Correction of altered plasma amino acid pattern in cirrhosis of the liver by somatostatin

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SUMMARY The purpose of our study was to evaluate the effect of somatostatin (500 $\mu\text{g/h}$ intravenously) upon insulin, c-peptide, glucagon and plasma amino acids concentrations in patients with and without cirrhosis of the liver. The typical plasma amino acid pattern in cirrhosis is characterised by increased concentrations of the aromatic amino acids and decreased concentrations of the branched chain amino acids and of alanine and glycine. After administration of somatostatin insulin, c-peptide and glucagon concentrations decreased and those of the branched chain amino acids in both groups increased; in addition in patients with cirrhosis the plasma concentrations of threonine, serine, glycine, alanine, lysine, and arginine increased also. Infusion of somatostatin plus insulin in patients with cirrhosis succeeded in preventing the increase in the branched chain amino acid concentrations, while the infusion of somatostatin plus glucagon decreased threonine, serine, glycine, alanine, phenylalanine, tyrosine, lysine and arginine concentrations. It is therefore suggested that the effect of somatostatin on the plasma amino acids may be because of the reduction of insulin and glucagon concentrations; however, other effects of somatostatin cannot be excluded at present.

The existence of an altered amino acid pattern in the plasma of patients with advanced cirrhosis of the liver is well known. This pattern is characterised by an increase in the concentration of the aromatic amino acids tyrosine and phenylalanine and a decrease in the concentrations of the branched chain amino acids valine, leucine, isoleucine and of alanine and glycine.¹⁻⁴ High concentrations of aromatic amino acids may be causally related to hepatic encephalopathy leading to accumulation of false neurotransmitter in the central nervous system.¹ Attention has, therefore, been focused on the distorted free amino acid patterns in the plasma of the cirrhotic patient. The reasons for the altered plasma amino acid pattern have been still incompletely understood, but two factors have been thought to contribute to the development of plasma amino acid imbalance in cirrhosis. The protein catabolism is increased⁵ and the concentrations of insulin and glucagon are increased;⁶⁻⁸ it has been proposed that hyperglucagonaemia causes an increase of the aromatic amino acids and hyperinsulinaemia a decrease of the branched chain

amino acids.⁹ In contrast a direct correlation of decreased branched chain amino acids concentrations with insulin concentrations and of raised aromatic amino acids concentrations with glucagon concentrations has not been observed.¹⁰

Somatostatin is known to inhibit insulin and glucagon secretion in normal man.¹¹ Somatostatin seems therefore to be a good medium for studying the inter-relationship between plasma amino acid and hormone imbalance. In order to elucidate this inter-relationship the influence of somatostatin on raised insulin and glucagon concentrations and on altered amino acid pattern in cirrhosis of the liver was investigated; moreover the effect of selective hyperinsulinaemia and hyperglucagonaemia on the plasma amino acid pattern was studied.

Methods

SUBJECTS AND PATIENTS

Ten male control subjects without liver disease and 10 male patients with histologically proven cirrhosis of the liver were included in this investigation. All patients had a history of excessive alcohol consumption; cirrhosis was regarded as the consequence of chronic alcoholism when no other cause of liver

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disease was shown. Upper gastrointestinal endoscopy revealed oesophageal varices in all patients. The patients were in stage Child B,¹² the serum creatinine was normal and the transferases below 60 IU/l (normal: <24 IU/l). All patients were abstinent for at least 14 days. All subjects gave written informed consent. The study had been approved by the local ethical committee.

The investigation was performed after an overnight fast and rest. On day 1 after a bolus injection of 250 µg somatostatin (Serono, Freiburg) somatostatin (500 µg/h) was infused intravenously for four hours. On day 2 somatostatin (500 µg/h after a bolus injection of 250 µg) was administered together with glucagon (45 nmol/h) (Glukagon, Lilly) and on day 3 together with insulin (11 nmol/h) (Insulin, Hoechst) over the same time period. Blood samples were drawn before and 240 minutes after starting the infusion. Plasma amino acids, insulin, c-peptide and glucagon were determined in all samples. Plasma immunoreactive insulin was measured¹³ using Sephadex bound antibodies (Phadebas, Pharmacia). C-peptide was determined¹⁴ by radioimmunoassay using a commercially available kit (Novo, Copenhagen). For the determination of plasma immunoreactive glucagon 4.0 ml samples of blood were transferred to chilled tubes containing 0.2 M EDTA-Na₂ and 0.2 ml aprotinin (Trasylol, Bayer). Plasma immunoreactive glucagon was determined using Unger's antibody 30 K.¹⁵ Free plasma amino acids were measured by ion exchange chromatography on an amino acid autoanalyser (Biotronik) using norleucine as internal standard; the plasma was deproteinised by 6% sulphosalicylic acid. All samples were stored at -20°C until analysed. Student's *t* test and where applicable the paired *t* test were used to determine statistical significance. Data are expressed as mean ± SEM.

