Published in final edited form as:

Horm Metab Res. 1984 December ; 16(Suppl 1): 47–50. doi:10.1055/s-2007-1014896.

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

A. Francavilla¹, A. Di Leo¹, S.Q. Wu², P. Ove³, D. Van Thiel⁴, C. Sciascia⁵, and T.E. Starzl²

¹ Department of Gastroenterology, University of Bari, Bari, Italy

² Department of Surgery, School of Medicine, University of Pittsburgh, Pittsburgh, PA, U.S.A

³ Department of Anatomy, School of Medicine, University of Pittsburgh, Pittsburgh, PA, U.S.A

⁴ Department of Gastroenterology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, U.S.A

⁵ Department of Surgery, University of Torino, Torino, Italy

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.

Keywords

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 “hepatotrophic” 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 biochemical 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 $\text{Na}^{+}/\text{K}^{+}$ ATPase activity likewise were not statistically significant when normal and regenerating hepatocyte membranes were compared (Pezzino, Vigneri, Cohen and Goldfine 1981).

Binding of ^{125}I -insulin and ^{125}I -glucagon

Pork insulin and glucagon were purchased from Novo. Biologically active ^{125}I -insulin ($100\text{ }\mu\text{Ci}/\mu\text{g}$) and ^{125}I -glucagon ($100\text{ }\mu\text{Ci}/\mu\text{g}$) were obtained from New England Nuclear, Boston, Mass. Plasma membranes ($50 \pm 10\text{ }\mu\text{g}$) were diluted to $100\text{ }\mu\text{l}$ with 1 mM KHCO_3 , and incubated with or without unlabelled hormone (insulin: $100\text{ }\mu\text{g}/\text{ml}$, glucagon: $100\text{ }\mu\text{g}/\text{ml}$) 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 $70\text{ }\mu\text{l}$ aliquots were removed and layered into $250\text{ }\mu\text{l}$ of Krebs-Ringer phosphate buffer, pH 7.5 containing $10\text{ mg}/\text{ml}$ of albumin in plastic microtubes. After centrifugation for 5 minutes at 0°C at $12,000\text{ rpm}$, the supernatants were discarded and the pellets washed with $250\text{ }\mu\text{l}$ of 10% sucrose. The radioactivity in the pellet was counted in a Beckman Scintillation Counter (Leffert, Alexander, Faloona, Rubalcava and Unger 1975). Specific binding was monitored to $100\text{ }\mu\text{g}$ of protein.

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, $50\text{ }\mu\text{Ci}$ of ^3H thymidine solution, specific activity $80\text{ Ci}/\text{mmol}$, was injected intraperitoneally. Livers were removed and ^3H 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.

(Salas, 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).

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 Jimgermann 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 accomodate special requirements such as those of regeneration is pure speculation.

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 intraportally 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.

Acknowledgments

Supported by research grants from the Veterans Administration; by project grant AM-29961 from the National Institutes of Health and by grant RR-00084 from the General Clinical Research Centers Program of the Division of Research Resources, National Institute Health, and grant 82/0031096 from Consiglio Nazionale Delle Ricerche (CNR).

References

- Bonney RS, Hopkinds HA, Walker PR, Potter VR. Glycolytic isoenzymes and glycogen metabolism in regenerating liver from rats on controlled feeding schedules. *Biochem J* 1973;136:115–124. [PubMed: 4772620]

