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THE INACTIVATION OF GLYCOGEN PHOSPHORYLASE IS NOT A PREREQUISITE FOR THE ACTIVATION OF LIVER GLYCOGEN SYNTHASE

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1. Introduction

The mechanism of activation of hepatic glycogen synthase has received much attention in the last years. According to [1–3], glycogen synthase is activated, that is, converted into the I form, only if the concentration of glycogen phosphorylase *a* is lowered below a threshold value ≃ 10% of total (*a* + *b*) phosphorylase activity. This theory is based mainly on two facts:

(1) The activation of glycogen synthase by glucose is preceded by the inactivation of glycogen phosphorylase (1,2,4,5);

(2) Glucose stimulates phosphorylase phosphatase (6,7) while phosphorylase *a* is a powerful inhibitor of glycogen synthase phosphatase [8].

Thus, according to these authors, glycogen synthase phosphatase could only convert the inactive form of glycogen synthase (D-form) into the physiologically active I form after the disappearance of its inhibitor, phosphorylase *a*, the inactivation of phosphorylase being a prerequisite for the activation of glycogen synthase. The lag period that precedes the activation of glycogen synthase by glucose would then correspond to the time required for the inactivation of phosphorylase.

Although this theory was originally proposed to explain the effects of glucose, it has also been invoked to explain the action of other agents such as insulin and K^+ on hepatic glycogen metabolism [9,10].

A preliminary report of these results was presented at the XVIIth Meeting of the Spanish Biochemical Society, Madrid, 2–3 October 1978

Here we present evidence that the inactivation of glycogen phosphorylase is not essential for the activation of liver glycogen synthase since fructose, in isolated rat hepatocytes, provokes the simultaneous activation of glycogen synthase and phosphorylase.

2. Materials and methods

Male Wistar rats (220–250 g), maintained on a standard laboratory diet were deprived of food 24 h prior to hepatocyte isolation which was carried out as in [11,12]. Cells were finally resuspended in Krebs-Ringer bicarbonate buffer (pH 7.4) free of glucose and pre-gassed with O_2/CO_2 19:1. Aliquots (3.5 ml, 6–9 × 10^6 cells/ml) were poured into stoppered 30 ml vials and incubated in a water bath with shaking (100 strokes/min). Sugars were in all cases dissolved in water at 300 mM. Variable volumes of these solutions were added to the cell suspensions to attain the desired final concentrations. At the end of the incubation the contents of each vial were centrifuged (3000 × *g*, 20 s) and the cell pellet was immediately homogenized with 250 μl ice-cold medium containing 150 mM KF and 15 mM EDTA (pH 7.0). At the same time an aliquot of the cell medium was added to an equal volume of 10% trichloroacetic acid and its glucose content determined with hexokinase and glucose 6-phosphate dehydrogenase after centrifugation and ether extraction. The cell homogenates were centrifuged for 15 min at 10 000 × *g* and the supernatants assayed for enzymatic activities. Glycogen synthase was measured at 30°C by the method in [13]; I activity was determined in the absence of

glucose 6-phosphate and total activity in the presence of 7.2 mM glucose 6-phosphate. Phosphorylase *a* activity was measured at 30°C by the method in [14], in the absence of 5′-AMP. One unit of enzyme activity is the amount of enzyme that incorporates 1 μmol [^{14}C]glucose from UDP-[^{14}C]glucose (glycogen synthase) or from [^{14}C]glucose 1-phosphate (glycogen phosphorylase) to glycogen per min. Protein was determined by the biuret method [15] as in [16]. (–) D-Fructose was from Sigma Chemical. Co. and (+) D-glucose from Baker. No glucose could be detected in the fructose solutions (300 mM) when assayed with hexokinase and glucose 6-phosphate dehydrogenase.

3. Results

When hepatocytes were incubated in the presence of glucose a decrease in glycogen phosphorylase *a* activity which slightly preceded the activation of glycogen synthase was observed (fig.1). These effects were dependent on the concentration of glucose. They were already apparent at 10 mM glucose and they were maximal at 50 mM glucose (data not shown).

