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European Journal of Pharmacology 521 (2005) 156–163
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Effects of R-102444 and its active metabolite R-96544, selective 5-HT_{2A} receptor antagonists, on experimental acute and chronic pancreatitis: Additional evidence for possible involvement of 5-HT_{2A} receptors in the development of experimental pancreatitis

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Received 3 June 2005; accepted 10 August 2005
Available online 22 September 2005

Abstract

The effects of R-102444 ((2*R*, 4*R*)-4-lauroyloxy-2-[2-[2-[2-(3-methoxy)phenyl]ethyl]phenoxy]ethyl-1-methylpyrrolidine hydrochloride) and its active metabolite R-96544 ((2*R*, 4*R*)-2-[2-[2-[2-(3-methoxy)phenyl]ethyl]phenoxy]ethyl-4-hydroxy-1-methylpyrrolidine hydrochloride), potent and selective 5-hydroxytryptamine 2A (5-HT_{2A}) receptor antagonists, on development of pancreatitis were investigated in experimental models of acute and chronic pancreatitis. Rat acute pancreatitis was induced by caerulein (20 μg/kg) intraperitoneal injection and by pancreatic duct ligation. In both the models, serum amylase and lipase activities were markedly increased. R-102444 dose-dependently reduced these enzyme activities at a dose range of 10 to 100 mg/kg (p.o.) for the caerulein model and 0.3 to 10 mg/kg (p.o.) for the ligation model. In a mouse model of acute pancreatitis induced by a choline-deficient, ethionine (0.5%)-supplemented diet, subcutaneous administration of R-96544 (10–100 mg/kg, bid) reduced serum amylase activity. Histological analysis showed that R-96544 dose-dependently attenuated pancreatic necrosis, inflammation and vacuolization. The effect of R-102444 was further examined in male Wistar Bonn/Kobori rats (4–9 months of age) which spontaneously show pancreatic fibrosis and parenchymal destruction compatible with human chronic pancreatitis. In Wistar Bonn/Kobori rats (from 3 to 9 months of age) fed a diet containing 0.017% and 0.17% of R-102444, pancreatic weight, pancreatic protein and amylase content were higher compared to those in non-treated pancreatitis control rats. Histological analysis showed that R-102444 suppressed parenchymal destruction and replacement with adipose tissue, indicating inhibition of pancreatic atrophy. These results clearly indicate that R-102444 and R-96544 inhibit the progression of acute and chronic pancreatitis and support the contention of possible involvement of 5-HT_{2A} receptors in the progression of experimental pancreatitis.

Keywords: 5-HT_{2A} receptor; Serotonin; Pancreatitis; R-102444; R-96544

1. Introduction

Acute pancreatitis is a disease with high morbidity and mortality (Mergener and Baillie, 1998). Chronic pancreatitis is an inflammatory disease in which progressive and irreversible structural changes to the pancreas result in permanent impairment of both its exocrine and endocrine functions (Mergener and Baillie, 1997). It has been suggested that several factors such as intra-acinar activation of trypsinogen, oxygen-derived free radicals and many cytokines (e.g., interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor-alpha and platelet activating factor) play roles in the onset and progression of pancreatitis in several experimental models (Sugiyama et al., 1996; Geokas et al., 1972; Sakorafas and Tsiotou, 2000; Konturek et al., 1992), but the mechanisms

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doi:10.1016/j.ejphar.2005.08.033

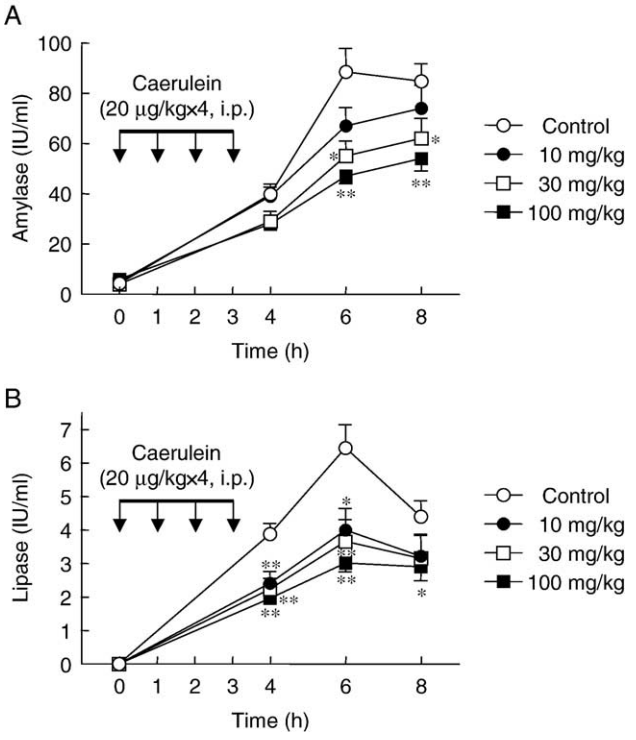

Fig. 1. Time courses of serum amylase (A) and lipase activities (B) in rats treated with caerulein. Four hourly intraperitoneal injections of caerulein (20 μg/kg) were given during the first 3 h of the experiment. R-102444 (10–100 mg/kg) was orally administered 30 min before the first caerulein injection. Data represent mean±S.E.M. of 9–10 animals. *$P<0.05$, **$P<0.01$ vs. control (vehicle-treated group).