Results

In patients with cirrhosis of the liver the preinfusion concentrations of insulin and glucagon (Table 1) and of the aromatic amino acids tyrosine and phenylalanine were raised whereas the concentrations of the branched chain amino acids valine, leucine, isoleucine and of alanine and glycine were decreased when compared with controls (Table 2). No differences were seen in the concentration of c-peptide (Table 1).

Somatostatin infusion resulted in a significant decrease of insulin, glucagon and c-peptide concentration in both groups (Table 1). At the end of somatostatin infusion the concentration of the branched chain amino acids were significantly increased in both groups, while the concentrations

Table 1 Plasma insulin, c-peptide and glucagon concentrations in control and cirrhotic subjects in response to somatostatin (500 µg/h after a bolus injection of 250 µg) administration for 240 minutes

	Controls (10)		Cirrhotics (10)	
	0 min	240 min	0 min	240 min
Insulin (pmol/l)	72±21	21±14*	259±86†	93±28*
C-peptide (nmol/l)	0.4±0.2	0.1±0.05*	0.55±0.25	0.1±0.05*
Glucagon (pmol/l)	21±3.4	12±1.7*	73±6.6†	50±10.3*

* Significantly different from basal values for the same subjects (*p*<0.01 or less).

† Significantly different from basal values obtained in the control group (*p*<0.01 or less).

of the aromatic amino acids remained unchanged (Table 2). In contrast with the controls somatostatin had an additional effect on the so called gluconeogenic amino acids threonine, serine, glycine, alanine, lysine, and arginine in patients with cirrhosis of the liver. Infusion of somatostatin led to a significant increase of these amino acids. The most prominent rise induced by somatostatin concerned the concentration of alanine, which reached normal values (Table 2).

The combination of somatostatin and glucagon in patients with cirrhosis led to a decrease of insulin from 230±70 to 79±40 pmol/l and of c-peptide from 0.6±0.22 to 0.1±0.05 nmol/l (*p*<0.01), whereas the concentration of glucagon increased from 71±7 to 229±28 pmol/l (*p*<0.001). In addition, the increase of the gluconeogenic amino acids induced by

Table 2 Plasma amino acids (µmol/l) in control and cirrhotic patients before and after infusion of somatostatin (500 µg/h after a bolus injection of 250 µg) for 240 minutes

	Controls (10)		Cirrhotics (10)	
	0 min	240 min	0 min	240 min
Threonine	83±24	87±20	80±20	117±24*
Serine	67±16	71±17	65±15	95±23*
Glycine	195±15	190±20	150±12†	185±10*
Alanine	235±10	246±25	161±35†	235±32*
Valine	160±11	216±12*	107±22†	145±10*
Leucine	122±8	156±10*	63±10†	104±9*
Isoleucine	68±11	90±13*	33±12†	50±8*
Phenylalanine	41±9	50±10	60±8†	68±7
Tyrosine	55±10	64±11	90±9†	99±12
Lysine	125±30	130±15	100±25	140±20*
Arginine	72±15	80±12	65±10	100±10*

* Significantly different from basal values for the same group (*p*<0.05 or less).

† Significantly different from basal values obtained in the control group (*p*<0.05 or less).

somatostatin alone was not observed. In contrast the concentrations of the gluconeogenic and aromatic amino acids decreased significantly (Table 3).

Infusion of somatostatin plus insulin led to a decrease of glucagon from 63 ± 2.8 to 45 ± 5 pmol/l and of c-peptide from 0.65 ± 0.2 to 0.1 ± 0.05 nmol/l ($p < 0.01$), but the insulin concentrations remained raised (129 ± 21 pmol/l). No increase of the branched chain amino acids was observed.