Incubation of hepatocytes with fructose produced a rapid increase in the activity of phosphorylase which was maximal 2–4 min after the addition of the sugar (fig.2). A concomitant activation of glycogen

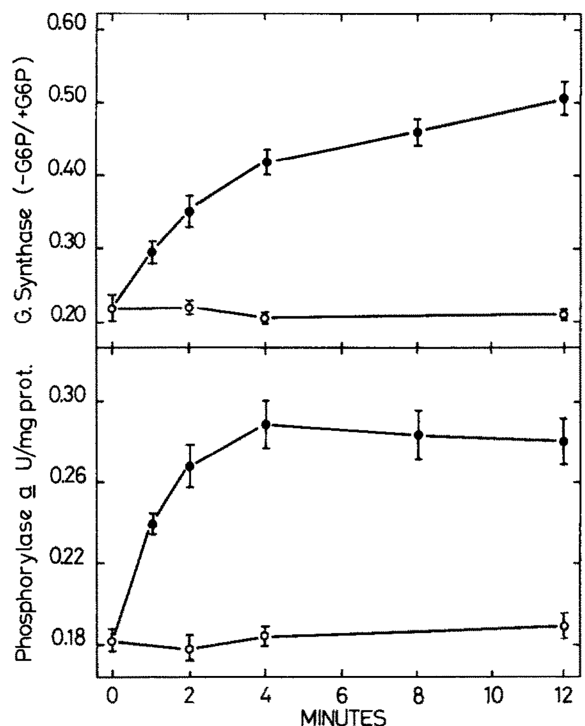

Fig.2. Time course of the effect of 50 mM fructose (•) on glycogen synthase activity ratio and on phosphorylase *a* activity in hepatocytes isolated from starved rats. (○) Control cells treated with saline. Other conditions were as in fig.1. Values are mean ± SE from at least 4 experiments performed on different days.

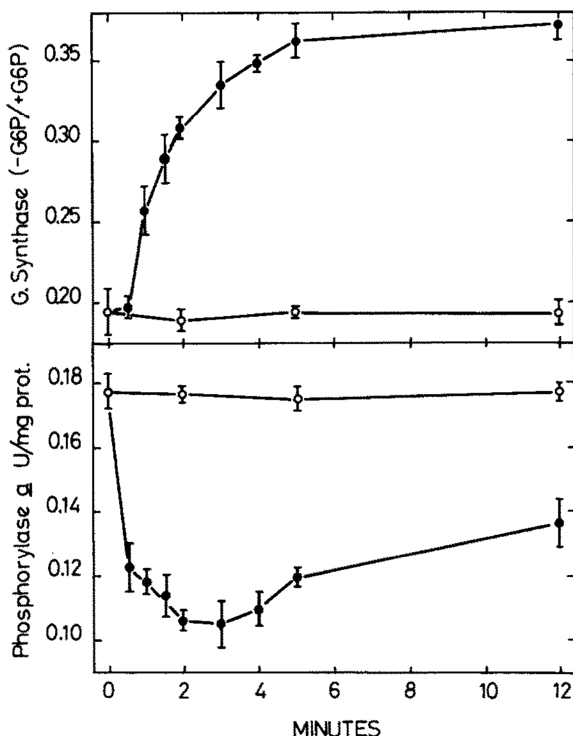

Fig.1. Hepatocytes isolated from starved rats were incubated in Krebs-Ringer bicarbonate buffer at 37°C as in section 2. After a 15 min preincubation period, 20 mM glucose (•) or saline (○) were added and incubations continued for the indicated periods of time. Total glycogen synthase activity ((+)-glucose 6-phosphate) was 7.52 ± 0.48 munits/mg protein, and remained constant under all conditions. Values are mean ± SE from 4 experiments performed on different days.

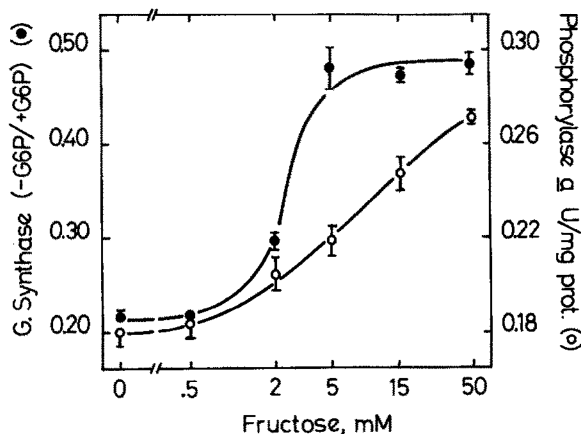

Fig.3. Dose dependence of the effect of fructose on glycogen synthase activity ratio and phosphorylase *a* activity. Hepatocytes were incubated as in fig.1 for 12 min with the indicated concentrations of fructose. Data are mean ± SE from 4 experiments performed on different days.

