EFFECTS OF ANAEROBIOSIS, GLUCOSE, INSULIN AND GLUCAGON ON GLYCOGEN METABOLISM IN ISOLATED PARENCHYMAL RAT LIVER CELLS

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1. Introduction

Glycogen metabolism in the liver is controlled by metabolites and hormones in a very complex and as yet incompletely understood manner [1–3]. The role of insulin is particularly obscure. This hormone promotes the accumulation of liver glycogen in vivo [4, 5], and inhibits glycogenolysis in the perfused liver [6–10]. However, the evidence has been very conflicting with regard to a rapid action of insulin on the activation of glycogen synthetase in vivo [5, 11–13] and in the perfused liver [10, 14], particularly because glucose alone can activate the synthetase [1, 14, 15]. Glucagon, on the other hand, inactivates this enzyme [11, 14].

In a previous report [16] it was shown that glucose stimulated glycogen synthesis in isolated rat liver cells, and that insulin promoted glycogen synthesis in cells from 72 hr-starved rats but not in cells from 16 hr-fasted rats. In the present report the control of glycogen metabolism in isolated liver cells from 16 hr-fasted rats is further investigated.

2. Materials and methods

Suspensions of isolated, parenchymal liver cells were prepared from male Wistar rats (250–300 g) as previously described [17–19] and incubated in suspension buffer at pH 7.6 (37°C) [16]. Metabolic analyses were performed as previously described [16]. Insulin and glucagon were gifts from NOVO Industries, Copenhagen.

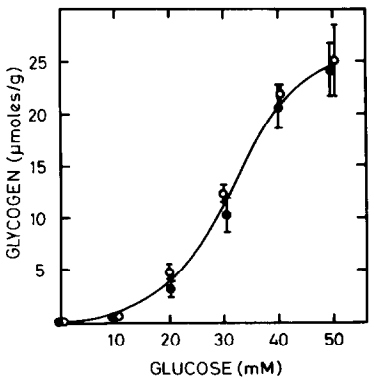

Fig. 1. Effect of insulin on glycogen synthesis at various concentrations of glucose. Isolated parenchymal rat liver cells were incubated for 90 min at various initial concentrations of glucose in the presence (○) or absence (●) of insulin (10^{-5} M), and the net accumulation of glycogen during this period was measured. Each point is the mean ± S.E. of 3 cell samples.

3. Results

It was previously shown that glycogen synthesis in isolated cells was a function of the glucose concentration [16]. At a high concentration of glucose, insulin had no effect on glycogen synthesis in cells from 16 hr-fasted rats [16], but the hyperbolic shape of the dose–response curve suggested that insulin might have an effect at lower glucose concentrations. However, as shown in fig. 1, insulin was ineffective throughout the glucose concentration range 10–50 mM. Insulin at concentrations ranging from 10^{-11} to 10^{-5} M failed to show any effect on either glycogen

