

Full Paper

Possible Involvement of Endogenous 5-HT in Aggravation of Cerulein-Induced Acute Pancreatitis in Mice

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Abstract. The aim of the present study was to elucidate the pathogenic role of endogenous 5-HT in pancreatitis. Injections of cerulein at hourly intervals caused edematous pancreatitis in mice characterized by hyperenzymemia and histological alterations. While the cerulein-induced hyperenzymemia was attenuated in mice pretreated with p-CPA, a 5-HT depletor, it was exaggerated by the preferential 5-HT_{2A} agonist (DOI), but not by the preferential 5-HT_{2B} agonist (BW723C86) or the preferential 5-HT_{2C} agonist (mCPP). Selective 5-HT_{2A} antagonists (risperidone, spiperone, ketanserin, AMI-193, and MDL 11,939) dose-dependently attenuated the hyperenzymemia; and their potency order, excepting that of ketanserin which has considerable affinity at the 5-HT_{2C} receptor as well, paralleled their reported pK_i values at the 5-HT_{2A} receptor. Selective 5-HT_{2B} (SB204741) and 5-HT_{2C} (SB242084) antagonists hardly affected the hyperenzymemia. Although the non-selective 5-HT_{2A/2B/2C} antagonists (metergoline, ritanserin, and methysergide) dose-dependently attenuated the hyperenzymemia, they were relatively less potent compared to their high pK_i values at the 5-HT_{2A} receptor. In another set of experiments, risperidone, but not SB204741 and SB242084, dose-dependently reversed the cerulein-induced histological alteration of the pancreas (inflammatory cell infiltration). These results suggest that endogenously released 5-HT activates 5-HT_{2A} receptors to aggravate cerulein-induced pancreatitis. We propose that selective 5-HT_{2A} antagonists may provide a new therapy for acute pancreatitis.

Keywords: serotonin (5-HT), 5-HT_{2A}-receptor activation, cerulein-induced acute pancreatitis, hyperenzymemia, histological alteration

Introduction

Serotonin (5-HT) is widely distributed throughout the body with 90% localized in the gastrointestinal tract, 8% in platelets, and 2% in the central nervous system. It has

been a matter of speculation why such a large amount of 5-HT is stored in the gastrointestinal tract and mostly in the enterochromaffin (EC) cells of the mucosal epithelia. Past experimental studies have unveiled the role of 5-HT in mediating peristalsis and emesis, which are physiological excretive responses to mechanical and chemical stimulation of the mucosa (1–3).

More recently, 5-HT has been shown to play an important role in the endocrine-exocrine system. Ingested food on reaching the duodenal lumen stimulates

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the release of not only cholecystokinin (CCK) but also 5-HT from EC cells, which in turn activates the respective receptors on the vagal afferent fibers rather than on the pancreatic acinar cells to mediate pancreatic enzyme secretion (4–7). Thus CCK, acting synergistically with 5-HT, causes robust postprandial pancreatic secretion despite a modest increase in plasma CCK (7).

The physiological control mechanism of pancreatic enzyme secretion, if deranged for various reasons, could trigger acute pancreatitis (8). In fact, animals treated with supramaximally stimulating doses of the CCK analog cerulein rapidly develop acute edematous pancreatitis characterized by hyperenzymemia and histological alterations of the pancreas (8–10). This cerulein-induced pancreatitis is ameliorated not only by the CCK antagonists proglumide (11) and loxiglumide (12), but also by the 5-HT₂-receptor antagonist ketanserin (13). The beneficial effect is not restricted to ketanserin and cerulein-induced pancreatitis. Yoshino and Yamaguchi (14) reported that 5-HT₂ antagonists attenuated hyperenzymemia, morphologic changes, and mortality of mice with necrotizing acute pancreatitis induced by a choline-deficient ethionine-supplemented (CDE) diet.

Recently, Ogawa et al. (15) have proposed that an activation of 5-HT_{2A} (formerly known as 5-HT₂) receptor is involved in the development of experimental pancreatitis. However, their speculation is based on the effect of the 'selective' 5-HT_{2A} antagonist R-109444 and its active form R-96544 which have not been well characterized for the selectivity for three 5-HT₂-receptor subclasses (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}), and this contradicts their own and others' findings that R-109444/R-96544 and ritanserin are less potent than ketanserin in ameliorating cerulein-induced pancreatitis (13, 15), while they are more potent than ketanserin in blocking 5-HT_{2A} receptors (16–18). In order to address these issues, we examined the effect of drugs of known selectivity at 5-HT₂-receptor subclasses in cerulein-treated mice.

Materials and Methods

Animals

Female 4-week-old ICR mice weighing about 14–20 g were purchased from Japan SLC (Shizuoka). The animals were kept in our laboratory for 3 days under conditions of 22 ± 1°C and 12-h light and dark cycles with lights on at 8:00 and then used for the acute pancreatitis studies. All experimental procedures described were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

Blood sampling

Acute pancreatitis was induced by intraperitoneal injections of 50 µg/kg cerulein (Sigma-Aldrich, Poole, UK) at hourly intervals. At a predetermined time, blood was taken from the orbital sinus into heparinized tubes and centrifuged at 5000 rpm. The obtained plasma was subjected to the analysis of amylase and lipase levels. For the determination of platelet count, blood samples were collected into EDTA-containing tubes which were provided by SRL Inc. (Kyoto).

1) In the time-course study, mice were randomly assigned to 4 groups (n = 5). While one group was served as the control (0), each of the remaining 3 groups was given a different treatment (1, 3, or 5 cerulein injections), and blood was taken at 1, 3, or 5 h after the first cerulein injections, respectively. The plasma amylase and lipase levels were determined simultaneously in one set of experiments, and the results of two series of experiments were combined and expressed as the mean of 10 animals.

