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The Cause of Hyperaminoacidemia in Completely Pancreatectomized Dogs

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Summary. **The rate of the hepatic glucose production was isotopically determined in combination with the measurement of plasma amino acid concentrations in completely pancreatectomized dogs.**

Marked hyperaminoacidemia mainly consisting of elevated glucogenic amino acids was observed regardless of the postoperative insulin replacement. Reduction of the elevated amino acids (except for the branched chain amino acids) by the exogenous glucagon was also observed.

Particularly in cases where the plasma glucose level had been maintained at normal levels by the insulin, the decreasing of the plasma amino acid concentrations was associated with enhanced hepatic glucose production, indicating that the cause of hyperaminoacidemia in totally pancreatectomized dogs is inhibited gluconeogenesis from amino acids due to glucagon deficiency.

INTRODUCTION

Since the report by Muller et al.[1] in 1979, hyperaminoacidemia in totally pancreatectomized patients has been recognized by several authors.[2-4] Although these authors assumed that the hyperaminoacidemia might be caused by impaired hepatic gluconeogenesis due to glucagon deficiency, the mechanism for the hyperaminoacidemia has not been fully elucidated.

In order to clarify the cause of the hyperaminoacidemia in the present study, we measured the rate of hepatic glucose production using radioisotopes in combination with the measurement of plasma amino acid concentrations in totally pancreatectomized dogs. In other words, the effects of total pancreatectomy and exogenous administration of insulin and/or glucagon to these animals on hepatic glucose production rate and on the plasma amino acid profile were studied. This study also provides information on how glucagon and insulin may affect plasma concentrations of each amino acid.

MATERIALS AND METHODS

1. Experimental animals and operative procedures

Twenty-six male mongrel dogs weighing about 10 kg were used. In 13 dogs of Groups A and B, removal of the entire pancreas was performed together with duodenectomy, splenectomy, and total gastrectomy. Reconstruction included end to end esophagojejunostomy and cholecystojejunostomy after ligating the distal cut end of the choledochus. In the other 13 dogs in Groups C, splenectomy was carried out as a sham operation.

2. Experimental protocol

On the three days preceding the experiment, the dogs were anesthetized with small amounts of pentobarbital sodium, and 2 indwelling catheters (multipurposes tube, 2.75 mm, i.d., ATOM, JAPAN) were inserted into the external jugular vein and femoral artery for infusion and for drawing serial blood samples, respectively. Both catheters were flushed with 6 ml of heparinized (20 units/ml) 0.9% NaCl solution and locked by a 3-way stopcock until the day of the experiment.

Food was withdrawn 20 h before the experiment and an infusion of 0.9% NaCl was started at the rate of 70 ml/kg-min. On the day of experiment, blood samples were withdrawn for the baseline measurement of the plasma glucose level, plasma immunoreactive insulin (IRI), glucagon (IRG), and plasma amino acid concentrations. Simultaneously, the hepatic glucose production rate (Ra: rate of appearance, mg/min per kg) was determined by the primed-constant infusion method of [6-^{3}H] glucose.[5]

Following the measuring of the baseline metabolic paramenters described above, the dogs were anesthetized with pentobarbital sodium (25 mg/kg and additional doses) and intubated. Each operation described above was thus done under anesthetized conditions with mechanical ventilation. Physiological saline infusion was continued and no nutrients, including glucose, were given postoperatively.

Pancreatectomized dogs were divided into 2 groups depending whether they were given insulin: dogs in Group A were not given insulin, while dogs in Group B received a continuous infusion of short-acting porcine insulin (Insulin Novo actrapid, Novo, Denmark) of 0.2 U/kg per day. Dogs in Group C served as the control.

Blood samples were withdrawn for the measurement of the plasma glucose level, IRI, IRG, and for the plasma amino acid concentrations on the morning of the 1st postoperative day. The Ra was then isotopically determined. After the measuring of baseline Ra, the same metabolic parameters described above—including Ra—were repeatedly measured 120 min after starting intravenous glucagon infusion (30 ng/kg. min, Glucagon, Novo, Denmark). The same experimental protocol was carried out on the 3rd postoperative day.

3. Tracer methods and calculations (Fig. 1)

Endogenous glucose production (Ra) was measured by the primed-constant infusion method of [6-^{3}H]-glucose. The priming dose of [6-^{3}H]-glucose (24 Ci/mmol, Amersham, England) was given as a 5 μCi/kg bolus injection followed by continuous infusion at a rate; F of 0.06 μCi/kg. min. Samples for the determination of steady-state plasma glucose specific activity (SA) were taken 60, 75, and 90 min after the priming bolus injection. Based on these specific activities, baseline Ra was calculated by the equation: Ra=F/SA. Thereafter, glucagon infusion was started. As glucose specific activity is not in a steady state condition during glucagon infusion, the Ra was calculated by Steele's non-steady state equation.[6] In this case, specific activities in the plasma taken 180, 195, and 210 min after the initial priming were used, and 40 ml/kg was adopted as the volume of glucose distribution according to Allsop et al.[5] Incidentally, Ra values obtained by either the steady state or Steele's non-steady state equations showed almost negligible differences for 180 to 210 min after the initial priming. In addition, aliquots of the solution of tritiated glucose prepared for infusion were treated in the same way as plasma samples and counted. Based on the actual counts obtained, the infusion rate (F) was determined.

