Long term regulation of glycogen metabolizing enzymes by insulin in H4 Hepatoma cells

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Abstract

Insulin alone at concentrations of less than 1 to 5 uU/ml increased the enzyme activities of glycogen synthase, synthase phosphatase, phosphorylase, and phosphorylase phosphatase in hepatoma H4 cells in culture in the presence and absence of serum. Increase in total and active forms of glycogen synthase and phosphorylase were observed. Cycloheximide blocked the action of insulin on glycogen synthase, glycogen synthase phosphatase and phosphorylase phosphatase. The enzymes with the exception of glycogen synthase phosphatase were expressed with greater hormonal sensitivity in the absence as compared to the presence of serum in terms of hormone concentration required and or time of onset.

These results demonstrate that these glycogen metabolizing enzymes are under long term control by insulin, with glycogen synthase being the most sensitive of the enzymes studied to the action of the hormone.

Introduction

Interconversion of enzymes between active and inactive forms as a result of phosphorylation and dephosphorylation reactions is a major mechanism for controlling many cellular processes (1–4). Enzymes in biodegradative pathways are generally activated by phosphorylation, whereas enzymes in synthetic pathways are inactivated (5). This has become evident particularly from studies of the short term control of glycogen metabolism (6). Glycogen synthase [UDP-glucose; glucan 4-α-D-glycosyltransferase (EC 2.4.1.11)] is activated by glycogen synthase phosphatase [UDP-glucose-α-glucan glycosyltransferase D phosphohydrolase (EC 3.1.3.42)] and inactivated by a number of protein kinases while the inverse is true in the case of phosphorylase. [1,4-α-D-glucan:orthophosphate α-glycosyltransferase (EC 2.4.1.1)]. Numerous studies have indicated that glycogen synthase phosphatase and phosphorylase phosphatase [phosphorylase α phosphohydrolase (EC 3.1.3.17)] are subject to hormonal and metabolic regulation. However the factors controlling enzyme activities in liver during the longer term are difficult to study in the intact animal, since many variables cannot be controlled. We have recently demonstrated that the substrate induced activation of glycogen synthase by glucose and gluconeogenic precursors in the isolated perfused liver is proportional to glycogen synthase
phosphatase activity which in turn is significantly and positively correlated with circulating insulin concentrations in vivo (7). These results clearly indicate that insulin may regulate glycogen synthase phosphatase activity at the level of transcription or translation. In the present investigations we have used a hormone sensitive hepatoma cell line, H4-II-E-C3 (H4) cells, to study the role of insulin in the long term regulation of synthesis of four key glycogen metabolizing enzymes. We have used this line because of its great sensitivity to insulin in terms of mRNA accumulation (8) and have compared the responses in the presence and absence of serum.

Materials and methods

Cells

H4-II-E-C3 (H4) cells, derived from the H35 Reuber Hepatoma cells were obtained from ATCC (#CRL 1600).

Chemicals

Rabbit muscle phosphorylase a was obtained from Sigma Chemical Company, St. Louis, MO and dialyzed against 60 mM glycylglycine buffer, pH 7.8, to remove glycerophosphate and ethylenediamine tetraacetic acid (EDTA). Glycogen (from rabbit liver, type III, Sigma Chemical Company) was further purified by passing through an Amberlite MB-3 mixed ion exchange resin to remove nucleotides. Beef insulin was obtained from Eli Lilly and Co. Indianapolis, IN. All other chemicals were of analytical grade and were obtained from standard chemical supply houses.

Glycogen Synthase D was purified to essential homogeneity (specific activity 40 U/g from dog hind leg muscle by the method of Takeda and coworkers (9). Before use as substrate, aliquots were dialyzed against 50 mM Tris, 50 mM mercaptoethanol and 25% glycerol, pH 7.4 to remove EDTA, ethylene glycol bis (aminoethyl ether)-N,N,N,N-tetraacetic acid (EGTA), phenylmethylsulphonyl fluoride (PMSF) and N-\(\alpha\)-tosyl-L-lysine chloromethylketone HCl (TLCK). The substrate was further diluted 20-200 fold before use depending on the concentration of substrate required, with 50 mM glycyglycine and 2% bovine serum albumin at pH 7.8. As shown by Newman and Curnow (10) liver and muscle glycogen synthase and phosphorylase were essentially equivalent substrates in the respective liver phosphatase assays.

Cell culture

H4 cells were dispersed in 0.125% trypsin for 5 minutes at 37°C, rinsed twice with Swim's S-77 medium and inoculated in 150 × 25 mm tissue culture dishes containing 40 ml growth medium. Growth medium consisted of modified Swim's S-77 medium containing 5% fetal calf serum, 5% horse serum, 2 mM glutamine and 0.02% gentamicin. Approximately 5 × 10^6 cells per dish were cultured. The cells were grown in a humidified incubator at 37°C under 95% air and 5% carbon dioxide. After 6 days, the medium was replaced with medium containing serum, insulin, or cycloheximide as indicated and cells were cultured for a further 24 h or as otherwise indicated.

At the end of the incubation, the medium was removed by aspiration, 5 ml of appropriate cell homogenizing buffer added, and cells removed by scraping with a rubber policeman. The cell suspension was centrifuged at 3000 × g for 5 min, pellets resuspended in homogenizing buffer, recentrifuged, dispersed in 400 μl buffer, homogenized in a glass teflon homogenizer and used directly for enzymatic assays.

Enzymes assays

For glycogen synthase and glycogen phosphorylase measurements, cells were washed and homogenized in ice-cold 0.5 M sucrose, 62.5 mM glycyglycine, 50 mM NaF and 10 mM EDTA-Na at pH 7.4, termed glycogen synthase buffer. Glycogen synthase activity was determined by the method of Thomas et al. (11) using 6.55 mM uridine