Serotonin reuptake transporter (SERT) plays a critical role in the onset of fructose-induced hepatic steatosis in mice

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Haub S, Kanuri G, Volynets V, Brune T, Bischoff SC, Bergheim I. Serotonin reuptake transporter (SERT) plays a critical role in the onset of fructose-induced hepatic steatosis in mice. Am J Physiol Gastrointest Liver Physiol 298: G335–G344, 2010. First published August 27, 2009; doi:10.1152/ajpgi.00088.2009.—Elevated dietary fructose intake, altered intestinal motility, and barrier function may be involved in the development of nonalcoholic fatty liver disease (NAFLD). Because intestinal motility and permeability are also regulated through the bioavailability of serotonin (5-HT), we assessed markers of hepatic injury in serotonin reuptake transporter knockout (SERT−/−) and wild-type mice chronically exposed to different monosaccharide solutions (30% glucose or fructose solution) or water for 8 wk. The significant increase in hepatic triglyceride, TNF-α, and 4-hydroxynonenal adduct as well as portal endotoxin levels found in fructose-fed mice was associated with a significant decrease of SERT and the tight-junction occludin in the duodenum. Similar effects were not found in mice fed glucose. In contrast, in SERT−/− mice fed glucose, portal endotoxin levels, concentration of occludin, and indices of hepatic damage were similar to those found in wild-type and SERT−/− mice fed fructose. In fructose-fed mice treated with a 5-HT3 receptor antagonist, hepatic steatosis was significantly attenuated. Our data suggest that a loss of intestinal SERT is a critical factor in fructose-induced impairment of intestinal barrier function and subsequently the development of steatosis.

intestinal serotogenic system; nonalcoholic fatty liver disease; endotoxin; sugar; tight junctions

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) comprises different stages of liver injury ranging from simple steatosis to liver cirrhosis. Usually NAFLD develops in the setting of obesity and insulin resistance (6). Steatosis is the earliest and most common type of NAFLD and has long been thought to be a relatively benign state of liver injury. However, it is clear that fatty livers are more vulnerable to injury from various causes (38) and can progress to steatohepatitis, increasing the probability of further liver-related morbidity and mortality (1). Because mechanisms involved in the development of NAFLD are not yet fully clarified, therapeutic options are still limited. Therefore, a better understanding of the biochemical and pathological changes involved in the onset of NAFLD (e.g., hepatic lipid accumulation) is desirable to improve prevention and intervention strategies.

High dietary carbohydrate intake has been suggested to be a key factor in the development of NAFLD. Indeed, results of recent human studies suggest that a diet rich in carbohydrates, and herein particularly fructose, may be a major etiological factor of NAFLD and may increase the odds of developing more severe pathophysiological phenotypes [e.g., nonalcoholic steatohepatitis (NASH)] (3, 16, 35). The hypothesis that the intake of fructose and sucrose might play a critical role in the pathogenesis of NAFLD is also supported by a number of studies performed in animals. In these studies, it was shown that an increased consumption of fructose (e.g., up to 60% of daily calories derived from fructose) may result in an increased accumulation of lipids in the liver accompanied by insulin resistance, elevated plasma triglyceride levels, and oxidative stress (2, 7, 17, 20, 24). We recently found that chronic fructose consumption can lead to an increased intestinal translocation of bacterial endotoxin, an induction of hepatic TNF-α and subsequently liver steatosis in mice (7). We also showed that concomitant treatment with antibiotics almost completely blocked the effect of fructose on mouse liver (7). Furthermore, Baraona et al. (5) reported that the bacterial flora of rodents can ferment carbohydrates to alcohol when intestinal stasis permits their overgrowth in the upper parts of the gastrointestinal tract. This further supports the hypothesis that elevated intake of sugars (e.g., fructose and sucrose) can contribute to the development of liver damage through mechanisms involving alterations at the level of the intestine. However, by which mechanism(s) fructose intake causes alterations of intestinal motility, bacterial overgrowth, and/or increased intestinal permeability has not yet been clarified.

