Changes in serotonin levels and 5-HT receptor activity in duodenum of streptozotocin-diabetic rats

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Changes in serotonin levels and 5-HT receptor activity in duodenum of streptozotocin-diabetic rats. Am J Physiol Gastrointest Liver Physiol 281: G798–G808, 2001.—Few previous studies have discussed the changes in serotonin receptor activity in the small intestine of diabetic animals. Therefore, we examined serotonin content in duodenal tissue and dose-dependent effects of serotonin agonists and antagonists on the motor activity of ex vivo vascularly perfused duodenum of streptozotocin (STZ)-diabetic rats. Serotonin content was significantly increased in enterochromaffin cells but not altered in serotonin-containing neurons in STZ-diabetic rats. Motor activity assessed by frequency, amplitude, and percent motility index per 10 min of pressure waves was reduced in the duodenum of diabetic rats, and this reduction was reversed by insulin treatment. Serotonin dose dependently increased the motor activity in control rat duodenum but only a higher concentration of serotonin increased the motor activity in diabetic rats. The 5-hydroxytryptamine (5-HT) receptor subtype 4 (5-HT4) antagonist SB-204070 dose dependently reduced motor activity in both control and diabetic rats, whereas the 5-HT3 receptor antagonist azasetron, even at a higher concentration in both control and diabetic rats, whereas the control rat duodenum. These results suggest that 5-HT3 receptor activity was impaired but 5-HT4 receptor activity dose dependently reduced motor activity in the duodenum of diabetic rats. Serotonin content was significantly increased in enterochromaffin cells but not altered in serotonin-containing neurons in STZ-diabetic rats. Motor activity assessed by frequency, amplitude, and percent motility index per 10 min of pressure waves was reduced in the duodenum of diabetic rats, and this reduction was reversed by insulin treatment. Serotonin dose dependently increased the motor activity in control rat duodenum but only a higher concentration of serotonin increased the motor activity in diabetic rats. The 5-hydroxytryptamine (5-HT) receptor subtype 4 (5-HT4) antagonist SB-204070 dose dependently reduced motor activity in both control and diabetic rats, whereas the 5-HT3 receptor antagonist azasetron, even at a higher concentration, failed to affect motor activity in diabetic rat duodenum but dose dependently reduced motor activity in control rat duodenum. These results suggest that 5-HT3 receptor activity was impaired but 5-HT4 receptor activity was intact in STZ-diabetic rat duodenum. Such an impairment of 5-HT3 receptor activity may induce the motility disturbance in the small intestine of diabetes mellitus.

In autonomic nerves, because similar motor patterns were seen (39) after truncal vagotomy or mechanical sympathectomy. However, because immunohistochemical studies in diabetic animals showed degenerative change in adrenergic but not cholinergic nerves, it has been proposed that nonadrenergic, noncholinergic neurotransmitters such as vasoactive intestinal polypeptide (VIP), nitric oxide (NO), or substance P induce diabetic gastrointestinal dysmotility (28, 35, 37). The number of NO synthase (NOS)-immunoreactive cells in the gastric myenteric plexus and NOS activity were reduced in the stomach and duodenum in streptozotocin (STZ)-treated (34) and spontaneously BB/W diabetic rats (48). The density of VIP-immunoreactive neurons and tissue content of VIP were increased in the ileum of STZ-diabetic rats (3, 4, 7) and in the duodenum of nonobese (insulin-dependent) diabetic mice (16) but unchanged in the duodenum of obese (insulin-independent) diabetic mice (14). VIP tissue levels were decreased in the antrum of both nonobese and obese diabetic mice (14, 15). Substance P level decreased in the antrum of nonobese and obese diabetic mice (14, 15, 17), increased in the duodenum of nonobese diabetic mice (17), and decreased in the duodenum of obese diabetic mice (14).

