Inactivation of basal glycogen synthase by glucagon and epinephrine in hepatocytes from fed rats

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Glucagon and epinephrine promote the inactivation of basal glycogen synthase in hepatocytes isolated from fed rats. However, this effect is only observable when the activation state of glycogen synthase is measured using the low glucose-6-P/high glucose-6-P activity ratio assay. This inactivation is the consequence of an increase in the kinetic parameters ($S_{0.5}$ for UDP-glucose and $M_{0.5}$ for glucose-6-P) of the enzyme. Therefore, this work demonstrates these hormones are also able to control glycogen synthase from fed animals.

(Rat hepatocyte) *Glucagon* *Epinephrine* *Glycogen synthase*

1. INTRODUCTION

Liver glycogen synthase (EC 2.4.1.11), the rate-limiting enzyme in glycogen biosynthesis, is subject to hormonal control [1,2]. Glycogenolytic hormones, such as glucagon and epinephrine, inactivate glycogen synthase as they are able to decrease the standard −glucose-6-P/+glucose-6-P activity ratio of this enzyme [3,4]. To date, these inactivating effects have been observed in hepatocytes from fasted rats but, surprisingly, no effects of these hormones on basal glycogen synthase activity have been detected when the hepatocytes are isolated from fed rats. Therefore, it has been concluded that neither epinephrine nor glucagon is able to inactivate glycogen synthase in liver cells from fed rats [3,5] unless the enzyme is previously activated by incubation of the hepatocytes with a high concentration of glucose, such as 30 mM [4].

This lack of effect has been attributed to the known inhibition of glycogen synthase phosphatase by the high levels of glycogen present in the liver from fed rats [6]. According to this theory the inhibition of glycogen synthase phosphatase would keep glycogen synthase in a highly phosphorylated state not able to be further increased by hormones [3].

Here, we have re-investigated the action of glucagon and epinephrine on glycogen synthase from liver cells isolated from fed animals, applying the low glucose-6-P/high glucose-6-P activity ratio to measure the activation state of this enzyme [7]. We show that these hormones inactivate glycogen synthase by changing its kinetic parameters.

2. MATERIALS AND METHODS

Suspensions of isolated parenchymal liver cells were prepared from fed male Wistar rats (200–250 g) as in [8]. Cells were finally resuspended in Krebs-bicarbonate buffer pre-gassed with O_2/CO_2 (pH 7.4) free of glucose or any other substrate.

Aliquots (5 ml, 8×10^6 cells/ml) were poured into stoppered vials and incubated at 37°C with shaking (100 strokes/min) with saline (control) or hormones. At the end of the incubations the con-

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tents of each vial was centrifuged (3000 × *g*, 20 s) and the cell pellet immediately homogenized with 150 μl ice-cold medium containing 300 mM KF and 30 mM EDTA (pH 7.0) using a motor-driven Potter-Elvehjem homogenizer. The cell homogenates were centrifuged at 10000 × *g* for 15 min and the supernatants filtered through Ultrogel AcA 202 columns (1 × 15 cm) equilibrated with 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 −glucose-6-P/+glucose-6-P standard assay (UDP-glucose concentration in reaction mixture, 4.4 mM) [9]; (ii) the low glucose-6-P/high glucose-6-P activity assay (UDP-glucose concentration in reaction mixture, 0.2 mM) [7] at 250 μM and 10 mM glucose-6-P, respectively.

Likewise, in this aliquot $M_{0.5}$ was estimated from Hill plots of $\log(V_a/V_{amax} - V_a)$ vs log[glucose-6-P] as in [10]. The second 300 μl aliquot was incubated for 30 min at 4°C with 2 U glucose-6-P dehydrogenase and 0.13 μmol $NADP^+$ to eliminate any trace of glucose-6-P left after column processing. $S_{0.5}$ 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 [11] as in [12]. All the chemical reagents utilized were of analytical grade and obtained either from Sigma or Merck. Collagenase came from Worthington, and bovine serum albumin (fraction V), glucagon and epinephrine from Sigma.

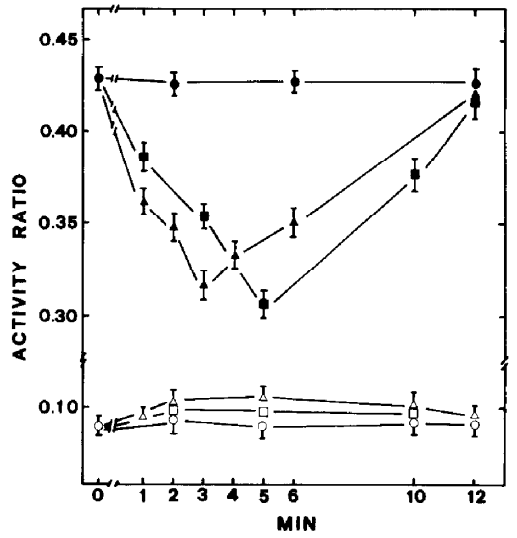

Fig.1. Effect of incubating hepatocytes from fed rats with 10^{-8} M glucagon (△, ▲), 10^{-6} M epinephrine (□, ■) or saline (○, ●) on the −glucose-6-P/+glucose-6-P (△, □, ○) and low glucose-6-P/high glucose-6-P (▲, ■, ●) activity ratios of glycogen synthase. Results are means ± SD of ≥3 experiments performed on different days.