Serotonin (5-hydroxytryptamine [5-HT]) is one of the key signaling neurotransmitters in the gut (for review see Ref. 18), which is involved in the initiation of motility, but has also been suggested to be involved in the control of intestinal permeability (37). A reduced function of the 5-HT-selective reuptake transporter (SERT), which regulates the bioavailability of 5-HT and thereby its physiological effects, has been associated with alterations of the gastrointestinal motility in rodents and humans (13, 14, 31). Furthermore, results of in vitro and animal studies suggest that 5-HT, probably through its receptor 5-HT3, may be a critical factor in the regulation of intestinal permeability and subsequently the translocation of intestinal endotoxin (4, 29, 37). For instance, it has been shown that 5-HT can stimulate the translocation of endotoxin in rat ileum by 5-HT3 receptor-dependent pathways. However, whether alterations of the intestinal serotogenic system are involved in the increased intestinal translocation of bacterial endotoxin found in mice chronically exposed to fructose, and subsequently in the onset of fructose-induced NAFLD, has not yet been clarified. Using a mouse model of sugar-induced steatosis, we investigated the role of 5-HT and SERT in the onset of fructose-induced NAFLD.

MATERIALS AND METHODS

Animals and treatments. Mice were housed in a pathogen-free barrier facility accredited by the American Association for Accreditation of Laboratory of Animal Care. All procedures were ap-
role of SERT in steatosis

Clinical chemistry and pathologic evaluation. Plasma alanine aminotransferase (ALT) activity was determined by a routine clinical chemistry lab procedure (Labor Enders, Stuttgart, Germany). Infiltration of neutrophils in hepatic tissue was evaluated by staining using an AS-D chloroacetate esterase kit (Sigma). The number of neutrophils in liver was counted in eight randomly selected fields (×200, 0.42 mm × 0.32 mm).

Endotoxin assay. Plasma samples were heated at 75°C for 20 min. Endotoxin plasma levels were then determined using a commercially available end-point chromogenic limulus amebocyte lysate assay with a concentration range of 0.015–1.2 EU/ml (Charles River, L’Arbæase, France). Plasma endotoxin levels of samples were calculated using a standard curve. In addition, recovery rates were calculated using plasma samples spiked with different concentrations of endotoxin. Recovery was between 85 and 95%.

Immunohistochemical staining for 4-hydroxynonenal adducts. Paraffin-embedded sections (5 μm) were cut and stained to detect 4-hydroxynonenal-protein adducts using a polyclonal antibody (AG Scientific, San Diego, CA) as described previously (5). In brief, to detect specific binding of primary antibody, tissue sections were incubated with a peroxidase-linked secondary antibody and diaminobenzidine (Peroxidase Envision Kit; DAKO, Hamburg, Germany). With the use of the image acquisition and analysis system described before, the extent of staining in liver sections was defined as percent of the field area within the default color range determined by the software. To determine means, data from five fields (×50) of each tissue section were used.

Measurement of the 5-HT content of the duodenum. Frozen duodenum was homogenized in 0.05 M HCl with 0.1% ascorbic acid and centrifuged at 14,000 revolution/min for 5 min at 4°C. 5-HT content (in ng/mg weight) was determined in the supernatant using an enzyme immunoassay kit according to the manufacturer’s instructions (IBL, Hamburg, Germany).

Immunoblot. To prepare total protein lysates, snap-frozen small intestine samples or CaCo-2 cells were homogenized with lysis buffer (20 mM MOPS, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) containing a protease inhibitor mix (Roche, Mannheim, Germany). Protein lysates (30 μg protein/well) were separated in a 10% SDS-polyacrylamide gel and transferred to Hybond-P polyvinylidene difluoride membranes. The resulting blots were then probed with antibodies against SERT (Santa Cruz Biotechnology, Heidelberg, Germany) or occludin (Zymed, San Francisco, CA), respectively, and bands were visualized using a Super Signal Western Dura kit (Pierce, Perbio Science, Rockford, IL). To ensure equal loading, all blots were stained with Ponceau red; signals were normalized to β-actin, which was detected using a commercially available antibody (New England Biolaboratories, Frankfurt, Germany).