Changes in the immunoreactivity or tissue levels of various neurotransmitters, including serotonin (6), neuropeptide Y (3, 6, 15), calcitonin gene-related peptide (6), or enkephalin (16), have been reported in the gastrointestinal tracts of diabetic animals. Serotonin seems to be the most important of these neurotransmitters responsible for motor disturbance in diabetes, because it primarily mediates the fasting phase III contractions of MMC or postprandial contractions (25, 27, 32, 44) and fasting and postprandial motor activities are impaired in diabetes mellitus. Changes in the serotonin levels as well as serotonin-immunoreactive cell density have been extensively investigated (11, 18, 33, 40–42, 46). The density of serotonin-containing enterochromaffin (EC) cells was reduced in the antrum and duodenum in STZ-treated (41), nonobese (18), or genetically (db/db or ob/ob) (40, 46) diabetic mice. Data

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on serotonin content in the intestine have been inconsistent. One study (35) found an increased level of serotonin in the small intestine of STZ-diabetic rats, but others showed decreased (11) or normal levels of serotonin (42) in the small intestine of alloxan- and STZ-induced diabetic rats.

Although several studies (11, 18, 33, 40–42, 46) have shown changes in the number of serotonin-containing cells or serotonin content induced by diabetes as described above, only a few studies (1, 51, 52) have shown an alteration in the characteristics of serotonin receptors in diabetic animals. In those studies (1, 51, 52), response to a serotonin agonist or antagonist was examined in a muscle strip from the gastrointestinal tract. Although such an in vitro preparation is suitable for examining the dose-dependent effects of agonists or antagonists on the receptor located on the smooth muscle, it may be not suitable for examining such effects on the receptor located on the enteric neurons. Recently, we (49) developed ex vivo perfused rat duodenum to examine the motor activity of intestinal segments with intact intrinsic and extrinsic nerve supplies. In this preparation, spontaneous contractions migrating in an oral-to-anal direction were observed (50). This ex vivo model permits a dose-dependent infusion of receptor agonists or antagonists directly into the intestinal segments, and the neuronally mediated response of the intestinal contraction can be examined (50).

In the present study, we investigate changes in the serotonin level in epithelial EC cells and enteric serotonin-containing neurons and compare the motor activity of ex vivo vascu1arly perfused rat duodenum in control and STZ-induced diabetic rats. We also investigate changes in the activity of serotonin receptor subtypes by administration of serotonin receptor agonists and antagonists into the perfused duodenum of control and STZ-diabetic rats.

MATERIALS AND METHODS

Animal preparation. Male Sprague-Dawley rats, weighing 250–300 g on the first day of the experiment, were used. Rats were divided into three groups: normal, STZ (Sigma Chemical, St. Louis, MO; 60 mg/kg)-treated, and STZ plus insulin-treated rats. STZ was injected intraperitoneally only on the initial day, and blood glucose level was measured 3 days after STZ injection. Insulin pellets (Linshin Canada; 29) were given for 3 wk after blood glucose level was detected at >400 mg/dl in STZ-treated rats. For the continuous delivery of insulin, an incision was made in the midsacral region and one piece of an insulin pellet (release rate of 1 U/day) was implanted into the back of the rat. Animals were maintained with free access to food and water for 24 days after the initial STZ injection and were fasted overnight before the experiment. On the day of the experiment, blood glucose levels were measured in all three groups.

Immunohistochemistry for serotonin. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg; Nembutal, Abbott) and perfused for 10 min via the left ventricle with 0.01 M PBS to wash out the blood. The rats were then perfused with a fixative containing 4% paraformaldehyde (PFA), 0.5% glutaraldehyde, and 0.2% picric acid (PA) in 0.1 M phosphate buffer (PB) at 4°C for 10 min at the speed of 30 ml/min. For immunohistochemistry of serotonin-containing nerve fibers, rats were treated with colchicine and pargyline as described previously (29). The duodenal segment between 10 and 30 mm from the pylorus was taken, immersed in a postfixative containing 4% PFA and 0.2% PA in 0.1 M PB at 4°C, and washed for 4 days with several changes of 0.1 M PB containing 15% sucrose. The duodenum was cut into 20-μm longitudinal or horizontal sections in a cryostat and collected in 0.1 M PBS containing 0.3% Triton X-100 (PBST). The sections were incubated with serotonin antibody (22) diluted 1:10,000 in PBST for 3 days at 4°C.

