INSULIN CONTROL OF RAT HEPATOCYTE GLYCOGEN SYNTHASE AND PHOSPHORYLASE IN THE ABSENCE OF GLUCOSE

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Received 26 July 1977
Revised version received 19 August 1977

1. Introduction

Most effects of insulin on glycogen metabolism in isolated hepatocytes that have been observed were in the presence of glucose, which by itself is capable of activating hepatic glycogen synthase and inactivating glycogen phosphorylase [1–4]. For example, activation of glycogen synthase by insulin in the presence of high concentrations of glucose has been reported in isolated rat hepatocytes incubated in a complex medium containing amino acids [2,5]. The ability of insulin to counteract the effect of glucagon on glycogen phosphorylase in liver cells has also recently been demonstrated [6].

In this report we present evidence that, in isolated rat hepatocytes, glucagon, in addition to activating glycogen phosphorylase, reduces basal levels of glycogen synthase I and that insulin is able to counteract these effects of glucagon in the absence of glucose.

2. Materials and methods

Female Wistar rats weighing 180–200 g and mantained on a standard laboratory diet were starved for 24 h prior to hepatocyte isolation. Hepatocytes were isolated by a modification of the method of Berry and Friend [7] without using hyaluronidase in the perfusion medium. Liver perfusion was initiated with Hank's Ca^{2+}-free buffer (pH 7.4, 38.5°C) containing 5 mM glucose, at 40 ml/min. After perfusion with 250 ml buffer, collagenase was added to a final concentration of 0.4 mg/ml (Worthington Biochemical Corp., Type I, Lots CLS 46K283P and CLS 46K286) and bovine serum albumin (Fraction V) (Sigma Chemical Co.) was added to a final concentration of 15 mg/ml. The recirculating perfusion with the enzyme was carried out at 60 ml/min for 12–15 min. The liver was then removed and gently teased apart in Hank's Ca^{2+}-free buffer. The resulting cell suspension was washed three times and finally resuspended (1/12) in Krebs-Ringer bicarbonate buffer (pH 7.4) free of glucose and any other substrate, and previously gassed with 95% O_2 and 5% CO_2. Three to four grams of cells were usually obtained from livers weighing 7–7.5 g. Once resuspended, 85% of these cells excluded trypan blue. Aliquots (3.5 ml) of this suspension (10^7 cells/ml) were poured into stoppered 30 ml vials and incubated in a water bath with shaking (100 strokes/min). These cells contained no ethanol-precipitable glycogen. At the end of the incubation with hormones the contents of each vial were centrifuged (3000 × *g* for 30 s) and the cell pellet was immediately homogenized in a Potter-Elvejhem homogenizer with 300 μl ice-cold medium containing 150 mM KF and 15 mM EDTA (pH 7.0) and assayed for enzymatic activities and protein content.

Glycogen synthase activity was measured at 30°C by the method of Thomas et al. [8], based on the incorporation of [^{14}C]glucose from UDP-[^{14}C] glucose into glycogen. Glycogen phosphorylase was measured at 30°C by the method of Gilboe et al. [9], based upon the incorporation of [^{14}C]glucose from [^{14}C]glucose 1-phosphate into glycogen.

Cell protein was determined by the method of Lowry et al. [10] and glycogen by the anthrone method of Carroll et al. [11].

Glucagon was obtained from the Sigma Chemical Co. and insulin from the Eli Lilly Co.

3. Results

Glucagon caused a clear decrease in glycogen synthase I activity and an increase in glycogen phosphorylase *a* activity. These effects were already apparent at a dose of 10^{-10} M glucagon (see fig.1 for effect on synthase), higher doses causing more evident and longer-lasting modifications of enzyme activity (data not shown).

