Different effects of glucagon and epinephrine on the kinetic properties of liver glycogen synthase

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Kinetic constants of glycogen synthase ($M_{0.5}$ for glucose-6-P and $S_{0.5}$ for UDP-glucose) were determined after hepatocytes isolated from starved rats were incubated with either glucagon or epinephrine. Incubation with these hormones resulted in an increase in both $S_{0.5}$ and $M_{0.5}$. However, the action of glucagon resulted in great modifications on $S_{0.5}$ whereas epinephrine affected mainly $M_{0.5}$. Therefore, glucagon and epinephrine alter the kinetic properties of glycogen synthase provoke the phosphorylation of glycogen synthase at different site(s) acting through different mechanisms.

Glycogen synthase *Epinephrine* *Glucagon* *Rat hepatocyte*

1. INTRODUCTION

Glycogen synthase has been recognized as an enzyme regulated by covalent phosphorylation and dephosphorylation [1]. It is now accepted that it contains several phosphorylation sites per subunit which can be phosphorylated by several protein kinases, each kinase acting on specific site(s) [2,3]. Cyclic AMP-dependent protein kinases and cyclic AMP-independent protein kinases have been recently described to be present in rat liver [4–8]. Phosporylation results in the increase in $S_{0.5}$ for the substrate (UDP-glucose) and $M_{0.5}$ for the activator (glucose-6-*P*) which lead to the inactivation of the enzyme [9,10].

Glucagon and epinephrine trigger the inactivation of the liver enzyme [11,12]. However, although the action of glucagon can be reasonably explained by the activation of the cyclic AMP-dependent protein kinase, no evidence on the mechanism of action of epinephrine is available. Therefore, it is not known whether or not the action of epinephrine results in the phosphorylation of the same sites of the enzyme as those phosphorylated by the action of glucagon.

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Here, we present data on the changes in the kinetic constants provoked by glucagon and epinephrine on glycogen synthase from rat hepatocytes. These data show that glucagon and epinephrine alter the kinetic properties of liver glycogen synthase in a different manner. It is then concluded that these two hormones provoke the phosphorylation of glycogen synthase at a different site(s).

2. MATERIALS AND METHODS

Suspensions of isolated parenchymal liver cells were prepared from starved (24 h) male Wistar rats (200–250 g) as in [13]. Cells were finally resuspended in Krebs bicarbonate buffer (pH 7.4) free from glucose or any other substrate, and pre-gassed with O_2/CO_2 (19:1). Aliquots (5 ml, $8–9 \times 10^6$ cells/ml) were poured into stoppered vials, incubated at 37°C and shaken (100 strokes/min). At the end of the incubations the content of each vial was centrifuged ($3000 \times g$, 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).

The cell homogenates were centrifuged at $10000 \times g$ for 15 min, and the supernatants were filtered through Ultrogel AcA 202 columns (1 cm × 15

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cm) and equilibrated with 150 mM KF and 15 mM EDTA (pH 7.0), 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 the −G6P/+G6P standard assay [14]. Similarly, in this aliquot, $M_{0.5}$ was estimated from Hill plots of $\log(V_a/V_{a,max} - V_a)$ *vs* log[glucose-6-P] as in [10]. The second 300-μl aliquot was incubated for 30 min at 4°C, with 2 units glucose-6-P dehydrogenase and 0.13 μmol NADP to eliminate any trace of glucose-6-P remaining after column process. $S_{0.5}$ was then estimated from Hill plots of $\log(V/V_{std} - V)$ *vs* log[UDP-glucose] as in [10].

Protein was determined by the biuret method [15] as in [16]. All the chemical reagents used were Analytical Grade and obtained from Sigma or Merck. Collagenase came from Worthington, and bovine serum albumin (fraction V), glucagon and epinephrine from Sigma.

3. RESULTS

When hepatocytes isolated from 24-h starved rats were incubated for 10 min with increasing concentrations of glucagon or epinephrine, dose-dependent increases in $S_{0.5}$ for UDP-glucose and $M_{0.5}$ for glucose-6-P were observed (fig.1). However, the observed increases in $S_{0.5}$ after incubation with glucagon were much greater than those produced after incubation with epinephrine. On the other hand, epinephrine provoked greater increases in $M_{0.5}$ than did glucagon.