responsible for the development of pancreatic lesions have not yet been fully elucidated.

Serotonin (5-hydroxytryptamine; 5-HT), a potent vasoconstrictor and platelet activator, has been suggested to be involved in the progression of acute pancreatitis. Ketanserin and ritanserin reduced serum amylase concentration in rats treated with caerulein (Oguchi et al., 1992). Yoshino and Yamaguchi (1997) reported that 5-HT blockers, especially 5-HT_{2A} (formerly known as 5-HT_2) receptor antagonists, attenuated mortality of mice fed a choline-deficient, ethionine-supplemented diet. However, the agents used for evaluation in these acute pancreatitis models have relatively low selectivity for the 5-HT_{2A} receptor (Fozard, 1982; Cohen et al., 1983; Vanhoutte et al., 1983). In addition, involvement of a 5-HT_{2A} receptor antagonist in the progression of chronic pancreatitis has been uncertain. These observations led us to examine whether a selective 5-HT_{2A} receptor antagonist could attenuate the severity of acute and chronic pancreatitis.

Recently, we reported R-96544 ((2*R*, 4*R*)-2-[2-[2-[2-(3-methoxy)phenyl]ethyl]phenoxy]ethyl-4-hydroxy-1-methylpyrrolidine hydrochloride) as a potent antagonist of the 5-HT_{2A} receptor (Tanaka et al., 2000). R-96544 has 600–2800-fold higher affinity for the 5-HT_{2A} receptor than for the 5-HT_3 and dopamine D_2 receptors, and the α- and β-adrenoceptors (Tanaka et al., 2000). In addition, in radioligand binding studies using cells expressing human 5-HT receptors, R-96544 showed 100-fold higher affinity for the human 5-HT_{2A} receptors than for the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{5A}, 5-HT_6 and 5-HT_7 receptors, and the 5-HT transporter (Ogawa et al., 2002). Its ester type prodrug, R-102444 ((2*R*, 4*R*)-4-lauroyloxy-2-[2-[2-[2-(3-methoxy)phenyl]ethyl]phenoxy]ethyl-1-methylpyrrolidine hydrochloride), is orally available and has potent antiplatelet and vasodilating actions (Ogawa et al., 2002, 2004). When orally administered to rats, R-102444 was metabolized in the intestine and absorbed into the portal vein as the active R-96544. We found that these selective 5-HT_{2A} receptor antagonists are effective in not only three distinctive acute pancreatitis models, but also in male Wistar Bonn/Kobori rats in which chronic pancreatitis occurs spontaneously (Ohashi et al., 1990).

2. Materials and methods

2.1. Experimental animals

All animal procedures were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee at Sankyo Research Laboratories (Tokyo, Japan). Male Wistar Bonn/Kobori rats (initial body weight: about 300 g, 3 months old), male Wistar rats (weighing about 200 g), and

