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Blockade of bradykinin B_2 receptor suppresses acute pancreatitis induced by obstruction of the pancreaticobiliary duct in rats

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1 The involvement of bradykinin (BK) B_2 receptor in acute pancreatitis induced by pancreaticobiliary duct ligation was investigated in rats.

2 The activities of amylase and lipase in the serum, the water content of the pancreas, and vacuolization of the acinar cells were significantly increased 2 h after obstruction of the duct in Sprague-Dawley rats.

3 Elevated serum amylase activity, increased pancreatic oedema, and damage of the pancreatic tissue were significantly less marked in plasma kininogen-deficient, B/N-Katholiek rats than in the normal strain, B/N-Kitasato rats 2 h after the ligation.

4 Obstruction of the pancreaticobiliary duct augmented the level of (1-5)-BK (Arg[1]-Pro[2]-Pro[3]-Gly[4]-Phe[5]), a stable BK metabolite, in the blood from 73.0 ± 21.7 pg ml^{-1} at 0 h to 149.8 ± 38.0 pg ml^{-1} at 2 h after the induction of pancreatitis in SD rats.

5 Administration of a BK B_2 receptor antagonist, FR173657 (100 mg kg^{-1}, p.o.) or Hoe140 (100 nmol kg^{-1}, s.c.), reduced the elevation of amylase and lipase activities in the serum and of pancreatic water content in a dose-dependent manner. The effective attenuation of oedema formation and vacuolization by the antagonists was also confirmed light-microscopically. In contrast, treatment with gabexate mesilate or indomethacin did not cause significant suppression of the pancreatitis.

6 These findings suggest a possible involvement of kinin B_2 receptor in the present pancreatitis model. Furthermore, they point to the potential usefulness of the B_2 receptor in clinical acute pancreatitis.

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Keywords: Bradykinin antagonist; Brown Norway Katholiek; FR173657; Hoe140; kinin B_2 receptor; pancreatitis; oedema; pancreaticobiliary duct

Abbreviations: (1-5)-BK, Arg[1]-Pro[2]-Pro[3]-Gly[4]-Phe[5]; BK, bradykinin; BN, Brown Norway; CCK, cholecystokinin; ELISA, enzyme-linked immunosorbent assay; SD, Sprague-Dawley

Introduction

The clinical features of acute pancreatitis range over a broad spectrum of severity, from a mild self-limiting character to a rapid mortality rate. The disease is characterized by a massive oedema of the pancreas and the adjacent retroperitoneal tissue, interstitial activation of proteolytic enzymes, increases of serum amylase and lipase activities, hypovolaemia, hypoalbuminaemia, pulmonary oedema and severe pain. To induce experimental pancreatitis in animals, a number of procedures have been used. These include ligation of the pancreatic duct, injection of bile salts into the duct, infusion of oleic acid, or infusion of the cholecystokinin (CCK) analogue caerulein (Adler *et al*., 1985). The latter procedure produces hyperstimulation of the exocrine secretion of pancreatic enzymes and leads to morphological changes similar to those seen in acute pancreatitis in man (Willemer *et al*., 1990). Some of the clinical symptoms of acute pancreatitis have been attributed to the activation of the kallikrein-kinin system (Ruud *et al*., 1985), since serum bradykinin and kallikrein levels in ascites are elevated during the course of the disease (Satake *et al*., 1973a, b). A few bradykinin (BK) receptor antagonists have been tested in secretagogue-induced acute pancreatitis (Félétou *et al*., 1995; Griesbacher & Lembeck, 1992; Griesbacher *et al*., 1993; Hoffman *et al*., 1996; Kanbe *et al*., 1996). In contrast, pharmacological information on the antagonist in models of obstruction-induced pancreatitis is sparse. Therefore, in the present work, the involvement of BK was examined in pancreatitis induced by the pancreaticobiliary duct ligation in rats using the B_2 receptor antagonists and a rat strain deficient in the kinin-precursor protein kininogen.

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Methods

Acute pancreatitis induced by duct ligation in rats

Animals were housed in the Experimental Animal Center of the Kitasato School of Medicine for 1 week and acclimatized in the laboratory for 1 h before the commencement of each experiment. All of the following procedures were conducted in accordance with the guideline principles of the Animal Care Committee of Kitasato University for the care and use of laboratory animals.

Male 8-week-old Sprague-Dawley (SD) rats (SLC Japan, Shizuoka, Japan), as well as inbred male Brown Norway Katholiek rats (plasma kininogen-deficient) and Brown Norway Kitasato rats (the normal strain), both 8–10 weeks old, kept in the Experimental Animal Center of Kitasato University School of Medicine, were used.

