

Discordance between Glucokinase Activity and Insulin and Glucagon Receptor Changes Occurring during Liver Regeneration in the Rat

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Summary

During regeneration of rat livers following 70% hepatectomy, insulin binding sites on hepatocyte plasma membranes are increased after 24-48 hours, glucagon binding sites are reduced on days 2-8, and the resultant insulin/glucagon binding ratio is markedly increased. An apparent paradox was the finding of a depression of the activity of an insulin associated enzyme, glucokinase, at a time when the number of insulin binding sites was increased.

Key-Words: *Glucokinase - Insulin Glucagon Receptors - Liver Regeneration - Rat*

Introduction

Many recent investigations and reviews (Starzl, Porter and Francavilla 1978; Starzl and Terblanche 1979; Bucher 1976; Leffert and Koch 1977; Leffert, Koch, Mozan and Rubalcava 1979) have focused upon the permissive effect, or possibly the initiating role of hormones in hepatic regeneration. Insulin and glucagon were the first two hormones whose so-called "hepatotropic" action was tied to liver regeneration (Starzl, Francavilla, Halgrimson, Francavilla, Porter, Brown and Putnam 1973; Francavilla, Porter, Benichou, Jones and Starzl 1978). Since then, many studies have shown that an increase in hepatocyte insulin binding and/or a decrease of glucagon binding (Starzl, Francavilla, Porter and Benichou 1978; Leffert, Alexander, Faloon, Rubalcava and Unger 1975; Freychet, Roth and Neville 1971; Pezzino, Vigneri, Cohen and Goldfine 1981; Morley, Kuke, Rubenstein and Boyer 1975) is a characteristic change which occurs during the first 48 hours of liver regeneration in the rat.

We report here an extension of these earlier observations in which the insulin/glucagon ratio of rat hepatocyte binding sites was determined for a full 10 days after partial liver resection. In addition, glucokinase was measured as a bio-

chemical marker of insulin activity (Salas, Vinuela and Sols 1963). Paradoxically, the glucokinase activity of the regenerating hepatocytes fell at the same time as the insulin binding sites and the insulin/glucagon binding ratio increased on the hepatocyte membrane.

Material and Methods

Animals

Male Sprague-Dawley rats (180-220 g) received food and water *ad libitum*. They were maintained in a temperature and light controlled room. Partial hepatectomy was performed according to the method of Higgins and Anderson (1931). In sham-operated animals, the liver was manually manipulated and returned into the abdomen. Operations were performed between 7.30 and 9.00 AM under ketamine[®] anesthesia (10 mg/100 g b.w.). The animals were sacrificed at 3 and 12 hours, and after the 1st, 2nd, 3rd, 4th, 6th, 8th and 10th day following hepatic resection or sham surgery.

Plasma Membrane Preparation

Rat liver plasma membranes were prepared according to Neville's procedure (Neville 1968). Once extracted, they were stored at -20°C. Their purity was monitored by both electron microscopy and assay of the 5'-nucleotidase activity (Goldfine, Smith, Wong and Jones 1977). There was no significant difference in the morphological appearance nor a statistically significant difference in the 5'-nucleotidase activity when preparation from normal and regenerating rat liver were compared. Changes in the Na⁺/K⁺ ATPase activity likewise were not statistically significant when normal and regenerating hepatocyte membranes were compared (Pezzino, Vigneri, Cohen and Goldfine 1981).

Binding of ¹²⁵I-insulin and ¹²⁵I-glucagon

Pork insulin and glucagon were purchased from Novo. Biologically active ¹²⁵I-insulin (100 µCi/µg) and ¹²⁵I-glucagon (100 µCi/µg) were obtained from New England Nuclear, Boston, Mass. Plasma membranes (50 ± 10 µg) were diluted to 100 µl with 1 mM KHCO₃ and incubated with or without unlabelled hormone (insulin: 100 µg/ml, glucagon: 100 µg/ml) for 20 min. at 30°C. Labelled hormones were suspended in Calcium free Krebs-Ringer phosphate buffer, pH 7.5 containing 3% bovine albumin (Fraction V). Fifty µl of the labelled hormone solutions were added to the incubation tubes to give a final concentration of 0.9 nM. At 0, 15, 30, 60, 90 and 120 minutes of incubation, duplicate 70 µl aliquots were removed and layered into 250 µl of Krebs-Ringer phosphate buffer, pH 7.5 containing 10 mg/ml of albumin in plastic microtubes. After centrifugation for 5 minutes at 0°C at 12,000 rpm, the supernatants were discarded and the pellets washed with 250 µl of 10% sucrose. The radioactivity in the pellet was counted in a Beckman Scintillation Counter (Leffert, Alexander, Faloon, Rubalcava and Unger 1975). Specific binding was monitored to 100 µg of protein.