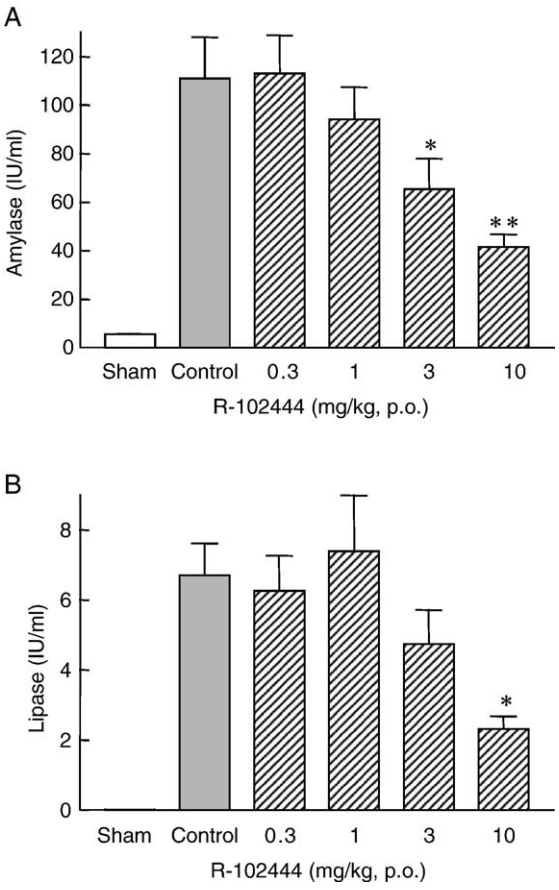

Fig. 2. Effects of R-102444 on serum amylase (A) and lipase (B) activities of pancreatic duct ligation-induced pancreatitis rats. Pancreatitis was induced by hepatic bile duct and common bile duct ligation. R-102444 was orally administered 30 min before the ligation. Data represent mean±S.E.M. of 8 animals. *$P<0.05$, **$P<0.01$ vs. control (vehicle-treated group).

male Sprague-Dawley rats (weighing about 250 g) were obtained from Japan SLC (Shizuoka, Japan). Female ICR mice (weighing 10–15 g) were obtained from Charles River Inc. (Atsugi, Japan). The animals were housed in temperature-controlled (23±2 °C) rooms with a 12-h light/dark cycle.

2.2. Caerulein-induced acute pancreatitis

Male Wistar rats were fasted for 16–18 h before the experiment. Rats received four intraperitoneal injections of caerulein (20 μg/kg) at hourly intervals. Blood was collected for measurement of serum amylase and lipase activities from the jugular vein under light ether anesthesia. R-102444 was suspended in 0.5% gum tragacanth (vehicle) and orally administered to rats in a volume of 1 ml/kg 30 min before the initial caerulein injection. In our preliminary results, vehicle (0.5% gum tragacanth) had no effect on amylase or lipase activity.

2.3. Pancreatic duct ligation-induced acute pancreatitis

Pancreatic duct ligation-induced pancreatitis in rats was produced according to the methods previously reported (Samuel et al., 1994; Taniguchi et al., 1997) with partial modification. After overnight fasting, Sprague-Dawley rats were anesthetized with ether and the abdomen was opened by midline incision. The hepatic bile duct and the common bile-pancreatic duct were ligated with silk thread, and then the abdomen was closed. Six hours after the ligation, blood for measurement of serum amylase and lipase activities was collected from the abdominal aorta under pentobarbital anesthesia (40 mg/kg, i.p.). R-102444 was suspended in 0.5% gum tragacanth (vehicle) and orally administered to rats in a volume of 1 mL/kg 30 min before the ligation.

2.4. Choline-deficient, ethionine-supplemented diet-induced acute pancreatitis

After overnight fasting, mice were fed a choline-deficient, 0.5% D,L-ethionine-supplemented diet (Oriental Yeast, Tokyo, Japan) for 64 h. R-96544 was dissolved in saline and subcutaneously administered to rats in a volume of 1 mL/kg twice a day (9:00 and 17:00), starting with 1 h before starting the choline-deficient, ethionine-supplemented diet. After the end of the study period, blood was collected for measurement of serum amylase via heart puncture under light ether anesthesia. Pancreatic tissues were isolated, fixed in 10% formaldehyde, embedded in paraffin, stained with hematoxylin-eosin, and