The rats were anaesthetized with 50 mg kg^{-1} of pentobarbitone sodium (Nembutal, Abbott Laboratories, North Chicago, IL, U.S.A.) given intraperitoneally. A left paramedian incision was made and the pancreaticobiliary duct was carefully exposed at its orifice in the duodenal wall. A tourniquet of polypropylene suture material (Ethicon 5-0 Prolene, Ethnor Pty Ltd., Sydney, Australia) was looped around the pancreaticobiliary duct adjacent to the duodenal wall without ligation. Care was taken to avoid trauma to blood vessels and the pancreas and to prevent drying of the pancreatic tissue. A 2-cm-long transverse incision in the skin at the anterior end of the back was made. A polyethylene cannula (Intramedic PE50, Clay-Adams/Becton Dickinson, Sparks, MD, U.S.A.) was tunnelled subcutaneously from the incision in the back to the abdominal cavity. One end of the cannula was passed through the right abdominal wall, and a loop of the suture material to be used as a tourniquet, was inserted into the cannula, and tied and secured outside the other end to prevent its being drawn back through. The other end of the cannula was placed subcutaneously in the incision in the back. Abdominal and back wounds were closed by suturing. Rats were allowed to recover, and to have free access to food and water for 7 days. For obstruction of the pancreaticobiliary duct, the skin on the anterior end of the back was incised again under light anaesthesia with diethyl ether. The cannula embedded in the back was exposed and the tourniquet in the cannula was drawn out firmly and secured. As a result, the pancreaticobiliary duct was constricted and obstructed. The cannula was firmly tied with the 5-0 Prolene tourniquet. This caused the pancreaticobiliary duct to be continuously obstructed. Rats, which were treated with the same procedures except for obstruction by the tourniquet, were used as the sham-operated control group.

Collection of blood samples

Blood was taken from the abdominal aorta of the rats under light anaesthesia with diethyl ether by using a needle with a polypropylene syringe. The blood was allowed to coagulate and was then centrifuged for 15 min at $2000 \times g$. The serum was collected and stored at -80°C until use. For measurement of (1-5)-BK, 2 ml of blood was taken into a polypropylene syringe containing 8 ml of ice-cold absolute ethanol. The mixture was transferred from the syringe into a polypropylene tube, and was centrifuged at $3000 \times g$ for 20 min at 4°C. The supernatant was evaporated to dryness in a vacuum centrifuge, and the dried residue was stored at -20°C.

Quantification of pancreatic oedema

Pancreatic oedema was estimated as water content. After exsanguination of the rat, a portion of the pancreas about 1 g in wet weight was excised and weighed. The tissue was dried in a vacuum centrifuge at 60°C and reweighed after 2 days. The difference between wet weight and dry weight was calculated. The increased water content of the tissue was expressed as a percentage of the water content of a normal rat pancreas.

Determination of serum enzyme activities

The colorimetric measurements were used for pancreatic enzyme activities in the serum. The serum activities of amylase (Caraway, 1959) and lipase (Williamson, 1976) were measured by modified methods using Amylase-Test Wako (Wako Pure Chemical Industries Ltd., Osaka, Japan) and Lipase Kit S (Dainippon Pharmaceutical Corp., Osaka, Japan), respectively, according to the manufacturers' instructions.

Determination of an enzyme-linked immunosorbent assay (ELISA) for the stable BK metabolite (1-5)-BK (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5).

The ethanol extract was washed three times with 5 ml of diethyl ether to remove lipid and prevent lipid interference with the assay. The washed sample was dissolved in 4 ml of distilled water acidified with 0.2 ml of 0.01 N HCl, and the mixture was applied to a Sep-Pak C_{18} cartridge column (Waters, Milford, MA, U.S.A.). After being washed with 12 ml of distilled water and 4 ml of 0.1 M acetic acid, (1-5)-BK was eluted with 6 ml of 80% (v v^{-1}) acetonitrile containing 0.1 M acetic acid. The kinin fraction was evaporated at reduced pressure, and the residue was dissolved in 500 μl of the assay buffer. The level of (1-5)-BK was determined with an ELISA kit for (1-5)-BK (Markit M (1-5)-BK, Dainippon Pharmaceutical Corp.) (Majima *et al*., 1996).

Microscopic observation

A portion of the pancreas was immediately fixed with 10% paraformaldehyde in 0.1 M phosphate buffered saline (pH 7.4). After the fixation, the tissue was dehydrated with a graded ethanol series, and embedded in paraffin. A thin section of paraffin-embedded tissue 3 μm in thickness was cut with a microtome, mounted on a glass slide, deparaffinized with xylene, and then stained with hematoxylin-eosin for light microscopy. Quantitative analysis was done using a Pixera 120es digital camera system (model no. PVC100N, Pixera Corp., Los Gatos, CA, U.S.A.) at a magnification of $\times 400$. Images of randomly photographed fields were processed in a Scion Image (Scion Corp., Frederick, MD, U.S.A.). Analysis of vacuolization was performed by a researcher blind to the specific treatment conditions. The numbers and the areas of vacuoles were quantified for an area of the photographic field corresponding to 0.1 mm^2 of pancreatic tissue.