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DNA Synthesis

Hepatic DNA synthesis was estimated during the 1st, 2nd, 3rd, 4th, 6th, and 8th day after hepatectomy or sham surgery. Two hours before sacrifice, 50 μ Ci of 3 H thymidine solution, specific activity 80 Ci/mmol, was injected intraperitoneally. Livers were removed and 3 H thymidine incorporation into DNA was determined (Ove, Coetzee and Morris 1971; Ove, Coetzee and Morris 1973). DNA was estimated by the diphenylamine method (Giles and Myers 1965) with calf thymus DNA as the standard. The results were expressed as cpm/mg DNA.

Glucokinase Activity Determination

Glucose phosphorylation by glucokinase, enzyme conversion number 2.7.1.2, was assayed by the glucose-6-phosphate-dehydrogenase spectrophotometric method described by Sols et al. (Solas, Vinuela and Sols 1963). The results were expressed as nanomoles of glucose phosphorylated/min/mg of cytosolic protein.

Protein Determination

Protein concentrations were determined by the method of Lowry (Lowry, Rosebrough, Farr and Randall 1951) using serum bovine albumin as standard.

Weight of Liver Determination

The weight of livers was determined at each experimental time after hepatectomy, and expressed as a percentage of predicted normal liver weight. The predicted normal weight was 4.52 ± 0.58 (SD)% of body weight and was determined by sacrificing 10 normal animals and examining the relationship between liver and body weight.

Statistical Analysis

Comparisons were made using the Student's non-paired t-test. Scatchard analyses were carried out with plasma membranes to determine whether decreased binding was due to either a decrease in binding capacity or a decrease in binding affinity (Scatchard 1948).

Results

There was a rapid decrease in glucagon binding beginning early during the first day after partial hepatectomy which reached a nadir on the third day (Figure 1A). In contrast insulin binding increased significantly at the end of the first day following hepatectomy and returned to normal values on the second day without undergoing further fluctuation (Figure 1B). DNA synthesis rates at various times after partial hepatectomy were similar to those reported previously in rats with maximum synthesis occurring on the first postoperative day (Figure 1C). The return of glucagon binding to normal levels following the initial decrease correlated with restoration of liver mass (Figure 2).

The ratio of insulin binding/glucagon binding for 100 μ g of hepatic plasma membrane protein increased markedly at one day and persisted for an additional four days (Fig. 3).

A typical time course of specific insulin and glucagon binding to plasma membrane in control and hepatectomized rats at 24 hours after partial hepatectomy is shown in Figure 4A. By 60 minutes after incubation, a steady state was reached and longer incubations resulted in no additional changes. Scatchard plot of insulin and glucagon binding to hepatic plasma membrane prepared from control and hepatectomized rats 24 hours after operation indicates a decrease in the number of binding sites for glucagon and an increase for insulin (Figure 4B).

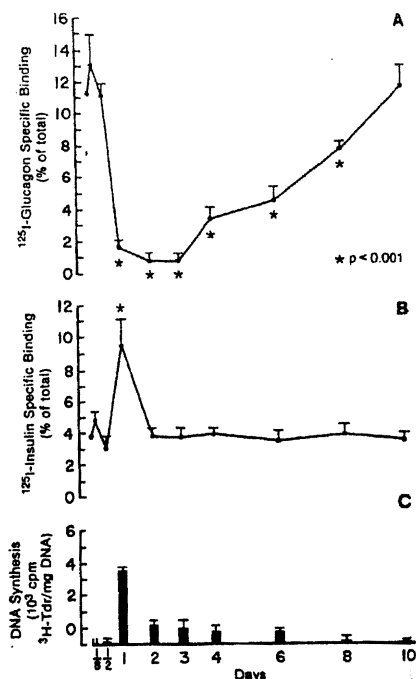


Fig. 1 Response to partial hepatectomy in terms of: (A) Specific binding to plasma membrane of the 125 I-glucagon, (B) Binding of 125 I-insulin and (C) DNA synthesis.