4. Laboratory methods

For the determination of the glucose specific activity, plasma glucose was extracted by the previously

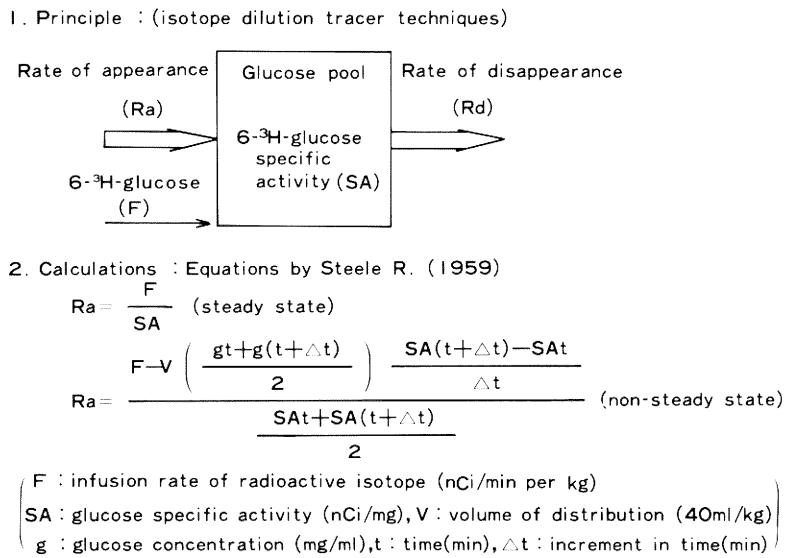

Fig. 1. Tracer methods and calculations: determination of hepatic glucose production rate (Ra: rate of appearance, mg/min-per kg) by the primed constant rate infusion of [6-^{3}H]-glucose

reported[7] "batch method" of ion-exchange resin. Plasma was deproteinized by the Somogi method of Nelson modification[8] (plasma: water: 0.3 N $Ba(OH)_2$: 5% $ZnSO_4$ = 1 : 5 : 2 : 2). Three milliliters of the protein-free supernatant was mixed with about 200 mg of "conditioned", moist Dowex 50 W-X8 (Bio Rad Laboratories, U.S.A.) and 400 mg of Amberlite IR-45 (Rohm & Haas Co. USA), then incubated with constant shaking at 37℃ for 40 min. Aliquot (0.5 ml) of the resin-treated protein-free supernatant was transfered into the counting vial and evaporated to dryness in order to remove any tritiated water, and then redissolved in 1 ml of distilled water. Samples were counted in ACSII (Amersham, England) by a liquid scintillation counter (Tricarb 460 CD, Packard, USA).

Plasma gucose concentration was measured by the glucoseoxidase method (Automated Glucose Analyzer; Glucoroder S, Analytical Instruments Co. JAPAN). Plasma amino acids were determined by the automated amino acid analyzer (Hitachi Co. 835 type, JAPAN) on deproteinized plasma with sulfosalicylic acid. IRI was measured by radioimmunoassay using INSULIN RIABEAD[9] (*Dainabot Co.*, JAPAN). IRG was measured by radioimmunoassay using antisera against the C-terminal fragment of pancreatic glucagon.[10]

5. Statistics

Statistical comparisons were done by Student's t test. Significant p values were those at <0.05. Data are expressed as the mean ± SD.

RESULTS

1. Changes in plasma IRI and IRG levels (Fig. 2 and 3)

Preoperative IRI levels in Groups A, B, and C did not show any differences. In Group A, in which no insulin