synthase was observed (fig.2) although the (−) glucose 6-phosphate/(+) glucose 6-phosphate activity ratio increased after glycogen phosphorylase had reached its maximum. These effects of fructose were dose dependent (fig.3). Activation of both glycogen synthase and phosphorylase was already observed at as low as 2 mM fructose. However glycogen synthase seemed to be more sensitive to activation by fructose than glycogen phosphorylase. The amount of glucose released by cells incubated with fructose was also measured. After a 12 min incubation with 50 mM fructose, medium glucose was 0.87 ± 12 mM (mean ± SD).

In order to prove that the activation of these enzymes by fructose was not due to the presence of a low molecular weight activator in the crude extracts, i.e., glucose 6-phosphate in the case of glycogen synthase, 0.5 ml of the extracts were filtered through 1 X 12 cm columns of Sephadex G-50 Fine (Pharmacia) then assayed for both enzymatic activities. This procedure proved to remove > 99% of [^{14}C]glucose or [^{14}C]glucose 6-phosphate added to the extracts. As can be observed in table 1, filtered extracts showed a slightly lower glycogen synthase activity ratio when compared with unfiltered aliquots diluted to equalize their protein concentration with their filtered counterparts. This fact is most probably due to the removal of the small amounts of glucose 6-phosphate present in the extracts. However, the difference in glycogen synthase activity ratio between fructose-treated and control cells was not affected by the procedure. Glycogen phosphorylase activity was slightly increased as a result of the filtration but this process did not alter the differences observed in the non-filtered extracts.

Table 1
Effect of dilution or filtration through Sephadex G-50 on glycogen synthase and phosphorylase activities in crude extracts from hepatocytes

Additions to the cells	Crude extracts treatment	Enzyme activities Glycogen synthase (−G6P/+G6P)	% Δ over control	Phosphorylase *a* units/mg protein	% Δ over control
Saline (control)	None	0.22	–	0.17	–
	1:1.3 dilution extract:buffer	0.17	–	0.16	–
	Sephadex G-50	0.15	–	0.19	–
Fructose 50 mM	None	0.34	+ 55	0.30	+ 76
	1:1.3 dilution extract:buffer	0.27	+ 59	0.28	+ 75
	Sephadex G-50	0.24	+ 60	0.34	+ 79

Hepatocytes incubated for 3 min with 50 mM fructose and from untreated hepatocytes. Extracts were diluted 1:1.3 (extract: buffer) in order to equalize protein concentration with their Sephadex-filtered counterparts. Results are the mean from 3 determinations in a representative experiment

4. Discussion

The experiments in this report show that the activation of liver glycogen synthase can take place with a concomitant increase in the levels of glycogen phosphorylase *a* activity. This is a definite demonstration that the inactivation of glycogen phosphorylase is not a prerequisite for the activation of glycogen synthase as it is widely accepted.

The activation of glycogen phosphorylase by fructose in perfused rat liver has been reported [17]. However the effects of this sugar on glycogen synthase were not observed. We cannot offer an explanation for the lack of responsiveness of glycogen synthase to fructose in [17], as the effects on isolated hepatocytes are clear and reproducible. It must be stressed that the activation of glycogen synthase in our experiments cannot be attributed to glucose released into the medium during the incubation, as the concentration of glucose (max. 1.1 mM) always remained far below the minimum concentration of this sugar (~10 mM) able to produce significant changes in the enzymatic activities.

It is worth noting that this study does not preclude that the mechanism postulated [1,2] is not operative in the liver. In fact our experiments with glucose confirm the observations in [1,2,10] as regards the temporal sequence of phosphorylase inactivation followed by synthase activation in response to glucose. Our results clearly prove that another mechanism exists in liver which serves to activate glycogen synthase independently of the inactivation of glycogen phosphorylase.

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