Peripheral Serotonin Enhances Lipid Metabolism by Accelerating Bile Acid Turnover

Hitoshi Watanabe, Daisuke Akasaka, Hideki Ogawara, Kan Sato, Masato Miyake, Kazuki Saito, Yu Takahashi, Takashi Kanaya, Ikuro Takakura, Tetsuya Hondo, Guozheng Chao, Michael T. Rose, Shyuichi Ohwada, Kouichi Watanabe, Takahiro Yamaguchi, and Hisashi Aso

Cellular Biology Laboratory (H.W., D.A., H.O., M.M., K.Sai., Y.T., T.K., I.T., G.C., S.O., K.W., T.Y., H.A.), Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan; Field Science Center (H.O.), Yakumo Experimental Farm, School of Veterinary Medicine, Kitasato University futami-gun, Hokkaido 049-3121, Japan; Animal Science (K.Sat.), Department of Biological Production, Tokyo University of Agriculture and Technology, Fuchu-shi, Tokyo 183-8509, Japan; Laboratory for Epithelial Immunobiology Research Center for Allergy and Immunology (T.K.), RIKEN, Yokohama-shi, Kanagawa 230-0045, Japan; and Institute of Biological, Environmental and Rural Sciences (M.T.R.), Abenystwyth University, Cardiganshire, SY23 3AL, United Kingdom

Serotonin is synthesized by two distinct tryptophan hydroxylases, one in the brain and one in the periphery. The latter is known to be unable to cross the blood-brain barrier. These two serotonin systems have apparently independent functions, although the functions of peripheral serotonin have yet to be fully elucidated. In this study, we have investigated the physiological effect of peripheral serotonin on the concentrations of metabolites in the circulation and in the liver. After fasting, mice were ip injected with 1 mg serotonin. The plasma glucose concentration was significantly elevated between 60 and 270 min after the injection. In contrast, plasma triglyceride, cholesterol, and nonesterified fatty acid concentrations were decreased. The hepatic glycogen synthesis and concentrations were significantly higher at 240 min. At the same time, the hepatic triglyceride content was significantly lower than the basal levels noted before the serotonin injection, whereas the hepatic cholesterol content was significantly higher by 60 min after the injection. Furthermore, serotonin stimulated the contraction of the gallbladder and the excretion of bile. After the serotonin injection, there was a significant induction of apical sodium-dependent bile acid transporter expression, resulting in a decrease in the concentration of bile acids in the feces. Additionally, data are presented to show that the functions of serotonin are mediated through diverse serotonin receptor subtypes. These data indicate that peripheral serotonin accelerates the metabolism of lipid by increasing the concentration of bile acids in circulation. (Endocrinology 151:4776–4786, 2010)
The first step in the synthesis of serotonin from tryptophan is the enzyme tryptophan hydroxylase (TPH), which is also the rate-limiting enzyme in its biosynthesis. TPH is known to have two isoforms sharing an overall identity of approximately 70%. These are called TPH1 and TPH2 (13). TPH1 is mainly present in the pineal gland, thymus, spleen, and enterochromaffin cells of the gastrointestinal tract. TPH2 is expressed solely in neuronal cells, such as the raphe nuclei of the brainstem. Mice lacking TPH1 contain little to no serotonin in the blood and gastrointestinal tract while maintaining normal levels in the brain. Peripheral serotonin in mice lacking TPH1 cannot be replaced with serotonin from the central nervous system synthesized by TPH2 (14). Furthermore, serotonin is thought not to be able to pass the blood-brain barrier. Serotonin was not found in the brain of mice given 5 mg/mouse or rats given 50 mg/kg after 10 or 30 min, respectively, after an ip injection (15, 16). Thus, there are two serotonin systems: one in the central nervous system and one in the periphery, with independent functions and pathways for biosynthesis.

It has been reported that increasing the circulating concentrations of serotonin decreases the blood glucose level via an induced secretion of insulin in mice (17, 18). In contrast, serotonin induces hyperglycemia by adrenaline release from the adrenal gland in rats, which is mediated by 5HT1A, 5HT2A, or 5HT7 receptors (19–21). Moreover, serotonin enhances net hepatic glucose uptake under hyperglycemic and hyperinsulinemic conditions (22) and stimulates glycogen synthesis at nanomolar concentrations (23). These results suggest that peripheral serotonin plays an important role in glucose metabolism and that serotonin may have a key role in lipid metabolism, even though there is no evidence for this presently. In the present study, we have therefore investigated whether serotonin affects not only glucose metabolism but also lipid metabolism and bile acid turnover. We are the first to show that peripheral administration of serotonin to mice enhances the concentration of bile acids in circulation and regulates the levels of various metabolites in blood as well as regulating the function of liver.