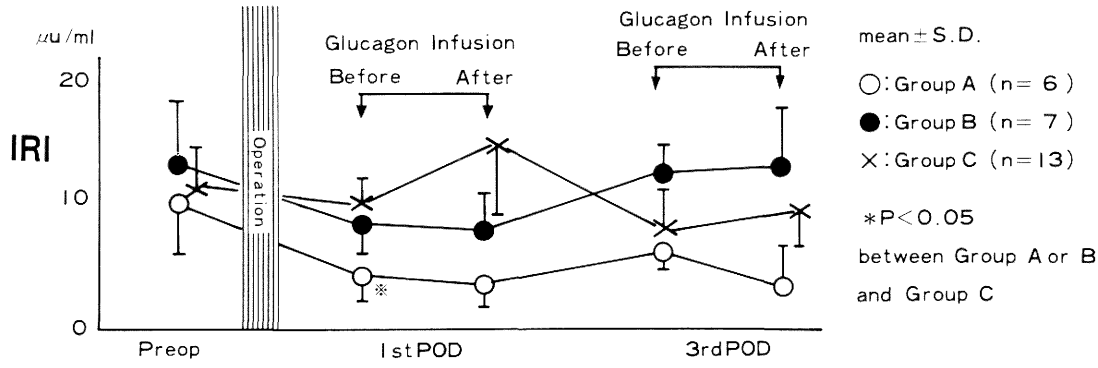

Fig. 2. Changes in the plasma IRI levels after total pancreatectomy.

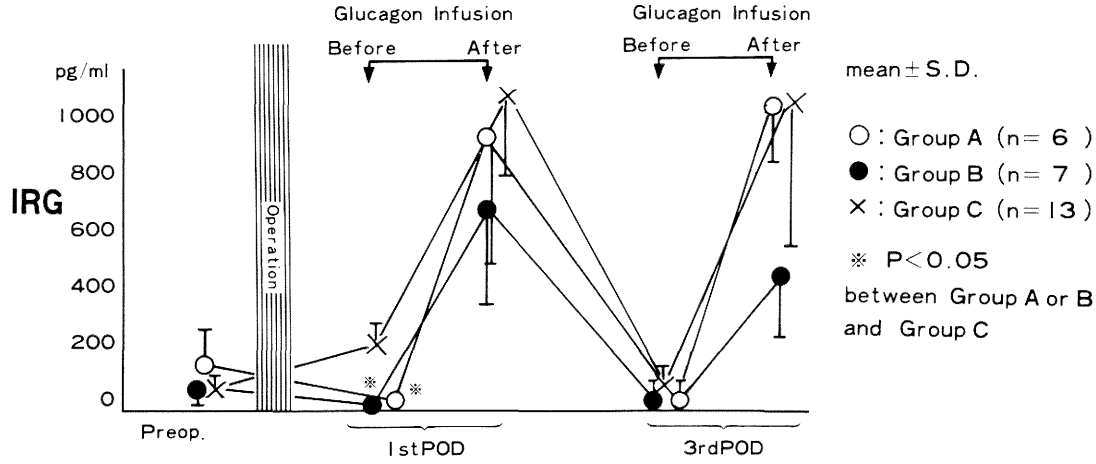

Fig. 3. Changes in the plasma IRG levels after total pancreatectomy.

was replaced after operation, postoperative IRI levels were almost at the lowest limitation for the measurement, although only the value on the 1st postoperative day (4.9±1.6 μU/ml) showed a statistically significant decrease than in Group C because of the wide variations of each IRI value. IRI levels (8.5±2.7 μU/ml) in Group B on the 1st postoperative day, in which dogs received insulin replacement, were maintained at the same levels as those in Group C (10.1±2.3 μU/ml). Exogenous glucagon administration did not produce any changes in IRI levels in any of the groups.

Preoperative IRG levels in Groups A, B, and C, were 133.3±111.5, 76.1±83.7, and 73.4±46.4 pg/ml, respectively; there were no significant differences between Groups A or B and Group C. IRG levels in Groups A and B on the 1st postoperative day, in which the dogs had undergone removal of the entire pancreas and stomach, were 28.4±11.3 and 29.2±12.4 pg/ml, respectively, and these values were significantly lower than those in Group C (196.8±162.0 pg/ml). After the glucagon infusion of 30 ng/kg. min for 120 min, IRG levels rose to 879±315, and 1060±198 pg/ml, respectively. Essentially the same patterns of change in plasma IRG levels were observed on the 3rd postoperative day.

2. Glucose metabolism

1) Changes in hepatic glucose production (Fig. 4)

Almost the same preoperative Ra values were observed in the 3 groups. Ra values in Groups A, B, and C on the 1st postoperative day were 10.0±2.5, 3.8±1.3, 3.4±1.3 mg/min per kg, respectively. Similar values of Ra on the 3rd postoperative day were noted in each group. Ra in Group A was significantly higher than that in Group C on both the 1st and 3rd postoperative days.

