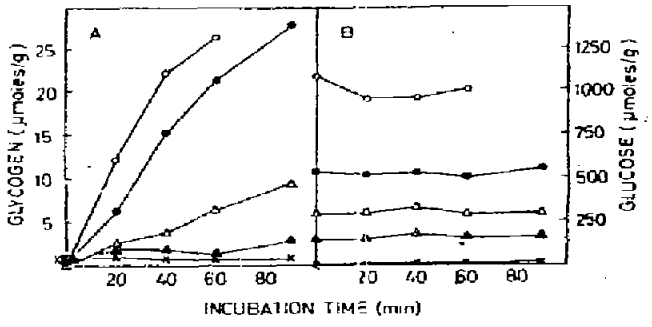

Fig. 1. Glycogen synthesis by isolated parenchymal rat liver cells. Freshly prepared cells were incubated in suspension buffer without glucose (X—X—X) or with the following concentrations of glucose: 10 mM (▲—▲—▲), 20 mM (△—△—△) 35 mM (●—●—●) or 70 mM (○—○—○). The content of glycogen (A) and glucose (B) in the total system (cells + medium) has been expressed as μmoles of glucose equivalents per g liver cells (wet weight).

The PCA-precipitates were used for analysis of protein [13], DNA and RNA [16] as previously described. Wet weights were obtained from cell samples pelleted by centrifugation at 3000 rpm for 5 min.

Insulin (beef insulin mono-component, lot no. MC-0-1070) was a gift from Novo. All other biochemicals were purchased from Sigma.

3. Results

The liver cells used in these experiments were prepared from animals taken at the end of a 16 hr fasting period, when glycogen levels are at a minimum [17]. Glycogen is further broken down during the cell isolation procedure, which involves perfusion, incubation and several centrifugations in the absence of substrate (altogether approx. 1 hr). Liver glycogen at the beginning of an experiment was therefore low (< 1 μmole/g) or undetectable.

Liver cells incubated with glucose (0—70 mM) accumulated glycogen in a dose-dependent manner (fig. 1A). The rate of glycogen formation at the higher concentrations of glucose (usually 50 mM) was in the range 15–30 μmoles/g per hr, which is comparable to rates found *in vivo* [1] and in the perfused liver [3]. In the absence of added glucose, no glycogen was synthesized. Instead the liver formed glucose at a rate of 5–7 μmoles/g per hr, which is only about 5% of the glucogenic rate found in glycogen-containing perfused livers [13].

Table 1
Effect of glucose on the metabolism of isolated rat liver cells.

	Metabolic change 0–90 min (μmoles/g)	
	− Glucose	+ Glucose
Glucose	+ 10.5 ± 1.1 (9)	− 86.2 ± 15.8 (10)
Glycogen (as glucose)	Undetectable	+ 25.0 ± 2.4 (10)
Lactate	+ 3.3 ± 0.7 (5)	+ 22.0 ± 0.9 (5)
Ketones (acetoacetate + β-hydroxybutyrate)	+ 36.4 ± 1.9 (5)	+ 32.6 ± 1.2 (5)
	Final value at 90 min (mg/g)	
DNA	1.83 ± 0.03 (5)	1.74 ± 0.05 (5)
RNA	11.2 ± 0.3 (5)	11.5 ± 0.1 (5)
Protein	261 ± 11 (5)	265 7 (5)

Freshly prepared parenchymal rat liver cells were incubated for 90 min in suspension buffer with or without 50 mM glucose, and the metabolite levels of the total system (cells + medium) were measured at 0 and 90 min. Metabolic changes during this period (+ denotes net accumulation; − denotes net consumption) have been expressed in the table as μmoles per g liver cells (mean ± S.E.) with the number of incubated samples in brackets. The content of DNA, RNA and protein was measured at the end of incubation.

There was no measurable net consumption of glucose by the liver cells at glucose concentrations below 50 mM (fig. 1B), which might suggest that endogenous substrates rather than glucose were being used for glycogen synthesis. However, glycogen formation accounted for only 2–3% of the total glucose present in the system, which is within the limits of experimental variability. At 50 mM glucose, a significant net utilization of glucose could usually be detected, accompanied by a significant increase in lactate formation (table 1). The rate of ketone body formation was not affected by glucose at this concentration, and the cellular content of DNA, RNA and protein remained constant during 90 min both in the presence and absence of glucose (table 1).

Glucose at 50 mM concentration *plus* possible endogenous substrate appeared to support near-maximal rates of glycogen synthesis, since further

Table 2
Effects of cycloheximide and lactate on glycogen formation in isolated rat liver cells.

Substance added	Glycogen formed (μmoles/g)	Significance of effect by the *t*-test
None	20.8 ± 2.9 (5)	
Lactate	22.1 ± 2.8 (5)	N.S.
Cycloheximide	12.1 ± 0.6 (5)	$P < 0.02$

Freshly prepared parenchymal rat liver cells were incubated for 90 min in suspension buffer containing glucose (50 mM) and lactate (5.6 mM) or cycloheximide (1 mM) as indicated. The amount of glycogen formed during the incubation period has been expressed as μmoles (glucose equivalents) per g liver cells (mean ± S.E.) with the number of incubated samples in brackets.

provision of gluconeogenic substrate by the addition of lactate (5.6 mM) did not affect glycogen formation (table 2).