**Materials and Methods**

**Animal experiments**

Seven-week-old male C57BL/6 mice (Japan SLC, Shizuoka, Japan) were used. All mice were housed in a temperature-controlled environment with 12-h light, 12-h dark cycle and fed standard rodent chow (CLEA Japan, Inc., Tokyo, Japan). Mice were fasted for 12–14 h and ip injected with serotonin (Sigma Chemical Co., St. Louis, MO) at 0.03, 0.1, 0.3 or 1 mg/mouse. In all experiments, we used six or eight mice at each time point indicated in the relevant figure; blood samples were taken each mouse once only. The mean volume of each blood sample was 600 µl (range, 500–800 µl). The experiments were permitted by the Tohoku University Environmental and Safety Committee and conducted in accordance with the Guidelines for Animals Experimentation of Tohoku University, which have been sanctioned by the relevant committee of the Government of Japan based on the Declaration of Helsinki.

**Plasma chemistry analysis**

Blood samples were collected from the carotid artery at periodic intervals after ip injection of serotonin into ice-cold tubes containing heparin (10 U/tube) (Mochida, Tokyo, Japan) and centrifuged at 20,000 × g for 15 min immediately thereafter. For the glucagon assay, samples were collected in ice-cold tubes containing heparin and aprotinin (500 KIU/tube) (Wako, Osaka, Japan). For the serotonin measurement, samples were collected in ice-cold tubes containing EDTA (1 mg/tube) and centrifuged at 200 × g for 15 min. To obtain platelet-free plasma, the supernatant was transferred to another tube and centrifuged at 4500 × g for 10 min. Plasma samples were stored at −80°C until analysis. Plasma concentrations of glucose, triglyceride, cholesterol, nonesterified fatty acids (NEFA), and bile acids were measured using commercial kits (Wako). Plasma serotonin, glucagon, insulin, and cholecystokinin (CCK) concentrations were determined by ELISA, which were purchased from Immunotech (Marseille, France), Yanaihara (Shizuoka, Japan), Morinaga Co. (Tokyo, Japan), and Phoenix Pharmaceuticals, Inc. (Burlingame, CA), respectively. All procedures were performed according to the respective manufacturer’s instructions.

**Table 1. Primers used in quantitative real-time PCR analysis**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence (5’–3’)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEPCK</td>
<td>Forward GGTTTTGAGAGAAACAGCATGAGA</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>Reverse GCCAGGTATTTGCCGAAGTTTGAG</td>
<td></td>
</tr>
<tr>
<td>GYS2</td>
<td>Forward AAGGGACATGTGCTGACCTTAC</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>Reverse TTGCCGGCCTGACTTTTA</td>
<td></td>
</tr>
<tr>
<td>PYGL</td>
<td>Forward TGCCGAGAATCTGCTCGGATA</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>Reverse CGCTGGGAGATCTGCTCGGATA</td>
<td></td>
</tr>
<tr>
<td>G6Pase</td>
<td>Forward GAAGGCCAAGGAGATGGTGTTGA</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>Reverse TGCAGCTTTTGCGTGATGAC</td>
<td></td>
</tr>
<tr>
<td>CYP7a1</td>
<td>Forward ACACATTACCAATAAGAAAGAC</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Reverse TGATGTAGACCAGAATAACCT</td>
<td></td>
</tr>
<tr>
<td>FGF15</td>
<td>Forward TGATACCCGCTGTTCCCTATGTC</td>
<td>142</td>
</tr>
<tr>
<td>ASBT</td>
<td>Forward CGCTGGATCTTGGACACAGAATACCT</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>Reverse CGCTGGATCTTGGACACAGAATACCT</td>
<td></td>
</tr>
<tr>
<td>18s</td>
<td>Forward CGGCTACCACATCCAAGGAA</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>Reverse GCTGGAATTACCCGCGCT</td>
<td></td>
</tr>
</tbody>
</table>
Histological analysis
Liver tissues and gallbladders were fixed in 4% paraformaldehyde/PBS (pH 7.2) and embedded in paraffin. The sections were stained with hematoxylin-eosin. For hepatic glycogen staining, livers were fixed in ethanol and embedded in paraffin. The sections were examined by the standard periodic acid-Schiff (PAS) staining reaction, followed by counterstaining with hematoxylin.