Statistical analysis

Results show mean $\pm$ s.e.mean, with the number of observations indicated in parentheses. For the comparison of data from two groups, Student's *t*-test was used to evaluate the

significance of differences. For comparison of data from multiple groups, ANOVA followed by *post-hoc* Fisher's PLSD test was used. A probability (P) value of less than 0.05 was taken to indicate statistical significance.

Drugs and chemicals

FR173657 ((E)-3(6-acetamido-3-pyridyl)-N-[N-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-N-methylamino-carbonylmethyl]acrylamide) was kindly provided by Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). It was suspended in 5% (w v^{-1}) of gum arabic and administered orally 1 h before the start of ligation of the pancreaticobiliary duct. Indomethacin (Wako Pure Chemical Industries Ltd.) was also suspended in 5% (w v^{-1}) of gum arabic and administered intraperitoneally 1 h before the obstruction. Hoe140 (D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg) was purchased from the Peptide Institute, Inc. (Osaka, Japan), and injected subcutaneously 30 min before the obstruction. Gabexate mesilate (FOY: ethyl-4-(6-guanidinohexanoyloxy) benzoate methanesulfonate) was purchased from Ono Pharmaceutical Co. (Osaka, Japan). It was injected intravenously 5 min before the obstruction. All other substances used were of analytical grade, and were purchased from commercial sources.

Results

Serum pancreatic enzymes and pancreatic oedema

The activity of amylase in the blood serum and the pancreatic water content had not changed significantly 20 min after the start of the obstruction of the pancreaticobiliary duct, but was markedly augmented at 2 h ($P<0.01$, Figure 1A,C), Lipase activity significantly increased 20 min after the obstruction ($P<0.01$, Figure 1B). The values of those inflammatory parameters showed peaks at 2 h, and had gradually decreased 8 h after the obstruction.

Comparison between acute pancreatitis in kininogen-deficient strain and that in the normal strain of rats

The activities of pancreatic enzymes in the serum and in the pancreatic oedema fluid were compared between plasma kininogen-deficient, B/N-Katholiek rats and the normal strain, B/N-Kitasato rats. At 2 h after the start of the obstruction of the pancreaticobiliary duct, serum amylase and lipase activities and the pancreatic water content in B/N-Kitasato rats were 1.9, 2.3, and 4.1 fold higher, respectively, than those in B/N-Katholiek rats ($P<0.05$, Figure 2). In the sham operation, no differences in those parameters were observed between the two strains of rats.

Change in level of (1-5)-BK in blood after obstruction of pancreaticobiliary duct

Blood was drawn at 0, 2, and 8 h after the obstruction of the pancreaticobiliary duct in SD rats. As a stable BK metabolite, (1-5)-BK was determined by ELISA. Its blood level increased significantly, from 73.0 ± 21.7 pg ml^{-1} ($n=12$) at 0 h to 149.8 ± 38.0 pg ml^{-1} ($n=5$) at 2 h after the induction of pancreatitis ($P<0.01$). It returned to the initial level at 8 h (69.9 ± 4.4 pg ml^{-1}, $n=8$).