Cell culture and treatment. CaCo-2 cells (carcinoma colon-2 cell, American Type Culture Collection, Manassas, VA), passages 8–20, were grown in DMEM media (PNV) supplemented with 10% FBS, 1% nonessential amino acids, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37°C in a humidified 5% CO₂ atmosphere. Analogous to the work of Courtois (15), cells were seeded on six-well Transwell cell culture chambers (0.4 μm pore size) (Greiner, Frickenhausen, Germany) at a density of 1–10⁵ cells/well and differentiated for 9 days. Starting from the day of confluence, transepithelial resistance was measured every second day using a Millicell-ERS instrument with Ag/AgCl electrodes (Millipore, Bedford, MA). Upon differentiation, cells were serum starved for 24 h. As described by Koayashi (23), basolateral media was replaced with a starvation media (without antibiotics) containing different concentrations of 5-HT (0–2.5 mM) for 20 min before apical media was removed and replaced with fresh serum and media supplemented with 5 μg LPS/ml. Cells were then incubated for 24 and 48 h, respectively, before media from the basolateral side was collected for endotoxin measurement and cells were
harvested for determination of protein concentration of occludin.

In a second set of cell culture experiments, differentiated CaCo-2
cells were incubated with 50 mM fructose or 25 mM glucose,
respectively, on the apical side for 24 h. Cells were then harvested
to determine SERT protein concentration. All cell culture experi-
ments were carried out in triplicate.

Statistical analyses. All results are reported as means ± SE (animal
studies: n = 5–7, cell culture: n = 3). One-way and two-way ANOVA
with Bonferroni’s post hoc test were used as appropriate to determine
statistical differences between groups. A P value ≤0.05 was selected
as the level of significance before the study.

RESULTS

Plasma and hepatic indices of liver damage. Representative
pictures of Oil Red O staining are shown in Fig. 1A,
whereas Fig. 1B comprises quantitation of hepatic triglyc-
eride levels after chronic intake of sugar-sweetened water.

In line with our earlier findings (7), using this mouse-model
chronic intake of 30% glucose solution resulted in a ~2.7-
fold increase in hepatic triglyceride levels compared with
controls (P ≤ 0.05). In livers of mice chronically fed with a 30%
fructose solution, triglyceride levels were approximately twofold higher than in glucose-fed mice and ~5.3-
fold higher than in controls (P ≤ 0.05). Similar results were
also found when determining other indices of liver damage
(e.g., liver weight and liver-to-body weight ratio, summa-
rized in Table 1). Because this model represents an early
phase of NAFLD, no differences were found in plasma ALT
levels (data not shown). However, a significantly increased
number of infiltrating neutrophils was found in fructose-fed
mice, but not in glucose-fed animals, compared with con-
trols (Table 1). Furthermore, the markedly higher increase
in hepatic triglyceride levels found in fructose-fed mice was
accompanied by significantly higher endotoxin levels in portal plasma and a higher concentration of 4-hydroxynonenal adducts, as well as expression of TNF-α in the livers of fructose-fed mice compared with controls (see Fig. 1, A and B, first columns; Fig. 3, first three columns; and Fig. 4, A, B, and C, first three columns). Similar changes were not found in mice fed with 30% glucose solution.

Serotonergic system in the duodenum. Because serotonin is involved in the regulation of intestinal motility and also permeability (29), which might contribute to the increased endotoxin levels found in fructose-fed mice and may therefore subsequently lead to the enhanced liver steatosis found in these animals, we determined intestinal motility, serotonin levels, and SERT protein content in the duodenum of mice after sugar feeding. Whereas serotonin levels were similar between all groups, intestinal motility was slightly increased in mice chronically exposed to 30% glucose solution (20% compared with controls, \( P < 0.05 \)) (Fig. 2, A and B). SERT protein levels were significantly decreased by 55% in mice fed water enriched with 30% fructose compared with plain water controls and animals fed with 30% glucose solution (Fig. 2C). Similar changes in the SERT protein concentration were not found in the ileum of fructose-fed mice (Supplemental Fig. S1; supplemental material for this article is available online at the American Journal of Physiology Gastrointestinal and Liver Physiology website).

