Effects of the bradykinin antagonist, HOE 140, in experimental acute pancreatitis

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1 The novel bradykinin antagonist, HOE 140, completely blocked the fall in rabbit blood pressure caused, not only by i.v. bradykinin, but also by i.v. kallikrein. This shows that both the effects of exogenously administered bradykinin and those of endogenously released kinins are antagonized by HOE 140.

2 Acute pancreatitis was induced in rats by i.v. infusion of the cholecystokinin analogue, caerulein. This treatment resulted in massive oedema of the pancreas, increased activities of amylase and lipase in serum and a characteristic, biphasic fall in blood pressure.

3 HOE 140 prevented the caerulein-induced pancreatic oedema and the second phase of hypotension whereas NPC 349, a widely used, but short-acting, bradykinin antagonist did not show a significant inhibition. HOE 140, in contrast to its inhibitory effects on caerulein-induced pancreatic oedema and hypotension, significantly augmented the increases in amylase and lipase activities in serum.

4 It is concluded that in this model of acute pancreatitis, the release of kinins induces pancreatic oedema and hypotension. Prevention by HOE 140 of the kinin-induced oedema allows the pancreatic enzymes to leave the tissue without hindrance and thus will diminish subsequent pathological events. It is suggested that the results obtained with the highly potent and long-acting bradykinin antagonist, HOE 140, provide a pharmacological basis for a clinical trial in acute pancreatitis.

Keywords: Bradykinin antagonists; HOE 140; pancreatitis (experimental); caerulein; hypotension; plasma protein extravasation; serum amylase; serum lipase

Introduction

Acute pancreatitis, which is associated with a considerable mortality rate, is characterized by a massive oedema of the gland 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 are used. These include ligation of the pancreatic duct, injection of bile salts into the pancreatic duct, infusion of oleic acid, or infusion of the cholecystokinin (CCK) analogue, caerulein (Adler *et al.*, 1986). 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). Therefore, in the present work, the effects of a novel, potent and long-acting bradykinin (BK) antagonist, HOE 140 (Lembeck *et al.*, 1991; Hock *et al.*, 1991; Wirth *et al.*, 1991; Griesbacher & Lembeck, 1991), were investigated in caerulein-induced experimental pancreatitis in the rat.

Methods

Rabbit blood pressure

Rabbits of either sex (3.5–5.0 kg) were anaesthetized with pentobarbitone sodium (35 mg kg⁻¹, i.v.). Blood pressure was monitored in a carotid artery with a Statham pressure transducer. The stability of the blood pressure and the corneal reflex were used as indices of the depth of the anaesthesia.

Bradykinin (BK; 1 or 3 nmol kg⁻¹) or kallikrein (1 or 3 U kg⁻¹) were injected into a jugular vein at intervals of 10 min. HOE 140 (3 nmol kg⁻¹) was then given i.v.. The injections of BK or kallikrein were repeated 5–15 min later.

Caerulein-induced pancreatitis in rats

Surgical procedure Female Sprague–Dawley rats (260 ± 30 g) were anaesthetized by simultaneous i.p. injection of pentobarbitone sodium (40 mg kg⁻¹) and phenobarbitone sodium (160 mg kg⁻¹). Captopril (50 µmol kg⁻¹, i.p.), an inhibitor of kininase II, was injected, at the same time, to augment the actions of any kinins released. Kininase II is present in many tissues (Lembeck *et al.*, 1990). Blood pressure in a carotid artery was monitored throughout the experiment with a Statham pressure transducer. A jugular vein was cannulated to allow the infusion of either caerulein (4 nmol kg⁻¹ h⁻¹) or of saline (0.034 ml min⁻¹) for a period of 120 min. The BK antagonists, HOE 140 (100 nmol kg⁻¹) or NPC 349 (1 µmol kg⁻¹), or a corresponding volume (0.5 ml kg⁻¹) of a 154 mM sodium chloride solution (saline) were injected s.c. 30 min before, or 10–45 min after, the start of the caerulein infusion. At the end of the experiment the animals were killed by decapitation and blood was collected for the determination of serum amylase and lipase activity.

Quantification of pancreatic oedema To quantify the pancreatic oedema, 5 mg kg⁻¹ of Evans blue, which binds quantitatively to serum albumin (Rawson, 1943), was injected i.v. immediately before the start of the i.v. infusion of caerulein or saline. At the end of the infusion, the rats were exsanguinated and perfused with 30 ml saline via the aorta. The portion of the pancreas adjacent to the duodenum was excised and weighed. It was then dried in a vacuum centrifuge, reweighed and the difference between wet and dry weight was used as a measure for the water content of the tissue. The dried tissues were then

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immersed for 24 h at 50°C in formamide in order to extract the Evans blue (Gamse *et al.*, 1980). The amount of dye in the supernatant was determined photometrically at 620 nm (Saria & Lundberg, 1983).