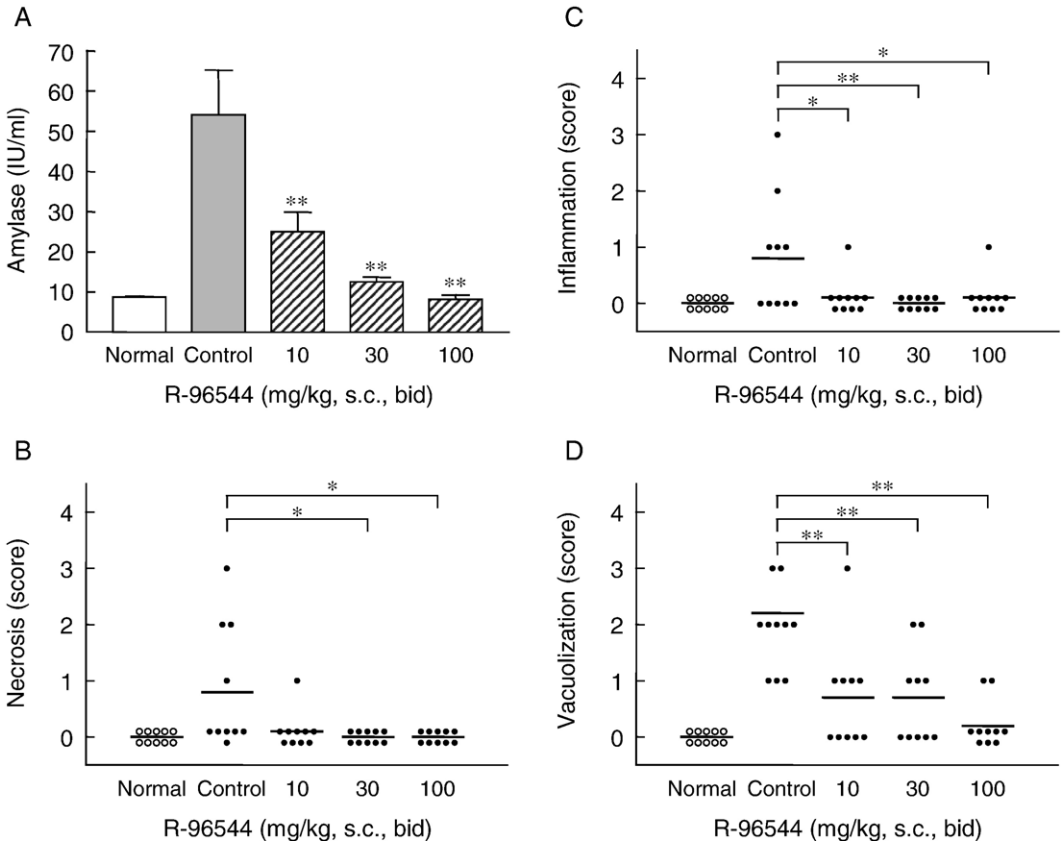

Fig. 3. Effect of R-96544 on serum amylase activity (A), necrosis (B), inflammation (C) and vacuolization (D) in choline-deficient, ethionine-supplemented diet-induced pancreatitis mice. Pancreatitis was induced by feeding the mice a choline-deficient, ethionine-supplemented diet for 66 h. R-96544 was subcutaneously administered to the mice twice a day. Data represent mean±S.E.M. (A) and individual scores of 10 animals (B–D). The histological grading of necrosis, inflammation and vacuolization refers to the approximate percentage of cells involved: 0=<5%, 1=5–15%, 2=15–35%, 3=35–50%, 4=>50%. *$P<0.05$, **$P<0.01$ vs. control (vehicle-treated group).

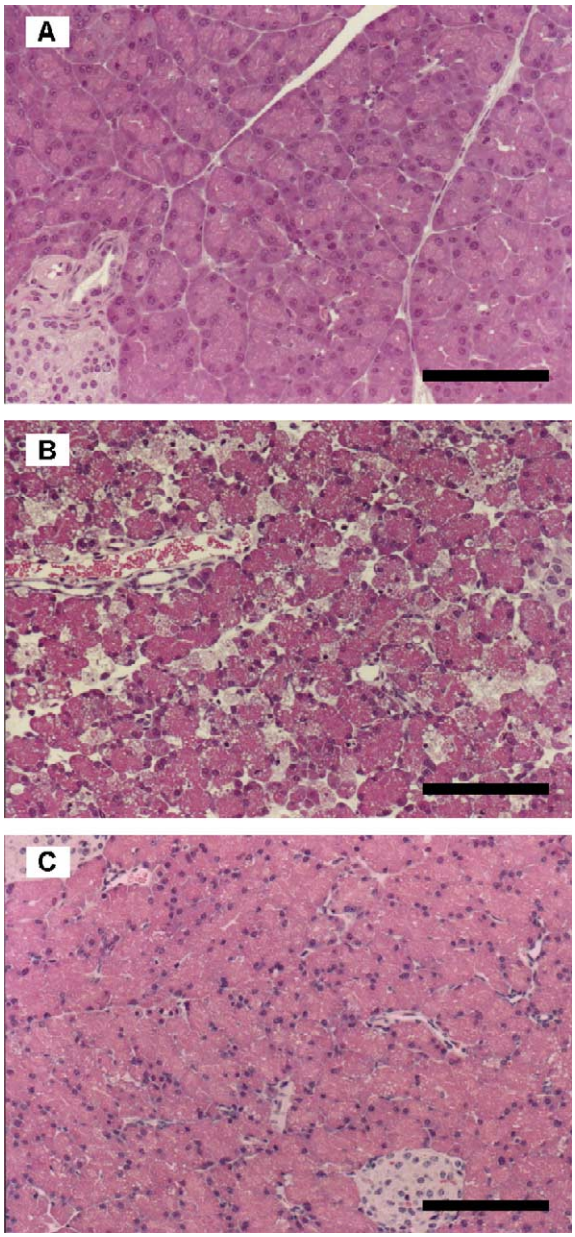

Fig. 4. Pancreatic histology of mice after 66 h on a choline-deficient, ethionine-supplemented diet. (A) Normal pancreatic histology of a mouse fed a standard diet. (B) Control pancreatic histology from a mouse fed the choline-deficient, ethionine-supplemented diet. Note marked necrotic cells and inflammatory cell infiltration, and numerous cytoplasmic vacuolization of acinar cells. (C) Pancreatic histology of a mouse that received subcutaneous injection of 100 mg/kg R-96544 (bid) while on the choline-deficient, ethionine-supplemented diet. Note suppression of the acute pancreatic lesions. Scale bar=5 μm.

