INSULIN ACTIVATION OF BASAL HEPATIC GLYCOGEN SYNTHASE

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1. Introduction

Insulin is widely believed to stimulate the synthesis of glycogen by increasing the activity of glycogen synthase, that is, by promoting the interconversion from the more phosphorylated forms to the dephosphorylated forms [1]. This process has been clearly observed in skeletal muscle [2], heart [3], adipocytes [4] and diaphragm [1]. On the contrary, in liver tissue, the ability of insulin to activate glycogen synthase has been perceived as questionable, as it has only been reported in experiments performed in isolated cells in the presence of glucose, whose sole presence already activates the enzyme [5–7], or by using perfused liver from fed diabetic rats under conditions where glucose should have accumulated [8]. With the hormone alone no activation of liver glycogen synthase measured as –G6P/+G6P activity ratio has been detected.

Glycogen synthase can be multiply phosphorylated in such a way that its kinetic properties vary with the phosphate content [9]. However, as reported in [10] the strongest changes in enzymic properties caused by phosphorylation are poorly reflected in the –G6P/+G6P assay (G6P, glucose-6-phosphate). However, the new activity ratio assay in [11], shows a greater sensitivity to changes in enzymic properties caused by phosphorylation–dephosphorylation in vitro or as provoked by physiological effectors.

The aim of this work was to investigate the effect of insulin on glycogen synthase in isolated hepatocytes from starved normal rats, in the absence of glucose. We have proven that by using the low G6P/high G6P activity ratio a noticeable effect of insulin on basal glycogen synthase activity can be demonstrated in such a way that few doubts can be cast as to the effectiveness of the hormone in liver tissue.

To further examine the changes in the enzymic properties induced by the hormone, we have also determined changes in $S_{0.5}$ for UDP-glucose and $M_{0.5}$ for G6P provoked by insulin in glycogen synthase from rat hepatocytes.

2. Materials and methods

Suspensions of isolated parenchymal liver cells were prepared from starved (24 h) male Sprague-Dawley rats (200–250 g) as in [12]. Cells were finally resuspended in Krebs bicarbonate buffer (pH 7.4) free of glucose or any other substrate, and pregassed with O_2/CO_2 (19:1).

Aliquots (5 ml, 9×10^6 cells/ml) were poured into stoppered vials and incubated at 37°C with shaking (100 strokes/min). At the end of the incubations the content of each vial was centrifuged (3000 × *g*, 20 s) and the cell pellet was immediately homogenized with 150 μl ice-cold medium containing 300 mM KF and 30 mM EDTA (pH 7.0). At the same time the cell medium was added to perchloric acid (0.3 N final conc.), centrifuged, and its glucose content determined by the glucose oxidase method [13].

The cell homogenates were centrifuged at 10 000 × *g* for 15 min, and the supernatants were passed through Ultragel AcA 202 columns (1 cm × 15 cm) equilibrated with the extraction buffer, all at 4°C.

The protein eluate was divided into two (500 μl + 300 μl) aliquots. The first was directly assayed for glycogen synthase activity using:

(i) The –G6P/+G6P standard assay [14];
(ii) The low G6P/high G6P activity ratio [11] at 200 μM and 10 mM G6P, respectively.

Likewise in this aliquot $M_{0.5}$ was estimated from Hill plots of log $(V_a/V_{a,max}-V_a)$ *vs* log [G6P] as in [10]. The second 300 μl aliquot was incubated, 30 min at 4°C, with 2 U G6P dehydrogenase and 0.13 μmol NADP to eliminate any trace of G6P left after column

process. $S_{0.5}$ was then estimated from Hill plots of log $(V/V_{std}-V)$ *vs* log [UDPG] as in [10].

Protein was determined by the biuret method (15) as in (16). All the chemical reagents utilized were Analytical Grade and obtained from Sigma or Merck. Insulin was obtained from the Eli Lilly. Collagenase came from Worthington Biochemicals and bovine serum albumin (fraction V) from Sigma.