Measurement of hepatic metabolite concentrations
Hepatic tissue (about 100 mg wet weight) was homogenized at 4°C in 5 ml of a mixture of CHCl₃-MeOH (2:1, vol/vol) using a Polytron homogenizer. After adding 2.5 ml dilute sulfuric acid (0.05%), the resulting suspension was vigorously mixed and centrifuged at 600 × g for 10 min. The chloroform layer containing lipid was dried under a flow of nitrogen. The triglyceride and cholesterol concentrations were determined by the same procedure as described above for the plasma chemistry analysis.

For the measurement of glycogen, approximately 100 mg (wet weight) of liver was treated with 1 ml 0.1 M KOH and sonicated. Liver samples were heated at 85°C for 40 min, acidified to pH 4.5 with acetic acid (3 M) and centrifuged at 20,000 × g for 10 min to remove the protein. Amyloglucosidase (0.15 U) was added to 100 μl of the supernatant, and the mixture was incubated at 40°C for 2 h. The glucose formed was measured by kit (Wako).

Total RNA preparation and quantitative real-time PCR analysis
Total RNA were extracted from the livers and ilea using Trizol reagent (Invitrogen, Co., Carlsbad, CA), and reverse transcribed with the Superscript III RT kit (Invitrogen) using random primers. The gene expression levels were analyzed by quantitative real-time PCR using the Thermal Cycler Dice Real Time System Single (Takara Bio Inc., Siga, Japan). After incubation for 10 sec at 95°C, the cDNA was followed by PCR for 40 cycles (95°C for 5 sec, 60°C for 30 sec). SYBR green fluorescence was detected at the end of each cycle to monitor the amount of PCR product formed during that cycle. At the end of each run, the melting curve profiles were recorded. The standard curve of each product followed the calculation of respective gene expression. Results are presented as the ratio of respective gene expression to 18S rRNA to correct for differences in the amounts of template DNA used. The sequence of primers and sizes of each PCR product are listed in Table 1.

Measurement of 2-deoxyglucose 6-phosphate (2DG6P) concentrations
Fasted mice were given 5 μmol 2-deoxy-D-glucose (2DG) (Sigma) through their tail vein and immediately ip injected with serotonin (1 mg/mouse) or PBS. After 60 min, blood samples were collected from the carotid artery of the mice and placed into ice-cold tubes containing heparin (10 U/tube) (Mochida, Tokyo, Japan). The samples were centrifuged at 20,000 × g for 15 min. The liver and gastrocnemius muscle were obtained and immediately frozen in liquid nitrogen. All samples were stored at −80°C until analysis.

The plasma samples were 30-fold diluted with 10 mM Tris-HCl and then boiled at 95°C for 15 min. After cooling and centrifugation at 18,000 × g for 15 min, the supernatant was diluted 4-fold by 10 mM Tris-HCl, and 20 μl of this diluted sample was placed into a well of a 96-well microplate. For the measurement of plasma 2DG, 65 μl reagent A [10 mM Tris-HCl (pH 8.1) containing

FIG. 1. Effects of serotonin on concentrations of plasma metabolites and hormones in fasted mice. Blood samples were taken from mice between 0 and 270 min after the ip injection of 1 mg serotonin (n = 6–8 mice per group). Plasma serotonin (A), glucose (B), triglyceride (C), cholesterol (D), NEFA (E), glucagon (F), and insulin (G) levels were measured. *, P < 0.05; ***, P < 0.01 relative to basal values at 0 min.
0.03% BSA, 195 μM nicotinamide dinucleotide (NAD), 7.5 U/ml hexokinase (Sigma), 2.6 mM ATP (Sigma), 1.3 mM MgCl₂, and 1.1 U/ml glucose-6-phosphate dehydrogenase (G6PDH) was added to each well, and the 96-well microplate was incubated overnight at room temperature. In this reaction, endogenous glucose was metabolized by the hexokinase and G6PDH in reagent A, and 2DG was phosphorylated to 2DG6P by the hexokinase.

