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Changes of Insulin and Glucagon Binding to Receptors in Hepatocytes During Liver Regeneration

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Summary

The binding of insulin (INS) and glucagon (GL) on isolated rat hepatocytes during the process of liver regeneration after partial hepatectomy was determined. Adult male rats were subjected to 65–70 % partial hepatectomy, control animals were sham-operated. The binding of radioiodine labelled INS and GL to isolated hepatocytes was determined 1, 2, 3 and 5 days after the surgery. The plasma levels of INS and glucose and microviscosity of liver plasma membranes were also measured. The decrease of INS receptor binding capacity was found 1, 2, and 3 days after operation. No differences in sham and partially hepatectomized groups in INS binding were noted 5 days after operation. A single insulin injection during the process of regeneration did not affect these changes of INS binding to hepatocytes. The increase of GL binding was observed on the third day after partial hepatectomy, however, on the 5th day no changes of GL binding to its receptors were noted. The plasma insulin and glucose levels were similar in both hepatectomized and sham-operated rats. The increase of plasma membrane microviscosity of hepatocytes during the process of liver regeneration and a negative correlation between INS binding and membrane microviscosity were found. These results demonstrated significant changes in binding parameters of both INS and GL receptors in hepatocytes during liver regeneration induced by partial hepatectomy.

Key words

Hepatocytes – Insulin – Glucagon – Receptors – Hepatectomy

Introduction

Both pancreatic hormones, insulin and glucagon, are known to exert strong effects on hepatocyte growth and metabolism. The presence of insulin is required in incubation media of primary hepatocyte culture (Michalopoulos 1990). Pancreatectomy is associated with diminished hepatic mass and with a decrease of DNA synthesis in the regenerating liver. Insulin and glucagon administration reversed these effects (Michalopoulos 1990). In spite of the evidence that insulin and glucagon promoted liver growth in partially hepatectomized rats (Bucher and Swaffield 1975, Bucher 1991, Šimek and Sobotka 1983) and have a permissive role necessary for optimal DNA synthesis and liver regeneration, there is no evidence that these hormones exert any direct mitogenic effects in the liver. However, both insulin and glucagon are required for optimal stimulation of mitogenesis induced by epidermal growth factor (Michalopoulos 1990). In relation to this significant role of insulin and glucagon in the stimulation of liver growth it was interesting to study how insulin and glucagon may produce their signals by interacting with cellular receptors. Leffert *et al.* (1979) demonstrated changes in insulin and glucagon binding during the early period (2–24 h) of regeneration by using membrane fraction of liver cells for binding studies. On the contrary, in our experiments the changes of receptor binding parameters for these two hormones were investigated during the later period (1–5 days) of regeneration by using the whole cell system instead of the membrane fraction of rat liver. In addition, the effect of insulin administration on insulin binding capacity of receptors during the regeneration was also studied.

Materials and Methods

Porcine monocomponent insulin for binding studies was obtained from Novo (Copenhagen, Denmark), insulin for injection was Superdep (Léčiva, Prague, Czech Republic), glucagone was from Elanco (Indianopolis, USA), EGTA from Sigma (St.Louis, USA), collagenase was from Sevac (Prague, Czech Republic), Pentobarbital, from Spofa (Prague, Czech Republic), $Na^{125}I$ was from Amersham (Little Chalfont, England). All other chemicals were reagent grade supplied by Sigma or Lachema (Brno, Czech Republic).

Adult male Wistar rats (body weight 320–360 g) were subjected to partial hepatectomy (65–70 %) as was described by Higgins and Anderson (1931). Sham-operated controls were subjected to laparotomy. Due to *in situ* isolation of hepatocytes, liver mass was not determined. In the first experiment (1, 2, 3 and 5 days after surgery), rats were anaesthetized with pentobarbital (9 mg/100 g) and liver cells were isolated by using an *in situ* perfusion method (Terris and Steiner 1975). Briefly, liver was first perfused *via* the portal vein with calcium-free Hank's balanced salt solution containing 1 mmol EGTA (pH 7.4) followed by perfusion with Hank's solution containing calcium (4.7 mmol/l) and 0.05 % collagenase. The liver was then excised, cut into small pieces and incubated with collagenase solution for 10 min. Isolated cells were collected by filtration. Cells were washed and pelleted by centrifugation (Terris and Steiner 1975). Cell viability was checked using trypan blue exclusion and it exceeded 95 % in all experiments.