### Table 1. Effect of chronic intake of sugar-sweetened water on weight gain, liver weight, liver-to-body weight ratio, and neutrophil infiltration in the liver

<table>
<thead>
<tr>
<th></th>
<th>Control WT‡</th>
<th>Glucose WT‡</th>
<th>Fructose WT‡</th>
<th>Control SERT−/−</th>
<th>Glucose SERT−/−</th>
<th>Fructose SERT−/−</th>
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<tr>
<td>( N )</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Weight gain, g</td>
<td>3.31 ± 0.34</td>
<td>6.48 ± 0.83*</td>
<td>4.27 ± 0.48</td>
<td>4.54 ± 0.36</td>
<td>7.69 ± 0.57*</td>
<td>7.4 ± 1.24*</td>
</tr>
<tr>
<td>Liver weight, in g</td>
<td>1.10 ± 0.05</td>
<td>1.07 ± 0.05</td>
<td>1.39 ± 0.07</td>
<td>1.24 ± 0.05</td>
<td>1.38 ± 0.09</td>
<td>1.58 ± 0.13*</td>
</tr>
<tr>
<td>Liver-to-body weight ratio, %</td>
<td>4.93 ± 0.16</td>
<td>4.97 ± 0.08</td>
<td>6.2 ± 0.16+†</td>
<td>5.03 ± 0.19</td>
<td>5.14 ± 0.15</td>
<td>6.45 ± 0.13+†</td>
</tr>
<tr>
<td>Neutrophil, number per field</td>
<td>0.15 ± 0.1</td>
<td>0.61 ± 0.19</td>
<td>1.32 ± 0.25+*</td>
<td>0.49 ± 0.05</td>
<td>1.16 ± 0.13*</td>
<td>1.38 ± 0.23+†</td>
</tr>
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Feeding of sweetened water is described in MATERIALS AND METHODS. Data are means ± SE (\( n = 5–7 \)). *\( P < 0.05 \) compared with mice fed water, †\( P < 0.05 \) compared with serotonin reuptake transporter knockout (SERT−/−) mice fed water. ‡Results of C57BL6 and SERT−/− [wild-type (WT)] mice did not differ; results of SERT−/− are shown to represent these 2 groups.

![Fig. 2](http://ajpgi.physiology.org/)

A: 5-HT content in the duodenum. B: intestinal motility. Representative Western Blot of SERT protein in the duodenum and \( \beta \)-actin as well as the quantitative analysis of the blots are shown in C. D: representative Western Blot of SERT and \( \beta \)-actin in CaCo-2 cells incubated with fructose or glucose and the quantitative analysis of the blots. Data represent means ± SE and are normalized to \( \beta \)-actin (A–C; \( n = 5–7 \) D; \( n = 3 \)). *\( P < 0.05 \) compared with mice fed with water or untreated cells, †\( P < 0.05 \) compared with mice fed 30% glucose solution.
Fructose downregulates SERT protein in a cell culture model of enterocytes. To determine whether the loss of SERT in the duodenum of mice was a direct result of the chronic intake of fructose, differentiated CaCo-2 cells, which have repeatedly been used by others as a model of enterocytes (15), were incubated with fructose and glucose, respectively, for 24 h. No differences were found in SERT protein concentration between untreated cells and cells treated with a media enriched with glucose (Fig. 2D). In contrast, incubation of CaCo-2 cells with fructose resulted in a significant reduction of SERT protein concentration (~−61% compared with controls).

Hepatic lipid accumulation in SERT−/− mice. To further investigate whether the loss of SERT found in fructose-fed mice is associated with the markedly higher triglyceride content and increased intestinal translocation of endotoxin found in fructose-fed mice, we repeated the feeding experiment with mice lacking SERT [i.e., SERT−/− mice] and compared them to the wild-type mice. Figure 1A comprises representative pictures of hepatic lipid accumulation as determined by Oil Red O staining, and Fig. 1B shows quantitation of triglyceride levels in livers of SERT−/− mice and wild-type animals after the chronic consumption of sugar-sweetened water. Hepatic triglyceride content was minimal in SERT−/− mice fed plain water and did not differ from wild-type mice. Chronic consumption of water enriched with 30% glucose resulted in a marked increase in hepatic triglyceride levels in SERT−/− mice, which were more than ~2.6-fold higher than those found in livers of wild-type mice fed glucose solution (P < 0.05). In livers of fructose-fed SERT−/− mice, a similar enhancement of triglyceride levels was not found; however, triglyceride levels were similar to those found in wild-type mice fed fructose, which were ~5.9-fold higher than those of controls.