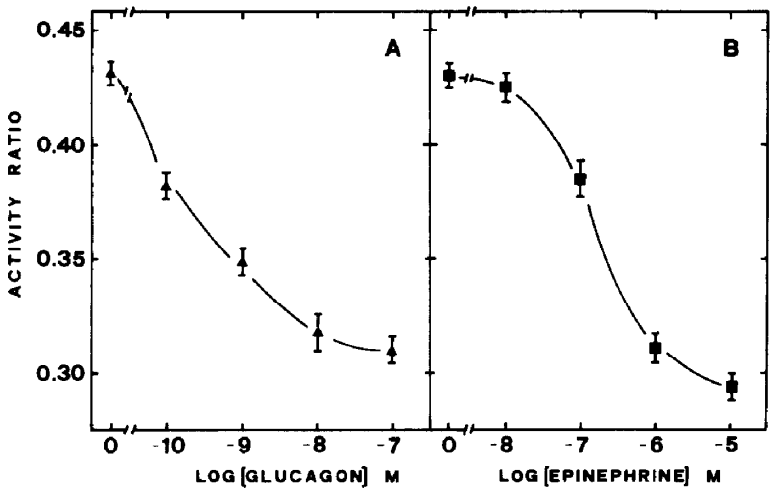

Fig.2. Concentration dependence of the effect of glucagon (▲) and epinephrine (■) on the low glucose-6-P/high glucose-6-P (0.25 mM/10 mM) activity ratio of glycogen synthase in hepatocytes from fed rats. Cells were incubated for 3 min (glucagon) or 5 min (epinephrine) at the indicated concentrations. Results are mean ± SD of ≥3 experiments performed on different days.

3. RESULTS

3.1. *Effects of glucagon and epinephrine on glycogen synthase activity of hepatocytes isolated from fed rats*

When hepatocytes from fed rats were incubated with 10^{-8} M glucagon or 10^{-6} M epinephrine, in the absence of glucose, no effect of these hormones on glycogen synthase activity could be observed if the activation state of the enzyme was measured applying the standard assay based on the −glucose-6-P/+glucose-6-P activity ratio. However, a clear-cut inactivation of glycogen synthase was demonstrated when the low glucose-6-P/high glucose-6-P activity ratio assay was used.

The inactivation of glycogen synthase by glucagon or epinephrine was time-dependent. Maximal effects were observed at 3 min (glucagon) and 5 min (epinephrine) of incubation. At 10–12 min the activity of the enzyme had returned to the original value (fig.1).

This effect was also concentration-dependent. The half-maximal effect was obtained at 5×10^{-10} M glucagon and 2×10^{-7} M epinephrine (fig.2).

3.2. *Effects of glucagon and epinephrine on the kinetic properties of glycogen synthase*

Upon cell incubation with either glucagon (10^{-7} M, 3 min), epinephrine (10^{-5} M, 5 min) or saline (control), kinetic constants of glycogen synthase, $S_{0.5}$ for the substrate UDP-glucose and $M_{0.5}$ for the allosteric activator glucose-6-P, were determined on hepatocyte extracts. The action of these hormones resulted in a noticeable increase in both $S_{0.5}$ and $M_{0.5}$ over the control values (table 1).

Table 1

Influence of glucagon and epinephrine on $S_{0.5}$ for UDP-glucose and $M_{0.5}$ for glucose-6-P of glycogen synthase in hepatocytes from fed rats

	$S_{0.5}$ for UDPG (mM)	$M_{0.5}$ for G6P (μM)
Control	39 ± 2	678 ± 30
Glucagon	61 ± 2	1010 ± 24
Epinephrine	66 ± 3	1155 ± 38

Cells were incubated with 10^{-7} M glucagon for 3 min or 10^{-5} M epinephrine for 5 min. Results are means ± SD of ≥5 experiments performed on different days

