Pathological events in experimental acute pancreatitis prevented by the bradykinin antagonist, Hoe 140

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1 In a previous investigation, Hoe 140, a specific and potent bradykinin B₂ receptor antagonist, prevented the pancreatic oedema and the hypotension observed during acute experimental pancreatitis; however, it augmented the associated rises in the serum activities of pancreatic enzymes. Therefore, we have now investigated the consequences of the pancreatic oedema for the fate of activated enzymes released into the tissue during the course of acute pancreatitis.

2 Acute oedematous pancreatitis was induced in rats, pretreated with captopril (50 µmol kg⁻¹, i.p.), by hyperstimulation of the exocrine function of the pancreas with the cholecystokinin analogue, caerulein (4 nmol kg⁻¹ h⁻¹, i.v.), for up to 120 min.

3 Pancreatic oedema began to develop 10 min after the start of the caerulein infusion, reached a maximum within about 45 min, and then declined slightly. The development of the oedema paralleled the second phase of the caerulein-induced fall in blood pressure found in earlier experiments. No further extravasation of plasma proteins occurred during the 2nd hour of the caerulein infusion. The oedema formation was completely blocked in animals pretreated with the bradykinin receptor antagonist, Hoe 140 (100 nmol kg⁻¹, s.c.). Pretreatment with aprotinin or soy bean trypsin inhibitor did not result in a significant inhibition of the oedema.

4 The haematocrit of animals with experimental pancreatitis showed a pronounced increase which started 10 min after the start of the caerulein infusion and reached maximal values at 60 min. The changes in haematocrit showed a reduction in total blood volume of 28% due to a 48% loss of plasma. This effect was completely blocked by Hoe 140.

5 In rats with caerulein-induced pancreatitis, there was a time-dependent increase in the activities of amylase and lipase in blood serum as well as in the pancreas. Pretreatment with Hoe 140 greatly augmented the caerulein-induced rise in enzyme activities in blood serum but potentially attenuated it in the pancreas. The activities of trypsin in both the blood serum and the pancreas were below or near the limit of detection in all experimental groups.

6 It is concluded that the second phase of hypotension in this model of acute pancreatitis is due to the liberation of kinins which cause a massive loss of blood plasma into the pancreas and into the retroperitoneal space. Activated enzymes are trapped in the pancreas, at least in part, by the oedema of the gland. Treatment with Hoe 140 prevents the oedema formation and greatly facilitates the egress of activated enzymes from the pancreas.

Keywords: Bradykinin antagonists; Hoe 140; pancreatitis (experimental); caerulein; hypovolaemia; plasma protein extravasation; amylase; lipase; trypsin; protease inhibitors

Introduction

Hyperstimulation of the exocrine function of the rat pancreas with the cholecystokinin (CCK) analogue, caerulein, leads to morphological changes in the pancreas which resemble those found in human acute interstitial oedematous pancreatitis (Willemer *et al.*, 1990). CCK antagonists have been shown to inhibit the occurrence of pathological events in several models of experimental pancreatitis thought to mimic certain forms of pancreatitis in man (see Woodruff & Hughes, 1991). Thus, a stimulation of CCK receptors is thought to play a causative role in the pathogenesis of the disease.

Since during the course of acute pancreatitis enzymes potentially capable of releasing kinins are released into the interstitial space of the pancreas (Watanabe *et al.*, 1984; Saluja *et al.*, 1987), we have previously (Griesbacher & Lembeck, 1992a) used the potent bradykinin (BK) B₂ receptor antagonist, Hoe 140 (Lembeck *et al.*, 1991; Hock *et al.*, 1991; Wirth *et al.*, 1991; Griesbacher & Lembeck, 1992b) in this model. It was demonstrated that pretreatment of rats with Hoe 140 prevented both the formation of the pancreatic

oedema and the second phase of the characteristic, biphasic fall in blood pressure induced by an infusion of caerulein. The fall in blood pressure was not reversed by Hoe 140 if the antagonist was administered at a time when the hypotension had already fully developed. Therefore, it appeared that kinin-mediated plasma extravasation is likely to be the cause of the observed hypotension rather than kinin-mediated vasodilatation. In contrast to the effects of Hoe 140 on blood pressure and oedema formation, this BK antagonist did not inhibit the caerulein-induced increases in the activities of amylase and lipase in serum, but augmented them. It was hypothesized that prevention of the development of pancreatic oedema would improve the egress of activated enzymes from the gland.

