INTRODUCTION

The accumulation of AAs in the pancreas has been demonstrated by positron emission tomography (PET) and macroautoradiography (1-3). The pancreas is an essential organ for the body through its functional endo- and/or exocrine roles. AAs would play significant physiological roles as the substrates for humoral factors (4, 5). In terms of the dynamics of free AAs, we previously found that the secretion of the branched chain AAs into the PJ was further induced by the branched chain AAs that were administered to the peripheral circulation (6). As for glutamate, its accumulation was observed in the pancreas and the intestine (7). The physiological role or the mechanism of the phenomenon has not been revealed. Nevertheless, glutamine-glutamate metabolism is recognized as a crucial mechanism for the maintenance of the homeostasis of the humoral pH in the kidneys, the production of energy in the intestine, the metabolism of NH3 in the liver, the neurotransmission in the central nervous system, and the activation of immune cells (8-13). With regard to the correlation of the insulin secretion and the AA metabolism in the pancreas, the glutamine-glutamate metabolism in the pancreatic β cells, which is enhanced by leucine through the activation of glutamate dehydrogenase, is related to the augmentation of the insulin production by the β cells (11, 14-17). Therefore, the glutamate dynamics of the pancreas would potentially play a significant physiological role in the homeostasis of the whole body and also reflect the pancreatic endocrine function. Thus, in the current study, we focused on the glutamate dynamics in the PJ of rats and also investigated the distribution of the glutamate transporters in the pancreas to clarify the physiological relevance of the pancreatic glutamate dynamics.

MATERIALS AND METHODS

Animals

8 week old male Cj: CD(SD) rats were purchased from Charles River Inc. (Yokohama, Japan). The rats were bred under the conditions of room temperature of 24±3°C, 12 hours light/dark cycle and ad libitum access to food and water. All the animal experiments were conducted under the approval of the Institutional Animal Care and Use Committee of Tohoku University.

Surgical procedure and sampling of the pancreatic juice

The rats underwent laparotomy under anesthesia with diethyl ether after 12 hours fasting. The details of the original cannulation
procedure were described previously (18). We modified the procedure and made the gastric or bile juice flow into the intestinal lumen in order to maintain the humoral homeostasis. In brief, a fine polyethylene tube (PE-10, Beckton Dickinson and Company, Sparks, MD) was inserted into the common duct, the interflow of the bile duct and the pancreatic duct. The bile duct was ligated at the cranial marginal level of the pancreas. Another fine tube was inserted into the hepatic side of the bile duct with its opposite end located in the duodenum to bypass the papilla. In order to eliminate the effect of gastric juice on the secretion of PJ, the pyloric portion of the stomach was ligated. The absolutely pure PJ, cooled with ice, was sampled from the extracorporeal end of the tube. After sampling of the PJ for an hour, blood was sampled from the aorta. For the intravenous administration of the AAs and glucose, the right cervical vein was cannulated with a fine silicon-rubber catheter 1.0 mm in external diameter (Kaneka Medics Inc., Osaka, Japan) 3 cm toward the proximal side after ligation at the distal side. The PJ was sampled after 60 min from the start of the intravenous infusion of saline or other substances. The intravenous administration was performed at the rate of 4 ml/hour. The concentrations of the substances were as follows: glucose (7.875%), alanyl-glutamine (AG) (50 mmol/l), alanine (50 mmol/l), and glutamate (50 mmol/l). All were purchased from Kyowa Hakko Inc. (Tokyo, Japan) (19).

Amino acid analysis and measurement of pancreatic juice volume

The plasma was obtained by conventional centrifugation of the sample blood. The plasma and the PJ were treated with 4% sulfosalicyclic acid after filtration. The amino acid analysis was performed with the plasma and PJ samples obtained from the same animal by a L-8500 AA analyzer (Hitachi Inc., Tokyo) for the evaluation of the 22 AAs. The whole glutamate amount excreted in the PJ was calculated by multiplication of each PJ volume and the corresponding glutamate concentration.

Immunohistochemistry

Immunohistochemistry was performed according to the standard protocol of the labeled streptavidin biotin or avidin-biotin complex method with 2.5% formaldehyde or 4% paraformaldehyde fixed and paraffin-embedded sections. After blocking the endogenous peroxidase of the samples by 0.3% hydrogen peroxide, thin sliced sections were incubated with the