examined microscopically by an experienced morphologist who was not aware of the treatment. We also collected blood and pancreatic tissues from mice fed a normal diet instead of a choline-deficient, ethionine-supplemented diet. Three features were assessed and scored: necrosis, inflammation and vacuolization. Each of these was graded from 0 to 4 as follows: 0, less than 5% of the cut section involved; 1, 5–15% of the section involved; 2, 15–35% of the section involved; 3, 35–50% of the section involved; and 4, more than 50% of the section involved.

2.5. Chronic pancreatitis model

Three-month-old Wistar Bonn/Kobori rats were divided into 3 groups: control (n=32), low-dose (n=24) and high-dose (n=24). Studies were performed for 0- (control group only), 1-, 4- and 6-month dosing periods. R-102444 was administered in the diet (F-2, Oriental Yeast, Tokyo, Japan) at 0.017% to the low-dose group and at 0.17% to the high-dose group. Doses of R-102444 calculated from food consumption averaged 6.4 mg/kg/day in the low-dose group (0.017%) and 76 mg/kg/day in the high-dose group (0.17%) throughout the experiments. After each dosing period, rats (n=8/group) were anesthetized with pentobarbital (40 mg/kg, i.p.), and pancreatic tissues were isolated. Wet weight of pancreatic tissues was measured and part of the pancreas was stored at −70 °C until assay. Pancreatic tissue (5–10 mg) for the assay for protein content and amylase and lipase activities was homogenized in saline using Branson's sonifier (model 450). At 9 months of age, histological examination was carried out. The pancreas (obtained from the lobes close to the spleen) was fixed in buffered formalin, sectioned into 5 parts, embedded in paraffin and stained with hematoxylin and eosin for light microscopic observation.

2.6. Determination of protein content, and amylase and lipase activities

Protein content was measured using a DC protein assay kit® (Biorad, CA, USA). Activities of amylase and lipase were measured by Amylase-Test Wako® (Wako, Osaka, Japan) and Lipase Kit S® (Dainippon Pharmaceutical, Osaka, Japan), respectively, according to the manufacturers' instructions. The values of serum amylase and lipase activities are expressed as units per milliliter (IU/ml).

2.7. Statistical analysis

Values are expressed as mean±S.E.M. Statistical analyses were performed by nonparametric Dunnett's test based on the joint ranking method for histological scores and parametric Dunnett's test for other values using an SAS statistical computer package (SAS Institute Inc., Cary, NC). A P value of less than 0.05 was considered significant.

2.8. Drugs and chemicals

R-102444 and R-96544 were chemically synthesized at Sankyo (Tokyo, Japan). Caerulein was purchased from Bachem AG (Bubendorf, Switzerland).

3. Results

3.1. Caerulein-induced acute pancreatitis in rats

An intraperitoneal injection of caerulein into rats at a dose of 20 μg/kg at hourly intervals over 4 h markedly increased serum amylase and lipase activities. The levels of serum amylase

activity in R-102444 (10–100 mg/kg, p.o.)-administered rats were significantly lower than those in control animals (Fig. 1A). Elevation of lipase activity, parallel to amylase, which reached a maximum 6 h after the first caerulein injection, was also significantly lower in R-102444-administered rats (Fig. 1B).

3.2. Pancreatic duct ligation-induced acute pancreatitis in rats

Serum amylase and lipase activities were measured 6 h after the ligation of both the hepatic bile duct and the common bile-pancreatic duct since time-dependent (2–6 h) increase in serum amylase activity (data not shown) had been observed in a preliminary experiment. In the control group, amylase and lipase activities were elevated compared to those in the sham operation group (Fig. 2). Oral administration of R-102444 (0.3–10 mg/kg) 30 min before the operation significantly reduced serum amylase and lipase activities in a dose-dependent manner.

3.3. Choline-deficient, ethionine-supplemented diet-induced acute pancreatitis in mice

We measured serum amylase activity of mice 66 h after feeding a choline-deficient, ethionine-supplemented diet, because of deaths among the mice after 70 h in a preliminary experiment (data not shown). R-96544 (10–100 mg/kg, s.c., bid), the active form of R-102444, dose-dependently reduced serum amylase activity (Fig. 3A). Pancreatic morphology by light microscopic observation indicated apparent acute pancreatic lesions such as necrosis, inflammation and vacuolization in mice fed a choline-deficient, ethionine-supplemented diet (Fig. 4A and B). Necrosis and inflammation were almost completely inhibited by R-96544 at the low dose of 10 mg/kg (Fig. 3B and C). Statistically significant inhibition by R-96544 (10–100 mg/kg) was also observed in vacuolization (Fig. 3D) although almost complete inhibition was observed at the relatively high dose of 100 mg/kg.