The purpose of the present investigation was: (1) to determine, in detail, the role of the pancreatic oedema in the development of the hypotension during acute pancreatitis, (2) to elucidate why Hoe 140 enhances the rise in enzyme activities in serum, and (3) to compare the effects of the BK antagonist with those of protease inhibitors. A preliminary account of these results has been presented to the British Pharmacological Society (Griesbacher & Lembeck, 1992c).

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Methods

Experimental procedure

Female Sprague-Dawley rats (200–280 g) were anaesthetized with pentobarbitone sodium (40 mg kg⁻¹, i.p.) and phenobarbitone sodium (160 mg kg⁻¹, i.p.). In order to inhibit the catabolism of endogenously released kinins, captopril (50 µmol kg⁻¹, i.p.), an inhibitor of kininase II, was injected at the same time. Hoe 140 (100 nmol kg⁻¹, s.c.), or a corresponding volume (0.5 ml kg⁻¹) of a 154 mM solution of sodium chloride (saline), was injected 10 min later. Caerulein was infused into a jugular vein at a rate of 67 pmol kg⁻¹ min⁻¹ for periods of 10, 45 or 120 min. Control animals received an infusion of saline (0.034 ml min⁻¹).

In similar experiments, the pretreatment with Hoe 140 was replaced by i.v. injections of aprotinin (20,000 u kg⁻¹) or of soybean trypsin inhibitor (SBTI, 16 mg kg⁻¹). These injections were made either 10 min before or 10 min after the start of the infusion of caerulein.

In all experiments, one group of animals was treated with Hoe 140 alone in order to exclude any possible intrinsic effects of the antagonist. No such effects were observed. The results from this group are not shown in the figures for the sake of clarity.

Quantification of pancreatic oedema

Water content A portion of the pancreas of about 2 g wet weight was taken, weighed, dried in a vacuum centrifuge and reweighed. The difference between wet weight and dry weight, expressed as a fraction of the dry weight, was taken as an estimate of the water content of the tissue.

Plasma protein extravasation In another set of experiments, the azo dye, Evans blue (5 mg kg⁻¹, i.v.), which binds quantitatively to plasma proteins, was injected 5 min before the infusion of caerulein. At the end of the experiment, the animals were perfused via the thoracic aorta with 40 ml of saline to remove intravascular Evans blue. After determining the water content of the pancreas as described above, the dried tissue was immersed in formamide for 24 h at 55°C to extract the dye (Gamse *et al.*, 1980). Evans blue was measured photometrically at 620 nm (Saria & Lundberg, 1983). The amount of Evans blue found in the tissue was taken as a measure of plasma protein extravasation.

Measurement of enzyme activities

For the measurements of the activities of amylase and lipase in serum, the rats were decapitated at the end of the experiment and the trunk blood was collected. The blood was allowed to coagulate and was then centrifuged for 15 min at 2×10^4 m s⁻². The serum was removed and stored at -20°C.

Part of the pancreas adjacent to the duodenum was excised, cut into small pieces and immersed in 2 ml of ice-cold saline. These samples were centrifuged at 2×10^5 m s⁻² for 25 min in order to extract the tissue fluid. The supernatant was removed and stored at -20°C until the determinants of the activities of amylase and lipase were carried out. For the measurement of trypsin activity, fresh samples of the supernatant were used. The pellet of pancreatic tissue was dried, as described above, to determine the dry weight.

Measurements of the activities of amylase and lipase were carried out on an Ektachem 700 Analyser. The enzymic amylase and lipase methods (Eastman Kodak, New York, U.S.A.) have a dynamic range of 5–1200 u l⁻¹ and 10–2000 u l⁻¹, respectively. Trypsin activity in the samples was determined by a colorimetric method (Test Combination Trypsin, Boehringer Mannheim, Germany). The limit of detection was 1 u l⁻¹.

Total protein in serum and pancreas

The total protein concentration in serum was determined by the Biuret method. The Coomassie Brilliant Blue G-250 method (Total Protein Test, Bio Rad, Hemel Hempstead, U.K.) was used to measure the total protein concentration in the tissue fluid samples.