Endotoxin levels in portal plasma of SERT−/−. Endotoxin concentration in portal plasma of wild-type and SERT−/− mice fed plain water was minimal and did not differ between these groups (Fig. 3). Similar to the effects on hepatic triglyceride accumulation, in the absence of SERT, chronic intake of glucose led to a significant increase in plasma endotoxin levels (+1.7-fold compared with wild-type glucose-fed mice and ~+4-fold compared with controls, P ≤ 0.05). Plasma endotoxin levels of fructose-fed SERT−/− mice did not differ from those of fructose-fed wild-type animals; however, compared with controls, plasma endotoxin levels were significantly elevated by approximately fourfold.

Hepatic concentration of 4-hydroxynonenal adducts and TNF-α mRNA expression in SERT−/− mice. Levels of 4-hydroxynonenal adducts in livers of wild-type and SERT−/− water controls were minimal and did not differ (Fig. 4, A and B). Similar to the results found for hepatic triglyceride accumulation and endotoxin, in SERT−/− mice, chronic intake of 30% glucose solution resulted in a significant approximately 3.5-fold increase in 4-hydroxynonenal adduct concentration in the liver, which was not found in wild-type mice exposed to glucose. 4-Hydroxynonenal adduct formation in livers of SERT−/− mice was similar to that found in livers of wild-type mice fed fructose (wild-type: ~4.8-fold, SERT−/−: 4.3-fold compared with controls, P ≤ 0.05). In line with the above summarized findings, chronic intake of water enriched with 30% glucose led to a significant approximately threefold increase in TNF-α mRNA expression in the livers of SERT−/− mice compared with wild-type mice fed glucose, whereas the expression of TNF-α in livers of fructose-fed SERT−/− mice was induced to a similar extent as in wild-type mice fed fructose (wild-type: ~3.1-fold, SERT−/−: ~5.6-fold compared with controls, P ≤ 0.05) (Fig. 4C).

Protein concentration of occludin in the duodenum of SERT−/− mice. Because others reported before that a loss of the tight junction protein occludin in the small intestine is associated with an increased translocation of intestinal endotoxin and subsequently the development of NASH in genetically obese mice (10), we determined protein levels of occludin in the duodenum of SERT−/− and wild-type mice after chronic intake of sugar-sweetened water (Fig. 5). In line with the findings for portal endotoxin levels and hepatic triglyceride content, occludin protein levels in the duodenum of SERT−/− mice chronically exposed to glucose and SERT−/− mice as well as wild-type mice that had ingested the fructose solution for 8 wk were significantly decreased by ~66% compared with controls and glucose-fed wild-type mice.

Effect of tropisetron on the SERT protein in sugar-fed mice. To further investigate whether the loss of SERT found in fructose-fed mice is associated with modulations of the intestinal serotonergic system, we repeated the feeding experiment with C57BL6 mice treated with sugar and tropisetron, a 5-HT3 receptor antagonist. Hepatic lipid and triglyceride content was minimal in livers of water-fed mice and water-fed mice concomitantly treated with tropisetron and did not differ between groups (Fig. 6, A and B). No differences in hepatic triglyceride accumulation were found between glucose-fed mice and glucose-fed mice concomitantly treated with the antagonist. In contrast, in livers of fructose-fed mice treated with tropisetron, triglyceride content was significantly lower than in mice only fed fructose.

Fig. 3. Effect of chronic consumption of sugar-sweetened water on portal endotoxin levels. Data are means ± SE and are performed in triplicate. aP < 0.05 compared with mice fed with water. bP < 0.05 compared with SERT−/− mice fed water.
Serotonin increases permeability in a cell culture model of enterocytes. As described above, a correlation between the loss of SERT, which may be implicative for an enhanced bioavailability of 5-HT, increased portal endotoxin levels, and hepatic triglyceride accumulation was observed after chronic intake of sugar in vivo. Therefore, the effect of 5-HT on intestinal permeability of endotoxin and the tight-junction protein occludin was determined in an in vitro model of enterocytes (see MATERIALS AND METHODS) (Fig. 7). The basolateral stimulation of cells with different 5-HT concentrations (0–2.5 mM) resulted in a dose-dependent increase of the translocation of endotoxin into the basolateral compartment of the Transwell system 24 and 48 h after the apical incubation of cells with endotoxin (Fig. 7A). In accordance with the increased translocation of endotoxin, protein levels of the tight-junction protein occludin were also significantly decreased in CaCo-2 cells treated with 5-HT (Fig. 7B).

