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Role of Pancreatic Blood Flow and Vasoactive Substances in the Development of Canine Acute Pancreatitis

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Bradykinin Involvement in the Aggravation of Acute Pancreatitis in Rabbits

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Key Words
Bradykinin
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Rabbit

Abstract
This study was designed to investigate the role of bradykinin in the aggravation of acute pancreatitis. After injection of bradykinin 2 μg/kg to anesthetized rabbits with cerulein-induced acute pancreatitis, the pancreatic blood flow through gastroduodenal and superior mesenteric arteries (GDAF and SMAF) was determined with electromagnetic blood flow meters, the serum amylase level was measured, and pancreatic tissue was observed histologically. In rabbits treated with a supramaximal dose of cerulein alone (20 μg/kg/h), pancreatic blood flow was decreased and the serum amylase level was increased significantly by the early phase, and histological examination showed acute edematous pancreatitis. In rabbits treated with cerulein and bradykinin, GDAF and SMAF were significantly diminished at 300 min (51 ± 5% and 50 ± 4%, respectively, $p < 0.05$), and the serum amylase level rose significantly at 180 and 300 min (730 ± 130% and 1,190 ± 200%, respectively, $p < 0.01$) compared with rabbits treated with cerulein alone, and histological examination revealed pancreatic necrosis and greater inflammatory cell infiltration. These findings suggest that bradykinin has an additive role in the aggravation of acute pancreatitis.

Kallikrein exists as inactivated prekallikrein in many tissues and body fluids, such as the salivary glands, kidneys, urine, plasma, stomach, small intestine, thyroid gland, and pancreas. Kallikrein is classified as plasma or glandular kallikrein. The former is a basic protein which releases bradykinin (BK) from high-molecular-weight kininogen, and the latter is an acidic protein which releases Lys-BK (kallidin) from high- and low-molecular-weight kininogens. Kinin (BK, Lys-BK, and Met-Lys-BK) mediates acute inflammation: it increases vascular permeability and induces vasodilatation and pain [1]. The kallikrein-kinin system has been considered to be involved in many clinical diseases, including essential hypertension [2], pulmonary edema [3], bronchial asthma [4], endotoxin shock [5], liver cirrhosis, acute viral hepatitis [6], and rheumatoid arthritis [7]. Since the pancreas contains glandular kallikrein and is able to release kinin [8], the kallikrein-kinin system is believed to be involved in the inflammatory process of acute pancreatitis.

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It is known that pancreatic ischemia is an initiating or promoting factor of acute pancreatitis, and systemic hemodynamics and pancreatic blood flow are impaired during acute pancreatitis [9, 10]. Various vasoactive substances or chemical mediators such as histamine [11], serotonin [12], myocardial depressant factor [13], prostaglandins [14], free radicals [15, 16], platelet-activating factor [17], and kinin [18] have been proposed as responsible agents in the hemodynamic and morphological changes in pancreatic tissue. It has been reported that kinin-forming enzyme, which is present in plasma and ascitic fluid, induces hypotension during severe acute pancreatitis [19].

However, the role of the kallikrein-kinin system on pancreatic blood flow during acute pancreatitis has not been clarified. The present study was designed to investigate the effect of BK on the aggravation of acute pancreatitis by measuring pancreatic blood flow after the injection of BK to rabbits with cerulein-induced acute pancreatitis.

Materials and Methods

Twenty male white rabbits weighing 2–2.7 kg were fasted for 24 h and anesthetized with intravenous sodium pentobarbital (30 mg/kg). Tracheostomy was performed, and they were ventilated mechanically with room air (15 ml/kg × 12/min). During the experiment, physiological saline was infused continuously through an external jugular vein (3 ml/kg/h) while anesthesia was maintained by the intravenous infusion of sodium pentobarbital (7.5 mg/kg/h). A catheter inserted into a femoral artery was connected to a pressure-transducer for the recording of mean arterial pressure (MAP). Two heparinized polyethylene tubes were inserted into the femoral veins for the infusion of cerulein (Ceosunin injection; Kyowa Hakko, Tokyo, Japan) and bradykinin (BK) (bradykinin acetate salt; Sigma, St. Louis, Mo., USA), and abdominal midline incision was performed.