Although insulin did not modify the basal levels of glycogen synthase I and glycogen phosphorylase *a*, the effects of 3×10^{-10} M glucagon were significantly reduced in cells previously incubated with insulin (fig.1). This action of insulin, however, was not observed with high glucagon doses (data not shown). At the third minute of incubation with glucagon, without previous exposure to insulin, the inactivation of glycogen synthase was maximal. The basal glycogen synthase I activity (5.8 ± 1.1 mU/mg protein, –G6P/+G6P ratio = 0.26 ± 0.05) was reduced by about 40%, the decrease being constant from experiment to experiment. At 10 min the activity had almost returned to the original level. The response to the same dose of glucagon in cells previously incubated 10 min with 8×10^{-9} M insulin was significantly less than that of cells incubated without insulin. In the insulin-treated cells, synthase I activity had already begun to recover by the third minute of incubation with glucagon, after having reached a maximal decrease in activity of only 25% from the original value.

In the same experiments, phosphorylase activity was also followed (fig.2). The activity of the *a* form of the enzyme (basal value 0.17 ± 0.01 U/mg protein) was rapidly increased by incubation with glucagon and began the return to basal level by about the second minute, almost reaching it by the fifth minute. When glucagon was added to cells previously incubated for 10 min with insulin, the amplitude of the effect on phosphorylase *a* activity was only about half of that observed with glucagon alone, but the rate of recovery was unaltered by insulin treatment.

The effect of insulin was dose-dependent. Cells pretreated with varying doses of insulin were then treated with 3×10^{-10} M glucagon for the periods of time at which maximal differences had been observed between insulin-treated and non-treated cells in the

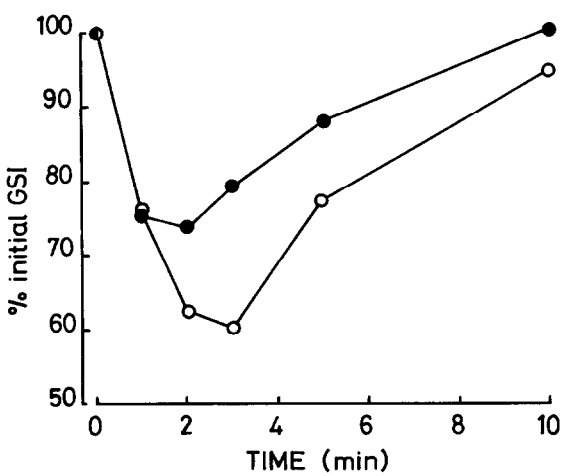

Fig.1. Hepatocytes isolated from rats starved for 24 h were preincubated for 10 min at 20°C in Krebs-Ringer bicarbonate buffer. They were then incubated for 10 min at 37°C with 8×10^{-9} M insulin (•) or without insulin (○). At the end of this period, 3×10^{-10} M glucagon was added and incubations were continued for the indicated periods of time. No changes in glycogen synthase I activity were observed in untreated cells during the incubation period. Activity of glycogen synthase I is expressed as a percentage of the I activity of control cells incubated without hormones (% initial GSI). The results are the average of four experiments.

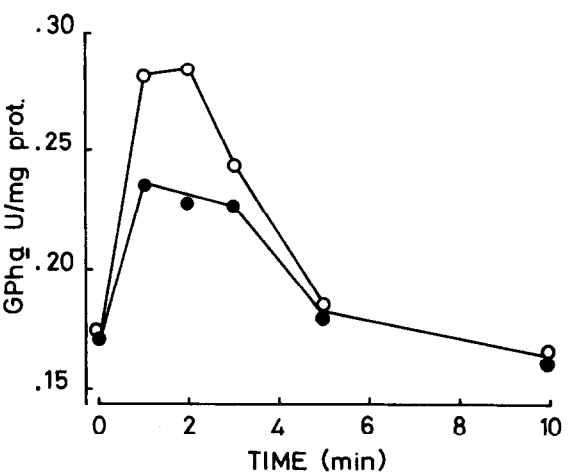

Fig.2. Time course of the effect of 3×10^{-10} M glucagon on glycogen phosphorylase *a* (GPh *a*) in hepatocytes (fig.1) incubated with (•) or without (○) insulin. No changes in glycogen phosphorylase *a* activity were observed in untreated cells during the incubation period. Data represented in figs.1 and 2 were obtained from the same four experiments.