The concentrations of 2DG in tissue samples were estimated by measuring the amount of 2DG6P, because 2DG6P phosphorylated from 2DG by glucokinase or hexokinase was not metabolized further. Briefly, 10 mg of pulverized frozen sample was put into a tube with 500 μL 10 mM Tris-HCl and then boiled at 95°C for 15 min. After cooling and centrifugation at 18,000 × g for 15 min, 20 μL of supernatant was put into a well of a 96-well microplate. To metabolize endogenous glucose-6-phosphate, 65 μL of reagent B [10 mM Tris-HCl (pH 8.1) containing 0.03% BSA (Sigma), 195 μM NAD (Oriental Yeast Co., Tokyo, Japan), 1.3 mM EDTA, and 1.1 U/ml G6PDH (Oriental Yeast)] was added.

After an overnight incubation at room temperature, 5 μL 1 N HCl was added to each well containing plasma and tissue reactions and incubated at 37°C for 15 min to inactivate the NAD. Five microliters of 1 N NaOH/50 mM Tris-HCl was added to each well and gently mixed to neutralize the sample. Furthermore, 5 μL reagent C [10 mM Tris-HCl (pH 8.1) containing 200 μM NAD phosphate (NADP) (Roche Applied Science, Indianapolis, IN), 40 mM EDTA and 860 U/ml G6PDH] was added to each well and incubated at 37°C for 1 h. In this reaction, as 2DG6P was metabolized by high concentrations of G6PDH, NADP was converted to reduced NADP. After NADP was inactivated by adding of 5 μL 1.5 N NaOH and incubating at 70°C for 1 h, 5 μL 1.5 N HCl/50 mM Tris-HCl was added to each well to neutralize the sample. Ninety microliters of reagent D [10 mM Tris-HCl (pH 8.1) containing 29 mM G6P (Oriental Yeast), 2.9 mM oxidized glutathione (Sigma), 2.9 mM EDTA, 124.7 U/ml G6PDH, 8.7 U/ml glutathione reductase (Oriental Yeast), and 20 mM 5,5′-dithiobis (Sigma)] was added to each well. The increasing absorbance of reduced NADP was measured at λ 450 nm every 1 min.

**Gallbladder volume measurements**

After mice were euthanized, the cystic duct was clamped off with forceps and the gallbladder was excised and placed in an Eppendorf tube. After puncturing the gallbladder with a needle, bile was collected by centrifugation at 800 × g for 10 min. The volume of bile was measured and normalized to the body weight of the animal.

**Measurement of fecal bile acid concentrations**

Stools from individually housed mice were collected every day during 3 d after serotonin injection. The fecal samples were lyophilized, weighed, and thoroughly mixed in 5 ml of a CHCl₃-MeOH (2:1, vol/vol) mixture. The measurement of the concentration of fecal bile acids was then followed by the same procedure as described above for the plasma chemistry analysis using kits (Wako).

**Western blot analysis**

Preparations of the membranes of the ilea of the mice were made for 0–240 min after the serotonin injection and homogenized in a lysis buffer (1 ml 20 mM Tris-HCl, 0.33 mM sucrose, 2 mM EDTA, 0.5 mM EGTA, 2 mM phenylmethylsulfonyl fluoride, 1% aprotinin) by a motor-driven Teflon pestle (Kontes, Vineland, NJ). Homogenates were centrifuged at 6000 × g for 5 min. Supernatant (300 μl) was added to the same volume of the buffer described above and centrifuged at 100,000 × g for 40 min. The membrane pellet was resuspended in Tris-Triton X buffer (10 mM Tris-HCl, 150 mM NaCl, 0.5% Triton X-100, 0.2 mM phenylmethylsulfonyl fluoride).

The proteins of the ilea membranes of the mice were mixed in buffer (62.5 mM Tris-HCl, 2% SDS, 20% sucrose, 0.02% pyronin Y, and 5% 2-mercaptopethanol) and boiled for 10 min.