3. Results

3.1. *Activation of liver glycogen synthase by insulin in the absence of glucose added*

When hepatocytes were incubated with 10^{-8} M insulin, glycogen synthase was activated in a time-dependent manner, maximal activation taking place at 10–20 min after adding the hormone (fig.1). The extent of observed activation varied with the activity ratio employed. Thus, at 10 min insulin produced a small increment in glycogen synthase activity when measured with the standard –G6P/+G6P activity ratio which increased by ~0.025. However, a larger increment could be observed using the low G6P/high G6P activity ratio at 200 μM and 10 mM G-6-P, respectively, the ratio thus increasing by ~0.11.

This activation of glycogen synthase by insulin was also found to be dose-dependent (fig.2). The half-maximal dose was ~5 X 10^{-10} M which falls within the range of physiological concentrations of the hormone.

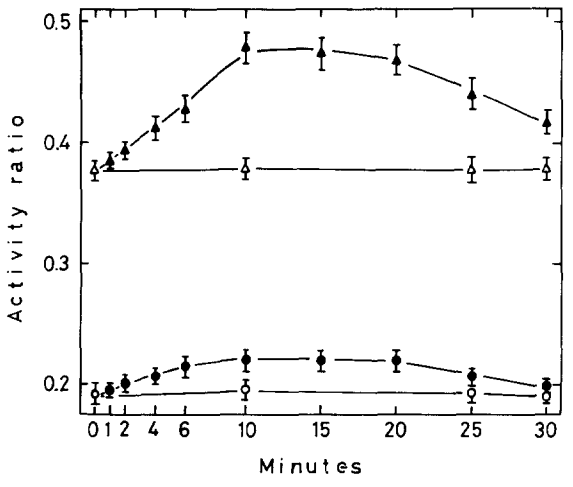

Fig.1. Changes in the standard –G6P/+G6P (○,●) and low G6P/high G6P (△,▲) activity ratios of glycogen synthase in rat hepatocytes incubated with 10^{-8} M insulin (●,▲) or saline (○,△) for the indicated times. Results are mean ± SD of ⩾6 expt performed on different days.

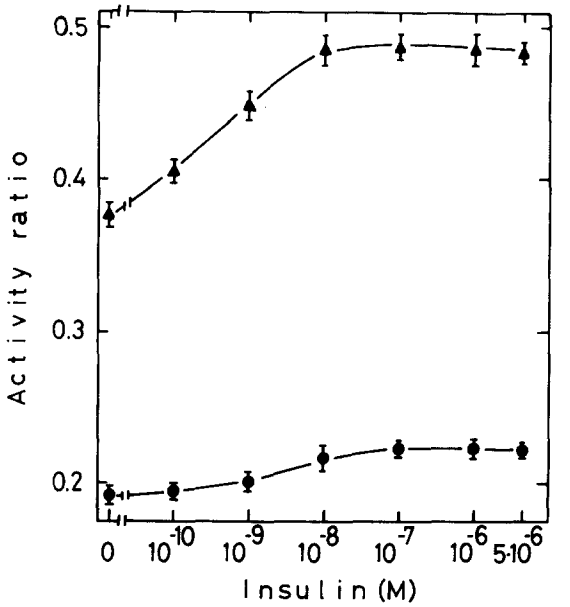

Fig.2. Dose-dependence of the effect of insulin on the standard –G6P/+G6P (●) and low G6P/high G6P (0.2 mM/10 mM) (▲) activity ratios of glycogen synthase in rat hepatocytes. Cells were incubated for 10 min at the indicated concentrations of the hormone. Results are mean ± SD of ⩾6 expt performed on different days.

It should be noted that in both cases, time course and dose–response, activation of glycogen synthase by insulin could hardly be detected by the –G6P/+G6P activity ratio, but was clearly observed using the low G6P/high G6P activity ratio.

The glucose level accumulated in the cell medium after 20 min incubation was in all cases <0.7 mM, far below the minimum concentration at which this sugar is able to produce significant changes in the enzymatic activity.

3.2. *Kinetic changes of liver glycogen synthase induced by insulin*

Kinetic properties, $S_{0.5}$ for UDP-glucose and $M_{0.5}$ for G6P were determined after hepatocytes were incubated with either saline or 10^{-8} M insulin for 10 min. The results, summarized in table 1, show that although the values for control cells were seen to vary from one experiment to another, incubation with insulin always resulted in a decrease in both $S_{0.5}$ for UDP-glucose and $M_{0.5}$ for G6P (table 1). No changes in V_{max} or $V_{a,max}$ between insulin and control cells were observed.