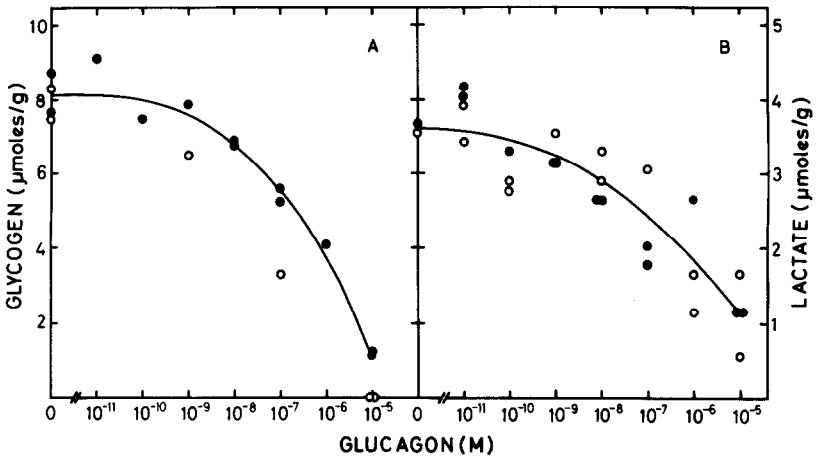

Fig. 2. Dose-dependent inhibition of glycogen and lactate formation by glucagon in the presence and absence of insulin. Isolated parenchymal rat liver cells were incubated from 90 min at 50 mM glucose and glucagon at the concentration indicated in the presence (○) or absence (●) of insulin (10^{-5} M), and the net accumulation of glycogen (A) and lactate (B) during this period was measured. Each point represents a single cell sample.

synthesis, glucose utilization or lactate formation (glycolysis) at 50 mM glucose.

Table 1
Effect of insulin, glucagon and glucose on glycogen degradation in isolated parenchymal rat liver cells.

		Glycogen content (μmoles/g)	
		Experiment 1 + glucose	Experiment 2 − glucose
0 min	No hormone	18.0 ± 0.4 (4)	13.9 ± 2.3 (5)
60 min	No hormone	17.8 ± 2.6 (5)	2.5 ± 0.4 (5)
	Insulin	13.3 ± 4.1 (5)	1.8 ± 0.2 (5)
	Glucagon	5.5 ± 0.8 (5)	0.66 ± 0.03 (5)
	Insulin + glucagon	2.9 ± 0.3 (5)	0.57 ± 0.05 (4)

Isolated liver cells were incubated with 50 mM glucose for 60 min in order to accumulate glycogen, pelleted by centrifugation, and resuspended in fresh suspension buffer. The cells were then incubated for another 60 min in the presence (experiment 1) or absence (experiment 2) of glucose (50 mM), with or without insulin (10^{-5} M) or glucagon (10^{-5} M) as indicated. The glycogen content (glucose equivalents) was measured at the beginning (0 min) and end (60 min) of the second incubation period. Values shown are the means ± S.E. of the number of cell samples given in parentheses. Experiments 1 and 2 represent two different cell preparations with different initial contents of glycogen.

In contrast, glycogen synthesis as well as glycolysis was inhibited by glucagon in a dose-dependent manner (fig. 2). The fact that a very high dose of hormone was required for maximal inhibition may be related to rapid glucagon degradation [20] as well as to the antagonistic action of glucose [14]. Insulin failed to antagonize the action of glucagon on either glycogen synthesis (fig. 2A) or glycolysis (fig. 2B).

In order to test the effects of hormones on glycogen degradation, cells were first loaded with glycogen during a 60 min-incubation in the presence of glucose, and then incubated for another 60 min with hormones (table 1). With glucose present during the second incubation period, the glycogen level was stable in the absence of hormones, whereas net glycogenolysis was observed in the presence of glucagon. In contrast to its action on the perfused liver [6, 7, 9, 10], insulin did not antagonize the action of glucagon.

With no glucose present, glycogen was degraded in the absence of hormones. Glucagon had a slight additional glycogenolytic effect, whereas insulin had no effect on either the basal or the glucagon-stimulated glycogenolysis.

It has been reported that insulin inhibits glycogenolysis in the perfused liver under hypoxic conditions [9]. As shown in fig. 3A, hypoxia (developed by stopping the shaking of the cells and thus their oxygen access) was a strong glycogenolytic stimulus.

