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Glucose has to be phosphorylated to activate glycogen synthase, but not to inactivate glycogen phosphorylase in hepatocytes

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2-Deoxyglucose and 5-thioglucose, in the same fashion as glucose, cause the inactivation of the rat hepatocyte glycogen phosphorylase and the activation of glycogen synthase. However, 6-deoxyglucose and 1,5-anhydroglucitol inactivate phosphorylase without increasing the activation state of glycogen synthase. With 3-*O*-methylglucose no changes in the activity of these enzymes occurred. These results prove that while glucose is the molecule that triggers the inactivation of phosphorylase, glucose 6-phosphate is the signal for glucose synthase activation and that a metabolite control of the activation state of glycogen synthase is operative in hepatocytes.

Glycogen synthase; Glycogen phosphorylase; Glucose; Glucose analog

1. INTRODUCTION

In liver, glycogen synthase is activated by glucose and other monosaccharides. The mechanism by which this enzyme is activated by glucose is still a subject of controversy. Stalmans et al. [1] proposed the so-called sequential mechanism. According to this mechanism glucose activates glycogen synthase following these steps: (1) glucose binds to glycogen phosphorylase rendering it a better substrate for phosphorylase phosphatase. Then, glycogen phosphorylase is dephosphorylated and inactivated [2,3]; (2) the dephosphorylation of phosphorylase releases the inhibitory effect of active phosphorylase on glycogen synthase phosphatase which now is able to activate glycogen synthase [4].

An intrinsic point in this theory is that it is glucose itself which triggers the activation of glycogen synthase by inactivating glycogen phosphorylase. However, we have shown that in rat hepatocytes the intracellular concentration of glucose 6-phosphate (P) shows a high positive correlation with the activation of glycogen synthase, suggesting that glucose 6-P rather than glucose could constitute the signal for glycogen synthase activation [5].

In this work we have used several glucose analogues with different capabilities to be phosphorylated to study the role of glucose 6-P on glycogen synthase activation. We present evidence that indeed glucose has to be phosphorylated in order to activate glycogen synthase, but not to inactivate glycogen phosphorylase. Thus, the activation of glycogen synthase is not necessarily coupled to the inactivation of glycogen phosphorylase.

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2. MATERIALS AND METHODS

Glucose, 2-deoxyglucose, 6-deoxyglucose, 3-*O*-methylglucose, 5-thioglucose, 1,5-anhydroglucitol, glycogen, glucose 6-P, glucose 1-P, leupeptin and bovine serum albumin were obtained from Sigma Chemical. Rabbit liver glycogen was treated with AG 501-X8 (Bio-Rad) before use. Sucrose and other common analytical grade reagents were from Merck. Collagenase was purchased from Boehringer. [^{14}C]Glucose 1-P was obtained from Amersham and [U-^{14}C]UDPglucose was prepared from [^{14}C]glucose by the method described in [6].

Hepatocyte suspensions were prepared as in [7] from 24 h-starved male Sprague-Dawley rats (200–250 g). Cells were finally resuspended in Krebs-Ringer bicarbonate buffer (pH 7.4) pre-gassed with O_2/CO_2 19:1. Aliquots of 6–8 × 10^6 cells/ml were incubated at 37°C with gassing and continuous shaking (100 strokes/min). Hepatocytes were allowed to equilibrate with the medium for 30 min before addition of the effectors. Sugars were dissolved in water at a concentration of 300 mM. Variable volumes of these solutions were added to the cell suspension to give the desired final concentrations. At the end of the incubations the contents of each vial were centrifuged (3000 × *g*, 20 s) and the cell pellet immediately homogenized on ice with the extraction solution, using a Polytron with a PT-7 rotor at setting 6 for 20 s. Cell homogenates were centrifuged (10 000 × *g*, 15 min) at 4°C and the supernatants processed for enzymic activities.