The receptor binding capacity of isolated hepatocytes for radioiodine labelled insulin and glucagon was determined by methods described in our previous papers (Zorad *et al.* 1986, Ficková and Macho 1988). Liver plasma membranes were prepared (Sakamoto *et al.* 1980) from 4 groups of animals. The microviscosity of membranes was determined by measuring fluorescence anisotropy with a DPH probe (1,6-diphenyl-1,3,5-hexatriene). The plasma was collected for determination of insulin levels (radioimmunoassay method, Novo, Nordisk, Denmark) and glucose (Biotest, Lachema, Brno, Czech Republic) in selected group of rats at various periods after surgery.

In the second experiment, insulin was administered subcutaneously to rats (2.5 IU/kg b.w., Dobozy *et al.* 1992) 24 hours after sham-operation or partial hepatectomy. The isolated hepatocytes were prepared 2 and 3 days after surgery and insulin binding was determined as mentioned above.

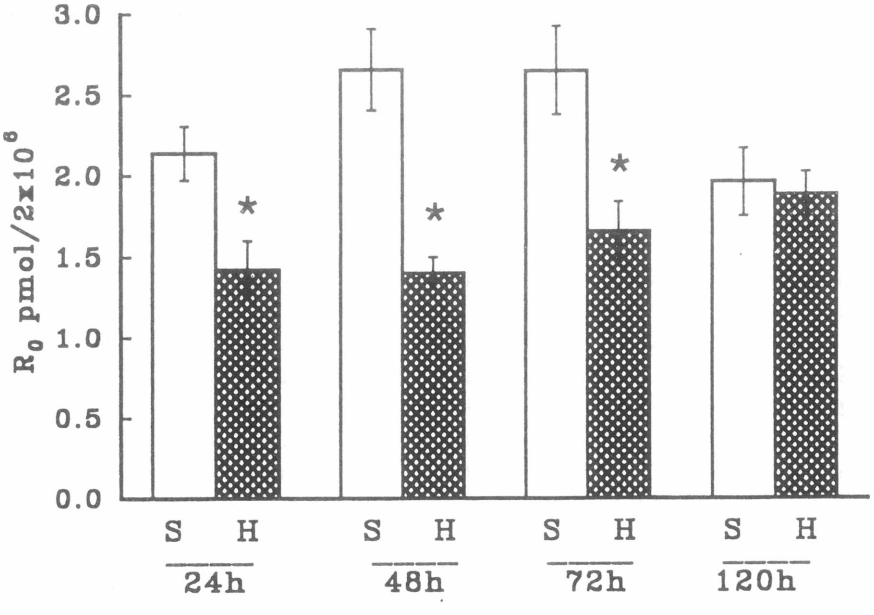

Fig. 1
Insulin binding capacity of isolated hepatocytes after partial hepatectomy. * sham-operated (S) vs partially hepatectomized (H) ($p<0.05$), period after surgery in hours. Number of animals at 24 h: S-6, H-10, at 48 h: S-10, H-9, at 72 h: S-11, H-12, at 120 h: S-10, H-10.

Results

The significant decrease of insulin receptor binding capacity and number of insulin receptors was found in hepatocytes of rats 1, 2 and 3 days after partial hepatectomy. Five days after surgery no significant differences in insulin binding to isolated hepatocytes of rats from the sham-operated group and that after partial hepatectomy were noted (Fig. 1).

The plasma insulin and glucose levels were not significantly different in sham-operated and partially hepatectomized animals 1, 2 and 3 days after surgery (Table 1).

Table 1
Insulin and glucose concentrations in rat plasma after partial hepatectomy.

	Sham-operated	Partial Hepatectomy		
Interval after surgery	24-72 h	24 h	48 h	72 h
Insulin μU/ml	20.2 ±2.3	21.8 ±2.3	20.8 ±2.3	22.3 ±2.5
Glucose mmol/l	5.19 ±0.14	4.97 ±0.15	5.26 ±0.32	5.00 ±0.16

Means ± S.E.M., 6 samples per group.