The gastroduodenal artery (GDA) and the superior mesenteric artery (SMA) were exposed, and noninvasive probes (1.0–2.5 mm in diameter) of electromagnetic blood flow meters (MFV-2100, 3100; Nihonkoden, Tokyo, Japan) were placed around them for the measurement of blood flow (GDAF and SMAF). A polyethylene tube (PE-50; Becton Dickinson, Parsippanny, N.J., USA) was inserted extraduodenally into the pancreatic duct and fixed. Pancreatic juice volume (PJV) was measured every 15 min by a fraction collector (Model 2110; Bio-Rad, Richmond, Calif., USA).

After a period of about 30 min for stabilization, PJV, MAP, GDAF, and SMAF were recorded as basal values, and blood was drawn for the measurement of basal pancreatic enzyme activities. The rabbits were divided into the following four groups: (1) control group (no cerulein, no BK; n = 5), physiological saline, 1 ml/kg was infused instead of BK; (2) BK group (n = 5), BK was dissolved in saline and 2 μg/kg was injected intravenously; (3) cerulein group (n = 5), cerulein, 20 μg/kg/h, was infused intravenously for 5 h, and (4) cerulein-plus-BK group (n = 5), BK 2 μg/kg was injected when the cerulein injection, 20 μg/kg/h, was started.

Hemodynamics were recorded continuously for 5 h and blood samples were taken from an external jugular vein 30, 60, 180, and 300 min after the start of the injection. Blood samples were centrifuged at 1,600 *g*, and the plasma was pooled and stored at below −40° C until assayed. Serum amylase activity was determined spectrophotometrically by the blue-starch method [20] and lipase activity by the enzymatic method [21]. Tissue was taken from the pancreas after 5 h of infusion and fixed with 10% neutral formalin, embedded in paraffin and stained with hematoxylin and eosin for histological examination. Semiquantitative analysis of the histological findings was performed. Interstitial edema was scored as follows: 0 = absent; 1 = interlobular space was expanded; 2 = interacinar space was expanded, and 3 = individual acini were separated. Inflammatory cell infiltration: 0 = absent; 1 = mild; 2 = moderate, and 3 = severe. Acinar cell vacuolization: 0 = absent; 1 = <20%; 2 = 20–50%, and 3 = >50% of acinar cells with cytoplasmic vacuoles in the examined area. Parenchymal necrosis: 0 = absent; 1 = focal (<5%); 2 = sublobular (5–20%), and 3 = lobular (>20%).

Values of PJV, serum enzyme activities and hemodynamics were expressed as percentages of the basal value, and presented as the mean ± standard error (SE), and analyzed by one-way analysis of variance. The histological score was presented as the mean ± SE and analyzed by the U test according to Wilcoxon, Mann and Whitney. A $p < 0.05$ was considered to be significant.

Results

Light-microscope examination of pancreatic tissue from rabbits treated with BK alone showed no significant difference from the control group. Histological changes in the cerulein group were interacinar and interlobular edema, acinar cell vacuolization, inflammatory cell infiltration in the interlobular edematous space, and minimal cell necrosis, demonstrating acute edematous pancreatitis. However, these findings were less marked than those in rats with cerulein-induced acute pancreatitis (fig. 1A). In the cerulein-plus-BK group, necrosis was present and inflammatory cell infiltration was more marked. These findings proved that BK together with cerulein caused and aggravated acute pancreatitis (fig. 1B, table 1).

No significant change of PJV was noted in the BK group. In the cerulein and cerulein-plus-BK groups, PJV decreased significantly starting in the early phase, demonstrating disturbed pancreatic exocrine secretion (at 15 min: cerulein, 66 ± 8%, $p < 0.01$; cerulein plus BK, 71 ± 6%, $p < 0.05$; control, 96 ± 4%; and 20 ± 9% and 24 ± 5% at 300 min; control, 92 ± 10%; $p < 0.01$). There was no significant difference between the two groups (fig. 2).

