Glucagon and glucose tolerance in liver cirrhosis

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Abstract. The present study was undertaken in order to establish the significance of glucagon in glucose intolerance in liver cirrhosis. The plasma glucose response to an oral glucose load (75 g) was determined in 10 control subjects and in 10 cirrhotic patients, after infusions of: glucagon (3 ng·kg⁻¹·min⁻¹) or saline (154 mmol/l); somatostatin (SRIH) (500 µg/h); and SRIH plus glucagon (3 ng·kg⁻¹·min⁻¹). Glucagon infusion did not impair glucose tolerance, neither in normal subjects nor in patients with cirrhosis. On the other hand, in both groups glucose tolerance was impaired by SRIH infusion, presumably owing to an absolute insulin deficiency. Both in normal subjects and in cirrhotic patients, SRIH plus glucagon infusion further impaired glucose tolerance, presumably as a result of excess glucagon and concomitant insulin deficiency. In conclusion, our data show that hyperglucagonemia is not an important factor in the development of the glucose intolerance in patients with hepatic cirrhosis.

Cirrhosis of the liver is characterized by an altered glucose tolerance (Megyesi et al. 1967; Samaan et al. 1969), an increased concentration of insulin in the peripheral blood (Johnston et al. 1977; Iwasaki et al. 1978; Greco et al. 1979), an increase in plasma glucagon (Marco et al. 1973; Greco et al. 1974; Sherwin et al. 1974; Smith-Laing et al. 1980) and resistance to exogenous and endogenous insulin, the latter being expressed as a reduction in insulin binding in the cell membrane (Greco et al. 1980a; Blei et al. 1982). Although the mechanisms involved in the genesis of glucose intolerance are still unclear, glucagon has been considered to be one of the principal causes of this intolerance.

According to Felig et al. (1976) the action of glucagon is evanescent and therefore not effective in maintaining the hyperglycemia. Instead, Unger et al. (1970), Gerich et al. (1975), claim that glucagon excess in combination with a relative insulin deficiency plays an important pathogenetic role in diabetes.

The present study was undertaken in order to determine whether hyperglucagonemia induced by an infusion of glucagon influences blood glucose regulation in normal and/or cirrhotic subjects. Using an infusion of SRIH, the pattern of the plasma glucose curve after administration of an oral glucose load in the presence or absence of glucagon was also studied.

Subjects and Methods

The study included a control group of 10 healthy males aged 34–52 years, with body weights within ± 15% of their ideal weights (Metropolitan Life Insurance Tables 1959). The test group had 10 non-diabetic cirrhotic subjects, without ascites, aged 39–56 years and with body weights within ± 10% of their ideal weights.

The diagnosis of cirrhosis was confirmed biopically. Patients with cirrhosis were graded according to a clinical scoring system and all 10 were evaluated as having 'advanced' cirrhosis (McCormick et al. 1973) (Table 1).

All patients were informed of the nature, purpose and possible risks of the study before participation. The three separate tests were performed on each subject on different days. For at least a week before the study the subjects received a daily diet of about 2500 Cal with at least 250 g of carbohydrates. All tests were performed after an overnight fast.
Catheters were introduced into the antecubital veins of both arms, one being used for drawing blood and the other for the infusion of SRIH, saline and glucagon. All infusions were delivered using Braun infusion pumps (Infusomat Secura, FRG). In the first test, either glucagon at a dose of 3 ng·kg⁻¹·min⁻¹ (Novo Industri %, Copenhagen, Denmark) or saline (154 mmol/l) was infused from 15 min after starting the study and until 180 min. An oral glucose tolerance test (OGTT) (75 g) was performed after 60 min. In the second test, an infusion of somatostatin (SRIH) (Serono, Rome, Italy) (500 µg/h) was begun 15 min after start of the infusion and continued until 180 min. The third test, which was performed under infusion of glucagon or glucagon plus SRIH, included an OGTT (75 g) 60 min after the start. Blood samples were taken at 0, 15, 60, 90, 120, 150, and 180 min. Heparinized specimens for insulin assay were centrifuged at 4°C and subsequently frozen at −20°C until assay. For the glucagon assay, 4-ml blood samples were placed in chilled tubes containing 2000 U of aprotinin and 0.2 ml of 2.4% EDTA-disodium solution. After centrifugation at 4°C, the plasma was separated and frozen until assayed.