Determinations of haematocrit

In addition to the surgical procedure described above, a carotid artery was cannulated with a short piece (1.5 cm) of polyethylene tubing (Intramedic PE-20, Clay Adams/Beckton Dickinson, Parsippany, N.J., U.S.A.). A small blood sample (40–50 µl) was withdrawn immediately before the i.v. infusion of caerulein to determine the basal haematocrit (H_{t0}). Further determinations of haematocrit (H_t) were made at 10, 25 and 45 min, and subsequently at regular intervals of 15 min until the end of the 2 h infusion of caerulein. Since no haemorrhagic lesions were observed, the % change in total blood volume (ΔV_B) was estimated from the equation:

$$\Delta V_B = 100 \left(\frac{H_{t0}}{H_t} - 1 \right)$$

and the % change in plasma volume (ΔV_P) was estimated as

$$\Delta V_P = 100 \left[\frac{\left(\frac{H_{t0}}{H_t} - H_{t0} \right)}{\left(1 - H_{t0} \right)} - 1 \right]$$

Substances used

Hoe 140 (D-Arg-[Hyp³,Thi²,D-Tic⁷,Oic⁸]-bradykinin) was a gift from Hoechst (Frankfurt/Main, Germany). Sulphated caerulein was obtained from Sigma (St. Louis, M.O., U.S.A.). Stock solutions of both peptides were prepared in a 154 mM solution of NaCl (saline). Pentobarbitone sodium (Nembutal) was obtained from Ceva (Germany). Phenobarbitone sodium (Apoka, Vienna, Austria) was dissolved in saline at a concentration of 80 mg ml⁻¹. Captopril was obtained from Squibb-von Heyden (Vienna, Austria). Evans blue was purchased from Sigma (U.S.A.). Aprotinin (Trasyol) was a gift from Bayer (Leverkusen, Germany). Aprotinin activity is expressed as kallikrein inactivator units; one unit will inhibit approximately 500 ng trypsin. SBTI was purchased from Sigma; 1 mg SBTI will inhibit approximately 1.6 mg trypsin.

Statistical analysis

Multiple nonparametric comparisons (Zar, 1984) were used to compare the effects of caerulein at the different time points and to evaluate the inhibition or potentiation of these effects by Hoe 140. All values are presented as means $\pm$ s.e.mean except those values obtained for the activities of trypsin in serum and in the pancreas. These values are given as median and range.

Results

Pancreatic oedema

Ten minutes after the start of the infusion of caerulein no increase in the water content of the pancreas was found. At the time of the maximum fall in blood pressure described earlier (Griesbacher & Lembeck, 1992a), i.e. at 45 min, the water content had increased by over fourfold (Figure 1a). At

the end of the infusion of caerulein (120 min), the mean value of the water content was slightly, but not significantly, lower.

A separate group of rats was injected with Evans blue, 5 min prior to the start of the caerulein infusion, in order to stain the plasma proteins. No Evans blue could be found in the pancreas after infusing caerulein for 10 min duration. However, 45 min after the start of the caerulein infusion there was over 500 μg Evans blue g^{-1} dry weight present in the pancreas (Figure 1b). Similar values were found at

Figure 1 Oedema of the pancreas during caerulein-induced acute pancreatitis in rats: caerulein ($4 \text{ nmol kg}^{-1} \text{ h}^{-1}$) was infused i.v. for 10, 45 or 120 min. Thirty minutes before the start of the caerulein infusion, the rats were injected either with saline (0.5 ml kg^{-1} , $\square$) or with Hoe 140 (100 nmol kg^{-1} , $\bullet$). Control animals received saline for both the s.c. injection and the i.v. infusion ($\square$). The oedema was quantified as the water content of the pancreas (in g g^{-1} dry weight, $n = 14\text{--}21$, a). For the quantification of the extravasation of plasma proteins, rats were additionally injected with the dye, Evans blue (5 mg kg^{-1} , i.v.), 5 min prior to the i.v. infusion, and the tissue content of Evans blue (in $\mu\text{g g}^{-1}$ dry weight, $n = 5\text{--}7$, b) was determined. The total protein content of the oedema fluid (in g ml^{-1} , $n = 7\text{--}14$, c) was measured in rats not injected with Evans blue. Symbols are mean values, vertical lines give s.e.mean; where no s.e.mean is given it was smaller than the symbol. Significance of inhibition by Hoe 140: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Significance of difference from controls: † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$.

120 min indicating that no further extravasation of plasma proteins was taking place during the second hour of the caerulein infusion. The total protein concentration in the oedema fluid (Figure 1c) showed a continuous increase during the 120 min of the caerulein infusion.