- Brinkmann A, Katz M, Sasse P, Jimgermann K. Increase of gluconeogenic and decrease of glycolytic capacity of rat liver with change of the metabolic zonation after partial hepatectomy. *Z. Physical Chem* 1978;359:1561–1571.
- Bucher, NLR. Insulin, glucagon and the liver. In: Weber, G., editor. *Advances in Enzyme Regulation*. Vol. 15. Oxford: Pergamon Press; 1976. p. 221-230.
- Cahill GF Jr. Physiology of insulin in man. *Diabetes* 1971;20:785–799. [PubMed: 4941092]
- Francavilla A, Porter KA, Benichou J, Jones AF, Starzl TE. Liver regeneration in dogs: morphologic and chemical changes. *J Surg Res* 1978;25:409–419. [PubMed: 213658]
- Freychet P, Roth J, Neville DM. Insulin receptors in the liver: Specific binding of (125I) insulin to the plasma membrane and its relation to insulin bioactivity. *Proc Natl Acad Sci USA* 1971;68:1833–1837. [PubMed: 4331561]
- Giles KW, Myers A. An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature* 1965;206:93. [PubMed: 14334364]
- Goldfine ID, Smith GJ, Wong KY, Jones AL. Cellular uptake and nuclear binding of insulin in human cultured lymphocytes: Evidence for potential intracellular sites of insulin action. *Proc Natl Acad Sci USA* 1977;74:1368–1372. [PubMed: 193098]
- Higgins GM, Anderson RM. Experimental pathology of the liver. I Restoration of the liver of the white rat following partial surgical removal. *Arch Pathol* 1931;12:186–202.
- Leffert H, Alexander NM, Faloona G, Rubalcava B, Unger R. Specific endocrine and hormonal receptor changes associated with liver regeneration in adult rats. *Proc Natl Acad Sci USA* 1975;72:4033–4036. [PubMed: 172891]
- Leffert, HL.; Koch, KS. Proliferation of hepatocytes. In: Porter, R.; Whelan, J., editors. *Hepatotropic Factors*; CIBA Foundation Symposium; Amsterdam: Elsevier/Excerpta Medica/North-Holland; 1977. p. 61-94.
- Leffert HL, Koch KS, Moran T, Rubalcava B. Hormonal control of rat liver regeneration. *Gastroenterology* 1979;76:1470–1482. [PubMed: 220134]
- Morley CGD, Kuke S, Rubenstein AH, Boyer JL. Serum hormone levels following partial hepatectomy in rat. *Biochem Biophys Res Comm* 1975;67:653–661. [PubMed: 1201045]
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–275. [PubMed: 14907713]
- Neville DM Jr. Isolation of an organ specific protein antigen from cell-surface membrane of rat liver. *Biochem Biophys Acta* 1968;154:540–552. [PubMed: 4967807]
- Ove P, Coetzee ML, Morris HP. DNA synthesis and the effect of sucrose in nuclei of host liver and Morris hepatomas. *Cancer Res* 1971;31:1389–1395. [PubMed: 4328733]
- Ove P, Coetzee ML, Morris HP. Separable DNA polymerase activities in host liver and Morris hepatomas. *Cancer Res* 1973;33:1272–1283. [PubMed: 4352366]
- Pezzino V, Vigneri R, Cohen D, Goldfine ID. Regenerating rat liver: Insulin and glucagon serum levels and receptor binding. *Endocrinology* 1981;108:2163–2169. [PubMed: 6262053]
- Royse V, Morley GD. A possible role for norepinephrine in the control of liver regeneration. *IRCS J Med Sci* 1980;8:411–412.
- Salas M, Vinuela E, Sols A. Insulin-dependent synthesis of liver glucokinase in the rat. *J Biol Chem* 1963;238:3535–3538. [PubMed: 14109182]
- Scatchard G. The attractions of proteins for small molecules and ions. *Ann NY Acad Sci* 1948;51:660–672.
- Starzl TE, Francavilla A, Halgrimson CG, Francavilla FR, Porter KA, Brown TH, Putnam CW. The origin, hormonal nature, and action of hepatotropic substances in portal venous blood. *Surg Gynecol Obst* 1973;137:179–199. [PubMed: 4353133]
- Starzl TE, Francavilla A, Porter KA, Benichou J. The effect upon the liver of evisceration with or without hormone replacement. *Surg Gynecol Obst* 1978;146:524–532. [PubMed: 205003]
- Starzl, TE.; Terblanche, J. Hepatotropic substances. In: Popper, H.; Schaffner, F., editors. *Progress in Liver Disease*. Vol. VI. New York: Grune & Stratton; 1979. p. 135-151.

Starzl, TE.; Porter, KA.; Francavilla, A., et al. 100 years of the hepatotrophic controversy. In: Porter, R.; Whelan, J., editors. Hepatotrophic Factors; Ciba Foundation Symposium No. 55; Amsterdam: Excerpta Medica (Elsevier North-Holland); 1978. p. 111-129.

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.

Fig. 4.
The time course (A) and Scatchard's plot (B) of specific ^{125}I -glucagon and ^{125}I -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.