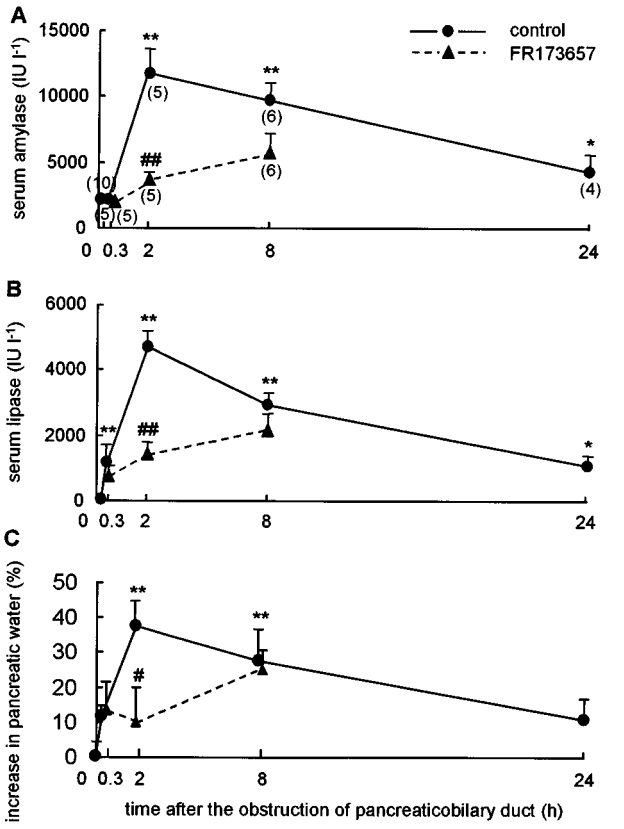

Figure 1 Changes in activities of amylase (A) and lipase (B) in serum and increase in water content in pancreas (C). Acute pancreatitis was induced in SD rats by the ligation of the pancreaticobiliary duct at 0 h. Serum and pancreatitis were collected at the indicated times to determine amylase activity (A) and lipase activity (B) in the serum, and of the amount of pancreatic water (C). Control rats received 5% (w v^{-1}) of gum arabic (3 ml kg^{-1}) orally 1 h prior to the start of occlusion. FR173657 (100 mg kg^{-1}) was administered orally 1 h before the obstruction and rats were sacrificed at the indicated times. Results show mean $\pm$ s.e.mean, with the number of observations indicated in parentheses. For comparison of data from multiple groups, one-way ANOVA followed by *post-hoc* Fisher's PLSD test was used. The significance of differences between sham-operated rats at 0 h and rats whose pancreaticobiliary duct was obstructed is indicated as follows: $*P<0.05$ and $**P<0.01$; and that between control rats and rats given FR173657 as follows: $\#P<0.05$; $\#\#P<0.01$.

Effects of BK B_2 receptor antagonist and other inhibitors on serum activities of amylase and lipase, and on pancreatic oedema in acute pancreatitis

The effects of an orally active BK B_2 receptor antagonist, FR173657, and a peptidic antagonist, Hoe140, on acute pancreatitis were evaluated. FR173657 (100 mg kg^{-1}) was administered orally 1 h before the start of the obstruction. At 2 h after the obstruction, the elevation of amylase and lipase activities in the serum had been significantly reduced ($P<0.01$, Figures 1 and 3). The excess of pancreatic water content at 2 h was significantly decreased by treatment with FR173657 ($P<0.05$). However, the reduction was not significant at 8 h. The suppressive effects of FR173657 on

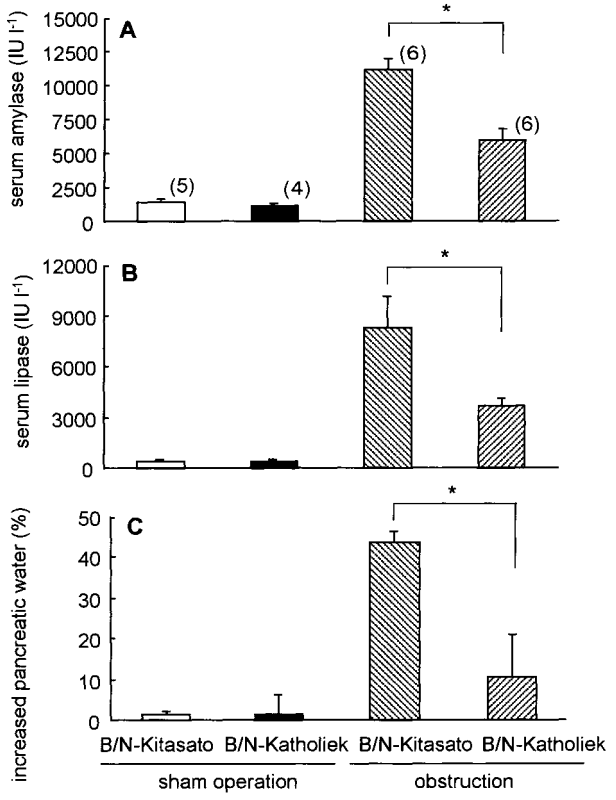

Figure 2 Acute pancreatitis induced by obstruction of the pancreaticobiliary duct in the kininogen-deficient (B/N-Katholiek) and normal (B/N-Kitasato) of rats. Rats were sacrificed at 2 h after the ligation of the pancreaticobiliary duct. Serum and pancreatitis were collected at the indicated times to determine amylase activity (A) and lipase activity (B) in the serum, and of any increase in pancreatic water (C). Rats treated with the same surgical procedures except for obstruction by the tourniquet were used as the sham-operated control group. Results are shown as mean ± s.e.mean with the number of observations indicated in parentheses. For comparison of the data between B/N-Katholiek and BN-Kitasato rats, Student's *t*-test was used to evaluate the significance of differences: $*P<0.05$.