Serum amylase levels were compared in the cerulein, control and BK groups. They were increased significantly

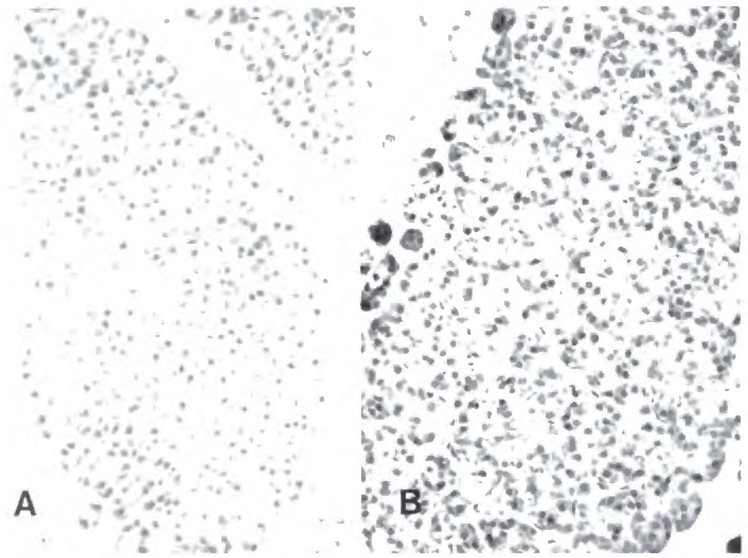

Fig. 1. Histology of the pancreas 5 h after starting injection of cerulein (20 μg/kg/h) alone (**A**) and cerulein (20 μg/kg/h) plus BK (2 μg/kg) (**B**). Marked interstitial edema with acinar cell vacuolization and inflammatory cell infiltration is found in the cerulein alone group. Parenchymal necrosis is present and inflammatory cell infiltration is more marked in the cerulein-plus-BK group.

Table 1. Histological changes

Group	Interstitial edema	Acinar cell vacuolization	Inflammatory cell infiltration	Parenchymal necrosis
Control (n = 5)	0	0	0	0
BK (n = 5)	0	0	0	0
Cerulein (n = 5)	2–3 (2.2 ± 0.2)	1–2 (1.6 ± 0.2)	1–2 (1.2 ± 0.2)	0–1 (0.2 ± 0.2)
Cerulein + BK (n = 5)	1–3 (1.8 ± 0.4)	1–2 (1.4 ± 0.2)	1–3 (2.0 ± 0.3)	1–3 (2.2 ± 0.4)*

Each value represents the mean ± SE. * $p < 0.05$ vs. the cerulein group.

at 30 min in the cerulein group (156 ± 17%; control: 108 ± 4%; BK: 100 ± 3%; $p < 0.01$). In the cerulein-plus-BK group, they were even higher, and at 180 min the difference from the cerulein group was significant (730 ± 120%; cerulein: 436 ± 35%; $p < 0.01$), and at 300 min they reached the highest level (1,190 ± 200%; $p < 0.01$). There was no significant difference between the control and the BK group (fig. 3).

In all the groups, serum lipase levels changed almost in parallel with the serum amylase levels. In the cerulein group, they were significantly increased at 30 min (256 ± 43%; control: 82 ± 4%, $p < 0.05$; BK: 78 ± 14%, $p < 0.01$), and reached a peak of 822 ± 69% at 300 min (control: 95 ± 25%; BK: 80 ± 20%; $p < 0.01$). In the cerulein-plus-BK group, they were higher than in the cerulein group at 60 min (660 ± 80%; cerulein: 402 ± 73%; $p < 0.01$), and 1,330 ± 170% at 300 min ($p < 0.01$). MAP in both the cerulein and the cerulein-plus-BK group decreased gradually; in the cerulein group it was significantly lower than in the control and BK group at 300 min (data not shown).

In the cerulein group GDAF fell significantly more, by degrees, than in the control and BK groups: at 30 min, 82 ± 5% (control: 101 ± 3%, $p < 0.05$; BK: 117 ± 8%, $p < 0.01$) and at 300 min, 67 ± 3% (control: 80 ± 4%, $p < 0.05$; BK: 83 ± 4%, $p < 0.05$). In the cerulein-plus-BK group, GDAF, instead of falling, was significantly higher than in the cerulein group at 30–60 min, because of the vasodilatory effect of BK, but then it decreased significantly more than in the cerulein group to 51 ± 5% at 300 min ($p < 0.05$; fig. 4).