2) In the study using 5-HT agonists, mice were randomly assigned to 3 groups (n = 10). Each group of mice was given either vehicle or a different dose of 5-HT agonists (1 or 10 mg/kg, s.c.) 15 min before the first cerulein injection, and blood was taken 1 h after the 2nd cerulein injection.

3) In the study using p-CPA, mice were randomly assigned to 3 groups (n = 10). One group was given saline, and the other groups were given p-CPA (200 or 400 mg/kg, p.o.) 48 and 72 h before the test. Each group of mice was subjected to 5 cerulein injections, and blood was taken at 1 h after the 5th cerulein injection.

4) In the study using 5-HT antagonists, mice were randomly assigned to 3 groups (n = 10). Each group of mice was given either vehicle or a different dose of 5-HT antagonists (0.32 or 3.2 mg/kg, s.c.) 15 min before the first cerulein injection, and blood was taken 1 h after the 5th cerulein injection. The results of two series of experiments were combined and expressed as the mean of 20 animals.

Biochemical analysis of blood

Blood samples were prepared according to the manual provided by SRL, Inc. (Kyoto) and analyzed by them according to the methods described below.

Plasma amylase activity was measured with an enzymatic calorimetric test, using 2-chloro-4-nitrophenyl-β-D-maltoheptaoside as a substrate. The colored product absorbance was measured at 405 to 660 nm with a Hitachi 7170 autoanalyzer (Hitachi, Tokyo).

Plasma lipase activity was measured with an enzymatic calorimetric test, using 1,2-*o*-dilauril-rac-glycer-3-glutaric acid-(6 methyl-resorufin)-ester as a substrate.

The colored product absorbance was measured at 570 to 700 nm with a Hitachi 7170 autoanalyzer.

Blood platelet counts were measured by a fully automated hematology analyzer (Sysmex SE-9000; Sysmex Corporation, Kobe).

Examination of pancreas

At a predetermined time, the pancreas was taken through an incision of the abdominal wall under ether anesthesia. The excised pancreas was determined for wet weight, fixed in a formalin solution, embedded in paraffin, sectioned at 5- μ m thickness, and examined under a microscope.

1) In the time-course study, mice were randomly assigned to 4 groups ($n=5$). While one group was served as the control (0), each of the remaining 3 groups was given a different treatment (1, 3, or 5 cerulein injections), and pancreas was taken at 1, 3, or 5 h after the first cerulein injections, respectively.

2) In the study using risperidone, SB204741 and SB242084, mice were randomly assigned to 4 groups ($n=5$). Each group of mice was given either vehicle or a different dose of the drugs (0.1–3.2 mg/kg, s.c.) 15 min before the first cerulein injection, and the pancreas was taken 1 h after the 5th cerulein injection. Pancreatic tissue samples were fixed in 10% buffered formalin. After paraffin embedding and sectioning, tissues were stained with hematoxylin and eosin. Two samples were randomly taken from each animal, examined blindly for the extent of edema, inflammatory cell infiltration, and acinar cell necrosis, and scored according to the following criteria: 0 = no change, 1 = slight change, 2 = moderate change, 3 = severe change, 4 = very severe change. The results of two series of experiments were combined and expressed as the mean of 10 animals.

Drugs

BW723C86, (\pm)DOI, ketanserin tartrate, m-CPP, metergoline, methysergide maleate, p-CPA, ritanserin, SB204070, SB204741, SB242084 dihydrochloride, and spiperone were purchased from Sigma-Aldrich. AMI-193 and MDL 11,939 were purchased from Tocris Cookson, Ltd. (Bristol, UK). Pindolol (Wako Pure Chemical Industries, Ltd., Osaka), granisetron hydrochloride (Kytril injection[®]; Chugai Pharmaceutical Co., Ltd., Tokyo), and risperidone (Risperdal oral solution[®]; Janssen Pharmaceutical K.K, Tokyo) were also obtained from the indicated commercial source. Granisetron hydrochloride, p-CPA, and risperidone were dissolved in saline, and the other drugs were suspended in 0.5% methylcellulose (Sigma-Aldrich). All drug solutions were prepared freshly and administered in a volume of 20 ml/kg. The control mice were given the respective

vehicles.

Statistical analyses

Results are expressed as mean \pm S.E.M. A one-tail paired *t* test was used to study the statistical significance of time course changes in plasma parameters. Dunnett's multiple comparison test was used to study the statistical significance of the drug effect on the plasma parameters. Dunn's multiple comparison test was used for the analyses of histological scores.

Results

Cerulein-induced changes of the pancreas

All the mice survived 5 intraperitoneal injections of cerulein (50 μ g/kg) at hourly intervals, and light microscopy revealed the typical picture of edematous pancreatitis. Acinar cell edema was seen as early as 1 h post-injection, and interstitial edema and separation of lobes and lobules became prominent at 3 and 5 h after the first cerulein injection. Acinar cell vacuolation and inflammatory cell infiltration were rarely observed at h 1, but clearly detected at h 3. The number of vacuolated acinar cells and inflammatory cells further increased at h 5. On the other hand, necrotic changes were scarcely found throughout the experimental period.

The body weight hardly changed during the experimental period, but the pancreatic weight relative to body weight increased to peak at h 3 and leveled off (Fig. 1). The values at 1 (0.0141 ± 0.0020), 3 (0.0173 ± 0.0023), and 5 (0.0156 ± 0.0013) h after the first cerulein injection were significantly different compared with the control (0.0112 ± 0.0008).