Plasma glucose levels on the 1st postoperative day

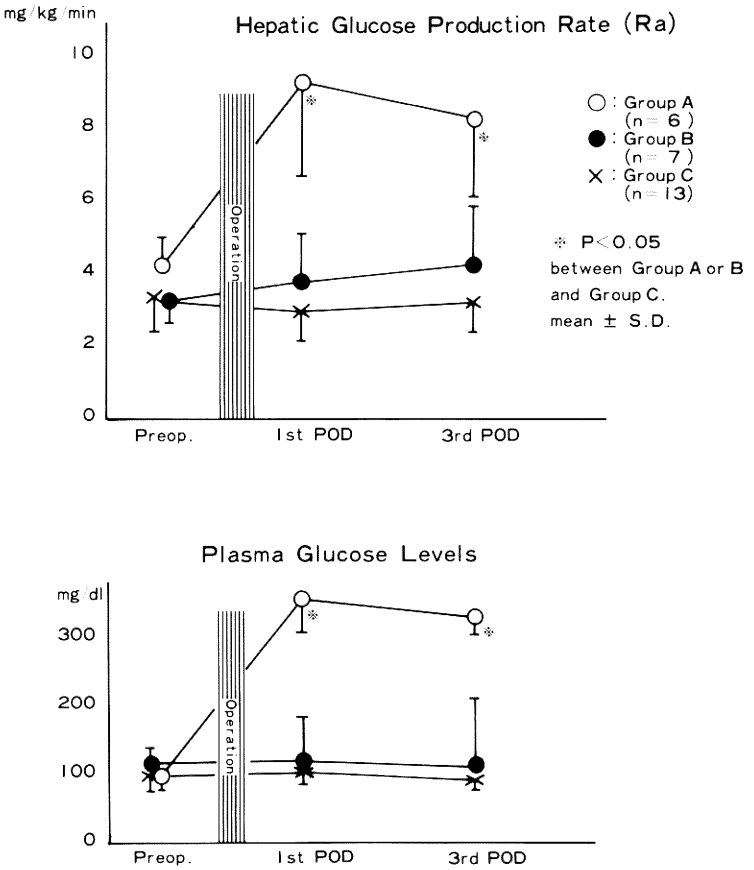

Fig. 4. Changes in the hepatic glucose production rate (Ra) and plasma glucose levels after total pancreatectomy.

in Groups A, B, and C were 362±62, 113±90, and 99±17 mg/dl, respectively. Almost the same values in each group were observed on the 3rd postoperative day. Values in Group A were significantly higher than those in Group C on both the 1st and 3rd postoperative days. These changes were consistent with the increased Ra values in Group A discribed above.

2) Effects of glucagon administration on hepatic glucose production and on plasma glucose levels (Fig. 5)

Ra in Group B rose significantly from 4.0±1.4 to 6.0±1.7 mg/min per kg after the 2-h glucagon infusion. In consistent with the Ra change, the plasma glucose level rose markedly from 121±71 to 216±83 mg/dl. Enhanced Ra by exogenous glucagon associated with increment in the plasma glucose level was also seen in Group C.

In contrast, in Group A, in which baseline settings of Ra and plasma glucose level had already been elevated, glucagon infusion was accompanied by a slight but significant drop in Ra (from 8.7±2.5 to 7.1±1.5 mg/min per kg). No remarkable change in plasma glucose level by glucagon was noted in this group.

3. Amino acid metabolism

1) Changes in concentrations of total plasma amino acids by the operations and the exogenous pancreatic hormones

Changes in concentrations of total amino acids (sum of the 20 measured plasma amono acids) caused by the operations and by the exogenous pancreatic hormones are summarized in Table 1. Significantly higher concentrations of plasma total amino acids were seen in Groups A and B than in Group C on both the 1st and 3rd postoperative days. A decrease in total amino acid concentrations by the exogenous glucagon administration was observed in all the groups.