Determination of serum enzyme activities Serum amylase activity was measured with a kinetic colour test using 2-chloro-4-nitrophenyl-D-maltoheptoside (Amylase test kit Roche, Germany). Serum lipase activity was measured by the reduction of the turbidity due to cleavage of triolein to monoglyceride and oleic acid (Monotest Lipase, Boehringer Mannheim, Germany). The limit of detection for amylase was 11 u l^{-1} and for lipase 16 u l^{-1} .

Substances used

HOE 140 (D-Arg-[Hyp³, Thi⁵, D-Tic⁷, Oic⁸]-bradykinin, a gift from Hoechst AG, Germany), NPC 349 (D-Arg-[Hyp³, Thi^{5,8}, D-Phe⁷]-bradykinin, NovaBiochem, Switzerland), caerulein (Sigma, U.S.A.) and bradykinin (Bachem, Switzerland) were dissolved in saline at a concentration of 1 mM. Porcine pancreatic kallikrein (Sigma, U.S.A.) was dissolved in 50% (v/v) ethanol and dilutions made with saline. Pentobarbitone sodium (Nembutal) was purchased from Sanofi Santé (France). Phenobarbitone sodium (Apoka, Austria) was dissolved in saline at a concentration of 80 mg ml⁻¹. Captopril was obtained from Squibb-von Heyden GmbH (Austria). Evans blue was purchased from Sigma (U.S.A.).

Statistical analysis

The hypotensive effects of BK and kallikrein in the rabbit before and after HOE 140 were compared by the Quade test (Conover, 1980). Comparisons between the different treatment groups in the rat were made by nonparametric multiple comparisons (Zar, 1984).

Results

Inhibition by HOE 140 of endogenously released kinins in the rabbit

The basal blood pressure of anaesthetized rabbits was about 110 mmHg. Intravenous injections of BK (1 or 3 nmol kg⁻¹) caused dose-dependent brief (about 1 min) falls in blood pressure. Comparable hypotensive effects could be elicited by i.v. injections of kallikrein (1 or 3 u kg⁻¹). HOE 140 (3 nmol kg⁻¹, i.v.) completely blocked the actions of these doses of BK and kallikrein; higher doses of BK (10 nmol kg⁻¹) and kallikrein (10 u kg⁻¹) induced only a minute fall in blood pressure when HOE 140 had been given previously (Figure 1). The inhibitory effect of HOE 140 was long-lasting: 120 min after the injection of HOE 140 the actions of BK or kallikrein were still reduced (not shown in the figure).

Caerulein-induced pancreatitis in rats

Blood pressure The basal blood pressure was 90–110 mmHg in all experimental groups. In control rats, which had received saline as s.c. injection and as i.v. infusion, the blood pressure fell by about 10–15 mmHg during the 2 h period of the i.v. infusion of saline. Pretreatment with HOE 140 (100 nmol kg⁻¹, s.c.) had no effect on the basal blood pressure or on the blood pressure during the i.v. infusion of saline. The i.v. infusion of caerulein caused a biphasic fall in blood pressure (Figure 2). The first hypotensive phase (about –20 mmHg) occurred during the first 15 min of the caerulein infusion. A further drop in blood pressure set in after 25–30 min, reaching minimal values of $70 \pm 10 \text{ mmHg}$ at 45–55 min. During the remaining time of the caerulein

Figure 1 Fall in mean arterial pressure in anaesthetized rabbits elicited by i.v. injections of bradykinin (a) or of kallikrein (b). Open symbols are values obtained before, closed symbols are values obtained 5–15 min after, an i.v. injection of HOE 140 (3 nmol kg⁻¹). Mean values with s.e.mean (vertical lines); $n = 3$. Significance of difference from control values: ** $P < 0.01$.

Figure 2 Fall in mean arterial pressure in anaesthetized rats during an i.v. infusion of caerulein (diamonds) or of saline (circles). Closed symbols are the values obtained from animals pretreated with HOE 140 (100 nmol kg⁻¹, s.c.); values obtained in control animals are given as open symbols. Mean values with s.e.mean (vertical lines); $n = 10$ in each of the 4 groups. Asterisks denote significant differences (* $P < 0.05$; ** $P < 0.01$) between groups above and below the asterisks.

infusion, the blood pressure recovered slowly. In animals pretreated s.c. with HOE 140, the caerulein infusion still induced the initial fall in blood pressure during the first 25 min. However, the second phase of the caerulein-induced hypotension was abolished by HOE 140.