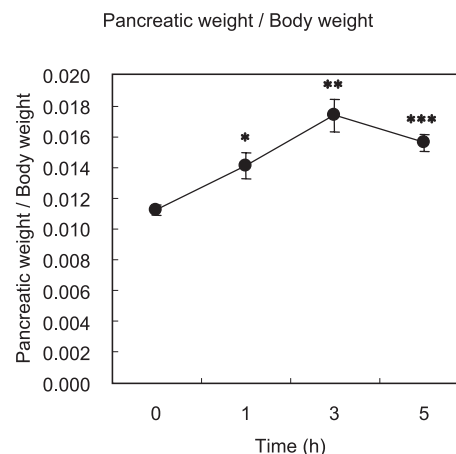


Fig. 1. Time-course changes in pancreas weight relative to body weight following cerulein injections at hourly intervals. * $P<0.05$, ** $P<0.01$, *** $P<0.001$: statistically significant compared with the control (0).

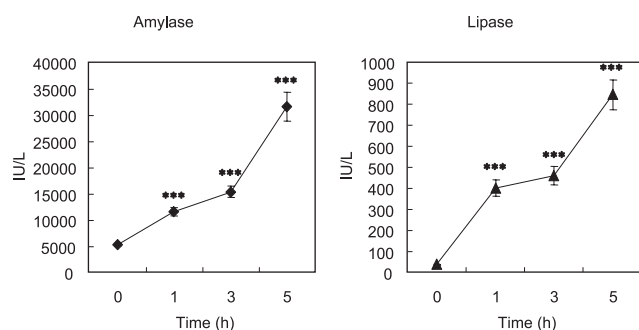


Fig. 2. Time-course changes in plasma amylase and lipase levels following cerulein injections at hourly intervals. *** $P < 0.001$: statistically significant compared with the control (0).

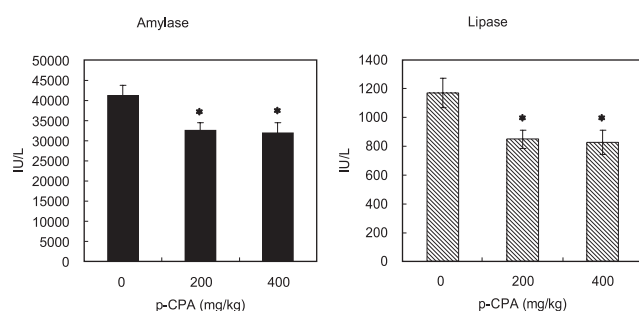


Fig. 3. Effect of p-CPA treatment on the plasma amylase and lipase levels in mice subjected to five cerulein injections. * $P < 0.05$: statistically significant compared with the control (0).

Blood parameter changes

As shown in Fig. 2, plasma amylase levels increased time-dependently to reach 11481 ± 776 , 15361 ± 1105 , and 31541 ± 2733 IU/L at 1, 3, and 5 h after the first cerulein injection, respectively. These values were significantly different compared with the control (5225 ± 199). Plasma lipase levels also increased time-dependently to reach 402.3 ± 40.4 , 460.0 ± 42.8 , and 846.0 ± 73.1 IU/L at 1, 3, and 5 h, respectively. These values were significantly different compared with the control (38.0 ± 1.8 IU/L).

In contrast to the changes in the plasma enzymes, platelet counts hardly changed throughout the experimental period, ranging from 97.0×10^4 to $100.1 \times 10^4/\text{mm}^3$ (data not shown).

Effect of p-CPA on the cerulein-induced hyperenzymemia

To assess the role of endogenous 5-HT in the cerulein-induced hyperenzymemia, we tested the effect of p-CPA, a 5-HT depletor. As shown in Fig. 3, 5 cerulein injections increased the plasma amylase levels to 41174 ± 2676 IU/L in the control mice, which was significantly reduced to 32498 ± 1897 and $31978 \pm$

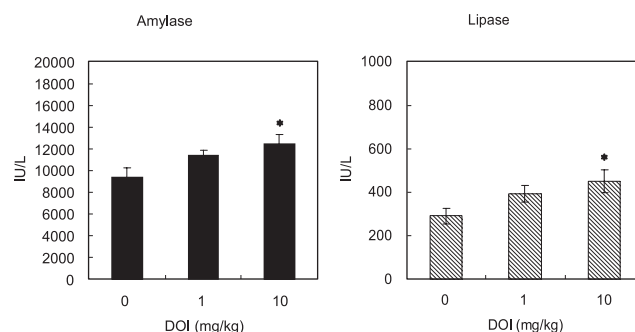


Fig. 4. Effect of DOI on the plasma amylase and lipase levels in mice subjected to two cerulein injections. * $P < 0.05$: statistically significant compared with the control (0).

2465 IU/L in the mice pretreated with 200 or 400 mg/kg of p-CPA, respectively. Similarly, cerulein injections increased the plasma lipase levels to 1171.3 ± 103.1 IU/L in the control mice, which was significantly reduced to 848.0 ± 64.8 and 828.4 ± 84.1 IU/L in the mice pretreated with 200 or 400 mg/kg of p-CPA, respectively.