![Fig. 1](image_url). Glutamate concentration in the PJ was distinctive and did not coordinate with the plasma glutamate. The basal composition of amino acids (AAs) in the rat pancreatic juice (PJ) (A), the composition of AAs of the plasma (B) the ratio of the concentration of AAs of the PJ to that of the plasma (C).
primary antibodies overnight at 4°C. For the incubation with the corresponding secondary antibodies and for visualizing the staining, either LSAB2 (DAKO) or Vectastain ABC kit (Vector Laboratories Inc. Burlingame, CA) was used. The images were recorded by an Olympus AH2 (Olympus, Tokyo, Japan) microscope after the slides were counterstained by haematoxylin and eosin. The primary antibodies were as follows: mouse anti-glutamate monoclonal (Chemicon International Inc., CA), ASCT2 polyclonal (Chemicon), rabbit anti-conjugated GABA polyclonal (Cell Sciences, MA), mouse anti-human LAT1 monoclonal (Transgenic Inc. Kobe, Japan), rabbit anti-rat BAT1 polyclonal (Transgenic Inc.), anti-rat 4F2hc polyclonal (Transgenic Inc.), anti-mouse xCT polyclonal (Transgenic Inc.), mouse anti-rat EAAC1 monoclonal (Chemicon), rabbit anti-rat GLT1 (Alpha Diagnostic International, TX), rabbit anti-rat GLAST (Alpha Diagnostic International) and rabbit anti-rat VGLUT1 polyclonal (Synaptic Systems, Goettingen, Germany).

Statistical analysis

The results are shown as the mean±standard error. The data were analyzed by SPSS software (SPSS Inc., Tokyo, Japan). The differences between the groups were tested by ANOVA followed by Tukey post hoc test. p values less than 0.05 were considered statistically significant.

RESULTS

The basal state of glutamate in the PJ

The average volume of the PJ sampled in the basal state was 59.5±8.2 µl (n=6). The plasma and the PJ concentrations were 253.6±23.7 nmol/ml vs. 9.4±4.2 nmol/ml for glutamine and 284.5±88.8 nmol/ml vs. 739.8±295.5 nmol/ml for glutamate (Fig. 1A & B). The ratio of glutamine/glutamate was 1.2±0.4 for the plasma concentration and 90.0±46.0 for the PJ concentration. The notably higher concentration of glutamate was observed in the PJ compared to the plasma (Fig. 1C).

In general, glutamine is hydrophobic and unstable alone. Therefore, in the current experiment, AG was substituted for glutamine. AG is a dipeptide of alanine and glutamine which is dissolved in the blood immediately to alanine and glutamine (19, 20). The peak elevation of the concentration of glutamine in the blood was confirmed at 30 min after the initial administration of AG (data not shown). The AG administration induced the secretion of glutamate into the PJ, which was statistically significant and distinct from alanine alone or glutamate administration (Fig. 2A & B). As for glucose, there were no significant effects on the secretion of glutamate into the PJ observed (Fig. 2C & D). The secreted volume of the PJ was not affected by the administration of either AG or glucose.

The dynamic state of glutamate in the PJ under the administration of glutamine

The expression of LAT1 was identified in the islet cells. 4F2hc was expressed in both of the islet cells and the acinar cells.

Table 1. Amino acid transporters in the pancreas.

<table>
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<th>Islet</th>
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<tbody>
<tr>
<td>LAT1</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>ASCT2</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>BAT1</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>4F2hc</td>
<td>+</td>
<td>+</td>
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<tr>
<td>GLT1</td>
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<td>GLAST</td>
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<td>VGLUT1</td>
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BAT1 and ASCT2 were stained in the cytosol of the islet cells and acinar cells. GLT1, GLAST, VGLUT and xCT were identified in the islet cells. EAAC1 was expressed in the acinar cells, especially in the cytosol. xCT staining was also confirmed in the epithelial cells of the pancreatic ducts, whereas 4F2hc was not (Fig. 3A). GABA, an AA produced from glutamate by glutamic acid decarboxylase, was localized in the islet cells (Fig. 3B). The results of the immunohistochemistry are summarized in Table 1.

DISCUSSION

In the current study, we revealed that glutamate was secreted in the rat PJ. The glutamate concentration of the PJ was higher than that of the peripheral blood, and was further augmented by AG. We previously reported that the ratios of most AA concentrations of the PJ to those of the plasma were below 1. And each concentration of the AAs in the PJ was increased coordinately by intravenous loading of the identical AAs. In the current study, the concentration of glutamate was prominent in the PJ and even three times higher than that in the plasma, while the concentrations of the other AAs tended to be lower in the PJ when compared to the plasma, as observed previously. Together with the relatively low concentration of glutamine in the PJ and pancreatic tissue, most of the glutamine that flowed into the pancreas would be utilized for the synthesis of biologically active molecules or metabolized to glutamate, which was further supported by the increase of glutamate in the PJ under the elevated plasma glutamine but not by glutamate. In addition, we did not find a modulation of the secretion of amylase or...
Glutamate to the extracellular spaces (29). The importance of the functions as a cystine/glutamate counter-transporter that secretes glutamate transporters is also represented by the glutamate re-

The side chains of the AAs defined the corresponding

or ductal cells. Previous research revealed that the structure of the pancreas.