**FIG. 2.** Dose-response effects of serotonin on concentrations of plasma metabolites and insulin in fasted mice. Blood was withdrawn from the mice at 60 or 120 min after the ip injection of serotonin (0.03, 0.1, 0.3, and 1 mg). Plasma glucose (A) and insulin (B) levels were measured at 60 min after the injection. Plasma triglyceride (C), cholesterol (D), and NEFA (E) concentrations were determined at 120 min after the injection. Each column is the mean of eight independent animals. Columns with a different letter are significantly different (P < 0.05).
Protein concentrations were determined using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA). The protein samples (7.35 μg/well) were applied on SDS-polyacrylamide gel (ATTO, Tokyo, Japan) and then transferred to membranes (Immobilon-P; Millipore, Billerica, MA). The membranes were blocked with 5% skim milk/Tris-buffered saline with Tween 20 for 1 h at room temperature. A goat polyclonal antibody against apical sodium-dependent bile acid transporter (ASBT) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA,) was diluted 100-fold and applied to the membranes, which were then incubated overnight at 4°C. The membranes were subsequently washed with Tris-buffered saline with Tween 20 three times for 10 min and incubated with peroxidase-conjugated secondary antibody (Invitrogen Co., Carlsbad, CA) for 1 h at room temperature. The signals were detected by ECL Plus Western blotting detection system (Bio-Rad Laboratories, Inc., Hercules, CA). The band intensities were quantified using NIH Image software.

**Effect of serotonin antagonists on serotonin action**

Ketanserin (Sigma), a 5HT2A antagonist, was dissolved in 0.1 M HCl, diluted with PBS, and administered in a dosing volume of 0.02 mg/mouse. SB-269970 (Sigma), a 5HT7 antagonist, and methysergide (Sigma), a 5HT1, 5HT2, and 5HT7 antagonist, were dissolved in PBS and administered in a dosing volume of 0.6 and 0.1 mg/mouse, respectively. All antagonists were ip injected 30 min before the injection of 1 mg serotonin. After 60 min, blood samples were collected from the carotid artery. Various plasma metabolite concentrations were measured using the same procedures described above in **Plasma chemistry analysis**.

**Statistical analysis**

Values are reported as means ± SD. Statistical analyses were performed using Student’s t test or one-way ANOVA followed by Tukey’s test to evaluate statistical differences among the groups. P values <0.05 were considered as statistically significant.

**Results**

**Effects of serotonin injection on concentration of plasma metabolites and hormones in fasted mice**

After an ip injection with 1 mg serotonin, plasma concentrations of metabolites and hormones were measured at the times indicated in Fig. 1. The average basal and physiological concentration of plasma serotonin was about 0.6 μM before the injection of the serotonin. After the serotonin injection, plasma serotonin levels reached a peak of 43.4 μM at 30 min, were half of peak values by 60 min, and gently decreased thereafter until 270 min after the serotonin injection (Fig. 1A). After the serotonin injection, plasma glucose levels were significantly elevated, reached a clear peak at 90 min, and gradually decreased until 270 min. Even so, the plasma glucose level at 270 min was still significantly higher than that noted before the sero-
tonin injection (Fig. 1B). In contrast, plasma triglyceride, cholesterol, and NEFA levels were significantly lower after the serotonin injection compared with the levels noted before the serotonin injection but returned to the baseline by 150, 270, and 180 min, respectively (Fig. 1, C–E). Serotonin injection caused a rapid decrease in the plasma concentration of glucagon (Fig. 1F); however, a high concentration of insulin was induced by the serotonin injection, which continued for 2 h (Fig. 1G). PBS injection (control) did not affect plasma glucose, triglyceride, cholesterol, and NEFA concentrations (Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org). In free-feeding mice, serotonin injection also increased plasma glucose concentrations and decreased the plasma concentrations of triglyceride, cholesterol, and NEFA, as for the fasted mice (Supplemental Fig. 2). However, the effect of serotonin was clearer in fasted mice than in free-feeding mice. Accordingly, fasted mice were used throughout the remainder of this study.

**Dose-dependent effect of serotonin injection on plasma biochemical metabolites and insulin**

Mice were ip injected with several doses of serotonin, and blood plasma was obtained from them. Plasma glucose concentrations increased by 0.3 and 1 mg serotonin (Fig. 2A). Injection of 0.3 and 1 mg serotonin increased plasma insulin levels; however, levels of insulin were slightly decreased by 0.03 mg serotonin (Fig. 2B). Serotonin injections decreased the concentrations of plasma triglyceride, cholesterol, and NEFA in a dose-dependent manner (Fig. 2, C–E). These data revealed that peripheral serotonin increased the plasma concentrations of glucose and insulin but decreased the plasma concentrations of fatty acid-related metabolites.