To inactivate endogenous peroxidase activity, the sections were incubated at room temperature for 20 min with 0.1% H2O2 in 0.1 M PBS and then for an additional 20 min with 0.1% phenylhydrazine in 0.1 M PBS. After being washed for 30 min with PBST, the sections were incubated at room temperature for 2 h in biotinylated anti-rabbit IgG (Vector, Burlingame, CA) diluted 1:1,000 in PBST. Sections were then washed and placed in avidin-biotin peroxidase complex (Elite, Vector) diluted 1:2,000 in PBST for 2 h at room temperature.

The immunoreactivity was visualized by incubating with 0.05 M Tris-HCl buffer (pH 7.6) containing 0.01% 3,3‘-diaminobenzidine, 1% ammonium nickel sulfate, and 0.0003% H2O2 for 30 min at room temperature. The stained sections were mounted on gelatin-coated glass slides, dehydrated with graded ethanol, and coverslipped with Entellan (Merck, Darmstadt, Germany). The number of serotonin-positive cells in the villus epithelium (EC cells) was counted throughout the entire length of the duodenum, and each value was divided by the corresponding length of the duodenum (cell no./mm of duodenum). Five sections were randomly selected from the antimesenteric side of the duodenum, and mean values were determined by sampling from five sections. In the same longitudinal sections, the villus height and crypt depth were measured separately, and mean values were obtained by sampling five sections from control and STZ-treated animals. The mean values of the diameter of the duodenum were also measured before cutting into cryostat sections.

Epithelial isolation and measurement of serotonin content in duodenum. While the rat was under deep anesthesia, the abdomen was opened and the duodenum was exposed. A nick was made with scissors in the antimesenteric site of the duodenum caudal to Treitz’s ligament. A 21-gage needle was inserted at the stomach, and the duodenum was flushed with 20 ml of 37°C Hanks’ solution (Nissui Pharmaceutical, Tokyo, Japan). The animals were then perfused for 2 min through the left ventricle at a rate of 30 ml/min with Hanks’ solution containing 30 mM EDTA and 1 mM pargyline hydrochloride (Nakarai Chemical) at 37°C. At the end of the perfusion, the duodenum between the pylorus and Treitz’s ligament was removed and placed in cold Hanks’ solution. The segment 10–30 mm from the pylorus was cut from the duodenum, gently everted, and secured to a rod (5 mm in diameter). The rod was attached to the shaft of an electric shaver and bursts of vibration given for 2 min; this removed most of the epithelium from the villi. The epithelial portions were collected in cold Hanks’ solution. After centrifugation for 5 min at 3,000 rpm at 4°C, the sediment of the epithelium was weighed and homogenized for 2 min in 1 ml ice-cold 0.1 N HClO4. After the epithelium was removed, the submucosal layer was scraped off by a razor and the muscle layer was homogenized for 2 min in 1 ml ice-cold 0.1 N HClO4. The homogenates of epithelium and muscle layer were centrifuged for 30 min at 3,000 rpm and 4°C, and 100-μl aliquots of
the supernatant were used for determination of 5-hydroxytryptamine (5-HT) by HPLC (21).

**Ex vivo isolated vascularly perfused rat duodenum and measurement of motor activity.** Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg; Nembutal, Abbott) and prepared for ex vivo isolated vascular perfusion of the duodenum. The duodenal segment between the pylorus and Treitz’s ligament was perfused vascularly as described previously (23). All vasculature apart from that leading into the duodenal segment was cut between double ligatures. The stomach, jejunum, ileum, colon, spleen, and pancreatic body and tail were removed. The pancreatic head was tied up by string to exclude the vascular supply. The vascular perfusate consisted of Krebs solution, 0.2% BSA (Sigma Chemical), and 5 mM glucose, saturated with 95% O₂-5% CO₂ gas to maintain pH 7.4. To rule out the side effects of rapidly changing the glucose concentration from hyperglycemia (in vivo) to normoglycemia (ex vivo) in STZ-treated animals, we used 20 mM glucose in the perfusate in some experiments. Arterial perfusion was achieved through a portal vein cannula with the tip lying adjacent to the celiac and superior mesenteric arteries, and vascular effluent was collected through a portal vein cannula. The perfusate and the preparation were kept at 36–38°C throughout the experiment, using a thermostatically controlled heating apparatus.