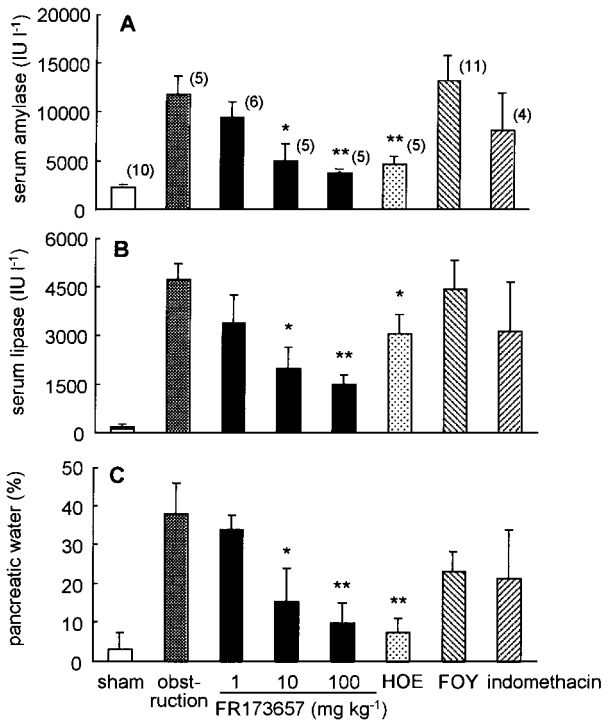

Figure 3 Effects of BK B_2 receptor antagonists and other inhibitors on serum activities of amylase (A), lipase (B), and pancreatic oedema (C) in acute pancreatitis. Acute pancreatitis was induced in SD rats by the ligation of the pancreaticobiliary duct. Serum and pancreatic tissue were collected at 2 h after the obstruction for the determination of amylase activity (A) and lipase activity (B) in the serum, and of the amount of pancreatic water (C). Control rats received 5% (w v^{-1}) of gum arabic (3 ml kg^{-1}) orally 1 h prior to the start of obstruction. FR173657 (10, 30 and 100 mg kg^{-1}) was administered orally 1 h before the obstruction and rats were sacrificed at 2 h after it. Hoe140 (100 nmol kg^{-1}) was subcutaneously injected 30 min prior to the obstruction. Gabexate mesilate (30 mg kg^{-1}, i.v.) or indomethacin (10 mg kg^{-1}, i.p.) suspended in 5% (w v^{-1}) of gum arabic was administered 5 min or 1 h, respectively, before the occlusion. Rats which received the same surgical procedures except the occlusion with a tourniquet were used as the sham-operated group. Results show mean ± s.e.mean, with the number of observations indicated in parentheses. For comparison of data from multiple groups, one-way ANOVA followed by *post-hoc* Fisher's PLSD test was used. The significance of the difference between the sham-operated rats that received gum arabic alone and rats given drugs is indicated as follows: $*P<0.05$; $**P<0.01$.

those parameters at 2 h were dose-dependent (Figure 3). Doses of more than 10 mg kg^{-1} of FR173657 significantly reduced the serum amylase and lipase levels and the water content. Another BK B_2 receptor antagonist, Hoe140 (100 nmol kg^{-1}), was subcutaneously injected 30 min prior to the obstruction. It also resulted in a suppressive effect on the pancreatitis (Figure 3). In contrast, administration of gabexate mesilate (30 mg kg^{-1}, i.v.) did not attenuate the enzyme activities or the oedema ($P=0.08$) in this acute pancreatitis. Treatment with indomethacin (10 mg kg^{-1}, i.p.) tended to suppress serum lipase activity and water content, but not significantly ($P=0.36$ and $P=0.181$, respectively) (Figure 3).

Effects of BK B_2 receptor antagonists on pancreatic morphology under light-microscopic observation

The possible involvement of BK in acute pancreatitis was evaluated in terms of the morphology of the pancreas at 2 and 8 h after obstruction (Figure 4). The ligation of the pancreaticobiliary duct resulted in the characteristic appearance of an oedematous variety of acute pancreatitis consisting of tissue oedema with widened interstitial spaces, and massive cytoplasmic vacuolization in the acinar cells (Figure 4B,C). The number and the area of the vacuoles after obstruction were quantified in an area of the photographic field corresponding to 0.1 mm^2 of pancreatic tissue under light microscopy. Significant increases in both number and area were observed at 2 and 8 h after obstruction ($P<0.01$, Figure 5A,B). The administration of FR173657 appeared to affect the morphologic changes seen in the pancreatic tissue, especially to reduce the formation of oedematous tissue (Figure 4D). Both FR173657 and Hoe140 significantly suppressed vacuolization in the pancreas ($P<0.01$, Figure 5C,D) at 2 h. On the other hand, the number and the area of the vacuoles in B/N-Katholiek rats were less than those in