Table 1
Influence of insulin on $S_{0.5}$ for UDP-glucose and $M_{0.5}$ for G6P of glycogen synthase in rat hepatocytes

Expt.	$S_{0.5}$ for UDPG (mM)		$M_{0.5}$ for G6P (μM)	
	Control	Insulin	Control	Insulin
1	21.8	17.6	538	449
2	21.9	18.9	507	239
3	19.9	13.1	633	525
4	20.2	15.4	466	353
5	19.3	18.0	509	446
6	18.7	16.6	629	474
Av. (±SD)	20.3 ± 1.3	16.6 ± 2.1	547 ± 69	414 ± 102

Isolated liver cells were incubated with 10^{-8} M insulin for 10 min. Results are from 6 different expt performed on different days. Decreases were highly significant ($p < 0.0025$)

4. Discussion

The results presented clearly demonstrate that insulin promotes activation of glycogen synthase from a basal state in isolated hepatocytes from normal starved rats in the absence of glucose. The effect of insulin is reflected in a stable change in the kinetic properties of glycogen synthase. The decrease in $S_{0.5}$ for UDP-glucose and $M_{0.5}$ for G6P would result in an activation of the enzyme in vivo.

The effect of insulin on liver glycogen synthase has only been reported in experiments in which the presence of glucose produced a concomitant activation of glycogen synthase [5–8] while no significant difference between insulin and control cells could be observed in experiments performed with liver cells in the absence of glucose [6,7]. As a result, it has been suggested [7] that insulin is only effective in the presence of glucose merely serving to augment the stimulation produced by the sugar. Our results differ notably from these conclusions, since a clear-cut activation can be observed with the low G6P/high G6P assay when glucose is not added to the medium. This fact greatly strengthens the argument for the direct effect of insulin on hepatic glycogenesis. In addition to the dubiety involved in the presence of glucose, it must be emphasized that large amounts of insulin have been used in most of the above experiments [6–8]. However, we have found clear responses even at 10^{-10} M insulin, well within the physiological range.

We have observed that incubation of the hepatocytes with insulin provokes a decrease in the $S_{0.5}$-value for UDP-glucose. As expressed in [10] the –G6P/+G6P activity ratio is a function of the $S_{0.5}$ for UDP-glucose. Thus, the observed decrease in $S_{0.5}$ produced by insulin (average from 20.3–16.6 mM) should theoretically correspond to an increase in the –G6P/+G6P activity ratio of only ~0.03 as the values of $S_{0.5}$ for both control and insulin are considerably higher than the concentration of UDP-glucose used in the standard –G6P/+G6P assay. This calculated value is in complete agreement with the increase observed in the experiments using the –G6P/+G6P activity ratio. Such a small increase can easily be dismissed and as a result we had overlooked its significance in [12,17], a phenomenon which could also account for the general lack of observation of the effects of insulin on basal hepatic glycogen synthase activity.

In addition to changes observed in $S_{0.5}$, $M_{0.5}$ for G6P also suffers a significant decrease when hepatocytes are incubated with insulin, (average from 547–414 μM) and these changes are faithfully reflected in the low G6P/high G6P assay of glycogen synthase. Thus, using the low G6P/high G6P activity ratio we find unmistakeable increments when hepatocytes are incubated with insulin, as the low G6P/high G6P is ~5-fold more sensitive to glycogen synthase activation by insulin that the –G6P/+G6P activity ratio (fig.2). Therefore, although the –G6P/+G6P activity ratio has been utilized extensively as a measure of the activation state of the enzyme, a more reliable index may be obtained by the $M_{0.5}$ calculation as proposed [11] and recommended [18], or by using a method like the low G6P/high G6P activity ratio sensitive to changes in $M_{0.5}$ for G6P.

These data offer new insight into the effects of insulin on hepatic glycogen metabolism, making it clearly evident that insulin not only counteracts the effects of the glycogenolitic hormones in liver cells [12,17] but is also able to control directly the activity of glycogen synthase under basal conditions.

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