Pancreatic oedema The infusion of caerulein led to a massive swelling of the pancreas which extended into the retroperitoneal area adjacent to the pancreas. A jelly-like oedema clearly separated the individual lobules and the pancreas had an almost transparent appearance. The water content of the tissue increased about three fold. The massive extravasation of Evans blue showed that a large amount of plasma proteins had accumulated in the interstitial space. In animals injected s.c. with HOE 140 (100 nmol kg⁻¹) 30 min before the caerulein infusion, caerulein did not induce any changes in the macroscopic appearance of the pancreas and the effects on the water content of the tissue and on plasma protein extravasation were completely abolished. HOE 140, when it was injected up to 25 min after the start of the caerulein infusion, also significantly inhibited the caerulein-induced pancreatic oedema (Figure 3). In contrast, NPC 349 (1 $\mu\text{mol kg}^{-1}$) was completely inactive when injected 30 min before caerulein. When NPC 349 was injected 10 min after the start of the caerulein infusion, the pancreatic oedema seemed to be smaller, but the effect was not statistically significant (Figure 3).

Figure 3 Oedema of the pancreas of rats quantified as water content (a) and as Evans blue content (b). Open columns show basal values obtained in animals receiving an i.v. infusion of saline, and solid columns show the effect of caerulein (8 nmol kg^{-1} infused i.v. within 120 min). Six further groups of rats were injected with bradykinin antagonists HOE 140 (100 nmol kg^{-1} , shaded columns) or NPC 349 (1 μmol kg^{-1} , stippled columns) either 30 min before, or 10, 25 or 45 min after the start of the caerulein infusion. Significance of difference from the effect of caerulein alone (solid column): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Column heights represent mean values, vertical lines show s.e.mean; $n = 6-13$.

Enzyme activities in serum After pretreatment with HOE 140 alone, the serum activity of amylase was the same as in control animals. In rats pretreated s.c. with saline, the i.v. infusion of caerulein increased the serum activity of amylase significantly and this caerulein-induced elevation in serum amylase activity was augmented approximately 6 fold in rats pretreated with HOE 140 (Figure 4).

The activity of lipase in serum was below the limit of detection in the two groups of rats which received an i.v. infusion of saline. Caerulein induced a rise in serum lipase activity which was about 10 times larger in rats pretreated with HOE 140 (Figure 4).

Discussion

HOE 140 is a selective antagonist of exogenously administered BK across a wide spectrum of *in vitro* and *in vivo* tests and has a much higher potency and a longer duration of action than all other BK antagonists previously available (Lembeck *et al.*, 1991; Hock *et al.*, 1991; Wirth *et al.*, 1991; Griesbacher & Lembeck, 1991). In experiments on the kallikrein-induced fall in the blood pressure of rabbits we have now demonstrated that the actions of endogenously

Figure 4 Serum activities of amylase (a) and lipase (b) in rats. Infusion of caerulein (8 nmol kg^{-1} within 120 min); pretreatment with HOE 140 (100 nmol kg^{-1} , s.c.) 30 min before the caerulein infusion. Control animals received corresponding volumes of saline instead of caerulein or HOE 140. Mean values with s.e.mean (vertical lines); $n = 10$ in each of the 4 groups. Significance of difference between the groups indicated by brackets: * $P < 0.05$.

released kinins are also antagonized by HOE 140. Kallikrein, an enzyme discovered in urine and pancreatic extracts (see Werle, 1970), splits BK or lysyl-BK from a precursor, kininogen. The kinins thus formed are responsible for the resulting fall in blood pressure. Rabbits were used for initial experiments comparing the effects of BK and kallikrein because in the rat, a different kinin, Ile-Ser-BK, is released from kininogen by other enzymes such as trypsin. Ile-Ser-BK has all of the characteristic actions of kinins such as fall in blood pressure or increase in vascular permeability (Greenbaum, 1989). HOE 140 also blocks the fall in blood pressure and the plasma protein extravasation evoked by i.v. injected BK in the rat (Lembeck *et al.*, 1991).

Activation of CCK receptors seems to be involved in several forms of experimental pancreatitis because CCK antagonists have been found to have alleviating effects in such models (see Woodruff & Hughes, 1991). Hyperstimulation of the exocrine function of the pancreas by the CCK analogue, caerulein, results in increases in the serum activities of amylase and lipase (Silverman *et al.*, 1989) as well as in morphological changes similar to those observed in human acute pancreatitis (Willemer *et al.*, 1990).