**Glycogen storage in liver**

Livers were obtained from fasted mice at the times indicated in Fig. 3, after an ip injection of serotonin. Liver sections were subjected to hematoxylin-eosin staining (Fig. 3A) and PAS staining (Fig. 3B). The hematoxylin-eosin staining shows that there was no difference in morphology between the livers from mice with and without the serotonin injection. In contrast, glycogen in the liver samples was clearly stained with PAS reagent at 240 min but only slightly at 0, 60, and 120 min after the injection. The hepatic glycogen content was significantly higher than the basal values at 240 min after the injection (Fig. 3C). At the same time, the hepatic triglyceride content was significantly lower than that before serotonin injection (Fig. 3D).

To provide additional evidence that serotonin is involved in glucose metabolism in the liver, we evaluated the mRNA expression in the liver involved in gluconeogenesis and blood glucose homeostasis. The mRNA expression of phosphoenolpyruvate carboxykinase (PEPCK), glycogen synthase 2 (GYS2), glycogen phosphorylase in liver (PYGL), and glucose-6-phosphatase (G6Pase) were significantly elevated at 240 min after serotonin injection compared with the levels noted before the serotonin injection (Fig. 3, E–H). These findings show that peripheral serotonin levels can also affect carbohydrate and lipid metabolism, including glycogen synthesis and storage, gluconeogenesis, and the concentration of triglycerides in the liver.

**Inhibition of glucose uptake by serotonin**

To determine the mechanism by which serotonin elevates plasma glucose concentrations, we investigated the effect of serotonin on the uptake of 2DG. The plasma concentrations of 2DG in serotonin-injected mice were 4-fold greater than that of PBS-injected mice (Fig. 4A). In

**FIG. 4.** Effect of peripheral serotonin on glucose uptake in liver and skeletal muscle. Mice were given 5 μmol 2DG through their tail vein and immediately ip injected with serotonin (5HT, 1 mg/mouse) or PBS. After 60 min, liver and gastrocnemius muscle samples were obtained from each mouse (n = 5–6 mice per group). 2DG contents were measured in plasma (A), liver (B), and muscle (C) as described in Materials and Methods.
contrast, serotonin did not affect the uptake of 2DG in the liver and muscle, which are major storage tissues of glycogen (Fig. 4, B and C). Therefore, the hyperglycemia induced by serotonin was likely caused by an inhibition of glucose uptake.

Induction of gallbladder contraction by serotonin

After ip injection of 1 mg serotonin, livers with gallbladders were obtained from mice at the times indicated after the injection. Gallbladders were photographed (A, top), and a section of them was stained with hematoxylin-eosin at 0, 60, and 240 min after the serotonin injection (n = 5 mice per group) (A, bottom). The volume of bile in the gallbladder (B), the plasma CCK concentration (C), FGF15 mRNA expression in ileum (D), hepatic cholesterol concentration (E), and the mRNA expression of CYP7a1 in liver (F) were measured in mice between 0 and 240 min after the injection. Each symbol is the mean of six to 10 independent animals. *, P < 0.05; **, P < 0.01 relative to basal values at 0 min. Graduation and bar, 1 mm.

Reabsorption of bile acids in ileum after serotonin injection

The concentration of bile acids in plasma was increased between 30 and 90 min after the serotonin injection, whereas levels were lower than the basal values between 240 and 270 min after the injection (Fig. 6A). In contrast, the concentrations of bile acids in feces were lower on the first and second days after the injection (Fig. 6B). To determine whether reabsorption of bile acids had increased or not, we examined changes in mRNA and protein expression of the ASBT in the ilea of mice after a serotonin injection. The mRNA expression of ASBT in ilea was significantly increased at 30 min, and this high level continued until 240 min after the injection (Fig. 6C). Moreover, ASBT protein levels were significantly increased at 240 min after injection (Fig. 6D). These data suggest that serotonin accelerates the turnover of bile acids, for example their excretion, biosynthesis, and reabsorption.