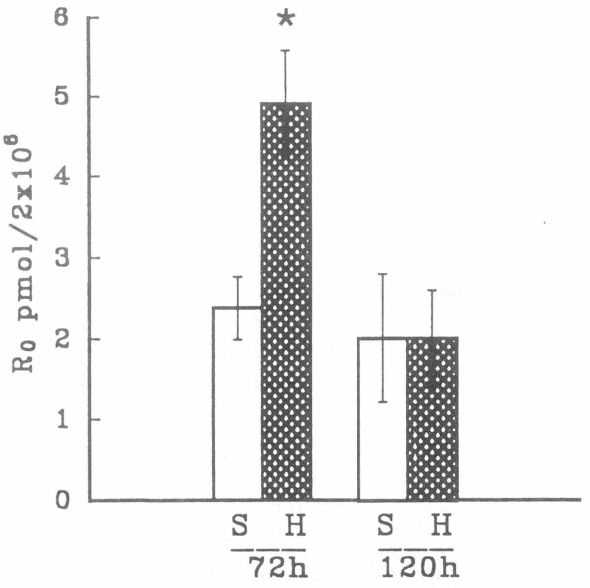

Fig. 2
Glucagon binding capacity of isolated hepatocytes from rats after partial hepatectomy. * sham operated (S) vs partially hepatectomized (H) ($p<0.05$), period after surgery in hours, each bar is a mean of 5 rats.

Glucagon receptor binding capacity of isolated hepatocytes was increased 3 days after partial hepatectomy. However, no changes in glucagon receptor binding capacity were observed on the 5th day of regeneration (Fig. 2).

Plasma membrane microviscosity in the regenerating livers was significantly increased during the regeneration period compared to livers from the sham-operated rats (mean values of fluorescence anisotropy (r) at 48 hrs 0.240+0.006 resp. 0.200+0.009). A negative correlation was found between insulin receptor binding capacity of isolated hepatocytes and microviscosity of the plasma membrane during the regeneration period suggesting that changes in fluidity of plasma membrane may have some role in the changes of insulin binding after partial hepatectomy (Fig. 3).

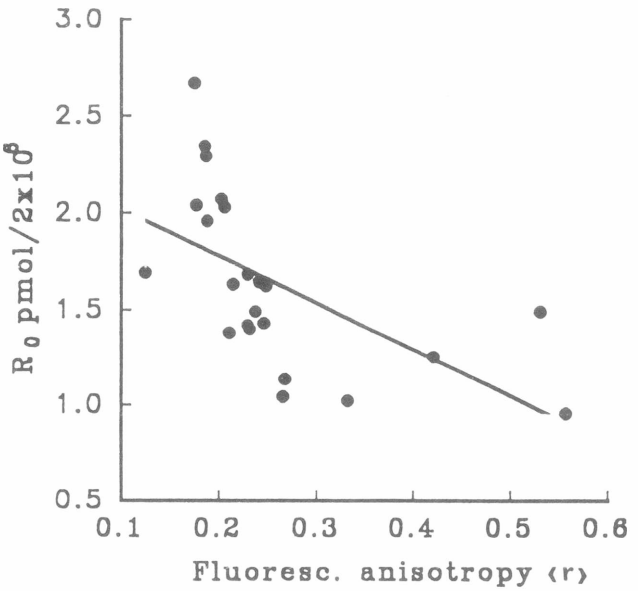

Fig. 3
Relationship between insulin binding capacity (Ro) and microviscosity of liver cell membranes fluorescence anisotropy (r) from sham-operated and partially hepatectomized rats. Correlation coefficient $r=0.5836$, $p<0.01$, $n=23$.

In the second experiment, the effect of a single insulin dose during the process of regeneration was studied. A similar decrease in the number of insulin receptors in regenerating liver was found in rats after insulin injection as was observed in rats without hormone administration (Fig. 4). These results clearly demonstrate that this dose of exogenous insulin has no effect on insulin binding capacity during the early stage of liver regeneration after partial hepatectomy.