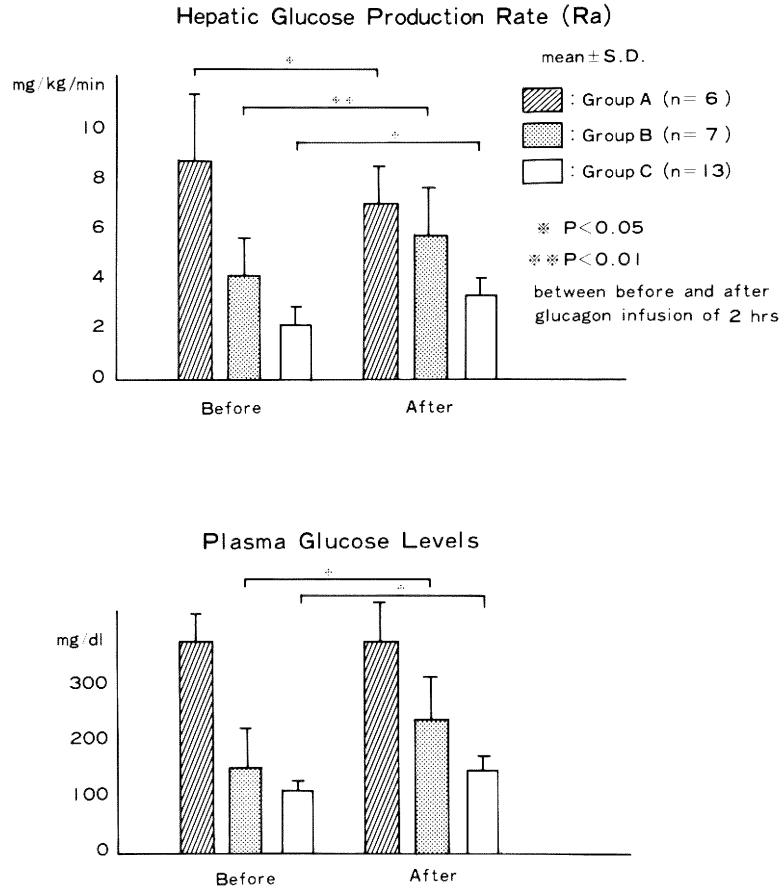

Fig. 5. Effects of glucagon administration on hepatic glucose production rate (Ra) and plasma glucose levels.

Table 1. Changes in concentrations of total plasma amino acids after glucagon administration

ean±S.D. nmol/ml

		Preop.	1stPOD	3rdPOD
Group A	Glucagon(−)	2152±808	5450±1321※	6408±1050※
(n=6)	Glucagon(+)		4457±1167☆	4579±1712
Group B	Glucagon(−)	2410±405	5840±2251※	5477±1532※
(n=7)	Glucagon(+)		4074±1669☆	3391±1120☆
Group C	Glucagon(−)	2254±355	2123±355	2173±172
(n=13)	Glucagon(+)		1557±449 ☆	1850±290

※P<0.01 between Group A or B and Group C
☆P<0.05 between before and after glucagon infusion of 2 hrs.

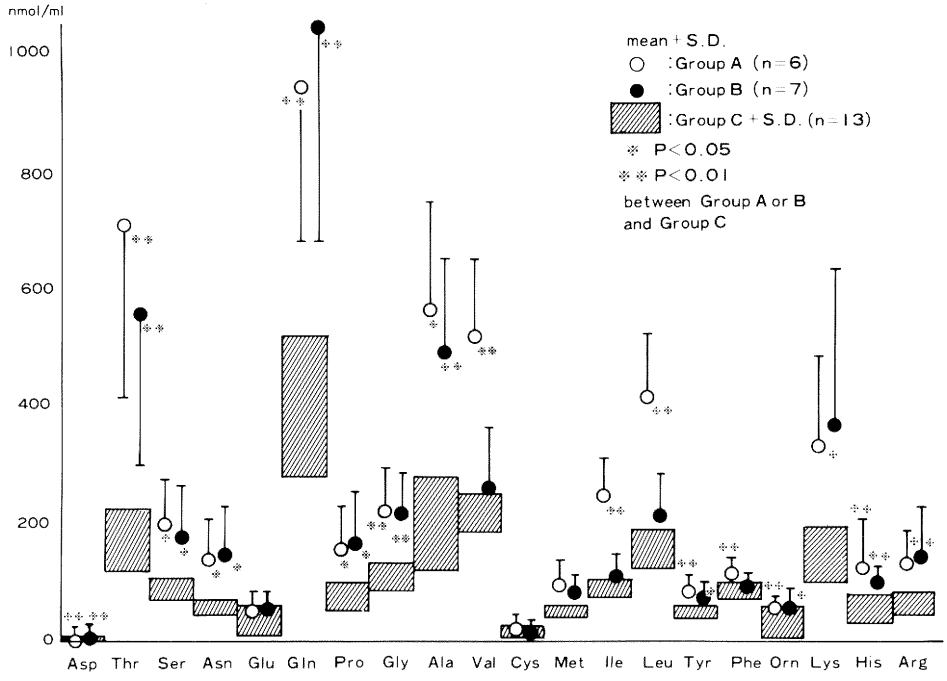

Fig. 6. Concentrations of plasma amino acids in totally pancreatectomized dogs on the 1st postoperative day.

2) Plasma amino acid profile in totally pancreatectomized dogs (Fig. 6)

Plasma levels of aspartic acid, threonine, serine, glutamine, proline, glycine, alanine, tyrosine, ornithine, histidine, and arginine were significantly elevated in Groups A and B in comparison with those in Group C on the 1st postoperative day. Similar changes in the plasma amino acid profile in both pancreatectomized groups were noted on the 3rd postoperative day. Elevation of branched chain amino acids (leucine, isoleucine, and valine) was observed only in Group A, in which postoperative insulin replacement was omitted.

