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## Abstract

The effects of insulin and glucagon administration on serum amino acid levels were investigated in patients with severe liver disease, since simultaneous injection of pancreatic hormones has been recently introduced as a therapeutic approach. The changes in serum amino acid concentrations, as observed 3 h after ceasing a 3 h infusion of insulin and glucagon in 500 ml glucose solution, were an elevation of serum branched chain amino acid (BCAA) levels and of the molar ratio of BCAA/aromatic amino acid (AAA) levels in patients with liver cirrhosis. Similar increases of serum BCAA levels during the infusion were also observed in patients with fulminant hepatitis. The results suggest that insulin-glucagon therapy for severe liver disease has no harmful side effects at least with respect to alterations in the serum aminogram.

**KEYWORDS:** glucagon, insulin, amino acid, liver disease

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## EFFECTS OF INSULIN AND GLUCAGON ON SERUM AMINO ACID CONCENTRATIONS IN LIVER DISEASE

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*Abstract.* The effects of insulin and glucagon administration on serum amino acid levels were investigated in patients with severe liver disease, since simultaneous injection of pancreatic hormones has been recently introduced as a therapeutic approach. The changes in serum amino acid concentrations, as observed 3 h after ceasing a 3 h infusion of insulin and glucagon in 500 ml glucose solution, were an elevation of serum branched chain amino acid (BCAA) levels and of the molar ratio of BCAA/ aromatic amino acid (AAA) levels in patients with liver cirrhosis. Similar increases of serum BCAA levels during the infusion were also observed in patients with fulminant hepatitis. The results suggest that insulin-glucagon therapy for severe liver disease has no harmful side effects at least with respect to alterations in the serum aminogram.

*Key words :* glucagon, insulin, amino acid, liver disease.

Simultaneous administration of insulin and glucagon has recently been reported to be an effective therapeutic approach for patients with fulminant hepatic failure (1). This therapy has been believed to be valid for accelerating the repair process of damaged hepatocytes; several investigations have been already reported in clinical (2, 3) and experimental studies with acute hepatic failure (4). It is well known, however, that the hyperinsulinemia and hyperglucagonemia frequently seen in hepatic failure (5) can produce impairment of serum aminograms and be involved in the pathogenesis of hepatic encephalopathy (6). Sherlock, therefore, raised a question about insulin-glucagon therapy for fulminant hepatitis, since exogenous administration of insulin and glucagon may induce hepatic encephalopathy by impairing serum aminogram abnormalities (7).

In this communication, alterations in neutral amino acid levels following insulin and glucagon were evaluated to assess the validity of this therapy with respect to serum aminograms.

### MATERIALS AND METHODS

Two cases with fulminant hepatitis, 3 patients with liver cirrhosis and 3 control subjects

were adopted in this study. Cirrhotic patients were admitted to Okayama University Hospital, and the diagnoses were based on liver biopsy. None of the patients suffered from diabetes mellitus.

Two patients with fulminant hepatitis each received 1 mg glucagon (Novo Ind., Denmark) and 10 U regular insulin (Kodama Ltd., Tokyo) in 250 ml 5% glucose solution for 2 h. Serum amino acid concentrations were determined before and 30 min after the start of the infusion at a flow rate of 8  $\mu$ g glucagon and 0.08 U insulin/min, respectively. Three types of 10% glucose solution with a final volume of 500 ml containing 10 U regular insulin, 1 mg glucagon or 10 U regular insulin plus 1 mg glucagon were given intravenously to 3 control subjects and 3 patients with compensated cirrhosis for 3 h in the early morning. In these studies, serum aminograms were examined immediately after and also 3 h following the end of the 3 h infusion of the solutions. Plasma glucagon and serum insulin concentrations were determined by radioimmunoassay and the insulin/glucagon molar ratios were calculated (8). Serum amino acid, plasma cAMP and blood ammonia levels were determined according to previous methods (9, 10). All the results were expressed as mean  $\pm$  S.D.