Plasma glucose was determined by the glucose oxidase method in a Beckman analyzer.

Plasma insulin was estimated by RIA with the double-antibody method (Hales & Randle 1968). The CV within and between assays was 10%.

Glucagon was measured radioimmunologically with Unger's 30 K antiserum (Unger et al. 1962). Sensitivity of the assay was about 20 ng/l; an intra-assay CV of 9% and an inter-assay CV of 13% were obtained at 150 ng/l. Each sample was incubated without antibody to test the degradation and non-specific binding of the tracer for each plasma.

All data were expressed as mean ± SEM. The two-tailed U-test of Mann-Whitney for non-parametric data was used for the statistical analysis.

Table 1.
Laboratory information on patients with cirrhosis. The severity of the liver disease has been assessed by the clinical grading system of McCormick et al. (1973).

<table>
<thead>
<tr>
<th>N</th>
<th>Age years</th>
<th>Weight % ideal body weight</th>
<th>Fasting plasma glucose (mmol/l)</th>
<th>Fasting IR1 (mU/l)</th>
<th>Bilirubin (mmol/l)</th>
<th>Albumin (g/l)</th>
<th>Pro-thrombin time %</th>
<th>Cholesterol (mmol/l)</th>
<th>Clinical score</th>
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Mean 47.2 ± 5.0  | 94.3 ± 5.7  | 5.40 ± 0.69  | 18 ± 7.7  | 17.2 ± 6.5  | 24.9 ± 4.5  | 51.0 ± 12.0  | 2.76 ± 0.48

Controls 45.6 ± 4.8  | 103.8 ± 6.0  | 4.54 ± 0.37  | 8.5 ± 5.6  | 14.8 ± 3.2  | 48.6 ± 6.7  | 92.8 ± 6.4  | 5.70 ± 0.73
level at 180 min (7.6 ± 0.4 mmol/l). Small plasma insulin increases were seen in both controls and cirrhotic patients.

Figs. 2 and 3 show plasma glucose, glucagon and insulin concentrations in controls and cirrhotic patients before and after oral glucose ingestion during a 165-min infusion of saline or glucagon. In both groups the plasma glucose response during the glucagon infusion did not differ from that observed during saline infusion. In addition, the plasma insulin response was unchanged in both the controls and the cirrhotic patients.

During infusion of SRIH (Fig. 4), the cirrhotic patients showed a reaction different from that of the controls. Fasting plasma glucose levels were similar in both groups. In the controls, plasma glucose had fallen to 3.11 ± 0.16 mmol/l after 75
Plasma glucose, glucagon and insulin before and after 75 g OGTT under saline infusion (154 mmol/l).
Legends as in Fig. 1.

Fig. 2.

Plasma glucose, glucagon and insulin before and after 75 g OGTT under saline infusion (154 mmol/l).

Legends as in Fig. 1.

min of SRIH infusion. The level then rose again, reaching 5.0 ± 0.2 at 180 min. In the cirrhotic patients, plasma glucose remained constantly low. Plasma glucagon and insulin levels were markedly suppressed and the differences between the two groups disappeared.

Figs. 5 and 6 show the changes of plasma glucose, glucagon and insulin in controls and in cirrhotic patients, before and after oral glucose ingestion during the course of a 165-min infusion of SRIH (500 µg/h) and SRIH plus glucagon. Plasma glucose after OGTT plus SRIH differs significantly between cirrhotic patients and controls (120 min: 9.8 ± 0.58 vs 5.9 ± 0.8 mmol/l, P < 0.002; 150 min: 11.5 ± 0.47 vs 8.1 ± 0.36, P < 0.002; 180 min: 13.1 ± 0.58 vs 9.41 ± 0.5, P < 0.002). The addition of glucagon produced a deterioration of glucose tolerance in both cirrhotic patients and in control subjects (120 min: 11.75 ± 0.51 vs 8.94 ± 0.97 mmol/l, P < 0.02; 150 min: 14 ± 0.55 vs 10.38 ± 0.87, P < 0.002; 180 min: 16.55 ± 1.3 vs 12.1 ± 0.81, P < 0.002).
Discussion