3) Effects of glucagon administration on plasma concentrations of each amino acid

As shown in Fig. 7, in Group A, plasma concentrations of aspartic acid, asparagine, glutamic acid, glutamine, proline, glycine, alanine, cystine, tyrosine, and histidine were reduced by the exogenous glucagon. In addition, glucagon infusion did not affect

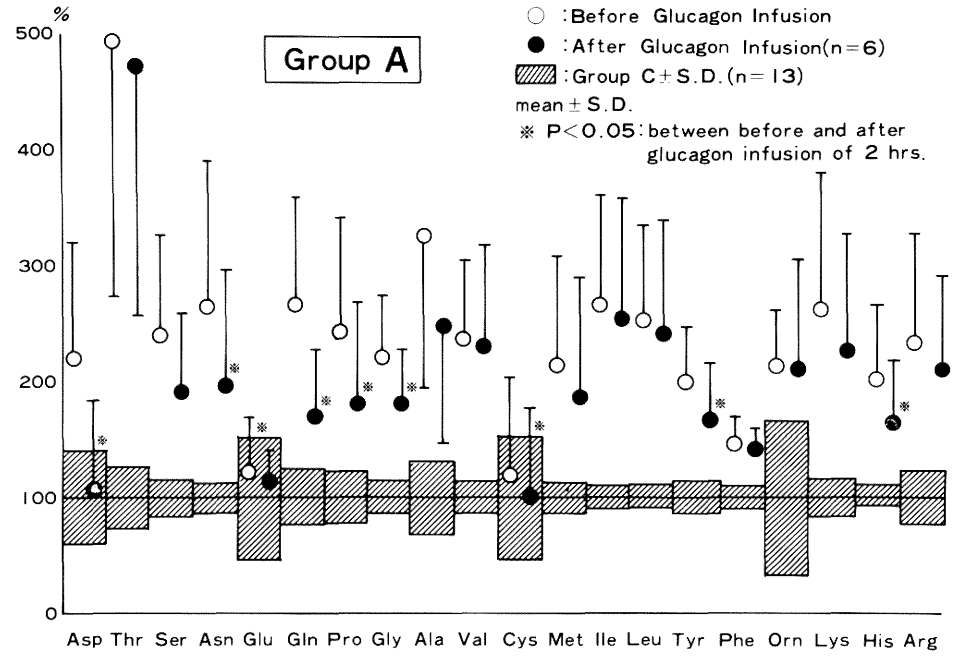

Fig. 7. Effects of glucagon administration on concentrations of plasma amino acids (Group A)—expressed as % of Group C values.

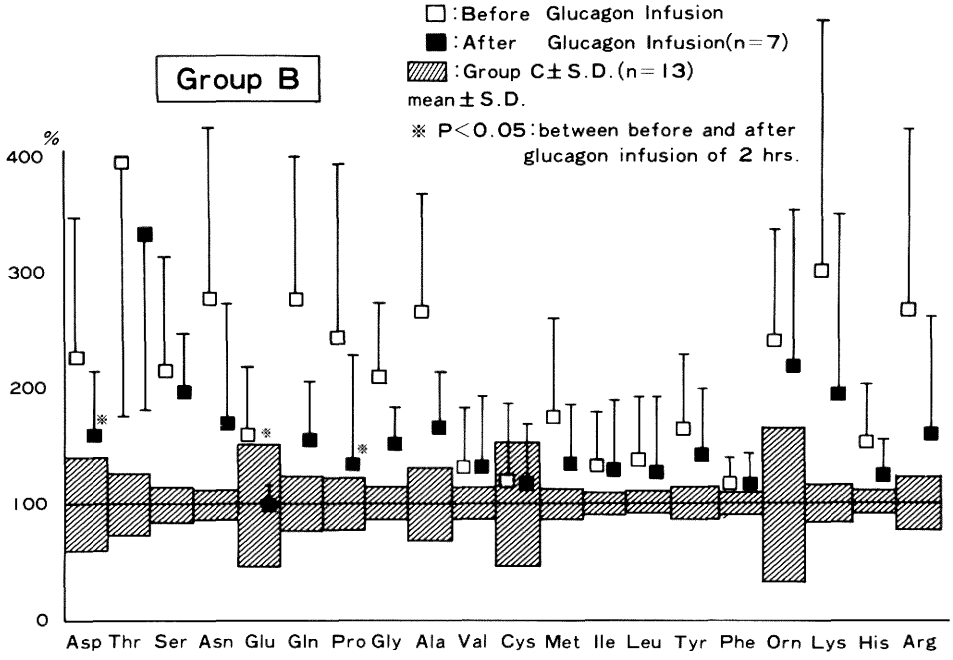

Fig. 8. Effects of glucagon administration on concentrations of plasma amino acids (Group B) — expressed as % of Group C values.

the plasma branched chain amino acids in this group. In Group B, a significant reduction in the plasma concentrations was noted in the aspartic acid, glutamic acid, and proline. The decreases by exogenous glucagon were also observed for serine, asparagine, glutamine, glycine, alanine, methionine, tyrosine, lysine, histidine, and arginine, although no statistical differences were obtained because of the widely scattered amino acid concentrations in this group (Fig. 8).