To measure the motor activity of the perfused duodenum, the manometric catheter (4 French, ATOM, Tokyo, Japan) with a single side hole was inserted through the pylorus, and the tip was placed 5 cm distal to the pylorus. The proximal end of the catheter was connected to the pressure transducer (TP-400T, Nihon Kohden, Tokyo, Japan). The catheter was infused with bubble-free distilled water at a rate of 1 ml/h by a low-compliance capillary infusion system using a heavy-duty pump (CFV-3100, Nihon Kohden). Intraluminal pressure was increased by an amplifier (AP-601G, Nihon Kohden), and simultaneously stored on a Mac Lab computer system (MacLab/8e, Apple Computer, AD Instruments). All data were analyzed using the Mac Lab system. After a 30-min equilibration period, spontaneously occurring pressure waves in the intestinal segment were recorded. These pressure waves were recorded for more than 15 min before drug administration. Each drug was introduced into the vasculature for 10 min with a side-arm infusion pump (CFV-3100, Nihon Kohden) at the following concentrations: 100 nM and 1 and 10 μM of serotonin creatinine sulfate (Sigma Chemical); 10 and 100 nM and 1 μM of azasetron [5-HT receptor subtype 3 (5-HT₃) antagonist, kindly supplied by Green Cross, Osaka, Japan]; and 10 and 100 nM and 1 μM of SB-204070 (5-HT₄ receptor antagonist, kindly supplied by Smith Kline Beecham Pharmaceutical, London, UK). All chemicals were dissolved in a distilled water.

To evaluate the motor activity, the percent motility index (MI) per 10 min, frequency, and amplitude of the pressure waves were compared. The mean values were obtained over a 10-min period with and without drug infusion. MI per 10 min was defined as the summation of the amplitudes of contractions for 10 min (49). Percent MI per 10 min was obtained by the percentage of MI per 10 min in STZ- or STZ plus insulin-treated animals compared with control animals or the percentage of MI per 10 min during drug infusion compared with saline-injected controls.

**Statistical analysis.** Comparisons between control and STZ-treated or STZ- and STZ plus insulin-treated rats were made with the Student’s t-test. Statistical analysis of drug effects was performed using one-way ANOVA followed by Fisher’s protected least significant difference test. $P < 0.05$ was considered statistically significant.

**RESULTS**

**Blood glucose levels in STZ-treated and STZ plus insulin-treated rats.** Blood glucose levels were significantly increased in STZ-treated rats compared with controls. As shown in Table 1, blood glucose level was reduced to the control level after treatment with insulin.

**Immunohistochemistry for serotonin.** Light microscopic observation in STZ-treated rat duodenum showed that the height of the villi increased significantly, but the depth of the crypts was unchanged (Fig. 1, A and B). The diameter of the duodenum in STZ-treated rats was significantly increased vs. controls (Fig. 1C). Serotonin-immunoreactive cells were found in the epithelium as well as in the lamina propria: the former cells are EC cells and the latter are mast cells. The number of EC cells in the epithelium appeared to be increased in the duodenum of STZ-treated rats (Fig. 1A). In the horizontal section of the duodenum, serotonin-immunoreactive nerve fibers were observed in the myenteric plexus; the distribution pattern and density appeared unchanged between control and STZ-treated rats (Fig. 1A).

**Number of EC cells and serotonin levels in villus epithelium of STZ-treated rats.** The number of serotonin-containing EC cells in the villus epithelium per unit length of the longitudinal section of the duodenum was counted. The number of EC cells per unit length of the duodenum in STZ-treated rats (11.66 ± 1.2 cells/mm, $n = 3$) was significantly higher than that in controls (6.4 ± 1.14 cells/mm, $n = 3$) (Fig. 2B). Serotonin content in the villus epithelium of STZ-treated rats (8.30 ± 1.07 ng/mg, $n = 3$) was significantly higher than that in controls (4.41 ± 1.34 ng/mg, $n = 3$) (Fig. 2A). However, serotonin content in the muscle layer in STZ-treated rats (3.61 ± 0.51 ng/mg, $n = 3$) was not altered from that in controls (2.91 ± 0.36 ng/mg, $n = 3$) (Fig. 2A).