For glycogen synthase and glycogen phosphorylase assays, pellets from 3.5 ml of cell suspension were homogenized with 250 μl of ice-cold buffer containing 150 mM KF, 15 mM EDTA, 500 mM sucrose, 3.5 μg/ml leupeptin, 50 mM 2-mercaptoethanol and 10 mM Tris-HCl, pH 7.0. Glycogen synthase was measured at 30°C by the method of Thomas et al. [8] in the absence or presence of 6.6 mM glucose 6-P. Phosphorylase was measured at 30°C by the method of Gilboe et al. [9] in the presence of 1 mM caffeine and 10 mM EDTA to specifically measure the amount of phosphorylase *a* [10].

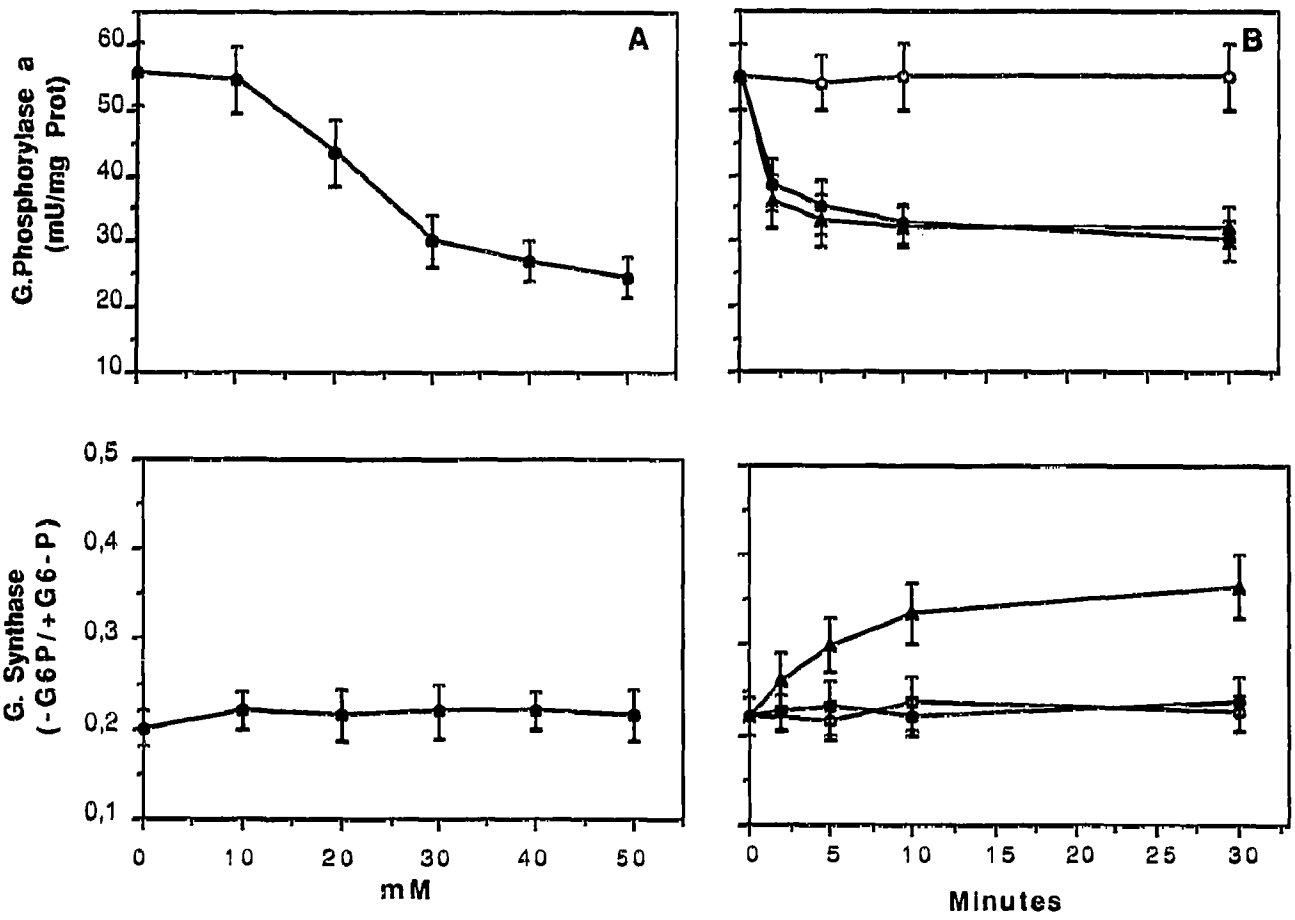

Fig. 1. (A) Concentration-dependence of the effects of 6-deoxyglucose on glycogen phosphorylase and glycogen synthase activities. Hepatocytes were incubated for 15 min with the indicated concentrations. Results represent the means of 3 experiments performed on different days. (B) Time-course of the effects of 30 mM 6-deoxyglucose (●) and 17 mM glucose (▲) on glycogen phosphorylase and glycogen synthase activities. Results represent the means of 3 experiments performed on different days. (○) Control cells incubated in the absence of the sugar.

3. RESULTS

Incubation of rat hepatocytes with glucose results in an inactivation of glycogen phosphorylase, with a simultaneous activation of glycogen synthase [11,12]. With the aim of dissecting the relationship between the changes in these enzymic activities and the role of free glucose and glucose 6-P on these processes, hepatocytes were incubated with the following glucose analogues: 6-deoxyglucose, 1,5-anhydroglucitol, 3-*O*-methylglucose, 2-deoxyglucose and 5-thioglucose.