DISCUSSION

Hyperaminoacidemia in patients who have undergone total pancreatectomy for various reasons has been recognized even in cases where blood sugar levels are closely controlled by daily insulin injections.[1-3] Although clinically apparent symptoms of hyperaminoacidemia have not been reported, potential harmful effects[11,12] of this metabolic derangement have recently been reported. There were also a few earlier reports which clarified the mechanism(s) for hyperaminoacidemia using simultaneous radioisotopic analysis of the glucose kinetics and the measurement of the plasma amino acid concentrations. Against such a background, we determined the hepatic glucose production by the primed-constant infusion with the measurement of plasma amino acid concentrations in totally pancreatectomized dogs.

When using in trying to achieve the hormonal condition of totally pancreatectomized humans differences in the A-cell distribution in organs between species has to be taken into account.

It is generally believed that endogenous insulin secretion disappears in patients whose entire pancreas is removed. On the other hand, regarding the endogenous "pancreatic" glucagon in totally pancreatectomized patients, some authors have found[1,13] no significant endogenous secretion, while other investigators[2,14] have noted small amounts of the secretion. However, in terms of the amounts reported, endogenous glucagon in totally pancreatectomized human could be regarded as negligible with no meaningful contribution to the carbohydrate metabolism. The facts that amount of glucagon originating gastrointestinal tract is far smaller in humans than in dogs and that human total pancreatectomy usually accompanies duodenectomy and partial gastrectomy, which result in the removal of a variable amount of the source of extrapancreatic glucagon, would explain the absence of detectable secretions.

In contrast, high concentrations of the IRG are found in the canine stomach. In addition, large amounts of A-cells which are ultrastructually indistinguishable from pancreatic A-cells can be identified particularly in the gastric fundus. Furthermore, canine gastric "true" glucagon is known to exhibit the function—for example, glycogenolytic activity —of glucagon.[15]

Therefore, in order to simulate the hormonal environment of the human in experimental dogs, endogenous glucagon secretion from the canine stomach has to be eliminated. Consequently, in the present study, the entire stomach was removed in addition to the total pancreatectomy. As seen in Fig. 3, an almost negligible baseline secretion of the glucagon was confirmed in both the pancreatectomized groups. In all 3 groups, the remarkable elevation of IRG upto supra-physiological levels was confirmed after the glucagon infusion of 30 ng/kg min for 2 h. IRI levels near the lower measurable limit were confirmed in the totally pancreatectomized dogs of Group A.

In the present study, Ra (rate of appearance) was obtained based on the tracer-dilution principles. [6-^{3}H]-glucose is reported to be the most suitable tracer for determining the hepatic glucose production by the primed constant infusion[16] method. Food was withdrawn 20 h before the experiment and no exogenous nutrients including glucose were given until the end of the study. Therefore, Ra obtained is considered to mostly represent the rate of gluconeogenesis in the liver.

In the dogs of Group A, in which insulin was not replaced, more than a twofold rise in the postoperative Ra values was observed, accompanied by remarkable hyperglycemia (Fig. 4). Catecholamine, cortisol, etc. would be responsible for the enhancement of the Ra in dogs with profound deprivation of insulin and glucagon. We observed these changes in the early postoperative days, while Muller et al.[1] reported remarkably elevated blood sugar levels after a 3-day insulin withdrawal test in patients long after the total pancreatectomy with negligible plasma IRG. Uki-Järvinen et al.[17] also observed an enhanced endogenous glucose production in patients suffering chronic glucagon deficiency after total pancreatectomy in whom a morning dose of insulin was withheld on the experimental day.

In Group A, a slight but significant fall of Ra without changes in plasma glucose levels was shown (Fig. 5). A similar phenomenon was reported by Nishimura et al.[18] They noted that the blood glucose levels decreased 90 min after 40 μg/kg subcutaneous bolus glucagon injection in a group of totally pancreatectomized dogs in which baseline (pre-injection)

levels of the blood sugar were greater than 400 mg/dl. These authors assumed that glycogen depletion in the liver would be responsible for these changes. As mechanisms for the reduced Ra by the glucagon in the present study, depleted hepatic glycogen, formation of the cyclic AMP antagonist in the liver,[19] down regulation of the glucagon receptors,[20] etc. might be the candidates. Further studies are required to clarify this curious phenomenon.

In contrast, in Group B, the same levels of Ra and plasma glucose as in group C were maintained by insulin supplementation. Increments in Ra associated with the elevation of plasma glucose levels were demonstrated by the glucagon infusion. The above mentioned report[18] also demonstrated that, in a group of totally pancreatectomized dogs with well controlled baseline blood sugar levels by insulin, the glycemic effect of the exogenous glucagon was remarkable. Recently, Bajorunas[21] et al. reported that a greater hyperglycemic response to the exogenous glucagon was observed in totally pancreatectomized patients than in type I diabetic patients. Results in Group B would indicate that the remarkable glycemic effect of exogenous glucagon observed in totally pancreatectomized patients with well controlled blood sugar levels is caused by enhanced hepatic glucose production (enhanced gluconeogenesis with or without glycogenolysis).