## RESULTS

Previous reports (1, 2) recommended that 1 mg glucagon and 10 U insulin in 500 ml 5% glucose solution are infused intravenously for 3 h to patients with fulminant hepatitis. Therefore, alterations in serum neutral amino acid levels before and 30 min after starting the drip infusion containing 10 U insulin and 1 mg glucagon were first examined in 2 patients with fulminant hepatitis. The levels of phenylalanine, tyrosine and methionine were not affected but slight increase of serum BCAA levels was observed during the infusion (Fig. 1).

Insulin, glucagon or insulin plus glucagon in glucose solution was infused intravenously to control and cirrhotic subjects as described under "Materials and Methods", and the serum aminograms were compared. In the cirrhotic patients, the decrease in neutral amino acids immediately after ceasing the 3 h infusion of insulin alone was smaller than in control subjects (Fig. 2). AAA

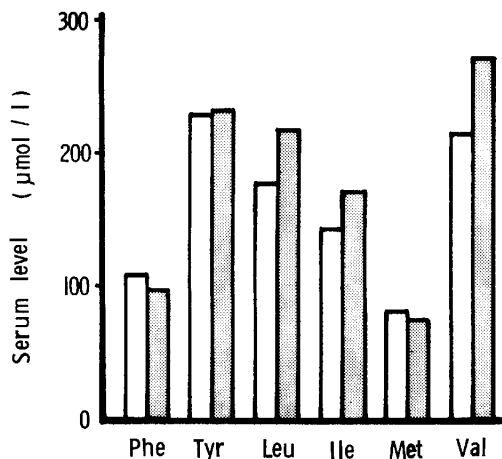


Fig. 1. Alterations in serum neutral amino acid levels before (□) and 30 min (▨) after initiating the drip infusion of 250 ml glucose solution (5%) containing 10 U regular insulin and 1 mg glucagon at a flow rate of 0.08 U and 8  $\mu$ g/ml, respectively, in two patients with fulminant hepatitis. Average values are shown.