**Motor activity in ex vivo vascularly perfused rat duodenum.** In the ex vivo vascularly perfused rat duodenum, spontaneously occurring pressure waves with regular rhythm, consisting of a period of intestinal quiescence followed by a period of contractile activity, were observed. The motor activity of the perfused duodenum from control, STZ-treated, and STZ plus insulin-treated rats was compared (Fig. 3A). In STZ-treated animals, we examined the effects of normal (5 mM) and high (20 mM) glucose levels in the perfusates on the

| Table 1. Characteristics of control and treated rats |
|-------------------|-------------------|-------------------|
|                   | $n$   | Body Wt, g | Blood Glucose Levels, mM |
| Control            | 12   | 393.3 ± 25.3 | 5.44 ± 0.66 |
| STZ treated        | 12   | 323.3 ± 29.6 | 22.16 ± 3.35 |
| STZ + insulin       | 4    | 357.5 ± 9.8  | 4.91 ± 1.15 |

Values are means ± SD; $n = $ no. of rats. * $P < 0.001$, † $P < 0.05$ compared with control; ‡ $P < 0.001$ compared with streptozotocin (STZ)-treated rats.
motor activity. No difference was found between 5 and 20 mM of glucose in the frequency (0.55 ± 0.05 and 0.61 ± 0.04 waves/min), amplitude (17.51 ± 1.03 and 16.25 ± 1.23 cmH2O), and percent MI per 10 min (53.90 ± 2.88 and 59.60 ± 3.63%) of the pressure waves. Therefore, a glucose concentration of 5 mM was mainly used in the present experiments. The frequency of the pressure waves in STZ-treated rats was significantly lower than that in controls, and this inhibitory response was reversed by treatment with insulin (Fig. 3B). The amplitude of the pressure waves in STZ-treated rats was significantly lower than that in controls, and this inhibitory response was not reversed by insulin treatment (Fig. 3C). Percent MI per 10 min in STZ-treated rats was significantly lower than that in controls, and this impaired motility was reversed by insulin treatment (Fig. 3D).

Effects of serotonin on motor activity of ex vivo perfused duodenum. We examined the effects of exogenously applied serotonin on the motor activity of the perfused duodenum in control and STZ-treated rats (Fig. 4A). Serotonin at 1 μM appeared to stimulate the motor activity of both control and STZ-treated rats (Fig. 4A). To examine in detail the effects of serotonin on motor activity, we evaluated motor activity using different components of pressure waves, such as frequency, amplitude, and percent MI per 10 min, and examined the effects of different serotonin concentrations on each component (Fig. 4, B, C, and D). In control animals, 1 μM of serotonin stimulated percent MI and frequency but did not affect amplitude. Serotonin at 10 μM stimulated amplitude and percent MI, but did not affect frequency (Fig. 4, B, C, and D). In STZ-treated rats, on the other hand, neither frequency, amplitude, nor percent MI per 10 min was affected by 1 μM of serotonin, but all of the components were stimulated by 10 μM of serotonin (Fig. 4, B, C, and D). These three parameters of motor activity in STZ-treated rats were always lower than those in controls, except in the case of 10 μM serotonin treatment, where frequency remained the same as that in controls (Fig. 4B). The stimulatory effects caused by 1 μM serotonin in controls and those caused by 10 μM serotonin in STZ-treated rats on the percent MI per 10 min were completely antagonized by atropine (Fig. 4E).