**DISCUSSION**

Increased fructose intake has been identified as one of the key factors in the development of obesity, metabolic syndrome (e.g., insulin resistance and dyslipidemia), and NAFLD (9, 34). Results of animal and human studies suggest that, similar to alcoholic liver disease, (for review see Ref. 8), bacterial overgrowth and increased translocation of bacterial endotoxins across the intestinal barrier may be involved in the pathogenesis of NAFLD (10, 34, 36). Furthermore, it has been shown before that carbohydrates can be further reduced to form ethanol (26, 27), when there is intestinal overgrowth of bacteria or yeast (5, 28) or if carbohydrates are consumed excessively (19), subsequently leading to increased ethanol levels in portal plasma but probably also increased portal endotoxin levels. However, mechanisms involved in the increased intestinal permeability and translocation of endotoxin in patients with NAFLD and mouse models of fructose-induced NAFLD are still poorly understood. Because it has been suggested by the results of animal and human studies that the intestinal serotonergic system may be a critical factor in the regulation of gastrointestinal motility and also permeability (11, 29, 37), the hypothesis that the intestinal serotonergic system might be involved in the onset of NAFLD was tested in a...
mouse model of sugar-induced hepatic steatosis. In line with the results of previous studies of our group (7), chronic fructose feeding resulted in the early phase of NAFLD characterized by hepatic steatosis, neutrophile infiltration, and also elevated formation of reactive oxygen species and induction of TNF-α expression in the liver. Furthermore, chronic intake of fructose resulted in a significantly increased translocation of bacterial endotoxins into the portal plasma. Similar alterations were not found in mice exposed to 30% glucose solution. The increased hepatic steatosis found in mice exposed to 30% fructose solution was associated with a marked reduction of SERT protein in the duodenum of these animals; however, intestinal motility, which was slightly increased in mice drinking glucose solution, was not affected in mice fed fructose. In contrast to the in vitro findings of Kim et al. (21), who reported that BON cells, a cell line derived from enterochromaffin cells, release markedly more 5-HT after being exposed to glucose, in the present study, neither chronic exposure of mice to glucose nor to fructose affected 5-HT levels in the duodenum. Differences between the results of Kim et al. (21) and our own study might have resulted from differences in the experimental setup (in vitro vs. in vivo). In line with our in vivo findings, SERT protein concentration was also markedly reduced in differentiated CaCo-2 cells after cells were exposed to fructose for 24 h, whereas glucose had no effect on SERT protein under the same conditions. These data suggest that the hepatic triglyceride accumulation that was found after the chronic intake of fructose, despite no changes in intestinal motility and total 5-HT content in the duodenum, is associated with an almost complete loss of SERT in the duodenum. Results of our in vitro data further suggest that the loss in SERT found in mice fed fructose may be a direct effect of fructose ingestion; however, mechanisms involved in the fructose-dependent regulation of SERT remain to be determined.

To further test the hypothesis that the reduction of SERT in the intestine may contribute to the development of fructose-induced hepatic steatosis, we fed SERT−/− mice with 30% glucose or 30% fructose solution. Contrary to the hepatic triglyceride accumulation found in wild-type mice fed 30% glucose solution, hepatic steatosis was markedly increased in SERT−/− mice. Indeed, hepatic steatosis in glucose-fed SERT−/− mice was comparable to that found in wild-type mice fed with 30% fructose solution. Furthermore, similar to mice having free access to 30% fructose solution, the elevated lipid accumulation found in SERT−/− mice fed with water enriched with 30% glucose was associated with significantly increased levels of endotoxin in portal plasma. In line with these findings, levels of 4-hydroxynonenal adducts, a marker for lipid peroxidation, and TNF-α mRNA expression in livers of SERT−/− mice fed glucose were also similar to those of mice fed 30% fructose solution. The hypothesis that the loss of SERT found in wild-type mice may play a crucial role in the development of sugar-induced NAFLD was further supported by the finding that hepatic damage and portal endotoxin levels were not extended in SERT−/− mice fed water enriched with 30% fructose. Furthermore, in wild-type mice treated with the 5-HT3 receptor antagonist tropisetron while being fed fructose, hepatic lipid accumulation found in mice only exposed to fructose solution was markedly attenuated. A role of the 5-HT3 receptor in the regulation of the intestinal translocation of endotoxins has been suggested before by the results of others (37). Taken together, these data suggest that the markedly greater damaging effect of fructose compared with glucose on the liver found in wild-type mice under the present conditions may, at least in part, result from a loss of SERT in the duodenum. The loss of SERT may subsequently alter the bioavailability of 5-HT in the small intestine, which in turn may result in an enhanced intestinal translocation of bacterial endotoxins and activation of hepatic Kupffer cells in the liver. Furthermore, these data also suggest that chronic intake of glucose may lead to alterations of the bacterial flora in the small intestine, similar to those found in fructose-fed mice; however, in the presence of a faultless serotonergic system, these alterations seem not to be of consequences in regard to intestinal translocation of bacterial endotoxins. Mechanisms by which fructose, but not glucose, leads to the loss of SERT in the small intestine and the role of the 5-HT3 receptor herein remain to be determined.