Discussion

In patients with cirrhosis of the liver high insulin concentrations have been documented.^{6,16} Results from studies dealing with the plasma c-peptide concentrations are found to be conflicting; some authors reporting normal^{6,7,17} and others increased concentrations of c-peptide.¹⁸ Insulin and c-peptide are secreted in equimolar amounts from the pancreatic cells.¹⁹ C-peptide is mainly metabolised in the kidney while insulin metabolism centres in the liver and in fat and muscle tissue.²⁰ Normal concentrations of c-peptide in cirrhosis would therefore indicate that hyperinsulinaemia in cirrhosis is mainly because of a diminished degradation of insulin.^{6,7} On the other hand raised c-peptide concentrations would suggest the presence of insulin hypersecretion.¹⁸ In our patients with cirrhosis insulin concentrations were increased and c-peptide concentrations were normal. In both groups of patients (those with and without cirrhosis) the infusion of somatostatin initially resulted in a marked reduction of insulin and c-peptide concentrations. After the somatostatin infusion, insulin levels nonetheless remained raised in the patients with cirrhosis, while the c-peptide concentrations

reached the same values as in the controls. This result would suggest that even in the suppressed state of insulin secretion the metabolism of the hormone may be impaired in cirrhosis, probably because of decreased hepatic degradation or secondary to portal-systemic shunting. This interpretation is supported by results, which show a reduced c-peptide insulin ratio.¹⁷

Glucagon concentrations are raised in advanced cirrhosis of the liver resulting from an increase of the so called pancreatic glucagon.²¹ In controls and in patients with cirrhosis glucagon concentrations were reduced by somatostatin, but the concentrations remained raised in the cirrhotic subjects. It is known that glucagon is degraded in the liver but substantial metabolism occurs in the kidneys as well.²² Under the influence of somatostatin a normal glucagon concentration could therefore be expected in cirrhotics, as somatostatin inhibits the pancreatic secretion of the hormone.¹¹ The failure of somatostatin to reduce raised glucagon concentrations to normal may be caused by an altered secretion of the hormone as suggested by Sherwin *et al.*²³

Infusion of somatostatin improved in part the deranged plasma amino acid pattern in cirrhosis in that the concentrations of the branched chain amino acids and of threonine, serine, glycine alanine, lysine, and arginine increased. The increase of the gluconeogenic amino acids may be because of the reduction of hepatic uptake and metabolism by the liver when glucagon concentrations are lowered by somatostatin, as it has been shown that suppression of glucagon secretion impairs hepatic extraction of these amino acids.²⁴

The concomitant infusion of somatostatin and glucagon led to a decrease of the insulin/glucagon ratio from 3.55 to 0.34 in patients with cirrhosis; this reduced insulin/glucagon ratio would favour the movement of plasma amino acids into the gluconeogenic pathways²⁵ and may therefore be responsible for the resulting decrease in plasma amino acid concentrations. It is notable that the rise in plasma glucagon concentrations during the infusion of somatostatin plus glucagon also decreased the aromatic amino acid concentrations. These results suggest that hyperglucagonaemia may not be responsible for the increased concentrations of the aromatic amino acids as suggested by James *et al.*⁹ Our interpretation is in accordance with data from the literature which have shown that glucagon stimulated the metabolism of the aromatic amino acids²⁶ and induced hypoaminoacidaemia.²⁷⁻²⁹

The combined infusion of somatostatin plus insulin in cirrhosis prevented the increase of the branched chain amino acids normally associated with somatostatin administration. It is therefore

Table 3 Influence of combined infusion of somatostatin (500 µg/h after bolus injection of 250 µg) and glucagon (45 nmol/h) on plasma amino acids (µmol/l) in patients with cirrhosis

	0 min	240 min
Threonine	88±20	66±17*
Serine	70±16	52±12*
Glycine	155±10	109±9*
Alanine	158±25	108±15*
Valine	109±21	104±15
Leucine	63±13	70±9
Isoleucine	35±12	36±10
Phenylalanine	64±6	45±9*
Tyrosine	87±10	70±8*
Lysine	105±20	85±20*
Arginine	63±10	47±6*

* Significantly different from basal values for the same subjects ($p < 0.05$ or less).

possible that the increase of these amino acids induced by somatostatin alone may be the result of a decrease in the insulin concentration. Some data support this hypothesis. The primary site of branched chain amino acid metabolism is the muscle and fat tissue, and amino acid entry is regulated by the insulin levels.⁹ In addition, in uncontrolled Type I diabetes mellitus branched chain amino acid concentrations are high³⁰ and are corrected by insulin treatment.^{31, 32}

In conclusion, the positive influence of somatostatin in improving the deranged plasma amino acid pattern normally found in cirrhotics may be because of its success in reducing raised insulin and glucagon concentrations. Other effects attributed to somatostatin (including inhibition of other hormone secretions), however, are possible causes and cannot be discarded at present.

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