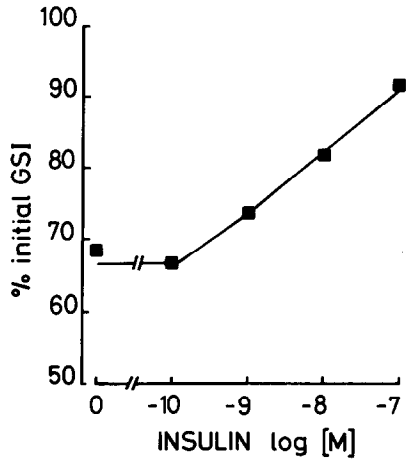

Fig.3. Hepatocytes (fig.1) were preincubated for 10 min at 20°C, then incubated with the indicated doses of insulin for 10 min at 37°C. At the end of this period, 3×10^{-10} M glucagon was added and incubation continued for 3 min. Results are expressed as in fig.1.

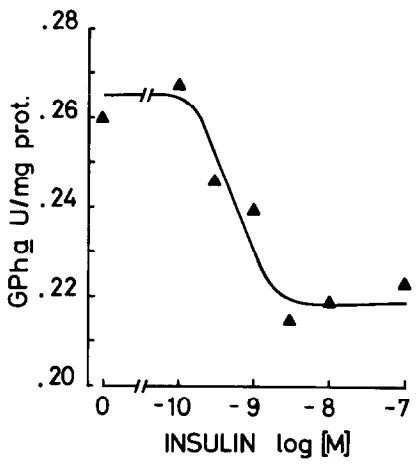

Fig.4. Dose dependence of the effect of insulin on glycogen phosphorylase *a* (GPh *a*) in hepatocytes incubated with glucagon. Conditions were the same as in fig.3 except that cells were incubated with glucagon for 1.5 min.

first series of experiments. Figure 3 shows the dose-dependence of insulin in counteracting glucagon-mediated glycogen synthase interconversion. Whilst the lowest dose at which an effect was observed was 10^{-9} M insulin, at a dose of 10^{-7} M insulin the effect of glucagon was almost completely abolished. Similarly, the dose-dependence of the effect of insulin on glycogen phosphorylase in cells incubated 1.5 min. with glucagon was studied (fig.4). The minimal dose of insulin producing an effect was approx. 3×10^{-10} M and doses higher than 3×10^{-9} M were saturating.

4. Discussion

The demonstration of a glucose control of hepatic glycogenesis and several negative reports [1,3] on the effect of insulin on hepatic glycogen synthase have combined to make the role of insulin in the regulation of glycogen synthesis in mammalian liver a controversial topic. The data presented in this paper argue strongly for a significant insulin control of liver-glycogen metabolism, as has been indicated from studies with perfused liver by Larner et al. [12,13]. Our results demonstrate that, in isolated rat hepatocytes, insulin counteracts the effects of glucagon on glycogen synthase and glycogen phosphorylase in the absence of glucose.

From the experiments in the absence of insulin, it is worth noting that an influence of glucagon alone, as described above, on the basal glycogen synthase I activity (that is, not previously elevated by glucose) has not been extensively documented for isolated cells; Hutson et al. [14] could observe clear effects of glucagon on synthase in hepatocytes from fed rats only after the % I form had been first increased by exposure to glucose, but briefly mention a glucagon effect on basal synthase levels in starved rats. Our observations of a glucagon effect even on low basal % I activities in the absence of glucose may correlate with our use of starved animals.

In conclusion, insulin treatment was able to modify the magnitude of the response of both glycogen synthase and glycogen phosphorylase to glucagon. It is also interesting to note that phosphorylase was considerably more sensitive to this action of insulin than synthase. This raises the question, therefore, as to how interdependent are the mechanisms by which insulin regulates these two key enzymes of glycogen metabolism.

Acknowledgements

The authors acknowledge the encouragement of the late Professor M. Rosell Pérez. They also wish to thank Dr Peter Roach for his helpful suggestions concerning the manuscript. J. M. is the recipient of a fellowship from the Plan de Formación de Personal investigador awarded by the Spanish Ministry of Education. J.J.G. is the recipient of a grant from the Agustí Pedro Pons Foundation (Barcelona, Spain).

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