Similarly, SMAF in the cerulein group was significantly diminished as early as 30 min (78 ± 5%; control: 98 ± 4%; $p < 0.05$) and fell to 64 ± 4% at 300 min (control: 83 ± 2%; $p < 0.05$). In the cerulein-plus-BK group, SMAF was significantly higher than in the cerulein group from 30 min (99 ± 6%, $p < 0.05$) to 120 min (87 ± 5%, $p < 0.05$), but at 300 min it had fallen significantly to 50 ± 4% ($p < 0.05$; fig. 5).

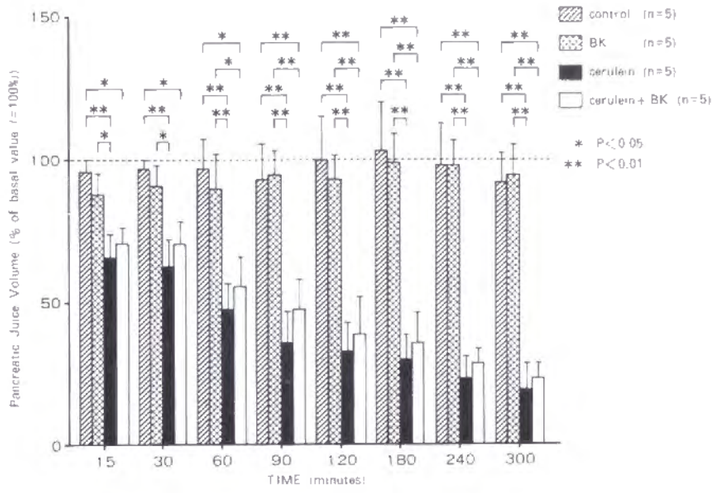

Fig. 2. Changes in PJV every 15 min. Results are expressed as the mean ± SE.

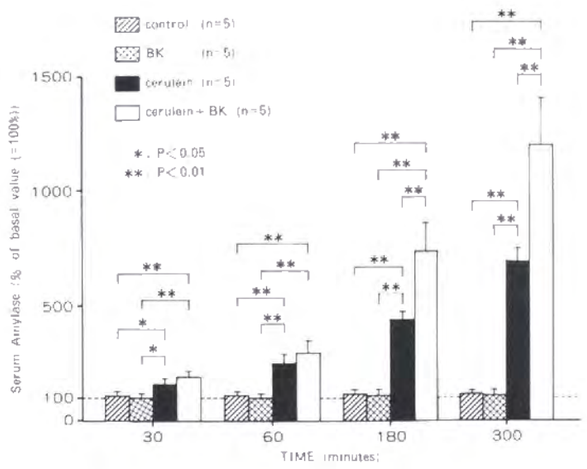

Fig. 3. Changes in serum amylase level. Results are expressed as the mean ± SE.

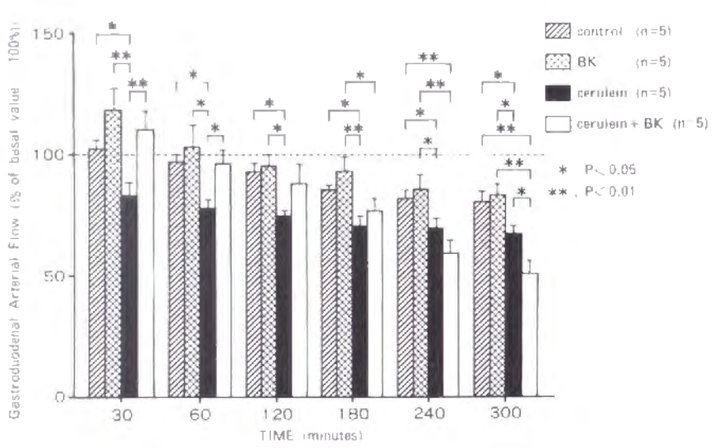

Fig. 4. Changes in GDAF. Results are expressed as the mean ± SE.