In order to clarify these results, the effect of a 120 min infusion of caerulein on oedema formation and plasma protein extravasation was compared in 2 groups of 8 rats: the first group was pretreated with Evans blue 5 min before the caerulein infusion, the second was injected with the dye 60 min after the start of the infusion. In both groups, the water content of the pancreas at the end of the experiment was similar ($7.73 \pm 0.53 \text{ g g}^{-1}$ dry weight and $6.99 \pm 0.66 \text{ g g}^{-1}$ dry weight, respectively). However, Evans blue in the pancreas was increased only in the first group ($501 \pm 71 \mu\text{g g}^{-1}$ dry weight) whereas the amount of Evans blue determined in the pancreas of the second group ($20 \pm 6 \mu\text{g g}^{-1}$ dry weight) was not different from that in the tissue blanks ($15 \pm 8 \mu\text{g g}^{-1}$ dry weight, $n = 4$).

Pretreatment with Hoe 140 completely prevented the formation of the pancreatic oedema. The water and Evans blue contents of the pancreas, as well as the protein content of the interstitial oedema fluid remained normal throughout the infusion of caerulein in these animals (Figure 1).

Since caerulein caused a significant extravasation of water and of proteins into the pancreas, the total protein concentration in the serum was also determined. During the 120 min of infusion of caerulein, no changes in serum protein levels were observed. All experimental groups showed protein concentrations of $45\text{--}55 \text{ mg ml}^{-1}$ in the trunk blood collected after decapitation.

Haematocrit

The basal haematocrit values of the rats in all experimental groups were $0.42\text{--}0.46$. Two hours i.v. infusion of saline ($0.034 \text{ ml min}^{-1}$) caused a slight reduction of these values (Figure 2a) due to the increase in blood and plasma volume (Figure 2b and c). Ten minutes after the start of the i.v. infusion of caerulein ($4 \text{ nmol kg}^{-1} \text{ h}^{-1}$), the haematocrit was not different from controls; subsequently, a progressive rise in haematocrit was observed which reached maximum values of 0.59 ± 0.01 at 60 min (Figure 2a). The rise in haematocrit values at this time point indicated a reduction in total blood volume of $28 \pm 3\%$ (Figure 2b) and a reduction in plasma volume of $48 \pm 4\%$ (Figure 2c). During the remaining course of the experiment, the haematocrit values declined slowly.

Figure 2 Haemoconcentration during experimental acute pancreatitis in rats: Haematocrit (a) of blood samples, drawn from a carotid artery, was measured repeatedly during a 2 h infusion of either caerulein ($4 \text{ nmol kg}^{-1} \text{ h}^{-1}$, circles) or of a corresponding volume of saline ($0.034 \text{ ml min}^{-1}$, squares); 30 min prior to the beginning of this infusion, the animals were injected s.c. either with saline (0.5 mg kg^{-1} , open symbols), or with Hoe 140 (100 nmol kg^{-1} , closed symbols). From the changes in haematocrit, the % changes in total blood volume (V_B , b) and in plasma volume (V_P , c) were calculated. Symbols represent mean values; vertical lines show s.e.mean. Where no s.e.mean is given it was smaller than the symbol; $n = 5\text{--}6$ in all experimental groups. Significance of difference from rats receiving caerulein only: * $P < 0.05$; ** $P < 0.01$.

The effect of caerulein was completely abolished in rats which had been pretreated with Hoe 140 (100 nmol kg^{-1} , s.c.). Hoe 140, administered to animals not receiving caerulein, did not have any effect by itself (not shown in Figure 2).

Enzyme activities in serum and in the pancreas

The activities of amylase and lipase in blood serum were not increased 10 min after the start of the infusion of caerulein, but showed a continuous rise thereafter (Figure 3a and c). In animals which had been pretreated with Hoe 140 (100 nmol kg^{-1} , s.c.), this rise in the serum activities of amylase and lipase was significantly augmented ($P < 0.05$) both at 45 min and at 120 min. The values for lipase activity obtained at 120 min were somewhat higher than those determined in our previous investigation (Griesbacher & Lembeck, 1992a). This is probably due to the differences in the methods used for the measurement of lipase activity.

Ten minutes after the start of the caerulein infusion, the activities of amylase and lipase in the pancreas were not increased. When caerulein was infused for periods of up to 120 min, the enzyme activities rose progressively to values that were about 30 times higher than the basal values (Figure 3b and d). In contrast to the effect of Hoe 140 on caerulein-induced changes in enzyme activities in the serum (see above), the caerulein-induced increases in enzyme activities in the pancreas were significantly attenuated by Hoe 140 both at 45 min ($P < 0.05$) and at 120 min ($P < 0.01$). At 120 min, the amylase and lipase activities in the pancreas were increased only by about five fold ($P < 0.05$).