Acute pancreatitis is characterized by pancreatic and retroperitoneal oedema and severe hypotension. In experimental models, the hypotension was associated with a major redistribution of body fluids. A great proportion of the measured loss in blood volume could be explained by the fluid found in

the peritoneal cavity (ascites) and the gain in volume of the pancreas itself (Ryan *et al.*, 1964). Since the transfusion of blood (Nugent & Atendido, 1966) or ascitic fluid (Ellison *et al.*, 1981) from animals with pancreatitis to healthy animals caused hypotension in the recipients, the involvement of vasoactive substances, such as kinins or histamine, has been postulated.

If kinins are released into the tissue they could also be responsible for the intense pain which is a major symptom of acute pancreatitis in man. BK is probably the most potent endogenous algesic substance and acts on capsaicin-sensitive afferent nerves (Juan & Lembeck, 1974). This type of nerve fibre is present in the pancreas (Sharkey *et al.*, 1984) and has also been studied in several experimental models for visceral pain (Ness & Gebhart, 1990).

Kinins in biological fluids have usually been measured by bioassay on the rat uterus or on the guinea-pig ileum. Similarly, kininogen was quantified as BK-like activity generated by trypsin. Using these methods, it has been found that, in experimental acute pancreatitis, the plasma levels of BK increase (Satake *et al.*, 1973) whereas those of kininogen decrease (Ryan *et al.*, 1964).

In order to prove that kinins are responsible for some of the clinical signs in acute pancreatitis, specific antagonists have to be employed. The failure of one of the earlier BK antagonists to protect against the hypotension in experimental pancreatitis (Berg *et al.*, 1989) had cast some doubt on the pathogenic role of kinins in this disease. However, the BK antagonist used in this earlier study belongs to a group of BK antagonists the effects of which are only very short-lasting *in vivo* (Griesbacher & Lembeck, 1987; Griesbacher *et al.*, 1989).

When the long-acting BK antagonist, HOE 140, became available, the proposed role of kinins in acute pancreatitis could be investigated in detail. Thus, the model of caerulein-induced pancreatitis in rats was chosen. The animals developed an acute interstitial oedema of the pancreas with hypotension and increased serum activities of amylase and lipase. This is comparable to the form of pancreatitis most frequently found in clinical situations in man (Büchler, 1991). No necroses were induced by the experimental procedure.

HOE 140 prevented the formation of the pancreatic

oedema and the second phase of the hypotension in rats given an i.v. infusion of caerulein. These effects may, thus, be attributed to the release of endogenous kinins. The extravasation of plasma proteins was observed only in the pancreas but not in other organs. This effect was abolished by HOE 140 and shows that liberation of kinins, under the experimental conditions used, only occurs within the pancreas. HOE 140 did not inhibit the oedema formation when it was administered at a time (45 min) when, in the absence of HOE 140, the caerulein-induced hypotension had fully developed (compare Figure 2 and Figure 3). Therefore, it is strongly indicated that the hypotension observed in acute pancreatitis is not due to a kinin-induced vasodilatation but rather to a massive loss of plasma into the pancreatic oedema. A widely used, short-acting BK antagonist, NPC 349, was almost inactive even when used at a dose that was 10 times higher than that of HOE 140.

The effect of HOE 140 on the caerulein-induced increases in serum amylase and lipase activities were surprising. Instead of reducing the increase in enzyme activities there was an augmentation of up to ten fold. An explanation of this result could be that, while the oedema is present, a large proportion of both enzymes is trapped in the pancreas but when the oedema is prevented by the BK antagonist, the enzymes can leave the gland without hindrance. Accumulation of these enzymes in the oedematous pancreas will, no doubt, eventually lead to further tissue damage.

The severity of acute pancreatitis in man does not correlate with the serum levels of amylase and lipase, but rather with the pancreatic oedema and acites, the extent of pancreatic and extrapancreatic necrosis, and subsequent bacterial contamination which finally determine the risk of mortality (Büchler, 1991). The mortality rate is also highly correlated with the degree of hypoalbuminaemia (Imrie *et al.*, 1976). Pancreatic oedema is an essential factor in the development of pancreatic necroses (Klöppel *et al.*, 1986) and of hypoalbuminaemia. A therapeutic procedure counteracting the oedema formation is, therefore, of paramount therapeutic value. Since the present investigation shows that HOE 140 potently blocks the hypotension and the formation of the pancreatic oedema, the results provide a pharmacological basis for a clinical trial of HOE 140 in acute pancreatitis.

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