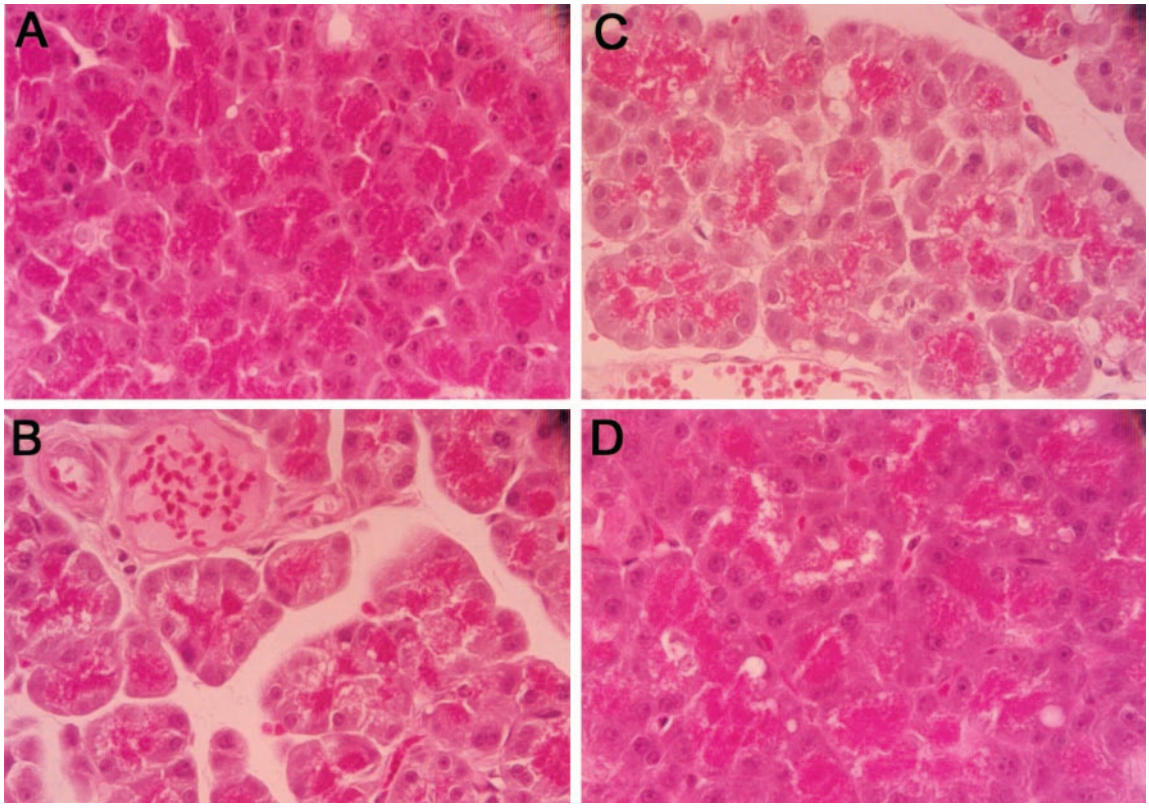

Figure 4 Typical histological features in light microscopic observation of acute pancreatitis induced by ligation of pancreaticobiliary duct and effects of FR173657 on development of pancreatitis. Pancreatic tissue was obtained at 2 (B) and 8 h (C) after the induction from SD rats with pancreatitis. FR173657 (100 mg kg^{-1}) was administered orally 1 h before obstruction and rats were sacrificed at 2 h after the obstruction of the pancreaticobiliary duct (D). Sham-operated rats (A) were treated with the same surgical procedures except for obstruction by the tourniquet. Magnification: $\times 400$.

B/N-Kitasato rats (Figure 5C,D) ($P<0.01$). The reduction of vacuolization by the BK B_2 receptor antagonists was comparable to that in B/N-Katholiek rats.