Totally pancreatectomized dogs in the present study (Groups A and B) showed hyperaminoacidemia mainly consisting of the elevation of the glucogenic amino acids and those of the Ornithine cycle-related amino acids. In addition, in Group A, significantly higher plasma levels of the branched chain amino acids (BCAAs) than in Group C were noted. Elevated plasma levels of the amino acids except those of BCAAs were reduced by the pharmacological amounts of exogenous glucagon in all groups.

Several authors have reported findings which indirectly support the concept that hyperaminoacidemia after the total pancreatectomy is caused by the reduced hepatic amino acid uptake associated with impaired gluconeogenesis. Muller et al.[3] recognized elevated plasma levels of the aspartic acid, serine, glutamine, asparagine, proline, citrulline, glycine, alanine, methionine, tyrosine, ornithine, lysine, and arginine in chronically glucagon-deficient patients who had undergone total pancreatectomy and had received daily insulin injections. They noted the decrease in the plasma concentrations of most of these above-mentioned amino acids after the glucagon administration (0.3 mg/24 h). Boden et al.[2] reported elevated concentrations of serine, alanine, arginine, glycine, threonine, citrulline, α-amino butyric acid, and tyrosine in 9 totally pancreatectomized patients with chronic glucagon deficiency 24 h after insulin withdrawal. In 2 of these patients, reduced concentrations of alanine, serine, threonine, arginine, and citrulline by exogenous glucagon was demonstrated. Del Prato et al.[4] reported that, even during the period immediately after the total pancreatectomy—i.e., the period of acute glucagon deprivation, plasma alanine levels were markedly elevated. Bajorunas et al.[21] speculated that the deficiency of the glucagon rather than that of insulin might be responsible for increased plasma concentrations of the glucogenic amino acids in pancreatectomized patients. Boden et al.,[22] studying the effects of acute glucagon deficiency induced by somatostatin infusion in 6 normal volunteers, noted that glucagon deficiency decreased—while glucagon excess increased—the urinary excretion of urea, suggesting that alterations in the rate of gluconeogenesis are responsible for the hypoaminoacidemic effect of the glucagon. Moreover, it is known that patients with glucagonoma have hypoaminoacidemia,[23] which is normalized after the removal of the tumor.

Normalization of the hyperaminoacidemia by the glucagon infusion associated with the rise in Ra—i.e., enhanced gluconeogenesis—was observed in the dogs of Group B in the present study. These results strongly support the concept that the cause of hyperaminoacidemia in "acute" glucagon deprivation is the reduced hepatic amino acid uptake associated with impaired gluconeogenesis. Regardlles of the brief duration of the glucagon deficiency in the present experiment, the mechanism(s) described above would also explain the hyperaminoacidemia in patients long after the total pancreatectomy with "chronic" glucagon deficiency.

On the other hand, branched chain amino acids responded uniquely to each pancreatic hormone. High concentrations of BCAA were observed in Group A, in which the dogs did not receive postoperative insulin, while dogs in Group B showed normal BCAA concentrations. Pozefsky et al.[24] reported that the output of such amino acids from the human forearm as leucine, isoleucine, tyrosine, phenylalanine, threonine, glycine, and α-aminobutyric acid were reduced by the insulin, while alanine output was not affected. Berger et al.[25] reported increased and decreased plasma BCAA levels in diabetic ketoacidosis and insulinoma, respectively. Felig et al.[26] observed a close relationship between insulin and plasma levels of BCAA, tyrosine, and phenylalanine in obese subjects. Our experimental

results and all the earlier reports mentioned above indicate that plasma BCAA concentrations are much more sensitive to insulin than to glucagon.

Several earlier experimental reports recommended the use of glucagon for totally pancreatectomized patients. For examplle, Yoshie et al.[27] showed its effectiveness for preventing hypoglycemia and a fatty liver. However, nutritional and metabolic management of these patients are today routinely done by insulin injection without supplementing the counter-regulatory hormone, glucagon. Although clinically apparent symptons have not been observed, such marked hyperaminoacidemia in patients after total pancreatectomy should be regarded as a severe metabolic derangement. Attention should be given to recently reported cholestatic effects of the arginine[11] or methionine[12] in association with the hyperaminoacidemia. The use of glucagon in addition to insulin would be advisable.

In conclusion, results of the present study in which the rate of hepatic glucose production was isotopically determined in combination with the plasma amino acid measurement indicated that the cause of hyperaminoacidemia in totally pancreatectomized patients is inhibited gluconeogenesis from amino acids due to glucagon dificiency.

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