4. DISCUSSION

Our results prove that glucagon and epinephrine are indeed able to inactivate hepatocyte glycogen synthase from fed rats. This effect had previously been overlooked because of a methodological problem. The activation state of glycogen synthase has been commonly determined by the ratio of the activities measured in the absence and presence of glucose-6-P using a concentration of substrate of 4.4 mM UDP-glucose. As we have shown in [7], what this ratio measures are the changes in the $S_{0.5}$ value for UDP-glucose. Hence, those effectors producing a decrease in $S_{0.5}$ for the substrate cause an increase in the activity ratio which results in activation of the enzyme. On the other hand, when $S_{0.5}$ is increased, the activity ratio decreases and then an inactivation is observed. As indicated in [7] the standard −glucose-6-P/+glucose-6-P activity ratio is sensitive to changes in $S_{0.5}$ when these take place at values close to that of the concentration of UDP-glucose used in the assay (4.4 mM). For example, an increase in $S_{0.5}$ for UDP-glucose from 4 to 8 mM changes the activity ratio from 0.52 to 0.35. However, a change in this kinetic constant from 40 to 80 mM only decreases the −glucose-6-P/+glucose-6-P ratio from 0.09 to 0.05. Such a decrease would be overlooked in most cases.

As shown in table 1, the $S_{0.5}$ value of glycogen synthase in hepatocytes from fed rats is approx. 40 mM and increases as a result of glucagon or epinephrine action to values between 60 and 70 mM. Changes in this range are almost undetectable by the standard −glucose-6-P/+glucose-6-P activity ratio and most likely for that reason effects of these hormones on hepatic glycogen synthase from fed rats had not been observed.

An alternative method of measuring changes in glycogen synthase activity is by using the low glucose-6-P/high glucose-6-P activity ratio assay described in [7]. This ratio is sensitive to changes in $M_{0.5}$ for glucose-6-P and has proven to be much more useful than the standard in showing the effects of hormones [7,13,14] and other agents [15–18] on glycogen synthase. It is by using this method that we have been able to show, for the

first time, the effect of glucagon and epinephrine on basal hepatic glycogen synthase from fed rats. Then, we conclude that, although glycogen synthase is already present in a very low activation state in these animals, it can still be further inactivated by glycogenolytic hormones.

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REFERENCES

[1] Ariño, J., Mor, A., Bosch, F., Baanante, I.V. and Guinovart, J.J. (1984) FEBS Lett. 170, 310–314.
[2] Ciudad, C.J., Camici, M., Ahmad. Z., Wang, Y., DePaoli-Roach, A. and Roach, P.J. (1984) Eur. J. Biochem. 142, 511–520.
[3] Hutson, N.J., Brumley, F.T., Assimacopoulos, F.D., Harper, S.C. and Exton, J.H. (1976) J. Biol. Chem. 251, 5200–5208.
[4] Strickland, W.G., Blackmore, P.F. and Exton, J.H. (1980) Diabetes 29, 617–622.
[5] Exton, J.H., Assimacopoulos-Jeannet, F.D., Blackmore, P.F., Cherrington, A.D. and Chan, T.M. (1978) Adv. Cyclic Nucleotide Res. 9, 441–452.
[6] Stalmans, W., De Wulf, H. and Hers, H.G. (1971) Eur. J. Biochem. 18, 582–587.
[7] Guinovart, J.J., Salavert, A., Massague, J., Ciudad, C.J., Salsas, E. and Itarte, E. (1979) FEBS Lett. 106, 284–288.
[8] Massague, J. and Guinovart, J.J. (1978) Biochim. Biophys. Acta 543, 269–272.
[9] Thomas, J.A., Schlender, K.K. and Larner, J. (1968) Anal. Biochem. 25, 486–499.
[10] Salavert, A., Itarte, E., Massague, J. and Guinovart, J.J. (1979) FEBS Lett. 106, 279–283.
[11] Gornall, A.G., Bardawell, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 751–766.
[12] Layne, E. (1957) Biochim. Biophys. Acta 540, 151–161.
[13] Ciudad, C.J., Bosch, F. and Guinovart, J.J. (1981) FEBS Lett. 129, 123–126.
[14] Stephenson, F.A. and Rogol, A.D. (1984) Arch. Biochem. Biophys. 234, 230–234.
[15] Tamura, S., Dubler, R.E. and Larner, J. (1983) J. Biol. Chem. 258, 719–724.
[16] Cheng, K., Creacy, S. and Larner, J. (1983) Mol. Cell. Biochem. 56, 177–182.
[17] Tamura, S., Brown, T.A., Dubler, R.E. and Larner, J. (1983) Biochem. Biophys. Res. Commun. 113, 80–86.
[18] Tamura, S., Brown, T.A., Whipple, J.H., Fujita-Yamaguchi, Y., Dubler, R.E., Cheng, K. and Larner, J. (1984) J. Biol. Chem. 259, 6650–6658.