Effects of 5-HT receptor antagonists on motor activities of ex vivo perfused duodenum. We examined the effects of exogenously applied 5-HT3 and 5-HT4 receptor antagonists on the motor activity of the perfused duodenum in control and STZ-treated rats (Figs. 5A and 6A). The 5-HT3 receptor antagonist azasetron at 100 nM appeared to suppress motor activity in control rats, whereas the same concentration of azasetron did not appear to alter motor activity in STZ-treated rats.
The 5-HT₄ receptor antagonist SB-204070 at 1 μM appeared to suppress motor activity in both control and STZ-treated rats (Fig. 6A). The effects of different concentrations of azasetron or SB-204070 on the three pressure wave components were examined (Figs. 5, B, C, and D, and 6, B, C, and D). In control animals, 10 nM azasetron inhibited percent MI per 10 min but did not affect frequency and amplitude; 100 nM and 1 μM azasetron inhibited all frequency, amplitude, and percent MI per 10 min (Fig. 5, B, C, and D). In STZ-treated rats, neither frequency, amplitude, nor percent MI per 10 min was affected by azasetron at any concentration (Fig. 5, B, C, and D). SB-204070 at 100 nM inhibited percent MI per 10 min and frequency but did not affect amplitude in both control and STZ-treated rats (Fig. 6, B, C, and D). SB-204070 at 1 μM inhibited frequency, amplitude, and percent MI per 10 min in both control and STZ-treated rats (Fig. 6, B, C, and D). In STZ-treated rats, amplitude and percent MI were always lower than in controls treated with SB-204070 (at any concentration), but no difference was found in the frequency of control and STZ-treated rats treated with 10 nM–1 μM of SB-204070 (Fig. 6, B, C, and D).

DISCUSSION

Enlargement of the diameter and mucosal thickness was observed in STZ-treated rat duodenum. Light microscopic observation showed that the height of the villi increased remarkably in the STZ-treated rats, although crypt depth was unchanged. Serotonin is included in EC cells in the epithelium, mast cells in the lamina propria, and enteric neurons. All of the nerve cell bodies are located in the myenteric plexus of the rat duodenum (22). Because EC cells and serotonin-containing nerve fibers are involved in the regulation of intestinal motility (10), we examined changes in serotonin levels in the epithelium and the myenteric plexus. The results showed that serotonin content of the epithelium increased in diabetic rats, whereas that in the muscle layer including myenteric plexus was unchanged. The number of serotonin-containing EC cells per unit length of the longitudinal sections increased remarkably in the diabetic rats, suggesting that the increased number of EC cells was responsible for the increase in serotonin content in the epithelium. Although hyperplastic change in the intestinal mucosa has been reported in animal models of diabetes (24, 41), data on EC cell density or serotonin content in intestinal tissue have been inconsistent. EC cell density was decreased in the duodenum in STZ-treated (41), nonobese diabetic (18), and ob/ob mice (46), but was not altered in the duodenum of db/db mice (40). In STZ-treated rats, one study (33) reported an increase, but another (42) found no alteration in serotonin content in the small intestine. Conversely, reduction in serotonin concentration in the gastrointestinal tract has been reported in alloxan-diabetic rats (11). The present results may not be comparable to the previous data, because serotonin content was measured in the isolated epithelium in the present study, whereas it was measured in whole intestinal segments in the studies previously cited (11, 33, 42). The total number of EC cells per unit length of the duodenum was counted in the present study, whereas cell density in the epithelium or mucosa was used as a parameter elsewhere (18, 40, 41). Because the volume of epithelium was also increased in the STZ-treated rats as shown in the present study, the total number of EC cells should be a better indicator than the cell density. It is known that serotonin released from EC cells regulates the peristaltic movement via 5-HT₄/5-HT₁₉ receptors on the primary afferent neurons and these afferent neurons are coupled with excitatory and inhibitory interneurons in the myenteric plexus (26). Furthermore, 5-HT₃ and 5-HT₄ receptors are known to localize on the cholinergic interneurons in the myenteric plexus (9). Therefore, serotonin derived from EC cells contributes to the intestinal motor activity.

In the present study, we used the ex vivo vascularly perfused duodenum to examine the effects of STZ on the motor activity and the involvement of the serotonergic mechanism in regulating motility. The ex vivo vascularly perfused intestine may be a suitable model to investigate the mechanism regulating intesti-
tinal motility, because it allows the study of dose-dependent effects of chemicals on motor activity (49, 50). Contractile activity observed in the ex vivo models was composed of different components such as frequency, amplitude, or percent MI, and these were not equally affected by drug treatment (50). The present results showed that frequency, amplitude, and percent MI per 10 min were all reduced in STZ diabetic rats, and these reactions were reversed by insulin treatment.