When hepatocytes were incubated with 6-deoxyglucose, which cannot be phosphorylated at C^6, glycogen phosphorylase was inactivated while no effects on glycogen synthase activity could be observed. The inactivation of phosphorylase by 6-deoxyglucose was dependent on both the concentration of sugar (Fig. 1A) and the time of incubation (Fig. 1B).

The inactivation of glycogen phosphorylase by 30 mM 6-deoxyglucose was similar to that provoked by 17 mM glucose and, as can be observed in Fig. 1B, glucose at this concentration already provokes a clear activation of glycogen synthase. Therefore, the lack of activation of glycogen synthase by 6-deoxyglucose cannot be attributed to its lesser potency to inactivate glycogen phosphorylase when compared with glucose. A similar result was obtained with 1,5-anhydroglucitol, a sugar that is also not a substrate for glucokinase [13], although with this sugar the effect on glycogen phosphorylase is more transient (Fig. 2).

3-*O*-Methylglucose, another sugar that cannot be phosphorylated does not modify the glycogen synthase activating state either. Furthermore, because of steric impediments this sugar is unable to bind glycogen phosphorylase and, therefore, is not capable of provoking the inactivation of this enzyme (data not shown).

All these experiments seemed to indicate that the phosphorylation of the sugar is not a requirement for the inactivation of glycogen phosphorylase, although it could be an absolute prerequisite for the activation of glycogen synthase. In order to check the latter point we performed another series of experiments using 2-deoxyglucose which is phosphorylated to 2-deoxyglucose 6-P without being further metabolized. When cells were