Effect of preferential 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} agonists

Subcutaneous doses (1 and 10 mg/kg) of DOI, a preferential 5-HT_{2A} agonist; BW723C86, a preferential 5-HT_{2B} agonist; and mCPP, a preferential 5-HT_{2C} agonist, were studied for their effects on hyperenzymemia in mice with cerulein-induced pancreatitis. Two cerulein injections in the control mice increased plasma amylase and lipase levels to 8396–9382 and 263.1–289.7 IU/L, respectively. The hyperamylasemia and hyperlipasemia were dose-dependently exaggerated by DOI, and the changes at 10 mg/kg were statistically significant (Fig. 4). BW723C86 and mCPP tended to enhance the plasma amylase and lipase levels, but the changes were not statistically significant (data not shown).

Effect of selective 5-HT_{1A/1B}, 5-HT₃, and 5-HT₄ antagonists

Because of the paucity of selective 5-HT agonists, further studies were conducted using selective 5-HT antagonists (0.32 and 3.2 mg/kg, s.c.). Five cerulein injections in the control mice increased plasma amylase and lipase levels to 26580–29117 and 876.0–1044.5 IU/L, respectively. The hyperamylasemia and hyperlipasemia are not significantly affected by pindolol, granisetron, and SB204070, which are antagonists selective for 5-HT_{1A/1B}, 5-HT₃, and 5-HT₄, respectively (data not shown).

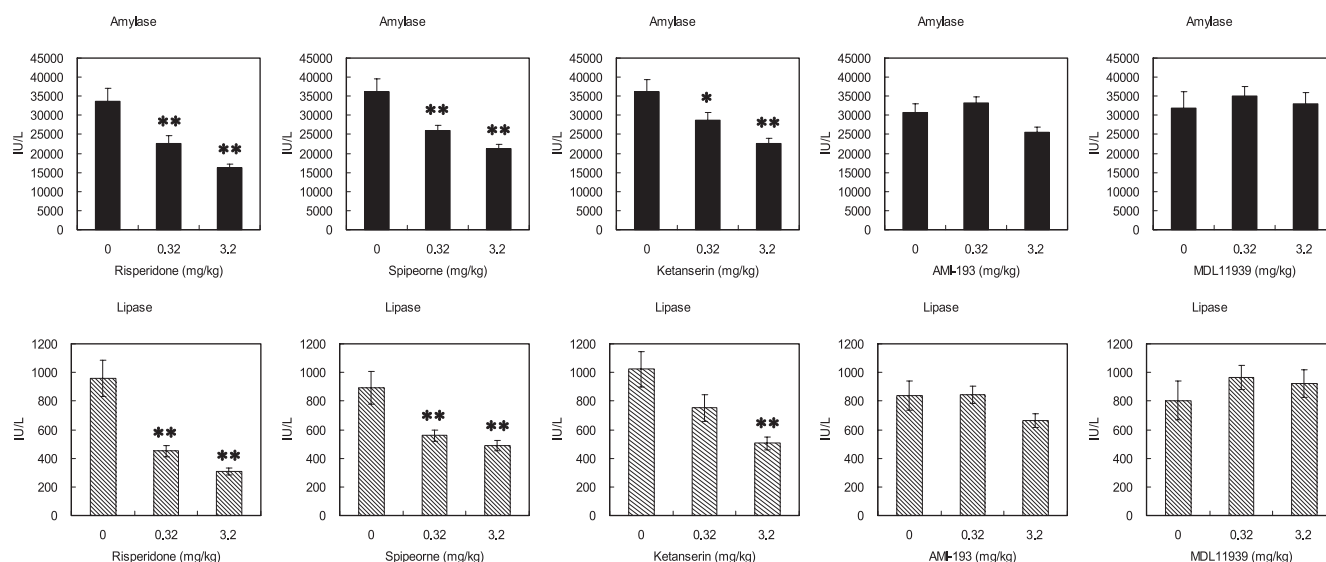


Fig. 5. Effect of risperidone, spiperone, ketanserin, AMI-193, and MDL 11,939 on the plasma amylase and lipase levels in mice subjected to five cerulein injections. * $P < 0.05$, ** $P < 0.01$: statistically significant compared with the control (0).

Table 1. Inhibitory activity of selective 5-HT_{2A} antagonists on the plasma amylase and lipase levels in mice subjected to five cerulein injections

Antagonists		Amylase (Inhibition %)			Lipase (Inhibition %)		
		0.32 mg/kg	3.2 mg/kg	Potency index	0.32 mg/kg	3.2 mg/kg	Potency index
5-HT _{2A} selective	Risperidone	33.0	52.0	42.5	53.0	67.9	60.5
	Spiperone	28.3	41.2	34.8	37.1	45.0	41.1
	Ketanserin	20.7	37.5	29.1	26.5	50.8	38.7
	AMI-193	-8.2	17.0	4.4	-0.7	21.1	10.2
	MDL11939	-9.7	-3.6	-6.6	-20.2	-14.9	-17.5

Each value represents the percent inhibition achieved by 0.32 and 3.2 mg/kg of the 5-HT_{2A} antagonists. Potency index represents the mean inhibition percent achieved by the two doses of each drug.

Effect of selective 5-HT_{2A} antagonists

Five cerulein injections in the control mice increased plasma amylase and lipase levels to 30720–36265 and 803.3–1024.1 IU/L, respectively (Fig. 5). The hyperamylasemia and hyperlipasemia were significantly and dose-dependently attenuated by risperidone, spiperone, and ketanserin. AMI-193 tended to attenuate both the plasma amylase and lipase levels at 3.2 mg/kg, but the changes were not statistically significant. MDL 11,939 did not significantly change the hyperenzymemia at the doses used.