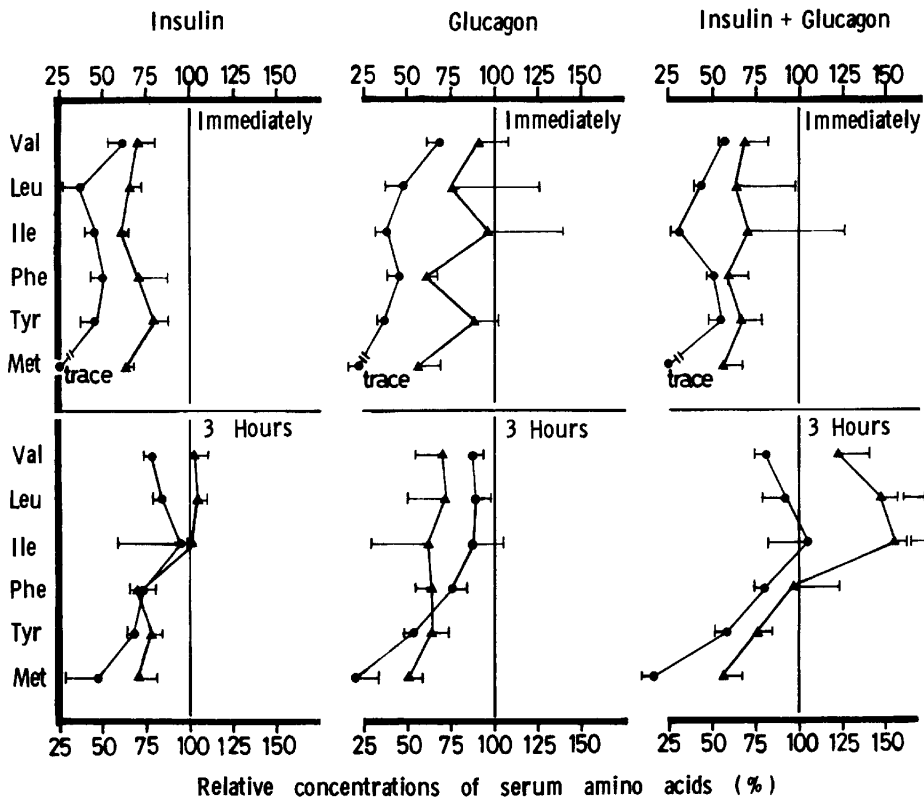


Fig. 2. Relative changes in serum neutral amino acid concentrations immediately and 3 h after the end of a 3 h infusion of 500 ml glucose solution (10%) containing 10 U regular insulin and/or 1 mg glucagon in 3 control subjects (●) and 3 patients with compensated liver cirrhosis (▲). The concentrations before the infusion are expressed as 100%. Horizontal bars indicate S. D. of the mean.

and methionine concentrations were still lower even 3 h after stopping the insulin injection, although other neutral amino acid levels recovered to the levels prior to the injection in cirrhotic and control subjects. The BCAA/AAA ratio increased both in control (average, 3.9 → 4.4) and cirrhotic subjects (1.4 → 1.6) 3 h after the drip infusion of insulin alone, and the insulin/glucagon ratio rose only immediately after the end of the 3 h injection, then decreased to nearly the basal levels (Fig. 3). The blood ammonia levels tended to rise slightly following insulin injection, although the changes were not significant.

Plasma glucagon levels following glucagon injection to control subjects increased to more than 2000 pg/ml, serum insulin also rose to an extent of 6 ~ 9-fold, and blood sugar concentrations decreased slightly after glucagon injection. In cirrhotic patients, however, blood sugar levels rose transiently to 3-fold only immediately after the glucagon infusion. Poor response of cAMP

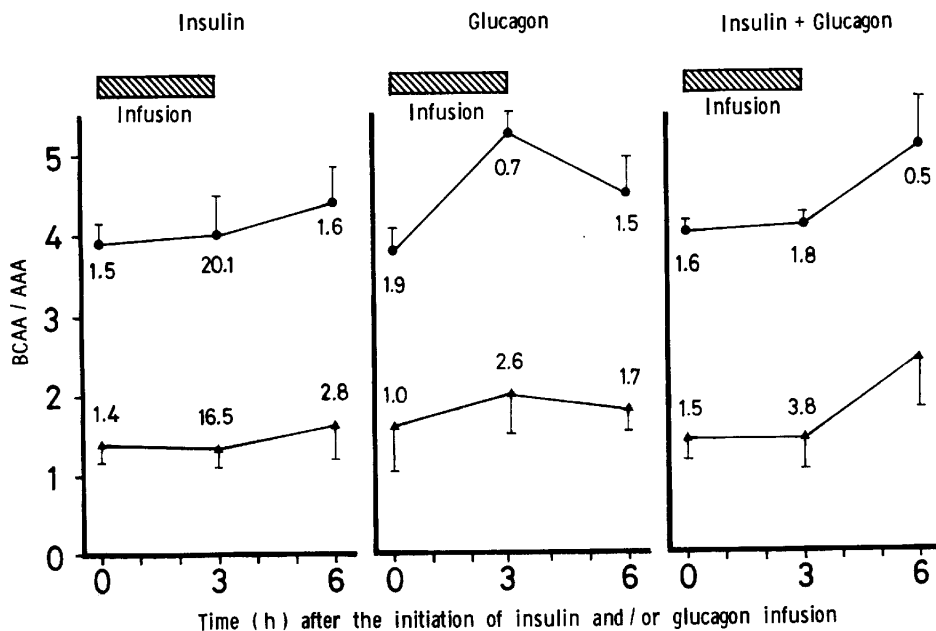


Fig. 3. Changes in the serum BCAA/AAA molar ratio and the serum insulin/glucagon molar ratio after initiating a 3 h' drip infusion of insulin and glucagon in 3 control subjects (●—●) and in 3 cirrhotic patients (▲—▲). Numbers indicate the molar ratio of insulin/glucagon.