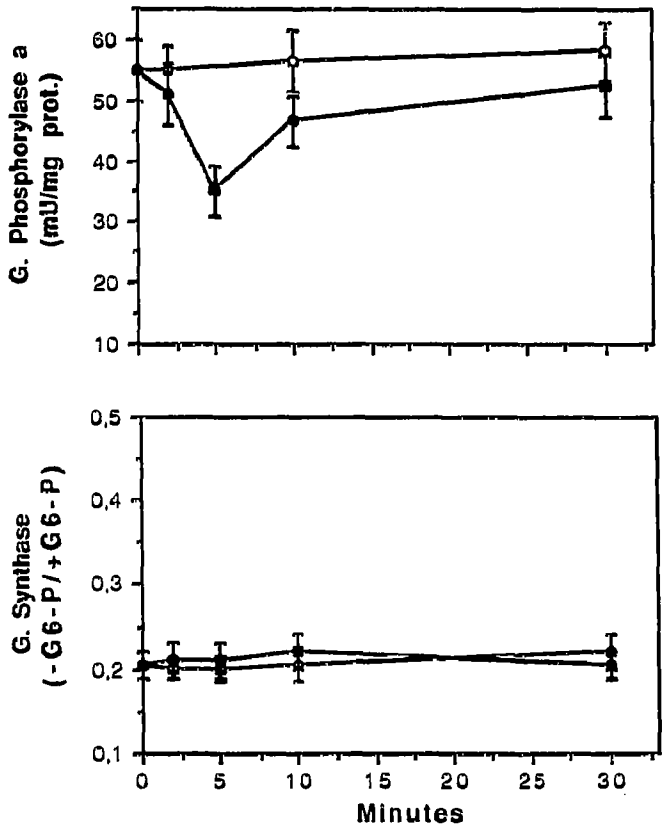

Fig. 2. Effects of 1,5-anhydroglucitol (●) on glycogen synthase and phosphorylase activities. Hepatocytes were incubated for the indicated times with 30 mM 1,5-anhydroglucitol. Results represent means of 5 independent experiments. (○) Control cells incubated in the absence of the sugar.

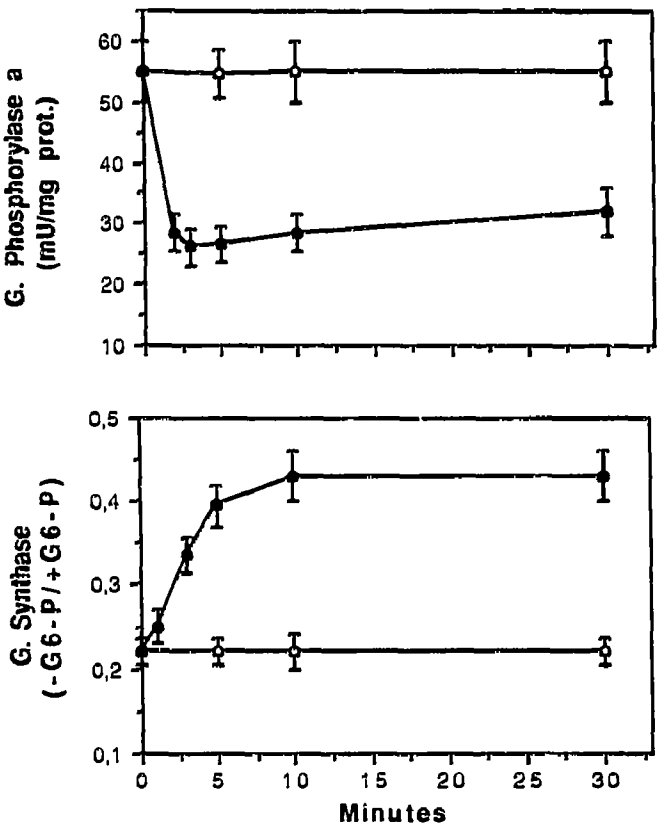

Fig. 3. Effects of 30 mM 2-deoxyglucose (●) on glycogen phosphorylase and glycogen synthase activities. Hepatocytes were incubated for the indicated times with 30 mM 2-deoxyglucose. Results represent the means of 3 experiments performed on different days. (○) Controls cells incubated in the absence of the sugar.

incubated with 2-deoxyglucose, the same pattern as that with glucose was obtained. That is, glycogen phosphorylase was inactivated while glycogen synthase was activated (Fig. 3). On a molar basis, 2-deoxyglucose was less effective than glucose in modifying both enzyme activities.