Table 1 summarizes the percent inhibition achieved by 0.32 and 3.2 mg/kg of the 5-HT_{2A} antagonists together with the potency index that represents the mean inhibition percent achieved by the two doses of each drug. The overall rank in order of potency as indicated by the potency index was risperidone, spiperone, ketanserin, AMI-193, and MDL 11,939, which, except for that of ketanserin, paralleled the reported pK_i values

for 5-HT_{2A} receptors (Table 2). Ketanserin has an affinity to 5-HT_{2C} (pK_i = 7.21) as well as to 5-HT_{2A} receptors (pK_i = 8.09). Risperidone, spiperone, AMI-193, and MDL 11,939 are selective for 5-HT_{2A} receptors, having pK_i values for 5-HT_{2A} receptors greater than those for 5-HT_{2B} and 5-HT_{2C} receptors.

Effect of selective 5-HT_{2B} and 5-HT_{2C} antagonists

Table 2 shows that SB204741 is selective for 5-HT_{2B} receptors, having pK_i values for 5-HT_{2B} receptors greater than those for 5-HT_{2A} and 5-HT_{2C} receptors. On the other hand, SB242084 is selective for 5-HT_{2C} receptors, having pK_i values for 5-HT_{2C} receptors greater than those for 5-HT_{2A} and 5-HT_{2B} receptors.

In the experiment using the selective 5HT_{2B} and 5-HT_{2C} antagonists, five cerulein injections in the control mice increased plasma amylase and lipase levels to 29317–44037 and 758.0–1394.0 IU/L, respectively. The hyperamylasemia and hyperlipasemia were not

Table 2. Receptor binding affinity (pK_i) of the drugs used in the present study at human recombinant 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors

	Antagonists	5-HT _{2A} $pK_i \pm S.E.M.$	5-HT _{2B} $pK_i \pm S.E.M.$	5-HT _{2C} $pK_i \pm S.E.M.$
5-HT _{2A} selective	Risperidone	9.7 ^a		7.49 ^a
	Spiperone	7.81 \pm 0.29	5.88 \pm 0.12	6.22 \pm 0.08
	Ketanserin	8.09 \pm 0.09	6.13 \pm 0.07	7.21 \pm 0.12
	AMI-193	7.65 \pm 0.08	6.01 \pm 0.02	5.81 \pm 0.16
	MDL-11,939	7.58 \pm 0.12	5.48 \pm 0.03	6.58 \pm 0.22
5-HT _{2B} selective	SB204741	<5.00	6.90 \pm 0.27	5.56 \pm 0.07
5-HT _{2C} selective	SB242084	6.07 \pm 0.18	6.84 \pm 0.28	8.15 \pm 0.10
Non-selective 5-HT ₂	Metergoline	8.64 \pm 0.07	8.75 \pm 0.08	8.75 \pm 0.11
	Ritanserin	8.34 \pm 0.09	8.67 \pm 0.09	8.18 \pm 0.15
	Methysergide	8.40 \pm 0.16	9.44 \pm 0.05	8.60 \pm 0.05

^aThese data were referred from two published reports (16, 18).

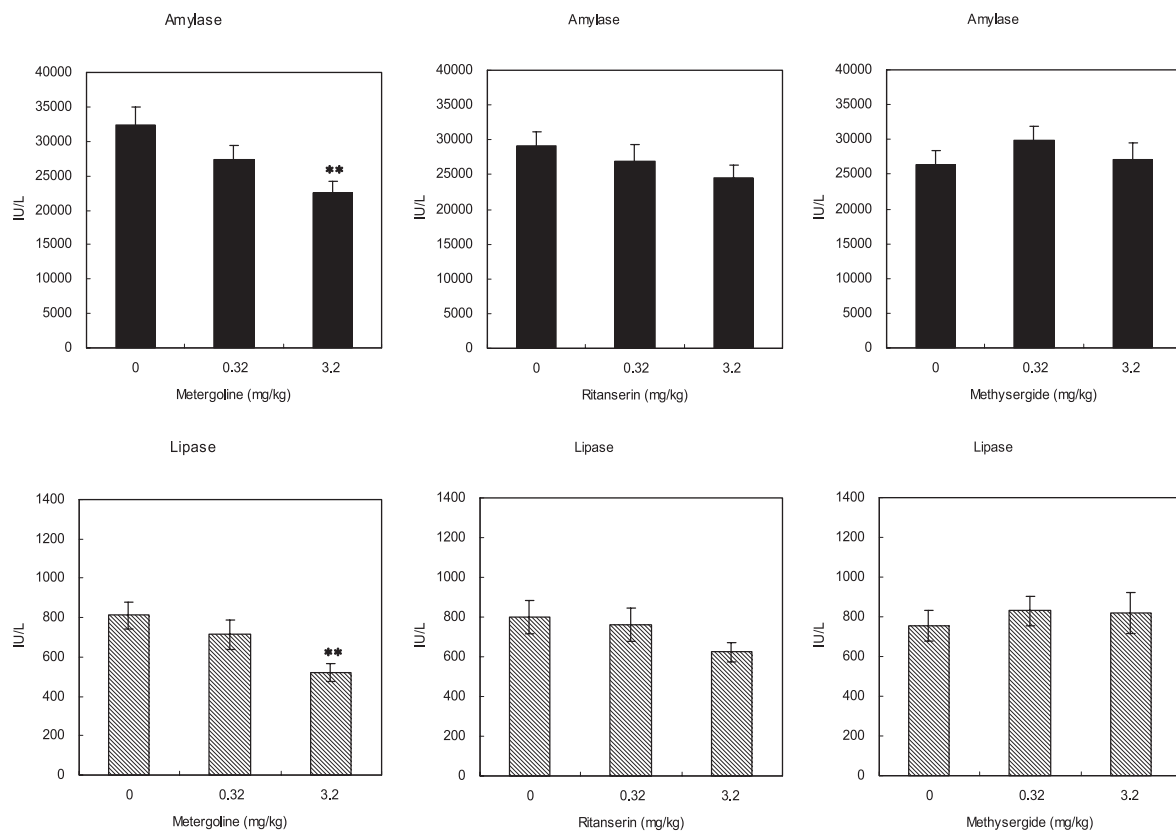


Fig. 6. Effect of metergoline, ritanserin, and methysergide on the plasma amylase and lipase levels in mice subjected to five cerulein injections. ** $P < 0.01$: statistically significant compared with the control (0).

significantly affected by any of the drugs (data not shown).