3.4. Chronic pancreatitis in Wistar Bonn/Kobori rats

The pancreas of male Wistar Bonn/Kobori rats shows distinct infiltration of inflammatory cells with interstitial edema in the acini as in early-stage acute pancreatitis (<3 months of age), which gradually progresses to chronic pancreatitis with widespread fibrosis and acinar atrophy leading to atrophy of the pancreas, similar to the lesions of chronic pancreatitis in humans (Su et al., 2000; Kakinuma et al., 1999; Ohashi et al., 1990). To assess the effect of R-102444 on the progression of chronic pancreatitis, R-102444 was administered to 3-month-old male Wistar Bonn/Kobori rats (~9 months old).

During the experiment, there were no significant differences in body weight between the control and R-102444-treated groups (Table 1). Although two deaths occurred, one was in the low-dose group at 4 months of age and the other was in the high-dose group at 6 months of age. These deaths were probably spontaneous since they were independent of dose and duration of administered R-102444.

There were no significant differences in pancreatic weight, pancreatic protein or amylase content in the control group between 3 and 4 months of age. At 7 and 9 months of age, significant reductions in the above pancreatic parameters were observed in the control group, suggesting progression to severe chronic pancreatitis. On the other hand, in the R-102444-treated groups, the values of these parameters were higher than those in the control group. In the high-dose group in particular, the values of all these parameters were ameliorated.

Table 1
Body weight and pancreatic wet weight, protein and amylase content in Wistar Bonn/Kobori rats treated with R-102444

	Age of rats			
	3 months	4 months	7 months	9 months
Body weight (g)				
Control	325.2±4.0 (n=8)	366.8±4.0[a] (n=8)	430.1±6.1[a] (n=8)	415.1±7.4[a] (n=8)
Low dose	–	364.0±3.1 (n=8)	411.4±7.7 (n=8)	432.6±7.8 (n=8)
High dose	–	357.6±3.1 (n=8)	408.9±3.7 (n=8)	436.7±6.0 (n=8)
Pancreatic weight (mg/100 g BW)				
Control	300.6±6.8 (n=8)	294.1±12.6 (n=8)	207.0±8.7[a] (n=8)	193.0±6.1[a] (n=8)
Low dose	–	256.2±10.9 (n=8)	242.5±7.1[b] (n=8)	215.3±5.3 (n=8)
High dose	–	281.7±11.4 (n=8)	240.5±9.3[b] (n=8)	224.9±10.0[b] (n=8)
Protein (mg/pancreas)				
Control	202.8±9.9 (n=8)	163.4±11.2 (n=8)	131.1±14.0[a] (n=8)	127.0±13.0[a] (n=8)
Low dose	–	169.8±11.1 (n=8)	187.0±8.3[c] (n=8)	175.3±4.9[c] (n=7)
High dose	–	186.0±19.5 (n=8)	179.5±8.9[c] (n=8)	194.2±10.7[c] (n=7)
Amylase (×1000 IU/pancreas)				
Control	17.1±1.3 (n=8)	13.7±3.1 (n=8)	6.1±2.6[a] (n=8)	3.3±1.4[a] (n=8)
Low dose	–	17.8±3.8 (n=8)	12.6±1.9[b] (n=8)	6.9±1.7 (n=7)
High dose	–	17.9±3.7 (n=8)	13.5±2.6[b] (n=8)	12.5±2.1[c] (n=7)

Values are expressed as the mean±S.E.M. (n=8 except for low dose and high dose groups in 9 months).
[a] P<0.01 vs. pre-administration value (3 months of age).
[b] P<0.05 vs. control.
[c] P<0.01 vs. control.