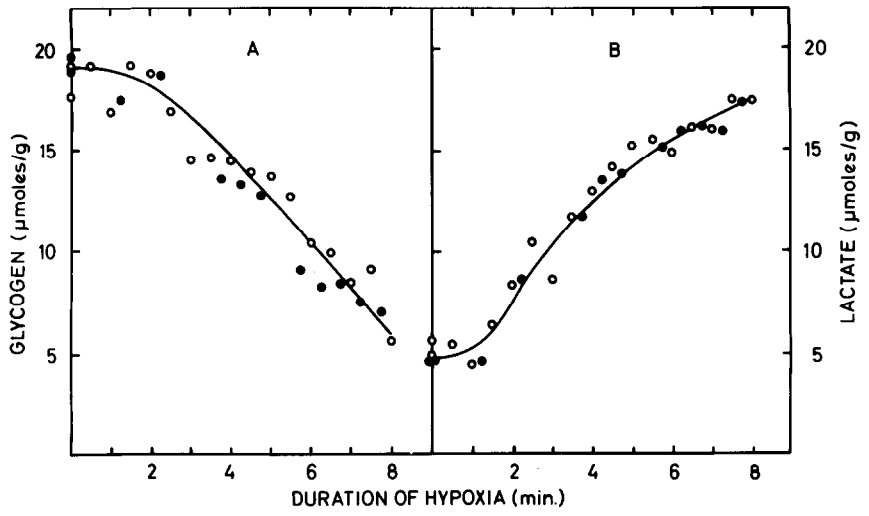

Fig. 3. Glycogen degradation and lactate formation during hypoxia in the presence or absence of insulin. Isolated parenchymal rat liver cells were incubated for 60 min at 50 mM glucose in the presence (○) or absence (●) of insulin (10^{-5} M). Hypoxia was then initiated by stopping the shaking of the cells, and samples were removed at subsequent time points for analysis of glycogen (A) and lactate (B). Each point represents a single sample.

Under hypoxic conditions glycogen was degraded despite the presence of glucose (50 mM), and the disappearance of glycogen was accompanied by an accumulation of lactate (fig. 3B). Insulin had no effect on either glycogen degradation or lactate accumulation.

4. Discussion

The data presented show that insulin, tested under a variety of conditions, has no effect on glycogen metabolism in isolated parenchymal liver cells from 16 hr-fasted adult male rats. In contrast, insulin significantly elevated the very low rate of glycogen synthesis in liver cells from 72 hr-starved adult male rats [16], and recent reports from other laboratories have indicated that insulin can antagonize the glycogenolytic effects of glucagon, epinephrine or dibutyryl-cyclic AMP in liver cells prepared by similar methods from fed, young male rats [21] and fasted young female rats [22]. Thus, the variability of the insulin response noted in vivo [4, 5, 11–13] and in perfused livers [6–10, 14] is not eliminated by the use of isolated liver cells, probably because the properties of the cells to some extent are determined by the biological condition of the liver donor (nutritional state, age, sex, strain etc.). Methodological differences must of course also be considered.

The present data show that glucagon in addition to its well-established glycogenolytic effect inhibits glycogen synthesis by a direct action on isolated liver cells, thus proving the functional significance of the reduced glycogen synthetase activity observed in vivo [11] and in the perfused liver [14]. Glycogen and glucose may be regarded as the primary regulators of glycogen metabolism, whereas insulin, which is known to influence liver protein synthesis [23] and degradation [24] may possibly potentiate the glucose effect by stimulating the formation of the glycogen synthetase phosphatase which is activated by glucose [1]. The rapid turnover of the latter enzyme [25, 26] is compatible with the disappearance of glucose stimulation and the appearance of glycogenic insulin sensitivity upon starvation [16], whereas the enzyme activity may be maximal in lightly-fasted rats.

The present experiments have disclosed an interesting aspect of non-hormonal control of liver glycogen metabolism. Whereas glycogenolysis is inhibited by glucose under aerobic conditions, exposure of the cells to anaerobic conditions (hypoxia) results in rapid glycogen degradation despite the presence of glucose. It thus appears that the control function of glucose

depends upon the presence of oxygen, a regulatory mechanism which may be related to the well-known Pasteur effect [27]. Presumably, under anaerobic conditions the combined effects of increased glycolysis (Pasteur effect) and the presently described removal of glycogenolytic control by glucose may provide a maximal access of carbohydrate precursors for energy production by means of substrate phosphorylation.

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