Effect of serotonin receptor antagonists on peripheral serotonin functions

To determine what kind of serotonin receptors were related to the peripheral serotonin functions, mice were pretreated with three kinds of serotonin receptor antagonist, ketanserin (5HT2A), SB-269970 (5HT7), and methy-
sergide (5HT₁, 5HT₂, and 5HT₇), at 30 min before serotonin injection. Sixty minutes after the serotonin injection, blood samples were obtained from the mice. Ketanserin, SB-269970, and methysergide all inhibited the hyperglycemia induced by serotonin (Fig. 7A). Methysergide significantly attenuated the serotonin-induced elevation of plasma insulin levels but not ketanserin or SB-269970 (Fig. 7B). Additionally, the triglyceride decrease after serotonin injection was avoided only by the administration of SB-269970 (Fig. 7C). The serotonin-induced reduction of plasma cholesterol concentration was fully impaired by methysergide (Fig. 7D). Ketanserin also antagonized the decrease of plasma NEFA concentrations induced by serotonin (Fig. 7E). Moreover, the serotonin-induced elevation of plasma bile acids was abolished by the administration of ketanserin and methysergide (Fig. 7F). These data indicate that the hyperglycemia and hyperinsulinemia induced by serotonin occur through independent receptors and mechanisms of action.

Discussion

There are two serotonin systems, one in the brain and one in the periphery. These are independently regulated and have distinct functions. In this report, we have demonstrated novel functions of serotonin in the periphery. Intraperitoneal serotonin injection affected the concentrations of metabolites related to glucose and lipid metabolism in the peripheral blood and liver and accelerated the turnover of bile acids.

Between 60 and 90 min after the serotonin injection, there was a significant increase in plasma glucose and insulin levels. However, in mice, it has been reported that serotonin induces hypoglycemia and hyperinsulinemia in mice at 30 min after injection (17) and that serotonin regulates insulin secretion by serotonin receptors within the pancreatic β-cells (24). In this study, the injection of 1 mg serotonin increased plasma glucose and insulin concentrations; however, 0.03 mg serotonin slightly decreased plasma insulin concentrations without hypoglycemia (Fig. 2). These data suggest that there may be different serotonin effects on insulin secretion in vivo between low and high rates of administration. Peripheral somatostatin, which is secreted from the stomach, intestine, and δ-cells in the pancreas, is known to inhibit the secretion of insulin and glucagon via somatostatin receptor 5 and somatostatin receptor 2, respectively (25), and to decrease plasma glucose but induce an increase of plasma NEFA (27). The serotonin-induced elevation of plasma glucose levels was attenuated by three kinds of serotonin receptor antagonist, (ketanserin, SB-269970, and methysergide) in the present study. In contrast, the serotonin-induced increase of plasma insulin concentrations was antagonized only by methysergide. In addition, there were other different effects of peripheral serotonin on fat metabolites (Fig. 7). Accordingly, serotonin may have independent functions through the different serotonin receptors.

We reveal here that serotonin inhibited the uptake of glucose into tissues from blood (Fig. 4). However, serotonin did not affect the glucose uptake activity of the liver or the skeletal muscle. It has been reported that hyperglycemia and hyperglucagonemia are induced by adrenaline released from the adrenal gland in rats after serotonin injection (19–21). These reports also indicate that adrenaline and glucagon convert hepatic glycogen into plasma glucose, which results in hyperglycemia. However, in our
experiments, serotonin injection decreased plasma glucagon concentration and increased hepatic glycogen. Although muscle normally uses glycogen for its energy needs, it does not normally release glucose to defend glycemia. Indeed, glycogen was almost expended in the skeletal muscle and liver after fasting for 12 h and was therefore not converted into glucose after injection with serotonin (Supplemental Fig. 3, A and B). Consequently, the mechanism by which plasma glucose concentrations are increased after serotonin injection remains to be determined.

As part of the enterohepatic circulation, most reabsorption (>90%) of bile acids occurs in the ileum. The bile acids are then returned via the portal vein to the liver. The liver then reuptakes about 80% of these, and the remainder flow on to the peripheral circulation. Serotonin induced the excretion of bile from the gallbladder, although the plasma concentration of CCK, a major determinant of bile excretion from gallbladder (28), was not influenced by the injection of serotonin. Considering the published reports that serotonin induces the excretion of bile from the gallbladder as well as the contraction of smooth muscle (9, 29), serotonin may have directly caused the excretion of bile acids from the gallbladder to the duodenum. It has additionally been reported that serotonin induced the contraction of longitudinal smooth muscle in the rat ileum through the 5HT2A receptor (30). As ketanserin and methysergide inhibited the increase of plasma bile acids after serotonin injection, 5HT2A and 5HT1 receptors may be involved in the effect of serotonin on turnover of bile acids (Fig. 7F).