Patients with hepatic cirrhosis have an increased plasma concentration of glucagon; the reason for this increase is still unclear. Marco et al. (1973) maintain that in cirrhosis the hepatic degradation of glucagon is decreased. Smith-Laing (1980) found that the hyperglucagonemia was related to the degree of liver damage and apparently unrelated to shunting. According to Sherwin et al. (1974) and Greco et al. (1979) hyperglucagonemia is a consequence of hypersecretion rather than a decreased hormonal catabolism. It has also been hypothesized that the increase in plasma glucagon is a result of an altered feedback mechanism owing to a decreased peripheral sensitivity to glucagon (Sherwin et al. 1978).

All these hypotheses offer an explanation as to why peripheral and portal glucagon levels are increased, but do not explain the role played by glucagon in the genesis of glucose intolerance.

Our results show that in liver cirrhosis, glucagon infusion produces only a small increase in plasma glucose. Glucose intolerance was evident in all the cirrhotic patients in our experiments (saline, glucagon, SRIH, SRIH plus glucagon).
No difference between the plasma glucose curve patterns of cirrhotic patients and normal subjects was seen when comparing glucagon and saline infusion. However, the addition of glucagon contributed to a further deterioration of glucose tolerance in both groups during infusion of SRIH.

A significant reduction in glucagon level after OGGT has been observed by several authors (Smith-Laing et al. 1980; Marco et al. 1973). Our present and previous data (Greco et al. 1974), however, demonstrate a stability of the glucagon level during the administration of glucose in cirrhotic patients. This difference could be explained by the notably higher basal level of plasmatic glucagon in our population. The administration of glucose alone is not sufficient to deactivate the mechanism responsible for the increased glucagon level whatever it might be (hypersecretion, decreased hepatic degradation, etc.).

In normal individuals, more than two-thirds of an oral glucose load escape splanchnic removal; quantitatively the peripheral tissues play the dominant role in glucose disposal (Katz et al. 1982). It is therefore improbable that glucagon,
Plasma glucose, glucagon and insulin before and after 75 g OGTT under infusion of somatostatin (500 µg/h). Legends as in Fig. 1.

Exton et al. (1971) observed that the apparent failure of glucagon to cause glucose intolerance in cirrhosis may be due to the fact that a major portion of pancreatic venous blood bypasses the liver, the prime site of glucagon action.

Felig et al. (1976) and Sherwin et al. (1976) emphasized the primary role of insulin deficiency in the diabetogenic action of glucagon. Hyperglucagonemia neither causes glucose intolerance in normal subjects nor brings about a deterioration of diabetic control when insulin is available. It is clear that the absence of insulin and the consequent increase in hepatic glucose production results in hyperglycemia; in this situation glucagon could become important as an aggravating factor of either the altered glucose tolerance or of the diabetic state.

Our present and past results (Greco et al. 1980b) demonstrate that in cirrhotic patients glucagon exerts a mild hyperglycemic action; this action is more pronounced during an OGTT in hypoinsulinemic conditions. Our data do not support the existence of glucagon resistance in hepatic cir-
rhosis (Danowski et al. 1956; Exton et al. 1971; Sherwin et al. 1978). It should be noted, however, that Projette et al. (1980) observed that hepatic glucose production rose promptly both in controls and in cirrhotic patients after glucagon administration, reaching a peak at 20 min. The acute response of hepatic glucose production to glucagon was somewhat lower in the cirrhotic patients than in the controls. However, this difference was not statistically significant.

In conclusion, these data demonstrate that a reduced glucose tolerance was a constant finding in the cirrhotic patients of our series and that glucagon, although producing a hyperglycemic effect, is not responsible for the impaired glucose tolerance.

References

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