Besides its role in regulating intestinal motility (11, 32, 39), 5-HT may also be involved in regulating permeability, as suggested by the results of several in vitro and in vivo studies (4, 29, 37). For instance, using goldfish intestinal epithelia, Bakker et al. (4) reported that vasoactive intestinal polypeptide and 5-HT reduce the ion selectivity of tight junctions through mechanisms involving an elevation of cAMP. Furthermore, it was reported that luminal hypotonicity increases the duodenal mucosal permeability through a 5-HT-dependent pathway acting on the intestinal 5-HT3 and 4 receptors and that the 5-HT3 receptor may also be crucially involved in intestinal translocation of endotoxin in setting of sepsis (29, 37). Here, using an in vitro model of enterocytes (e.g., confluent differentiated CaCo-2 cells), we show that serotonin increases the translocation of bacterial
endotoxin across a confluent monolayer of intestinal enterocyte and reduces concentrations of the tight-junction protein occludin in these cells in a dose-dependent way. Furthermore, the loss of SERT protein found in fructose-fed wild-type mice was associated with an almost complete loss of the tight-junction protein occludin in the duodenum. In support of the hypothesis that this tight-junction protein may be a crucial trigger of the increased intestinal translocation of endotoxin found in mice chronically fed with fructose solution, protein concentrations of occludin were markedly reduced in both glucose- and fructose-fed SERT knockout mice. Because a similar loss of this tight-junction protein was not found in SERT knockout mice fed water, these data suggest that additional factors such as bacterial side products (e.g., ethanol, acetaldehyde) may be involved in the loss of occludin and increased permeability. This possibility will need to be addressed in future studies.

In conclusion, the results of the present study suggest a novel mechanism in sugar (fructose)-induced NAFLD, in which a reduction or loss of SERT protein in the small intestine probably triggered through 5-HT3 receptor-dependent pathways leads to an increased intestinal permeability and translocation of endotoxin (see Fig. 8). Results of the present study further bolster recent findings that dietary fructose intake is associated with increased translocation of endotoxin, thereby being a key factor in the pathogenesis of NAFLD (7, 30, 34). However, the results of the present study also suggest that an increased consumption of sugar, be it glucose or fructose, may lead to NAFLD if the serotonergic system in the small intestine is disturbed. These results further suggest that therapies that...
more specifically target intestinal 5-HT release or enhance 5-HT reuptake might be useful therapies to treat NAFLD or prevent progression. Future studies will be needed to address this possibility.

**Fig. 7.** Effect of 5-HT on intestinal permeability and the tight-junction protein occludin in vitro. A: endotoxin concentration as determined in the basolateral media of the CaCo-2 cells (24 and 48 h). Representative Western Blots of occludin and β-actin for CaCo-2 cells (24 and 48 h) as well as the quantitative analysis of the blots are shown in B. Data are means ± SE and are normalized to β-actin (n = 3). N, naive controls; 0 mM, LPS no 5-HT; 0.05 mM, LPS and 0.05 mM 5-HT; 0.5 mM, LPS and 0.5 mM 5-HT; 2.5 mM, LPS and 2.5 mM 5-HT; aP < 0.05 compared with N, bP < 0.05 compared with 0 mM, cP < 0.05 compared with 0.05 mM.

**Fig. 8.** Proposed mechanisms by which the intestinal serotonergic system may be involved in the development of sugar-induced nonalcoholic fatty liver disease (NAFLD) in mice. ROS, reactive oxygen species.

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REFERENCES


DISCLOSURES

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