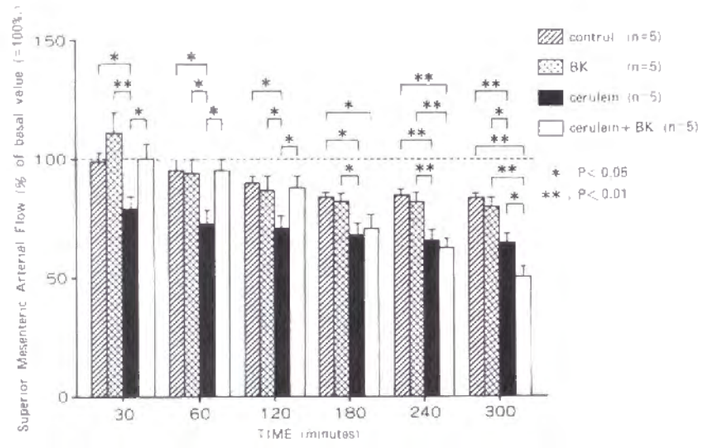

Fig. 5. Changes in SMAF. Results are expressed as the mean ± SE.

Discussion

The pancreas is one of the organs most susceptible to ischemia, and it has been shown that pancreatic ischemia plays an important role in the initiation and aggravation of experimental and clinical acute pancreatitis [10, 22]. In experimental pancreatitis, pancreatic blood flow is diminished along with lowered systemic blood pressure, cardiac output and oxygen consumption starting in the early stage [23, 24]. It has been suggested that impaired pancreatic blood flow comes from systemic circulatory disturbances due to hypovolemia, disseminated intravascular coagulation, myocardial depressant factor, or toxic substances in the plasma and ascitic fluid [25–27]. And various vasoactive substances, including kinin, have been proposed to be involved in the pancreatic circulatory disturbance and to lead to the development of acute pancreatitis.

It has been shown that BK, probably glandular kallikrein, is released from the pancreas into the portal vein during acute pancreatitis [28]. BK acts on the cardiovascular system, and its vasodilating potency is about 10-fold that of histamine [29]. BK has a direct effect on vascular smooth muscle which results in rapidly decreased systolic and diastolic blood pressure [30], tachycardia, and increased cardiac output. In this study we attempted to determine whether BK aggravates cerulein-induced acute pancreatitis in anesthetized rabbits. An intravenously injected supramaximal dose of 20 μg/kg/h of cerulein for 5 h significantly disturbed pancreatic exocrine secretion, diminished pancreatic blood flow, and elevated serum pancreatic enzyme levels in the early phase in anesthetized rabbits. Histological examination 5 h after starting the cerulein injection showed acute interstitial edematous pancreatitis. Thus, acute edematous pancreatitis was induced by a supramaximal dose of cerulein in anesthetized rabbits. In this study, blood pressure quickly decreased and pancreatic blood flow increased immediately after the injection of BK, 2 μg/kg (data not shown). In the cerulein-plus-BK group, pancreatic blood flow through GDAF and SMAF were better maintained than in the cerulein group in the early phase, at 60 min and at 120 min. However, pancreatic blood flow was significantly lower in the late phase, at 300 min, than in the cerulein group.

In the cerulein-plus-BK group, the serum amylase level was significantly higher than in the cerulein group at 180 min, and histological examination showed marked pancreatic necrosis and inflammatory cell infiltration in addition to interstitial edema and acinar cell vacuolization in the cerulein group. These results suggest that BK has a direct effect on pancreatic acinar cells. BK is a key enzyme in the activation of phospholipase A_2 and generates prostaglandins together with platelet-activating factor from biomembrane phospholipids [31]. Induced prostaglandins and leukotriene activate leukocytes, cause vasoconstriction or vasodilation, and increase vascular permeability [32]. It has been reported that the increase in vascular permeability caused by BK is about 15-fold that caused by histamine since BK also stimulates emigration of the leukocytes [33]. It is possible that free radicals and platelet-activating factor are generated in the activated leukocytes and injured vascular endothelial cells, resulting in pancreatic injury. The blood coagulation system activated by BK, and vascular endothelial cell injury caused by free radicals or platelet-activating factor may produce microthrombi in the microcapillaries, which can induce pancreatic necrosis.

In conclusion, in the present study, intravenously injected BK in rabbits with cerulein-induced pancreatitis significantly diminished pancreatic blood flow, increased pancreatic enzyme levels, and aggravated histological findings, indicating aggravation of acute pancreatitis. Thus, BK appears to play an important role in the aggravation of acute pancreatitis.

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