Effect of non-selective 5-HT_{2A/2B/2C} antagonists

Table 2 also shows that metergoline, ritanserin, and methysergide are non-selective towards 5-HT_{2A}, 5-HT_{2B},

and 5-HT_{2C} receptors, having pK_i values ranging from 8.18 – 9.44 for these receptors.

These non-selective 5HT_{2A/2B/2C} antagonists were studied for their effects on cerulein-induced hyperenzymemia (Fig. 6). Five cerulein injections in the control mice increased plasma amylase and lipase levels

Table 3. Inhibitory activity of non-selective 5-HT₂ antagonists on the plasma amylase and lipase levels in mice subjected to five cerulein injections

Antagonists		Amylase (Inhibition %)			Lipase (Inhibition %)		
		0.32 mg/kg	3.2 mg/kg	Potency index	0.32 mg/kg	3.2 mg/kg	Potency index
Non-selective 5-HT ₂	Metergoline	15.9	30.4	23.2	12.2	36.1	24.1
	Ritanserlin	4.4	15.2	9.8	-3.6	21.2	8.8
	Methysergide	-13.4	-2.6	-8.0	-9.7	-8.2	-9.0

Each value represents the percent inhibition achieved by 0.32 and 3.2 mg/kg of the 5-HT₂ antagonists. Potency index represents the mean inhibition percent achieved by the two doses of each drug.

Table 4. Effect of risperidone on the histologic alterations of the pancreas in mice subjected to five cerulein injections

Risperidone (mg/kg)	Histologic Score [#]		
	Edema	Inflammatory cell infiltration	Necrosis
0	1.95 ± 0.15	2.10 ± 0.14	0.15 ± 0.08
0.1	1.75 ± 0.10	2.05 ± 0.11	0.15 ± 0.08
0.32	1.95 ± 0.05	2.10 ± 0.07	0
1	1.80 ± 0.09	1.65 ± 0.11	0
3.2	1.90 ± 0.07	1.40 ± 0.11**	0

Pancreatic histology was evaluated 5 h after the first cerulein injection. [#]The edema, inflammatory cell infiltration, and necrosis were graded according to the degree of alterations as described in the Methods. Each value represents the mean ± S.E.M. (n = 10).

**P<0.01: statistically significant compared with the control (0).

to 26374 – 32453 and 756.0 – 813.3 IU/L, respectively. The hyperamylasemia and hyperlipasemia were dose-dependently attenuated by metergoline, and the changes at 3.2 mg/kg were statistically significant. Ritanserlin dose-dependently reduced the plasma amylase and lipase levels, although the changes were not statistically significant. Methysergide hardly affected the plasma amylase and lipase levels.

Table 3 summarizes the percent inhibition achieved by 0.32 and 3.2 mg/kg of the non-selective 5-HT_{2A/2B/2C} antagonists together with the potency index that represents the mean percent inhibition achieved by the two doses of each drug. The potency indices of metergoline (23.2 – 24.1), ritanserlin (8.8 – 9.8), and methysergide (-8.0 – -9.0) were less than those of spiperone and ketanserlin which have smaller pK_i values for 5-HT_{2A} receptors (Table 2).

Effect of risperidone, SB204741, and SB242084 on the pancreatic weight and histologic alterations

The pancreatic weight relative to body weight in mice given 5 cerulein injections (0.0163 ± 0.0006) was significantly different from that in normal mice (0.0112 ± 0.0008), but the cerulein-induced pancreatic weight gain was similarly observed in mice treated with 0.32 and 3.2 mg/kg of risperidone, SB204741, and SB242084 (data not shown).

The histologic alterations of the pancreas in cerulein-treated mice are shown in Table 4. The pancreas of all the control mice revealed edema and inflammatory cell infiltration, and their mean histologic scores were 1.95 ± 0.15 and 2.10 ± 0.14, respectively. On the other hand, only two out of ten control mice revealed slight necrotic changes, and the mean score of necrosis was 0.15 ± 0.08. Risperidone (0.1 – 3.2 mg/kg) hardly changed the edema, but dose-dependently attenuated the inflammatory cell infiltration. Although two mice of the 0.1 mg/kg group revealed slight necrotic changes, none of the mice treated with more than 0.32 mg/kg exhibited necrotic changes. In contrast, SB204741 and SB242084 hardly attenuated the histological alterations in cerulein-treated mice (data not shown).

Figure 7 shows the typical light microscopy of the pancreas taken from the cerulein-treated mice. While numerous inflammatory cells infiltrated to the interstitium of the control mouse (left), only a few inflammatory cells infiltrated to the interstitium of the mouse treated with 3.2 mg/kg of risperidone (right). In contrast, the degree of edema was similar between the two.