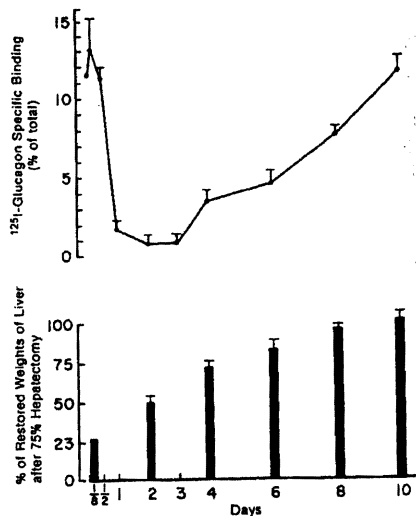


Fig. 2 Correlation between 125 I-glucagon binding changes and the restoration of liver mass.

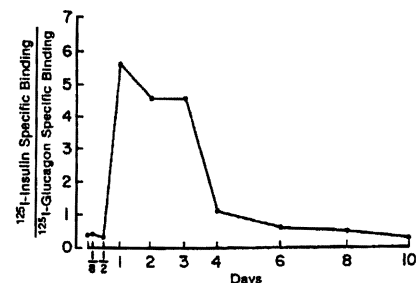


Fig. 3 The ratio between 125 I-insulin binding and the 125 I-glucagon binding to the plasma membranes from hepatectomized rats at different times after operation.

Glucokinase, Ins

Glucokinase activity was obtained from various times after hepatectomy. The enzyme activity decreased to the insulin binding site on the second day following hepatectomy and reached its nadir on the third day. It returned to normal on day 10 as observed in sham-operated rats.

Discussion

Previous studies to hepatic plasma membranes have been limited to insulin and glucagon binding (Benichou, Unger 1975) and DNA synthesis (Unger 1975) during this period. The results during this period are similar to those reported by Cohen and Gold (1975). It is possible that the decrease in glucagon binding immediately following hepatectomy is due to a decrease in binding capacity. In previous studies, these parameters returned to normal after completion of the regeneration process (restoration of liver mass). It is of interest that the changes in hepatic glucokinase activity observed following hepatectomy are those observed for the restoration of the liver mass. The previously been shown that plasma levels of insulin and glucagon rose during the first 10 days (Francavilla et al. 1984).

The unexpected fall in glucokinase activity after hepatectomy suggests that binding sites are increased in the liver. It is important to note that the increase in glucagon or epinephrine binding after insulin administration (Francavilla et al. 1984). However, the results of earlier biochemical studies (Jungermann 1978; Unger 1973) in regeneration of the liver show an increase in glycolytic activity 48 hours after hepatectomy. A possible explanation could be a preferential regulation of carbohydrate metabolism by insulin. Our experimental results indicate that insulin has many effects on the regulation of the liver, such as those

Glucokinase activity present in the cytosol of hepatocytes obtained from normal and hepatectomized animals at various times after the operation is shown in Figure 5. The enzyme activity underwent a paradoxical decrease relative to the insulin binding changes observed and the time course of the change was different, occurring later than the changes in insulin binding which were maximal during the first and second day following hepatectomy. Glucokinase activity decreased on the first day following partial hepatectomy, reached its nadir on day 3 after the operating and returned to normal on day five. None of these alterations was observed in sham-operated animals (data not reported).

Discussion

Previous studies concerning insulin and glucagon binding to hepatocyte plasma membranes during regeneration have been limited to the first 48 hours (Starzl, Francavilla, Porter and Benichou 1978; Leffert, Alexander, Faloona, Rubalcava and Unger 1975; Freychet, Roth and Neville 1971). Our results during this interval are consistent with those of earlier workers and demonstrate increased insulin binding, decreased glucagon binding and a major increase in the resulting insulin/glucagon binding ratio at hepatic plasma membranes. It is possible that the increased binding of insulin immediately following partial hepatectomy is at least partially due to a decreased rate of degradation (Pezzino, Vigneri, Cohen and Goldfine 1981). In contrast to those earlier studies, these processes in our studies were followed to their completion (restoration to normal levels) and were related to changes in hepatocyte insulin responsiveness measuring glucokinase activity, an insulin inducible enzyme. It was of interest that the changes in glucagon binding far outlasted those observed for insulin and appeared to correlate with restoration of the hepatic mass to normal levels. It has previously been shown that after 70% hepatectomy, the plasma levels of insulin underwent inconsistent changes, but glucagon rose first and returned to normal in seven days (Francavilla et al. 1978).