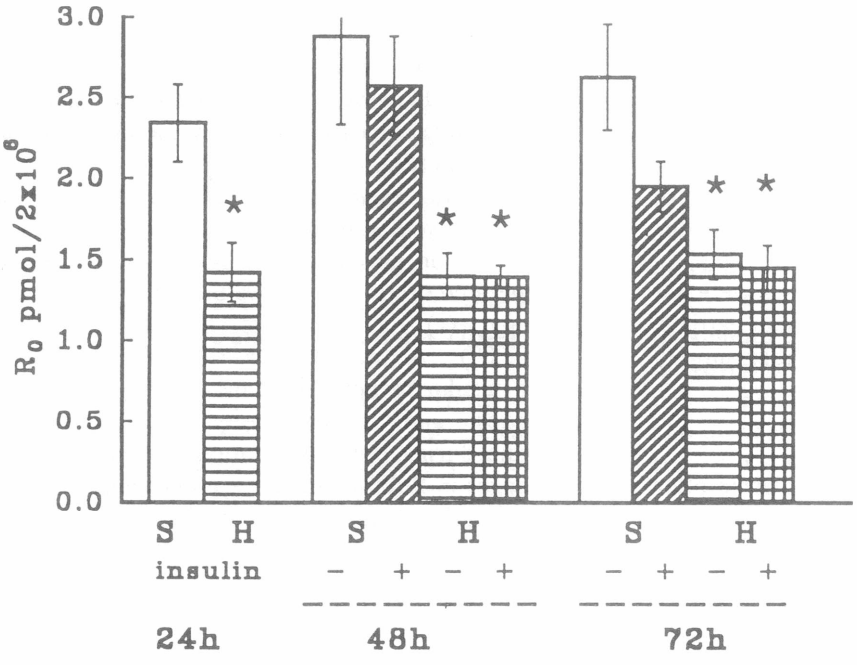

Fig. 4
Insulin receptor binding capacity of hepatocytes from sham-operated (S) and partially hepatectomized (H) rats without (-) and with (+) injection of insulin (1 day after the surgery). * S vs H ($p < 0.05$), period after surgery in hours, number of animals in sham-operated groups 6–10 per group, in partially hepatectomized group without insulin 10 rats per group, in sham-operated group and partially hepatectomized group after insulin injection 5 rats per group.

Discussion

The results of our experiments showed significant changes of insulin and glucagon receptors in liver cells during the regeneration period after partial hepatectomy. The decreased number of insulin receptors was found during the first three days after surgery. However, no differences were found on the fifth day after partial hepatectomy. Insulin receptor binding capacity was similar in sham-operated groups 1 and 5 days after the surgery. However, a slight but nonsignificant increase of insulin receptors was noted in sham groups on the 2nd and 3th days in comparison to the 1st day. This could be the result of transient fasting after surgery because a slight decrease of body mass was also noted in sham-operated groups (Cornell 1981) and an elevation of insulin binding was found in the liver of fasted rats (Yokono *et al.* 1982). This mechanism is not effective in partially hepatectomized rats. Insulin receptor binding capacity is regulated by plasma insulin levels, by physico-chemical properties of plasma membrane and by the action of several other hormones. The increase of plasma insulin levels (Lane 1981), or elevation of insulin concentration in the incubation medium of insulin-sensitive cell cultures is followed by a significant decrease of insulin binding due to mechanism of receptor down-regulation (Lane 1981). However, in the case of liver regeneration some discrepancies were observed in insulin plasma levels during different periods after partial hepatectomy. Bucher and Swaffield (1975), Leffert *et al.* (1975) and Cornell (1981) reported that plasma insulin levels in the first hours after partial hepatectomy are decreased. On the other hand, Morley *et al.* (1975), Rassmussen *et al.* (1992) as well asd Foss and Ahren (1991) showed that there are no changes in peripheral plasma insulin levels 6 hours to 3 days after partial hepatectomy. Moreover, hyperinsulinaemia in portal venous blood and an increased insulin secretion after a glucose load were described in fasting rats after partial hepatectomy (Cornell 1981, Tennoku *et al.* 1986). In our experiments, the unchanged insulin and glucose levels in peripheral plasma are in good agreement with those of Rassmussen *et al.* (1992) who also found no changes of insulin and glucose plasma levels after partial hepatectomy. Due to the transfer of all insulin produced in the pancreas by portal venous blood into the liver there is clearly an increased load of insulin to only one third of liver tissue, which might account for the decline of insulin receptors via the mechanism of down-regulation. It was reported that in spite of rapid regeneration there is still only about 60 % of liver mass on the third day after partial hepatectomy (Červinková *et al.* 1984). However, Leffert *et al.* (1975) found no changes in insulin binding and a diminution of glucagon binding to liver plasma membrane fraction 2, 8, 12, 16 and 24 hours after partial hepatectomy. We do not have the data on insulin binding during this early period after surgery and the discrepancy between our results 24 hours after surgery and those of Leffert *et al.* (1975) can be explained by a different technique used in the binding experiments. Whole cell system for

binding studies seems to be much more sensitive for detection of hormone-induced down-regulation than binding experiments done on isolated membrane preparation (Lane 1985, Krupp and Lane 1981).