Discussion

The present study confirmed that injections of supra-maximally stimulating doses of cerulein, a decapeptide

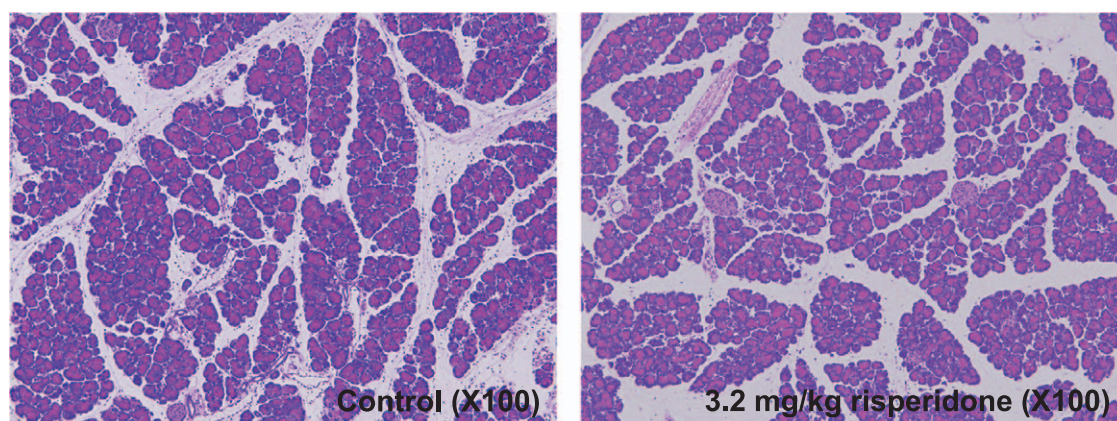


Fig. 7. Effects of risperidone on histologic alterations in cerulein-induced pancreatitis mice ($\times 100$). The pancreas was removed 5 h after the first cerulein injection. Left: Pancreatic histology of a control mouse that received 5 cerulein injections. Acinar cells and interstitium are edematous, and a large number of inflammatory cells infiltrated in the interstitium. Right: Pancreatic histology of a mouse that was pretreated with 3.2 mg/kg of risperidone 15 min before the first cerulein injection. Note that edema of acinar cells and interstitium are similarly observed, but the number of inflammatory cells is greatly reduced compared with the control.

analogue of CCK, caused mild edematous pancreatitis in mice; plasma amylase and lipase levels increased time-dependently, while in the pancreas, edema developed first, which was followed by inflammatory cell infiltration and then necrosis (8–10). It is a novel finding, however, that the 5-HT-depletor p-CPA attenuated but the preferential 5-HT_{2A} agonist DOI exaggerated the cerulein-induced hyperenzymemia. Although the changes induced by p-CPA and DOI were small, and Oguchi et al. (13) reported that exogenously given 5-HT did not aggravate cerulein-induced hyperenzymemia, there is a possibility that involvement of receptors other than 5-HT_{2A} reduced the final outcome of the 5-HT, DOI, and p-CPA actions. In this respect, it should be noted that 5-HT and DOI have more or less agonistic activities at 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors (18), and p-CPA depresses all the 5-HT-receptor activities. The possibility that 5-HT_{2A} and 5-HT_{2B}/5-HT_{2C} receptors play opposite roles in the pathogenesis of acute pancreatitis will be discussed later. Thus, we propose that CCK releases endogenous 5-HT, which in turn activates 5-HT_{2A} receptors and results in aggravation of the cerulein-induced hyperenzymemia. Our speculation coincides with the well-documented interactions between endogenous CCK and 5-HT regarding colonic motility, feeding behavior, and pancreatic enzyme secretion (7, 19, 20).

As for the source of 5-HT, one possibility is EC cells that contain a huge amount of 5-HT. It has been documented that CCK induces 5-HT release from intestinal EC cells (5, 6). On the other hand, Celiński et al. (21) reported that there was a significant increase in 5-hydroxyindoleacetic acid (5-HIAA) accompanied

by the relevant decrease of 5-HT in the stomach of cerulein-treated rats. It is thus reasonable to assume that cerulein induces 5-HT release from the gastric EC cells as well. Another possibility is platelets; Prinz et al. (22) reported that intravenously injected pancreatic fluid causes platelet aggregation and resulted in the release of 5-HT. In the present study, however, blood platelet counts did not change throughout the experimental period. Platelets may not be the source of 5-HT release. The last possibility is pancreatic acinar cells; there is evidence that pancreatic acinar cells uptake and store 5-HT, which is released when the cells are stimulated by secretagogues including CCK (23, 24). However, the previous authors demonstrated that both 5-HT and 5-HIAA decreased in the pancreas, liver, kidney, and brain of cerulein-treated rats (21). Therefore, synthesis and/or release of 5-HT appear to be depressed in these organs.

In the study, using various 5-HT antagonists, we found that the 5-HT_{1A/1B} antagonist pindolol, 5-HT₃ antagonist granisetron, or 5-HT₄ antagonist SB204070 hardly affected the hyperenzymemia. More importantly, the 5-HT_{2A} antagonists, but not the 5-HT_{2B} and 5-HT_{2C} antagonists, attenuated the hyperenzymemia in cerulein-treated mice. According to the potency index adopted in the present study, risperidone (42.5%–60.5%) was the most potent among the drugs used, followed by spiperone (34.8%–41.1%), ketanserin (29.1%–38.7%), AMI-193 (4.4%–10.2%), and MDL 11,939 (–6.6%–17.5%). The potency order except for ketanserin parallels the reported pK_i values at 5-HT_{2A} receptors (18, 25). One possible explanation for why ketanserin was dissociated from the relationship between the potency index and pK_i values could be that the difference

between spiperone and ketanserin is so small that it sometimes falls within the range of variations. In fact, Bonhaus et al. (16) reported that spiperone had a higher pK_i value (9.0) for 5-HT_{2A} receptors than ketanserin (8.5), whereas Knight et al. (18) reported that ketanserin had a higher pK_i value (8.09) than spiperone (7.81). Also in the present study, 3.2 mg/kg of spiperone was more potent than ketanserin in attenuating the hyperamylasemia (41.2% vs 37.5%), while the latter was more potent than the former in attenuating hyperlipasemia (50.8% vs 45.0%).