The unexpected finding in our studies was the paradoxical fall in glucokinase activity at a time when the insulin binding sites are increased. In the interpretation of these data, it is important to note that glucokinase is not influenced by glucagon or epinephrine while glucokinase disappears in diabetes and after fasting and reappears within a few hours after insulin administration or refeeding (Giles and Myers 1965). However, our observations are consistent with those of earlier biochemical studies (Brinkmann, Katz, Sasse and Jungermann 1978; Bonney, Hopkins, Walker and Potter 1973) in regenerating rat liver in which a significant decrease in glycolytic capacity was reported during the first 48 hours after hepatectomy.

A possible explanation for the apparent paradoxical findings could be a preferential utilization of insulin in metabolic pathways subserving regeneration as opposed to those regulating carbohydrate metabolism under the conditions of our experiments. In this regard, it is well known that insulin has many and varied actions (Cahill 1971), but the modulation of these actions to accommodate special requirements such as those of regeneration is pure speculation.

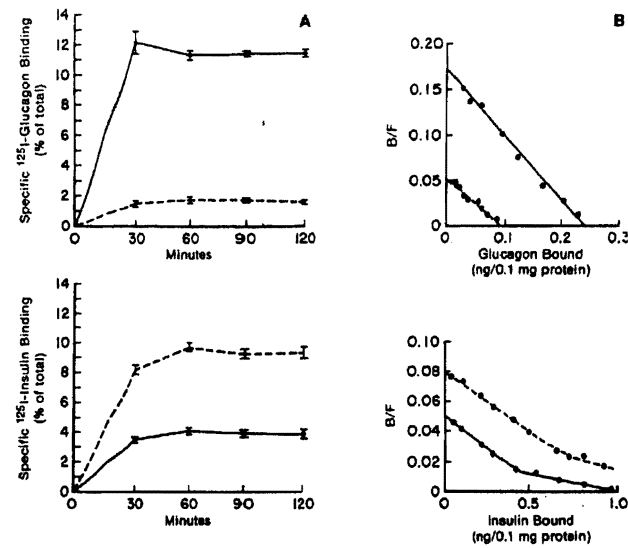


Fig. 4 The time course (A) and Scatchard's plot (B) of specific ¹²⁵I-glucagon and ¹²⁵I-insulin binding from sham-operated and hepatectomized rats 24 hours after the operation.

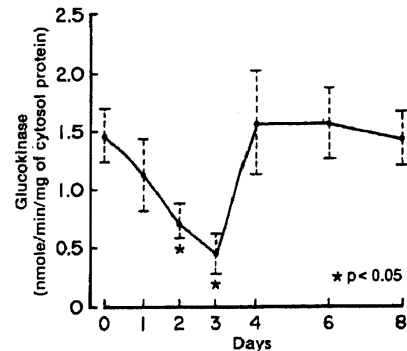


Fig. 5 Glucokinase activity assayed in the livers of hepatectomized rats at different times after operation.

Another hypothesis that might explain at least in part our observations is that during regeneration, the insulin binding sites might be occupied by insulin-like polypeptides (growth factors) other than insulin. Polyamines and a number of other substances concerned with regeneration might be reasonable candidates for such binding. It has been accepted generally that the hormonal events of hepatic regeneration are very complex and involve more than the simple interactions of two pancreatic hormones, insulin and glucagon (Starzl and Terblanche 1979; Bucher 1976; Leffert and Koch 1977; Leffert et al. 1979; Starzl et al. 1973; Francavilla et al. 1978; Starzl et al. 1978). The results of Royse and Morley (1980) have suggested that neurohumoral control could be a factor. They showed that 6-OH-Dopamine injected intraperitoneally into rats is able to abolish hepatocyte mitosis (through chemical sympathectomy) after 75% hepatectomy without affecting the well known glucagon and insulin modification in the blood.

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