Finally we also tested the action of 5-thioglucose. This glucose analog also caused the inactivation of glycogen phosphorylase and the activation of glycogen synthase (Fig. 4). We checked whether 5-thioglucose could be phosphorylated in isolated hepatocytes. We found that 5-thioglucose 6-P is a substrate for glucose 6-P dehydrogenase from *Leuconostoc mesenteroides*, although it is less efficient than glucose 6-P. Therefore, we were able to measure 5-thioglucose 6-P in acid extracts from cells incubated with 5-thioglucose. After 5 min incubation with 30 mM 5-thioglucose, hexose 6-P (5-thioglucose 6-P plus glucose 6-P) levels were 60 ± 3 nmol/g vs 33 ± 1 nmol/g in control cells, thus proving that 5-thioglucose is phosphorylated in isolated rat hepatocytes.

4. DISCUSSION

The experiments performed with glucose analogues prove that in rat hepatocytes glucose has to be phosphorylated in order to activate glycogen synthase. Only those hexoses able to give a phosphate ester in C_6 such as glucose, 2-deoxyglucose and 5-thioglucose activate the enzyme. Interestingly, experiments carried out with 6-deoxyglucose and 1,5-anhydroglucitol are very illustrative since these compounds do not provoke any change on hepatocyte glycogen synthase activity while they are able to inactivate glycogen phosphorylase. These results confirm that glucose is, indeed, the responsible molecule for the inactivation of glycogen phosphorylase and at the same time they clearly demonstrate that the inactivation of glycogen phosphorylase is not the signal for glycogen synthase activation. 3-*O*-Methylglucose is unable to interact with glycogen phosphorylase and, indeed, a lack of effect of 3-*O*-methylglucose on liver phosphorylase *a*-to-*b* conversion has been reported using purified enzyme [3] and also in in vivo experiments [14].

The picture emerging from the results presented here

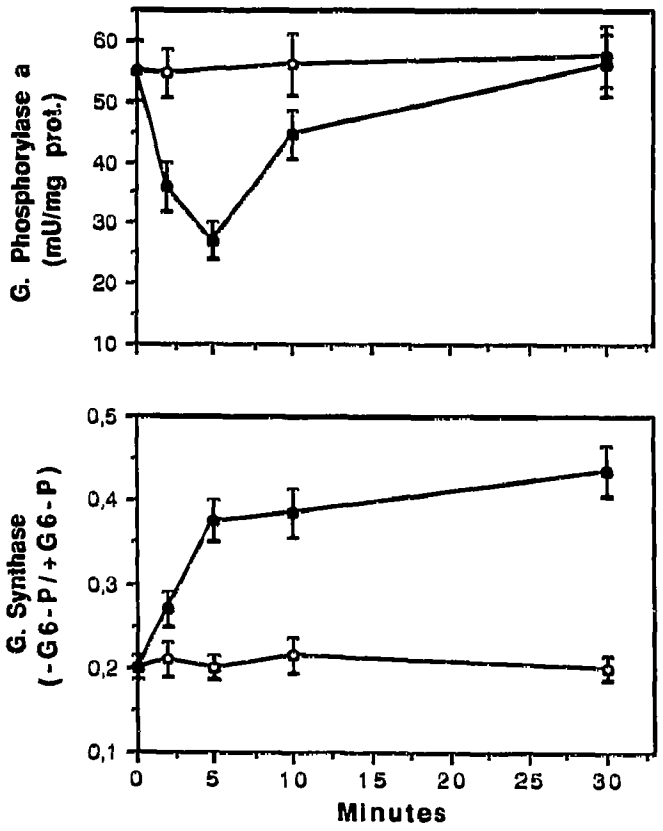

Fig. 4. Effects of 5-thioglucose (●) on glycogen synthase and phosphorylase activities. Hepatocytes were incubated for the indicated times with 30 mM 5-thioglucose. Results are means of 5 independent experiments. (○) Control cells incubated in the absence of the sugar.

is that the inactivation of glycogen phosphorylase is triggered by the glucose molecule itself, whereas glucose 6-P constitutes the signal for glycogen synthase activation.

Therefore, we must conclude that the sequential mechanism [1] is not responsible for the glucose-induced effects on glycogen synthase. Alternatively, our results indicate that a metabolite control, through glucose 6-P and AMP [15] concentrations, of the activation state of glycogen synthase is operative in isolated rat hepatocytes.

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