Another explanation is that endogenous 5-HT has a protective as well as aggravating activity for the pathology of acute hyperenzymemia. In this respect, it is interesting to note that ketanserin binds with not only 5-HT_{2A} but also 5-HT_{2C} receptors (16, 18, 25). Although these two receptors are very similar both in terms of structure and pharmacology (26), they are different in that 5-HT_{2A} receptor activation causes vasoconstriction (27–29), whereas 5-HT_{2C} (formerly named 5-HT_{1C}) receptor activation causes endothelium-dependent vasodilation (30, 31). Furthermore, a body of evidence has suggested that circulatory disturbance or ischemia are the pathogenic factors in acute pancreatitis; a pharmacological vasoconstrictor, phenylephrine, increased severity of cerulein-induced pancreatitis (32), and hemorrhagic shock converts cerulein-induced edematous pancreatitis to more severe hemorrhagic pancreatitis (33). Taken together, we speculate that activation of 5-HT_{2A} receptors in the smooth muscle cells decreases blood flow and aggravates hyperenzymemia, whereas activation of 5-HT_{2C} receptors in the endothelial cells increases blood flow and attenuates hyperenzymemia. Ketanserin appears to block both the protective and aggravating actions of 5-HT, while the selective 5-HT_{2A} antagonists risperidone and spiperone block only the latter action.

The present study also revealed that metergoline, ritanserin, and methysergide, non-selective 5-HT_{2A/2B/2C} antagonists, were less potent compared with their high pK_i values for 5-HT_{2A} receptors (16, 18). This could be explained by the functional antagonism between the 5-HT_{2A} and 5-HT_{2C} receptor blockades, mentioned above. Furthermore, it should be noted that activation of 5-HT_{2B} receptors also leads to endothelium-dependent vasodilation (27, 34–36). Therefore, a blockade of not only 5-HT_{2C} but also 5-HT_{2B} receptors by non-selective 5-HT_{2A/2B/2C} antagonists may counteract the beneficial effect of a 5-HT_{2A} blockade on cerulein-induced hyperenzymemia. This speculation could also explain why the effect of p-CPA is less pronounced than that of selective 5-HT_{2A} antagonists, but appears to contradict with the observation that both the preferential 5-HT_{2B}

agonist BW723C86 and preferential 5-HT_{2C} agonist mCPP did not significantly attenuate the hyperenzymemia. Although BW723C86 and mCPP are known as preferential 5-HT_{2B} and 5-HT_{2C} agonists, respectively, their 5-HT_{2A}/5-HT_{2B} and 5-HT_{2A}/5-HT_{2C} affinity ratios are less than those of DOI (18). Thus the former two drugs might have affected all the 5-HT₂-receptor subclasses, just as in the case of non-selective 5-HT₂ antagonists that induced minimal effects on hyperenzymemia.

Further studies were conducted using the most selective 5-HT_{2A} antagonist risperidone, and we found that risperidone did not attenuate cerulein-induced interstitial edema and the increase in pancreatic weight. An activation of CCK receptors, but not 5-HT_{2A} receptors, appears to be involved in the edema formation, a local inflammatory response. It has been shown that the cerulein-induced pancreatic edema and weight increase was attenuated by CCK antagonists (10–12). On the other hand, risperidone attenuated inflammatory cell (neutrophil) infiltration. Neutrophils are believed to play an important part in the inflammatory response to the pancreatic acinar cell injury and pathogenesis of pancreatitis (37); upon activation, neutrophils adhere to vascular endothelial cells near the inflammatory foci, transmigrate across endothelial barriers, activate digestive enzymes, and cause auto-digestion of the pancreas. Depletion of circulating neutrophils with antineutrophil serum (37–39) reduced the severity of acute pancreatitis, supporting this hypothesis. On the other hand, it has been reported that 5-HT enhances vascular permeability (40) as well as neutrophil chemoattractant production by the endothelial cells (41), which are attenuated by 5-HT_{2A} antagonists. Further to be noted, there are papers that 5-HT_{2A} antagonists attenuated 5-HT-induced IL-6 production by smooth muscle cells (42) or macrophages/lymphocytes (43). These results suggest that activation of 5-HT_{2A} receptors in the endothelial cells, smooth muscle cells, and macrophages/lymphocytes exaggerates the local inflammatory response that is initiated by CCK receptor activation.

Based on the above discussions we propose that selective 5-HT_{2A} antagonists such as risperidone could provide a potential new treatment for acute pancreatitis. In fact, risperidone strongly attenuated not only the mild edematous pancreatitis induced by cerulein but also severe necrotizing acute pancreatitis induced by a CDE diet (K. Hamada et al., unpublished data). Although identifying the precise mechanism of action is beyond the scope of the present study, risperidone appears to attenuate inflammatory responses through blockade of 5-HT_{2A} receptors in the endothelial cells, smooth muscle

cells, and/or macrophages/lymphocytes. In addition, risperidone may attenuate circulatory disturbance or ischemia through blockade of 5-HT_{2A} receptors in the smooth muscle cells.

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