The volume of bile in the gallbladder returned to normal values by 240 min after the serotonin injection. Because FGF15, a hormone produced by the small intestine, is required in order for the gallbladder to fill (31), expression of FGF15 may have been involved in this process after the injection of serotonin. Additionally, bile acids are synthesized from cholesterol by a metabolic pathway involv-
ing the rate-limiting enzyme cholesterol 7a-hydroxylase (CYP7a1) (32). After an increase in hepatic cholesterol in the present experiment, a significant increase in the expression of CYP7a1 mRNA was observed at 240 min after the serotonin injection. However, it has been reported that FGF15 represses hepatic bile acid synthesis through a mechanism that involves both FGF receptor 4 (FGFR4) and orphan receptor short heterodimer partner (33). This suppressive effect of FGF15 was examined after treatment with a farnesoid X receptor (FXR) agonist for 4 d. Our data show an effect of serotonin on bile acids synthesis within the short period of 240 min. Indeed, the concentration of bile acids in plasma increased temporarily at 60 min and returned to basal levels by 240 min after the serotonin injection, as for FGF15. In thrombocytopenic mice, such as those used in models of liver generation, the expression of serotonin 2A and 2B subtype receptors in the liver increased after hepatectomy (11). A serotonin agonist promoted hepatocyte proliferation, but an antagonist of serotonin 2A and 2B receptors inhibited liver generation. These data indicate that serotonin might directly affect the synthesis of bile acids in the liver.

After the excretion of bile acids from the gallbladder, bile acids were resorbed in the ileum, and thereafter, the plasma concentrations of bile acids increased. The concentration of bile acids in feces was significantly decreased for 2 d after the injection with serotonin. The expression of ASBT mRNA and ASBT protein levels were also significantly increased after the serotonin injection; this may be a novel function of peripheral serotonin. However, some recent studies have noted that bile acids and FGF15 induced negative feedback regulation of ASBT expression through the FXR-FGF15 signaling pathway (34–38). We observed that serotonin induced not only bile acid excretion and FGF15 expression in the ileum but also ASBT expression in ileum. These data also raise the possibility that serotonin may up-regulate ASBT expression via an FXR-FGF15-independent pathway.

We reveal here that the injection of serotonin decreased the concentration of triglyceride in the liver but increased the hepatic cholesterol concentrations. The plasma concentrations of serotonin were rapidly increased to more than 40 μM at 30 min after serotonin injection (Fig. 1). After this, plasma bile acids increased (Fig. 6A). It has been reported that serotonin stimulates glycogen synthesis at nanomolar levels but inhibits it at micromolar concentrations by serotonergic mechanisms in hepatocytes (23). In addition, bile acids are physiological ligands for FXR and activate genes by FXR-dependent and -independent mechanisms (39). Because FXR agonist catabolized cholesterol to bile acids, plasma cholesterol concentrations were significantly decreased by its treatment in mice (40). Therefore, the serotonin-induced elevation of plasma bile acids concentrations may indirectly lead the liver to preserve glycogen. These data also suggest that there are multiple functions of peripheral serotonin via several serotonin receptors.

Serotonin is secreted from enterochromaffin cells of the gastrointestinal tract by digestion-related luminal stimuli such as bile salts, amines, tastants, and olfactants (41–45). This suggests that serotonin secretion caused by food intake causes the excretion of bile acids and modulates the absorption of lipids.

Serotonin is an important gastrointestinal signaling molecule. In this study, we have investigated the peripheral effects of serotonin after ip injection. Finally, we note that peripheral serotonin affects glucose metabolism by inhibiting glucose uptake from blood to the tissues and accelerates lipid metabolism and the turnover of bile acids by both direct and indirect mechanisms.

Acknowledgments

This study was supported by two grants from the Japan Livestock Technology Association (17-355) and the Ministry of Agriculture, Forestry and Fisheries (2004).

Address all correspondence and requests for reprints to: Hisashi Aso, Cellular Biology Laboratory, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsuamiori-Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan. E-mail: asosan@bios.tohoku.ac.jp.

Disclosure Summary: The authors have nothing to disclose.

References


19. Chaouloff F, Laude D, Baudrion V 1990 Ganglionic transmission is a prerequisite for the adrenaline-releasing and hyperglycemic effects of 8-OH-DPAT. Eur J Pharmacol 185:11–18


42. Gerson MD 1999 Review article: serotonin roles played by 5-hydroxytryptamine in the physiology of the bowel. Aliment Pharmacol Ther 13(Suppl 2):15–30