levels to glucagon injection was also seen in cirrhotics. As shown in Fig. 2, serum BCAA remained at levels similar to the preinfusion values immediately after the injection, while in control subjects all neutral amino acids decreased to a greater extent. Three h after the end of glucagon infusion, diminished amino acid levels were recovered in controls but were still low in cirrhotics (Fig. 2). The BCAA/AAA ratios in control subjects and cirrhotic patients rose to 5.3 and 2.0 immediately after ceasing the glucagon injection, respectively, as illustrated in Fig. 3. The insulin/glucagon ratio both immediately, and 3 h, after the end of the injection decreased in control subjects but was elevated in cirrhotic patients.

BCAA levels were 120 ~ 155% of the original levels 3 h after ceasing the simultaneous injection of glucagon and insulin to cirrhotic patients (Fig. 2). The BCAA/AAA ratio was markedly elevated in both control (4.0 → 5.1) and cirrhotic patients (1.5 → 2.4) 3 h after the injection (Fig. 3). Slight increase of insulin/glucagon ratio was found in both groups immediately after the end of the injection. No adverse effects was observed in control and cirrhotic subjects during or after the infusion.

## DISCUSSION

Insulin has been reported to accelerate amino acid uptake by skeletal muscle (11); therefore, factors altering muscle uptake of amino acids from the plasma or their release into the circulating blood may influence the plasma levels of amino acids. The catabolic effects of glucagon with a decline of plasma amino acid levels are primarily due to enhancement of hepatic utilization of amino acids (12). Many other works on the direct relationship between amino acid and pancreatic hormones have been published already. To our knowledge, however, few studies have been made on the effects of simultaneous administration of insulin and glucagon on serum amino acid concentrations in human.

Serum BCAA levels in cirrhotic patients were increased 3 h after the end of the 3 h infusion of insulin and glucagon. Similar increases in serum BCAA levels during the infusion were observed in patients with fulminant hepatitis. The results obtained by insulin and glucagon administration suggest that glucagon plays an important role in the elevation of serum BCAA levels. Administration of insulin in glucose solution may induce accelerated secretion of endogenous glucagon from the pancreas, and the BCAA levels were slightly elevated 3 h after the end of insulin infusion. Since serum methionine and tyrosine levels diminish and BCAA/AAA rise in subjects treated with pancreatic hormones, a commercially available amino acid solution containing relatively larger amounts of these amino acids may not be toxic for these patients at least at the time of simultaneous addition of glucagon and insulin. Therefore, insulin-glucagon therapy for fulminant hepatitis has no harmful side effects at least with respect to the aminogram.

Changes in systemic insulin and glucagon levels may induce compensatory alterations in growth hormone, epinephrine, and adrenal corticoid, all of which may affect nitrogen metabolism (13). For direct investigation of hormonal effects on the target tissues, close intra-arterial infusion of hormones should be selected. These considerations complicate the problem by suggesting that the observed alterations in amino acid levels could be induced solely by hormonal actions of insulin or glucagon. However, the important finding in this communication is that the changes in the serum aminogram induced by simultaneous infusion of insulin and glucagon were not dangerous during treatment of severe liver disease.

Munro *et al.* (14) proposed that decrease in plasma BCAA levels in patients with liver cirrhosis was the result of excessive removal of these amino acids by muscle due to hyperinsulinemia delivered by portal-systemic shunting. However, the decrease in BCAA levels in patients with idiopathic portal hypertension was not accompanied by hyperinsulinemia, and BCAA levels were not correlated with plasma insulin levels (15). Therefore, decreased levels of BCAA in patients with liver cirrhosis as well as in those with idiopathic portal hypertension could not be ascribed to hyperinsulinemia. Further detailed studies

are needed to solve this problem.

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