Out of the hormonal factors affecting insulin receptors, glucocorticoids are the most important hormones involved in regulation of insulin receptors (Olefsky *et al.* 1975, Kahn *et al.* 1981, Macho and Ficková,1992, Macho *et al.* 1993). Elevated plasma glucocorticoid levels are followed by a decrease of insulin receptors in several tissues (Kahn *et al.* 1981, Macho and Ficková 1992). Increased plasma corticosterone levels in rats were observed from 1 hour up to 3 days after partial hepatectomy (Knopp *et al.* 1991, Leffert *et al.* 1979). This suggests that increased corticosterone plasma levels after partial hepatectomy might also participate in the decline of the number of insulin receptors in hepatocytes.

The increase of membrane microviscosity in regenerating liver cells was observed in our experiments together with a negative correlation between insulin receptor number and microviscosity of the cell membrane. Ginsberg *et al.* (1990) demonstrated that increased membrane fluidity is connected with the elevation of insulin receptor number. Thus our results suggest that the changes of membrane fluidity could also be partially responsible for the diminution of insulin binding to receptors.

It was demonstrated that hormone receptors mature only after primary interaction with the adequate hormone (hormonal imprinting, Csaba 1986). An appropriate quantity of the adequate hormone is necessary for normal hormone receptor maturation in the critical period, usually during the perinatal period. According to Csaba *et al.* (1989) the dedifferentiation and subsequent redifferentiation processes take place during liver regeneration. This is also the period when insulin receptors could undergo imprinting. Dobozy *et al.* (1992) reported that single insulin treatment (imprinting), applied in the initial stage of liver regeneration, was followed by a higher percentage of specific insulin binding to the liver plasma membrane 2–12 days after partial hepatectomy. This insulin injection did not affect basal glucose and insulin levels 2–12 days after partial hepatectomy. However, the above authors did not determine the insulin receptor binding capacity of whole liver cells. The administration of a single insulin dose performed 24 hours after the surgery in our experiment showed that there is no effect of this single insulin injection on the binding capacity and the number of insulin surface receptors in hepatocytes during 5 days of liver regeneration. It was also found that insulin-treated and control rats responded to insulin loading by the same decrease of blood glucose levels (Dobozy *et al.* 1992), suggesting that there was no effect of single insulin dose on the insulin sensitivity of target tissues.

Our findings of increased glucagon receptors in hepatocytes on the third day of regeneration are in accordance with the stimulatory role of glucagon on the regeneration process. At the present time, the explanation of this increase is difficult due to the ambiguous relationship between plasma glucagon levels and glucagon receptor number. Down-regulation (Fouchereau-Peron *et al.* 1976, Sricant *et al.* 1977) or up-regulation (Soman and Felig 1977) or even no changes (Balage *et al.* 1986) in receptor number due to changes of plasma glucagon levels were reported. In our experiments, the concentration of glucagon in the plasma was not determined, but Morley *et al.* (1975) clearly demonstrated an increase of plasma glucagon levels at 6, 24 and 48 hours after partial hepatectomy. Thus it seems likely that the increase of glucagon receptors on the third day after partial hepatectomy could be the result of up regulation by an elevated plasma hormone level (Soman and Felig 1977). However, Leffert *et al.* (1979) observed decreased glucagon binding to the liver cell membrane during the early period of regeneration (2 to 24 hours after surgery). In contrast to the above studies, the intact cellular system and later periods after surgery were used for assessing glucagon binding.

In conclusion, the results of our experiments demonstrated that there are significant changes in the binding capacity of insulin and glucagon surface membrane receptors in hepatocytes during liver regeneration after partial hepatectomy and that these changes could result from the regulation of receptors by insulin and glucagon plasma levels. The involvement of plasma corticosterone and changes of plasma membrane properties in the insulin receptor alteration are also predicted.

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Reprint Requests

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