physiological significance of the secretion of glutamate from the physiological significance of AA in the PJ, glutamate secreted into the intestine would not be only a source of energy, but also a protective factor for the intestinal epithelia (21, 22). AG is recognized also as effective for the maintenance of the nutritional absorbance of the intestinal epithelia of critically ill patients. Thus, the increased excretion of glutamate into the PJ caused by AG might exert a protective effect on the intestinal epithelia. From another point of view, glutamate metabolism is related to insulin production, which may suggest that the secretion of glutamate in the PJ reflects the endocrine status of the pancreas, including insulin production. Therefore, we tested this hypothesis by the administration of glucose. However, no significant changes were observed for glutamate secretion in the PJ, probably because of the lack of other factors like leucine.

Relationship between feed-regulating peptides (i.e., leptin, apelin, ghrelin, obestatin and orexins) and pancreatic exocrine secretion was controversy (23). The peptides and the oral intake of glutamate also improve appetite (21), which might suggest the physiological significance of the secretion of glutamate from the pancreas.

In terms of the molecular mechanisms of the secretion of glutamate, we investigated the potential transporters that might be responsible for the transport of glutamate in the acinar cells or ductal cells. Previous research revealed that the structure of the side chains of the AAs defined the corresponding transporters (24). In general, the transporters include ASCT2 that transports neutral AAs such as alanine, serine, cysteine, glutamine and asparagine via a sodium-dependent mechanism (25), and LAT1 that transports sodium-dependently the high molecular weight neutral AAs such as leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, and methionine under the coordination of the cofactor 4F2hc (26). BAT1 transports cystine, neutral and basic AAs (26). The transporters for the acidic AAs such as glutamate and aspartate are classified into 2 categories, sodium-dependent or independent (23, 28). The former category includes membranous EAAC1, GLT1 and GLAST for the cellular uptake of glutamate. VGLUT1 localizes to the intracellular secretory granules, which mediate the uptake of glutamate into the vesicles (29). As for the latter, xCT functions as a cystine/glutamate counter-transporter that secretes glutamate to the extracellular spaces (29). The importance of the glutamate transporters is also represented by the glutamate re-

uptake mechanisms for the subsynaptic interval, leading to the termination of neuronal signal transmissions (31). We observed the expression of potential importers such as GLT1, and GLAST, and exporters such as VGLUT1 and xCT in the islet cells, some of which were described previously (32). However, glutamate was not identified in the islet cells, while there was GABA staining in the cytosol of the islet cells. Glutamate was exclusively found in the acinar cells that accompany EAAC1 expression. The microcirculation of the pancreas, called the islet-acinar-axis, might support our hypothesis of glutamate production and distribution from the islets to the acinar cells in the pancreas (33, 34). The perinuclear staining pattern of EAAC1 in the current study would be explained by the local high concentration of glutamate, as the high concentration of glutamate could alter the subcellular localization of EAAC1 from the peripheral membrane to the Golgi apparatus (35). The expression of glutamate transporters such as GLT1 and GLAST identified in the duct cells would play a potential role in the re-

uptake of glutamate by the PJ (36). The glutamate in the PJ could enhance the regeneration of islet cells under some specific conditions (37, 38). The hypothetical schema for the transport system of glutamate in the pancreas is shown in Fig. 4.

The intestinal bile acid absorption and the enterohepatic circulation constitute the internal bile acid pool. A specific transporter, the apical sodium-dependent bile acid transporter (ASBT) of terminal ileum is the key player in the system. The function of the ASBT could be modulated by the lipid status, which would play a role for the maintenance of the lipid homeostasis (39) probably through the modulation of the bile acid pool. As for the AAs, glutamate is not only a metabolic fuel of small intestine, but also a substitute of hepatic gluconeogenesis (12). The serum glucose supplied by the gluconeogenesis is also metabolized to glutamine through the glucose-alanine-lactate-glutamine cycle in the skeletal muscles (40). Therefore, pancreatic glutamine-glutamate metabolism and the AA transporters are likely to constitute a huge AA pool through the entero-hepatic muscle-pancreatic circulation just as the bile acids.

In conclusion, we revealed the physiological secretion of glutamate in the rat PJ and some of the potentially responsible transporters for the local glutamate dynamics. However, the importers and the secretory molecules of glutamate in the acinar cells remain to be clarified. The hypothesis should be tested by some genetically engineered animals, if technically available, in the future.
CONCLUSIONS

The transporters for the AAs were identified together with their specific distribution in the rat pancreas. Glutamate was secreted into the PJ. The secretion of glutamate from the pancreas was modulated by glutamine in the plasma. Glutamine-glutamate metabolism and the secretory pathway of glutamate via the islet-acinar axis were speculated to be novel mechanisms in the pancreas.

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