Discussion

Many animal models have been used in attempts to reproduce human acute pancreatitis. Ligation of the pancreaticobiliary duct system is characterized by increased intraductal pressure (Kueppers *et al*., 1993; Ohshio *et al*., 1991; Steer, 1988), a possible bile reflux into the pancreas, absence of pancreaticobiliary fluids from the duodenum and elevated serum CCK levels (Larsen *et al*., 1991; Murayama *et al*., 1991). The rationale for the use of this model is that obstruction of the sphincter of Oddi occurs, as in human gallstone disease (Acosta & Ledesma, 1974). In these aspects, it appears that this model of pancreaticobiliary duct obstruction differs from the experimental model of mild acute pancreatitis induced by caerulein (Steer, 1992). The pancreas is one of the organs with the highest tissue concentrations of kallikrein. Consequently, the roles of the kallikrein-kinin system in acute pancreatitis have been extensively investigated since as early as the 1960s (Ryan *et al*., 1964). The use of kinin receptor antagonists provided experimental evidence for the involvement of the kallikrein-kinin system in pancreatitis. Attempts to measure components of the system have been made (Griesbacher, 2000; Orlov & Belyakov, 1978). Treatment with BK B_2 receptor antagonists has been shown to be effective in acute pancreatitis induced by caerulein (Griesbacher & Lembeck, 1992; Griesbacher *et al*., 1993), taurocholic acid (Bloechle *et al*., 1998; Kanbe *et al*., 1996), and in postischaemic pancreatitis (Hoffmann *et al*., 1996). However, involvement of BK in acute pancreatitis induced by pancreatic duct obstruction is unclear. Accordingly, kinin B_2 receptor antagonists and a rat strain deficient in the kinin-precursor protein, kininogen, were used for the present study.

Elevated activities of amylase and lipase in the serum, as well as pancreatic oedema, were observed in rats after obstruction with a pancreaticobiliary tourniquet. In order to ascertain whether BK is involved in those characteristics of acute pancreatitis in this model, the BN-Katholiek rats, which lack both high-molecular-weight and low-molecular-weight kininogens in the plasma (Hayashi *et al*., 1993), were selected for this experiment. In comparison with the normal strain, the B/N-Katholiek rats exhibited lower responses to increases of serum amylase and lipase activity and oedema formation. Furthermore, the concentration of a stable BK metabolite, (1-5)-BK (Shima *et al*., 1992) in the blood was significantly increased after obstruction in the SD rats. Elevation of immunoreactive BK in the circulation was also reported in caerulein-induced pancreatitis (Griesbacher *et al*., 1998; Shimizu *et al*., 1993). These results suggest activation of