Glucose still stimulated glycogen synthesis in the presence of cycloheximide (table 2), which means that new protein synthesis is not necessary for this effect, since cycloheximide at the concentration used (1 mM) inhibits protein synthesis by 90% (unpublished observation). However, the fact that cycloheximide reduced glycogen accumulation by 40% during 90 min indicates that protein components with fairly rapid turnover may be involved in glucose-stimulated glycogen synthesis.

Insulin had no effect on glycogen formation in the usual experimental system (table 3). The hormone was ineffective over a wide concentration range, and was also without effect when lower concentrations of glucose were used. However, if cells were prepared from rats starved for 3 days, a significant stimulation of glycogen formation by insulin could be observed at high concentrations of glucose (table 3). The rate of glycogen formation both in the presence and absence of insulin was conspicuously low in these starved cells, indicating a glycogen-synthetic lesion (e.g. the loss of a rapidly turning over protein component as mentioned above) which was only partially repaired by insulin.

Table 3
Effect of insulin on glycogen formation in liver cells from starved and non-starved rats.

Animal treatment	Glycogen formed (μmoles/g) − Insulin	+ Insulin	Significance of insulin effect by the *t*-test
Starved 72 hr	1.8 ± 0.1 (3)	3.1 ± 0.3 (3)	$P < 0.01$
Non-starved	41.8 ± 3.2 (3)	44.5 ± 2.3 (3)	N.S.

Freshly prepared parenchymal rat liver cells were incubated for 90 min in suspension buffer containing 50 mM glucose; with or without insulin (5×10^{-6} M). The amount of glycogen formed during the incubation period has been expressed as μmoles of glucose equivalents per g liver cells (mean ± S.E.) with the number of incubated samples in brackets.

4. Discussion

Glycogen synthetase, the rate-limiting enzyme in glycogen formation, exists in an inactive phosphorylated form and an active dephosphorylated form. Activation is catalyzed by the enzyme glycogen synthetase phosphatase, the activity of which is subject to control by glucose and hormones [6, 7]. According to Hers [6] this control is exerted at the level of glycogen phosphorylase phosphatase, which catalyzes the conversion of the active phosphorylase *a* (phosphorylated) to inactive phosphorylase *b* (dephosphorylated), and thereby relieves the direct inhibition of glycogen synthetase phosphatase by phosphorylase *a*.

The present study demonstrates that glucose stimulates glycogen synthesis by a direct effect on parenchymal liver cells in the complete absence of hormones and other exogenous agents. The glucose effect does not require protein synthesis, but the metabolic lability of a participating protein which is indicated by the partial cycloheximide inhibition may explain why the glucose effect almost disappears during starvation. The labile component may be glycogen synthetase phosphatase, which has been shown to disappear during starvation or cycloheximide treatment *in vivo* [2]. Glucose restored the enzyme activity in the starved animals; an effect which was blocked by cycloheximide [2]. This *in vivo*-effect of glucose may have been caused by insulin release, since the

hormone displays a similar effect – albeit of small magnitude – on glycogen synthesis in isolated liver cells *in vitro*. The absence of an insulin effect on normal liver cells may be due to an already maximal ability to activate glycogen synthetase.

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References

[1] H. De Wulf and H.G. Hers, European J. Biochem. 2 (1967) 50.
[2] K. Grühner and H.L. Segal, Biochim. Biophys. Acta 222 (1970) 508.
[3] H. Buschiazzo, J.H. Exton and C.R. Park, Proc. Natl. Acad. Sci. U.S 65 (1970) 383.
[4] W. Glinsmann, C. Pauk and E. Hern, Biochem. Biophys. Res. Commun. 39 (1970) 774.
[5] H. De Wulf, W. Stalmans and H.G. Hers, European J. Biochem. 15 (1970) 1.
[6] H.G. Hers, H. De Wulf and W. Stalmans, FEBS Letters 12 (1970) 73.
[7] J. Larner and C. Villar-Palasi, Curr. Top. Cell. Regul. 3 (1971) 195.
[8] M.N. Berry and D.S. Friend, J. Cell Biol. 43 (1969) 506.
[9] P.O. Seglen, Exptl. Cell Res. 74 (1972) 450.
[10] P.O. Seglen, Exptl. Cell Res., in press.
[11] T. Berg, D. Boman and P.O. Seglen, Exptl. Cell Res. 72 (1972) 571.
[12] T. Christoffersen, J. Mørland, J.B. Osnes, T. Berg, D. Boman and P.O. Seglen, Arch. Biochem. Biophys. 150 (1972) 807.
[13] P.O. Seglen, Biochim. Biophys. Acta 264 (1972) 398.
[14] H.U. Bergmeyer, Methods of Enzymatic Analysis (Academic Press, New York and London, 1965).
[15] O. Walaas and E.Walaas, J. Biol. Chem. 187 (1950) 769.
[16] T. Berg, J. Mørland and P.O. Seglen, Hoppe-Seyler's Z. Physiol. Chem. 351 (1970) 898.
[17] M. Watanabe, V.R. Potter and H.C. Pitot, J. Nutrition 95 (1968) 207.