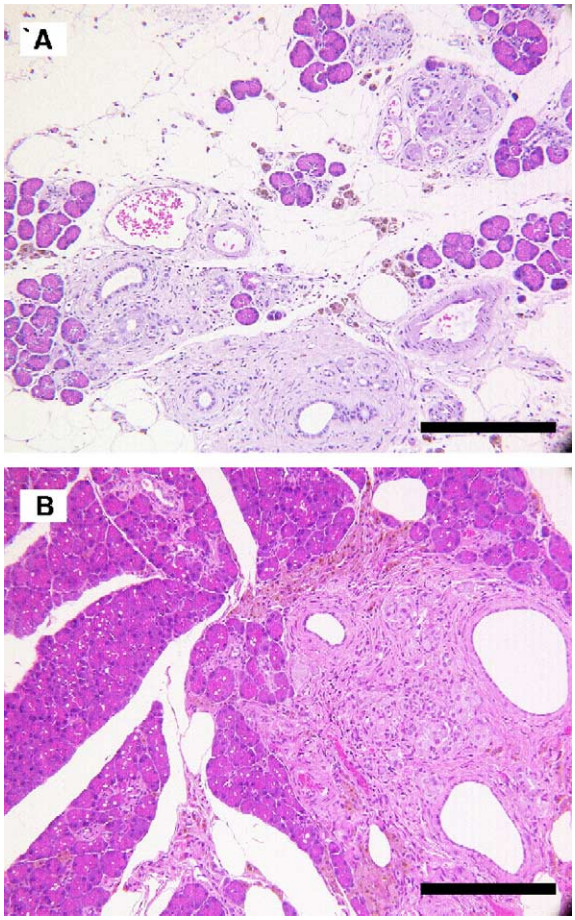

Fig. 5. Pancreatic histology of Wistar Bonn/Kobori rats at 9 months of age. (A) Control pancreatic histology of a rat receiving no additional treatment. Note marked fibrosis, parenchymal destruction and replacement with adipose tissue. (B) Pancreatic histology of a rat that received a diet containing 0.17% R-102444. Note suppression of the chronic pancreatic lesions. Scale bar=5 μm.

Histological examination showed progression of chronic pancreatitis lesions in the control group at 9 months of age (Fig. 5A). Parenchymal destruction, fibrosis and replacement with adipose tissue similar to that found in human chronic pancreatitis were evident. In contrast, these chronic pancreatic lesions were reduced in the R-102444 treated groups, especially the high-dose group (Fig. 5B). Table 2 summarizes the lesion scores of chronic pancreatitis in Wistar Bonn/Kobori rats and shows the parenchymal destruction and replacement with adipose tissue were significantly reduced by treatment with the high dose of R-102444. Although the decrease in fibrosis was not statistically significant ($P=0.059$ for the high dose group), a tendency of decrease was evident.

4. Discussion

In the present study, we investigated the effects of R-102444 and its active form R-96544, selective and potent 5-HT_{2A} receptor antagonists (Ogawa et al., 2002, 2004; Tanaka et al., 2000), on acute and chronic experimental pancreatitis models. Oral administration of R-102444 to rats inhibited progression of acute pancreatitis induced by caerulein and by pancreatic duct ligation. Subcutaneous injection of R-96544 reduced serum amylase activity and pancreatic lesions in choline-deficient, ethionine-supplemented diet-fed mice. In Wistar Bonn/Kobori rats, a model of spontaneously occurring chronic pancreatitis, the beneficial effects of R-102444 were obvious in reduction of pancreatic weight, pancreatic protein and amylase content. These beneficial effects are closely related to pancreatic histology. These findings suggest that R-102444 and R-96544 are effective against the progression of acute and chronic experimental pancreatitis.

In the present study, we showed the effectiveness of 5-HT_{2A} receptor antagonists in three distinctive experimental acute pancreatitis models. Oral administration of R-102444 reduced serum pancreatic enzyme activity in rats treated with caerulein. Subcutaneously administered R-96544 prevented progression of acute pancreatitis in choline-deficient, ethionine-supplemented diet-induced acute pancreatitis in mice. These results are consistent with the findings of previous reports on ketanserin treatment of acute pancreatitis induced by caerulein and a choline-deficient, ethionine-supplemented diet (Oguchi et al., 1992; Yoshino and Yamaguchi, 1997). We further demonstrated that a 5-HT_{2A} receptor antagonist reduced serum amylase and lipase activities, and pancreatic lesions in acute pancreatitis induced by pancreatic duct ligation. These results are additional evidence that 5-HT_{2A} receptor function is commonly involved in experimental acute pancreatitis.

In choline-deficient, ethionine-supplemented diet-induced acute pancreatitis, subcutaneously injected R-96544 inhibited not only necrosis and inflammation cell infiltration, but also cytoplasmic vacuolization, a pancreatic lesion appearing in early-stage acute pancreatitis in several animal models and in clinical pancreatitis (Adler et al., 1985; Niederau and Grendell, 1988; Saluja et al., 1987). This is inconsistent with the findings of a previous report (Yoshino and Yamaguchi, 1997), which described the ineffectiveness of a 5-HT_{2A} receptor antagonist on cytoplasmic vacuolization. This discrepancy might be due to the