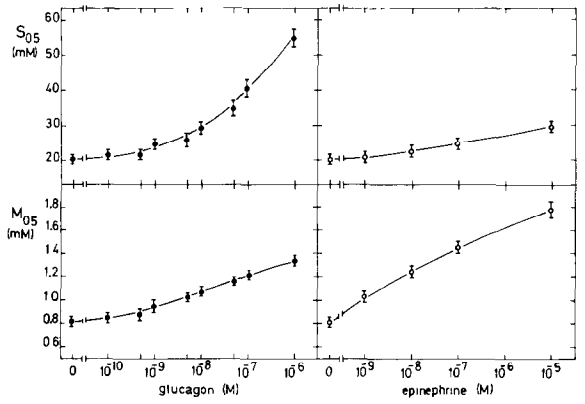

Fig.1. Dose-dependence of the effects of glucagon and epinephrine on $S_{0.5}$ for UDP-glucose and $M_{0.5}$ for glucose-6-P. Cells were incubated for 10 min with the indicated concentrations of the hormones. Results are mean ± SE of at least 5 expt.

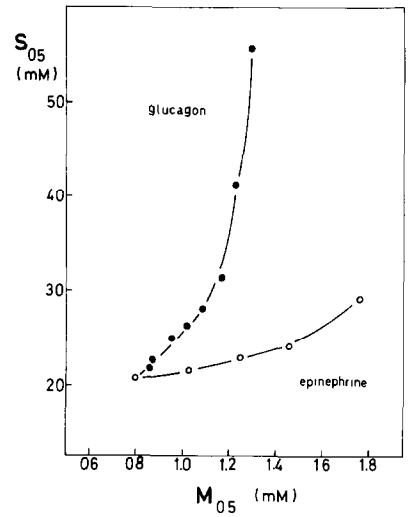

Fig. 2. Results in fig. 1 were replotted as $S_{0.5}$ *vs* $M_{0.5}$.

After replotting $S_{0.5}$ *vs* $M_{0.5}$-values for glycogen synthase of cells incubated with increasing concentrations of glucagon or epinephrine, the resulting curves were at variance (fig.2).

4. DISCUSSION

Glycogen synthase undergoes multiple phosphorylation. Phosphorylation results in an increase in the values of $S_{0.5}$ for UDP-glucose and $M_{0.5}$ for glucose-6-P [9,10]. Therefore, the more phosphorylated forms are less active than the dephosphorylated forms.

It seems logical to accept that UDP-glucose and glucose-6-P bind the enzyme at different sites. UDP-glucose binds at the active site, while glucose-6-P binds at an allosteric one. Therefore, the quantitative changes that the phosphorylation of a particular site exerts on $S_{0.5}$ and $M_{0.5}$ should be interpreted as the result of the modifications that the introduction of a phosphate in that phosphorylation site may exert on the configuration of both the active and the allosteric sites.

Although the phosphorylation of most sites seems to be able to exert effects on both the active and allosteric sites, it appears rather unlikely that the modifications produced by the phosphoryla-

tion of two different phosphorylation sites exert identical effects on the active and allosteric sites; i.e., it seems logical to assume that the phosphorylation of a site would produce deeper modifications on the active site than on the allosteric one, while the phosphorylation of other sites would have the opposite effect.

The plotting of $S_{0.5}$ *vs* $M_{0.5}$ for samples of glycogen synthase increasingly phosphorylated by a certain protein kinase expresses how the phosphorylation of the centers available to that kinase relatively alter the active and the allosteric sites. Therefore, it is likely that if two kinases follow different patterns of phosphorylation, the two plots of $S_{0.5}$ *vs* $M_{0.5}$ for the resulting species of glycogen synthase will be at variance.

Both $S_{0.5}$ and $M_{0.5}$ are affected by the incubation of rat hepatocytes either with glucagon or epinephrine. The action of glucagon results in great modifications on $S_{0.5}$ whereas epinephrine affects mainly $M_{0.5}$. Therefore, the plotting of $S_{0.5}$ *vs* $M_{0.5}$ for glycogen synthase from cells treated with increasing concentrations of glucagon differs from that obtained from cells treated with epinephrine.

These results suggest that the species of glycogen synthase resulting from the incubation of the cells with glucagon and epinephrine, are different as indicated by their different kinetic characteristics. This different behaviour can logically be attributed to the phosphorylation of different sites in response to glucagon or epinephrine.

Although it is widely accepted that the actions of glucagon and epinephrine on liver glycogen synthase metabolism have to be exerted through different mechanisms, this is the first time evidence has been obtained showing that the two hormones affect glycogen synthase in a different manner leaving their distinct imprints in the enzyme. Therefore, although both hormones inactivate glycogen synthase, the resulting inactive enzyme is not exactly the same in both cases.

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