It is widely known that impairment of gastrointestinal motility generally occurs in patients with diabetes mellitus (8, 19, 30, 35, 39, 43, 45). In diabetic patients, upper gastrointestinal symptoms are highly prevalent, induced by abnormalities of both fasting and fed motor activity in the stomach and upper small intestine (30, 35, 39, 45). Gastric emptying was prolonged and phase III MMC in the stomach and duodenum was disrupted in diabetic patients (8, 39, 43, 45). Such a disordered motility in diabetic patients has been attributed to the autonomic neuropathy in the gastrointestinal tracts. In most of the previous studies (2, 31, 48) examining the neuronal degeneration induced by diabetes mellitus, rats were used 6–8 wk after STZ injection. However, it is known that overgrowth of intestinal mucosa occurs within 3 wk after STZ injection (20) and changes in the enzyme activity, such as NOS in the duodenal tissue, occur within 4 wk after STZ injection (34). In the present study, we performed the experiment 24 days after STZ injection, because changes in the activity of receptors on the enteric neurons seem to occur earlier than the neuronal degeneration.

Previously (50), the effects of serotonin depletion induced by p-chlorophenylalanine on the duodenal motility have been examined. Results showed that frequency and percent MI, but not amplitude, were re-
Fig. 4. A: effects of 1 μM of serotonin 5-HT on the pressure waves of ex vivo vascularly perfused duodenum in control and STZ-treated rats. B–D: effects of different concentrations of serotonin on frequency (B), amplitude (C), and %MI/10 min (D) of pressure waves of ex vivo perfused duodenum in control (open bars) and STZ-treated rats (filled bars). E: effects of serotonin and serotonin + atropine on the %MI/10 min in control (open bars) and STZ-treated rats (filled bars). Values are means ± SE from 4 animals. *P < 0.05, **P < 0.01, ***P < 0.001. #P < 0.05, ##P < 0.01 compared with saline-infused control.
duced significantly in the duodenum on depletion of endogenous serotonin (50). Therefore, the change in the motor activity of the STZ-treated rat duodenum observed in the present study can be attributed to either the lack of endogenous serotonin or to impairment of serotonin receptor activity. Because serotonin content in the duodenum of STZ-treated rats was higher than in controls, the latter seems highly possible.

To examine the serotonin receptor activity, the effects of serotonin agonists and antagonists on the motor activity of the perfused duodenum were examined. The effective dose of serotonin needed to stimulate the frequency and percent MI of pressure waves in STZ-treated rats was 10 times higher than required for controls. This result suggests that the activity of serotonin receptors may be inhibited in the STZ-treated rat duodenum. Previously (25, 32, 36, 44), the involvement of serotonin receptor subtypes in the regulation of contractile activity in the small intestine has been extensively investigated. In conscious guinea pigs, 5-HT₃, 5-HT₁, and/or 5-HT₂c receptors are responsible for increasing duodenal contractility (36). In conscious rats, serotonin stimulates the cycling of MMC in the small intestine, and this action is mediated by 5-HT₃ receptors located on the cholinergic neurons (32, 44). 5-HT₂ receptors have also been shown (25) to be involved in the stimulation of MMC in the conscious dog jejunum. In the ex vivo perfused rat duodenum, neither 5-HT₁ nor 5-HT₂ receptor antagonists affected contractile activity, although both 5-HT₃ and 5-HT₄ receptor antagonists blocked the contractile activity (50). Because previous data indicated that 5-HT₃ and 5-HT₄ receptors are involved in the regulation of motor activity in the rat duodenum, the effects of 5-HT₃ and 5-HT₄ receptor antagonists on motor activity in the control and STZ-treated rat duodenum were examined in this study. The present results showed that the 5-HT₃ re-