Table 2
Pancreatic lesions in Wistar bonn/Kobori rats treated with R-102444

Group	Parenchymal destruction							Fibrosis							Replacement with adipose tissue						
	0	1	2	3	4	5	*P* value	0	1	2	3	4	5	*P* value	0	1	2	3	4	5	*P* value
Control	–	–	1	6	–	1		–	–	–	3	4	1		–	–	–	2	4	2	
Low dose	–	–	3	4	–	–	0.337	–	–	–	5	2	–	0.317	–	–	2	3	2	–	0.098
High dose	–	1	4	2	–	–	0.027	–	–	1	5	1	–	0.059	–	2	1	4	–	–	0.004

Histological grading of pancreatic lesions was scored by the approximate percentage of tissue involved: 0, absent; 1, focal; 2, <10%; 3, 10–30%; 4, 30–50%; 5, >50%.

agents used. Ketanserin and cyproheptadine, which have adrenalin α_1 antagonistic activity leading to decrease in blood pressure (Fozard, 1982; Cohen et al., 1983; Vanhoutte et al., 1983), were used in that study. Hypotensive effects of those agents probably reduced blood and drug supply to pancreatic microcirculation. In contrast, we used a selective 5-HT_{2A} receptor antagonist, R-96544, which has no effect on blood pressure (Ogawa et al., 2002). The discrepancy in the effects of 5-HT_{2A} receptor antagonists on cytoplasmic vacuolization could also be due to more efficient pancreatic blood flow by R-96544 compared with ketanserin and cyproheptadine. Taken together, 5-HT_{2A} receptor activation appears to be involved in acute pancreatitis from the early stage, and a highly selective 5-HT_{2A} receptor antagonist without hypotensive action would be highly effective for treatment of acute pancreatitis.

Chronic pancreatitis is an intractable disease featuring irreversible irregular scarring of the exocrine parenchyma characterized by acinar destruction and fibrosis subsequent to inflammation in the pancreas (Mergener and Baillie, 1997; Ammann and Muellhaupt, 1994). However, the mechanism of the progression of chronic pancreatitis, especially the involvement of 5-HT_{2A} receptor activation, has not yet been fully clarified. Previous findings in male Wistar Bonn/Kobori rats indicated pancreatic changes similar to those observed in chronic pancreatitis in humans (Ohashi et al., 1990), suggesting that the Wistar Bonn/Kobori strain of rat would be a useful model of human chronic pancreatitis. In fact, several promising agents for treatment of chronic pancreatitis have been evaluated in this model (Graf et al., 2002; Ito et al., 1998). Thus, we used Wistar Bonn/Kobori rats to examine the effect of a 5-HT_{2A} receptor antagonist on the progression of chronic pancreatitis. Treatment of Wistar Bonn/Kobori rats with R-102444 for 6 months inhibited the atrophy of the pancreas as indicated by changes in pancreatic weight, protein content and amylase activity. Histological examination also showed significantly lower parenchymal destruction replaced with adipose tissue in rats treated with R-102444. These results clearly show that R-102444 attenuated atrophy of the pancreas leading to chronic pancreatitis. To our knowledge, this is the first report indicating effectiveness of a 5-HT_{2A} receptor antagonist in the progression of chronic pancreatitis.

Pancreatic microcirculatory impairment has long been recognized as one of the etiological events of acute pancreatitis (Kaska et al., 2000; Zhou et al., 2002; Gullo et al., 1996; Klar et al., 1990). This might be related to leaked enzyme from the damaged pancreas inducing platelet activation (Prinz et al., 1984), leading to impairment of microcirculation. Indeed, elevation of serum 5-hydroxyindoleacetic acid, a metabolite of 5-HT which is a potent activator of platelet aggregation and vasoconstriction, was observed in mice with acute pancreatitis (Yoshino and Yamaguchi, 1997). Therefore, the mechanism underlying the effect of a 5-HT_{2A} receptor antagonist on the progression of pancreatitis seems to be improvement in pancreatic blood flow by interfering with receptor-mediated platelet activation and vasoconstriction. Chronic pancreatitis is also associated with a reduction in pancreatic blood flow (Schilling et al., 1999; Patel et al., 1995). In Wistar Bonn/Kobori rats, enhanced blood coagulation and platelet aggregation have been observed at 6–9 months of age and chronic pancreatic lesions have been speculated to be associated with pancreatic ischemia (Nobukata et al., 2000). These findings strongly suggest that effectiveness of a 5-HT_{2A} receptor antagonist in chronic pancreatitis in Wistar Bonn/Kobori rats is also due to the prevention of thrombus formation in pancreatic microcirculation.

The present study provides additional evidence for the involvement of 5-HT_{2A} receptor activation in the progression of experimental pancreatitis. The findings point to the potential usefulness of a 5-HT_{2A} receptor antagonist in acute and chronic pancreatitis, although further experiments are necessary to clarify this role.

Acknowledgements

We thank Ms. Tamami Shimoji and Ms. Takako Nagasawa for their expert technical assistance.

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