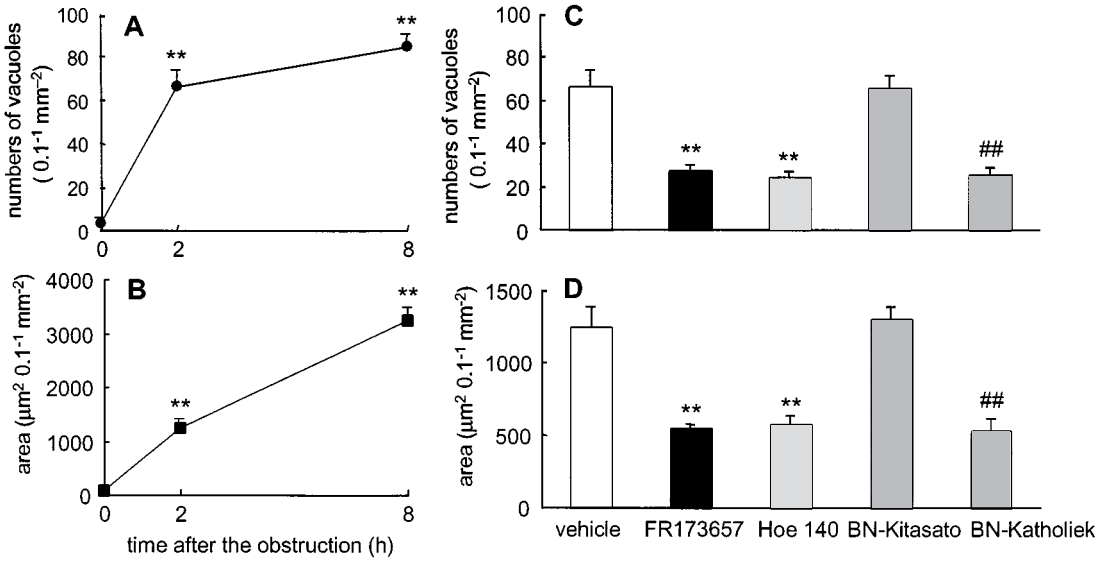

Figure 5 Vacuolization in pancreatic acinar cells induced by the obstruction of pancreaticobiliary duct. Pancreatic tissue was obtained from SD rats at 0, 2, and 8 h (A and B) after ligation of the pancreaticobiliary duct. A portion of the pancreas was immediately fixed with 10% paraformaldehyde in 0.1 M phosphate buffered saline (pH 7.4). The paraffin-embedded tissue was stained with hematoxylin-eosin for light-microscopic observation. For analysis of the vacuolization of acinar cell, the numbers (A and C) and the area (B and D) of vacuoles (in an area of the photographic field corresponding to 0.1 mm^2 of pancreatic tissue) was quantified by using Pixera 120es digital camera system at a magnification of $\times 400$. Images of randomly photographed fields were processed in Scion Image. Evaluation was performed by a researcher blind to the specific treatment conditions. FR173657 (100 mg kg^{-1}, p.o., closed column), Hoe140 (100 nmol kg^{-1}, s.c., dotted column), or the vehicle solution (open column) was treated in the same way as in Figure 3 in SD rats (C and D). SD, BN-Kitasato (left-upward hatched column), and BN-Katholiek (right-upward hatched column) rats were sacrificed at 2 h after the obstruction (C and D). Values are expressed as mean $\pm$ s.e.mean of five experiments. For comparison of data from SD rats, one-way ANOVA followed by *post-hoc* Fisher's PLSD test was used. Significance of difference: $^{**}P<0.01$. For comparison of data between B/N-Katholiek and BN-Kitasato rats, Student's *t*-test was used to evaluate the significance of differences: $\#\#P<0.01$.

the kallikrein-kinin system followed by release of kinin, and possible implication of BK in the acute pancreatitis model used in this study. In conformity with these results, administration of FR173657 or Hoe140 effectively limited the progression of acute pancreatitis at 2 h after the obstruction in a dose-dependent manner. FR173657 has been thoroughly characterized as a potent, highly selective, and orally active BK B_2 receptor antagonist both *in vitro* (Aramori *et al*., 1997; Asano *et al*., 1997; Rizzi *et al*., 1997) and *in vivo* (Griesbacher & Legat, 1997; Griesbacher *et al*., 1997; Hayashi & Majima 1999; Majima *et al*., 1997). Another B_2 receptor antagonist, Hoe140, reproduced the suppressive effect of BK on pancreatitis. Surprisingly, the profile of suppression by the B_2 receptor antagonists in the present model appeared to differ from that in the caerulein-induced pancreatitis reported previously (Griesbacher & Lembeck, 1992). In the latter model, caerulein-induced elevation of both amylase and lipase activities in the serum were dramatically augmented by up to 10 times by pretreatment with Hoe140, while the formation of pancreatic oedema was inhibited. The elimination of pancreatic oedema by Hoe140 releases the pancreatic enzymes trapped in the gland back into the circulation. However, in the present study, both B_2 receptor antagonists reduced the increase in the enzyme activity in the serum. It appears that kinin release may occur before acinar cell damage. This is supported by the finding that the B_2 receptor antagonists reduced the formation and development of vacuoles in the acinar cells. Furthermore, the number and area of vacuoles per area of photographic field in B/N-Katholiek rats were less than those in B/N-Kitasato rats. The reduction of vacuolization in the acinar cells in the rats treated with the antagonists was comparable to that in B/N-Katholiek rats. Therefore, the enzyme activities in the blood were attenuated. However, there may have been some other, as yet unknown, regulatory mechanism due to BK that caused the release of the pancreatic enzymes. At least, BK is known not to cause contraction of Oddi's sphincter (Harada *et al*., 1986); and in any case, the caerulein-induced model and the present model of acute pancreatitis may well have different characteristics.

Gabexate mesilate, which has been reported to strongly inhibit several key enzymes in the pathogenesis of acute pancreatitis (Dobosz *et al*., 1989; Tamura *et al*., 1977; Yang *et al*., 1987), tended to have a protective effect in the present model, but not to a significant extent. However, in the clinical trial of gabexate mesilate, some of the investigations did not demonstrate any positive effects on parameters such as mortality rate (Valderrama *et al*., 1992). Taken together, these results suggest that distinct and complicated mechanisms may be involved in the progression of the various experimental forms of acute pancreatitis. Further study is required on this. Treatment with indomethacin was also shown to be ineffective. This is in agreement with the report in that E type prostaglandins prevent the development of caerulein-induced pancreatitis in rats (Robert *et al*., 1989). Upon inhibition of cyclo-oxygenase with indomethacin, amylase secretion has

been reported to be upregulated in comparison with the level in the pancreatic acini without indomethacin (Zabel-Langhennig *et al*., 1999). This suggests that no protection is offered by indomethacin against acute pancreatitis.

A therapeutic procedure counteracting oedema formation is, therefore of paramount therapeutic value. The present investigation shows that a BK B_2 receptor antagonist potently blocked the releases of pancreatic enzymes and the formation of pancreatic oedema. These results suggest that this antagonist may be of use in clinical acute pancreatitis, if administered in the early stages of the disease. Acute pancreatitis induced by the obstruction of the pancreaticobiliary duct may not offer a simple solution in terms of surgical procedure. However, the use of this model rather than a secretagogue-induced model may well be of benefit in the evaluation of therapeutic agents for clinical acute pancreatitis, since the effects of BK B_2 receptor antagonists on the pancreatic enzymes in the present model were different from those in the caerulein-induced model.

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