Fig. 5. A: effects of 100 nM azasetron on the pressure waves of ex vivo vascularly perfused duodenum in control and STZ-treated rats. B–D: effects of different concentrations of azasetron on frequency (B), amplitude (C), and %MI/10 min (D) of pressure waves of ex vivo perfused duodenum in control (open bars) and STZ-treated rats (filled bars). Values are means ± SE from 4 animals. *P < 0.05, **P < 0.01, #P < 0.05, ##P < 0.01, ###P < 0.001 compared with saline-infused control.
Receptor antagonist azasetron dose dependently inhibited frequency, amplitude, and percent MI per 10 min of pressure waves in control rats, but neither frequency, amplitude, nor percent MI per 10 min was affected by azasetron at any concentration in STZ-treated rats. The 5-HT4 receptor antagonist SB-204070, on the other hand, dose dependently inhibited the frequency, amplitude, and percent MI per 10 min of pressure waves in both control and STZ-treated rats. The rank order of potency was quite different in 5-HT3 and 5-HT4 receptor antagonists in STZ-treated rat duodenum. This result suggests that the activity of the 5-HT3 receptor might be decreased in the STZ-diabetic rats, while that of the 5-HT4 receptor might be not altered.

Despite the increase in serotonin levels in the duodenal mucosa, serotonin-mediated motor events were impaired in STZ-treated rats in the present study. This phenomenon might be explained by the desensitization of 5-HT receptors in the STZ-treated rats by a high concentration of mucosal serotonin. However, such desensitization may not occur equally in all 5-HT receptors, because motor activity of the duodenum in STZ-treated rats was reacted to 10 μM serotonin. It has been shown (12, 13) that serotonin evokes biphasic contractions in the guinea pig ileum, and both phases are mediated neuronally via 5-HT3 and 5-HT4 receptors. One phase of contraction is evoked by low concentrations of serotonin (10−8 to 10−7 M) via the 5-HT4 receptor, and another phase of contraction is evoked by high concentrations of serotonin (10−7 to 10−5 M) via the 5-HT3 receptor (12, 13). Therefore, 10 μM of serotonin may desensitize the 5-HT3 receptor but not the 5-HT4 receptor in STZ-treated rat duodenum. During the infusion of 10 μM serotonin in the ex vivo perfused duodenum, serotonin-mediated motor events were impaired in control rats. The 5-HT4 receptor antagonist SB-204070, on the other hand, dose dependently inhibited the frequency, amplitude, and percent MI per 10 min of pressure waves in both control and STZ-treated rats. The rank order of potency was quite different in 5-HT3 and 5-HT4 receptor antagonists in STZ-treated rat duodenum. This result suggests that the activity of the 5-HT3 receptor might be decreased in the STZ-diabetic rats, while that of the 5-HT4 receptor might be not altered.

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duodenum, the effective concentration of serotonin at the level of neuronal receptors might be 10 μM, because concentration-effect curves shown in Fig. 4 were consistent with those obtained in the in vitro longitudinal muscle/myenteric plexus preparation from guinea pig ileum (13).

The present results showed that the stimulatory effect of exogenously applied serotonin on the motor activity was completely antagonized by atropine in both control and STZ-treated animals. This result suggests that the effect of serotonin on motor activity is mediated via 5-HT3 and/or 5-HT4 receptors located on the cholinergic neurons, and cholinergic neurons stimulate the motility via muscarinic receptors on the smooth muscles. It remains controversial whether diabetic neuropathy causes impaired cholinergic transmission in the myenteric plexus. It has been demonstrated (38) that cholinergic neurotransmission is impaired in the myenteric plexus in STZ-treated rat small intestine, whereas other reports have shown the normal release of ACh in STZ-treated rat ileum (5) and in spontaneously diabetic BB/W rat stomach (47). The present results suggest that the impairment of the activity of serotonin receptors located on intact cholinergic neurons may cause gastrointestinal dysmotility in STZ-treated rats.

In conclusion, an increase in the number of EC cells and serotonin content in the duodenum was observed in STZ-diabetic rats. Motor activity measured in the ex vivo perfused duodenum of diabetic rats was reduced, and this reduction was reversed by insulin treatment. The rank order of potency of serotonin agonists and antagonists in the motor activity of the perfused duodenum was examined, and the results indicated that 5-HT3 receptor activity was impaired in STZ-diabetic rats.

REFERENCES


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5-HT RECEPTOR ACTIVITY IN DIABETIC RATS