Trypsin activity was below the limit of detection in the serum of control animals. After a 2 h infusion of caerulein, a small, but significant ($P < 0.05$), trypsin activity was found (median 0.005 u ml^{-1} , range $0.002\text{--}0.011 \text{ u ml}^{-1}$, $n = 8$). The trypsin activities found in the serum of rats pretreated with Hoe 140 (median 0.008 u ml^{-1} , range $0\text{--}0.012 \text{ u ml}^{-1}$, $n = 7$) were not significantly different from those obtained from rats treated with caerulein alone. In the pancreas of control animals, trypsin activity was found in only 1 animal (0.301 u g^{-1} dry weight), whereas no trypsin activity was detectable in the other 4 controls. The infusion of caerulein did not induce a significant elevation of active trypsin in the pancreas (median 0.049 u g^{-1} dry weight, range $0\text{--}0.672 \text{ u g}^{-1}$ dry weight). Hoe 140 had no influence on these values (median 0 u g^{-1} dry weight, range $0\text{--}0.277 \text{ u g}^{-1}$ dry weight).

Effects of protease inhibitors

To investigate the effects of protease inhibitors on the pancreatic oedema in caerulein-induced pancreatitis, aprotinin ($20,000 \text{ u kg}^{-1}$), or SBTI (16 mg kg^{-1}), was injected i.v. either 10 min before, or 10 min after, the start of the caerulein infusion. All animals were given an i.v. injection of Evans blue (5 mg kg^{-1}) 5 min before caerulein in order to stain the plasma proteins. The effect of aprotinin was inconsistent when it was injected 10 min after the beginning of the infusion of caerulein. In 3 out of the 8 animals in this experimental group both the water content and the Evans blue content of the pancreas were almost normal, whereas in the other 5 animals caerulein-induced oedema was as pronounced as in animals that had not been given a protease inhibitor. In this group, the water content of the pancreas (Figure 4a) was not statistically different from both the basal values and from the values found in the group treated with caerulein alone. Pretreatment with aprotinin 10 min prior to the caerulein infusion had no effect on the caerulein-induced extravasation of water (Figure 4a) or on extravasation of plasma proteins (Figure 4b). SBTI also had no effect on the caerulein-induced extravasation of water or plasma proteins into the pancreas.

Figure 3 Activities of amylase (a and b) and of lipase (c and d) in serum (in u ml^{-1} , a and c) and in the pancreas (in u g^{-1} dry weight, b and d): acute pancreatitis was induced in rats by an i.v. infusion of caerulein ($4 \text{ nmol kg}^{-1} \text{ h}^{-1}$) for periods of 10, 45 and 120 min; 30 min prior to this infusion, the rats were injected s.c. either with saline (0.5 ml kg^{-1} , ○) or with Hoe 140 (100 nmol kg^{-1} , ●). Control animals received saline as s.c. injection and as i.v. infusion (□). Symbols represent mean values, vertical lines show s.e.mean; where no s.e.mean is given it was smaller than the symbol. $n = 7\text{--}15$. Significance of difference between the effects of caerulein in rats injected with Hoe 140 and in rats injected with saline: * $P < 0.05$; ** $P < 0.01$. Significance of difference from results in control animals infused with saline: † $P < 0.05$; ‡ $P < 0.01$; ‡‡‡ $P < 0.001$.

Figure 4 Effects of aprotinin and soybean trypsin inhibitor (SBTI) on the formation of the pancreatic oedema in caerulein-induced acute pancreatitis in rats: caerulein (8 nmol kg⁻¹ administered over 120 min) was given as an i.v. infusion. The pancreatic oedema was measured as the water content of the tissue (in g g⁻¹ dry weight of the pancreas, a) and the extravasation of plasma proteins was quantified as the tissue content of Evans blue (in µg g⁻¹ dry weight, b). Aprotinin (20,000 u kg⁻¹, hatched columns), or SBTI (16 mg kg⁻¹, cross-hatched columns), was injected i.v. either 10 min before, or 10 min after, the start of the caerulein infusion. Solid columns show the effects of caerulein alone; open columns give control values determined in animals given an i.v. infusion of saline (0.034 ml min⁻¹) instead of caerulein. Column heights represent mean values, vertical lines show s.e.mean; $n = 8-14$. Values obtained after treatment with aprotinin or SBTI were not significantly different from those obtained with caerulein alone. Significance of difference from control values: $\dagger P < 0.05$; $\dagger\dagger P < 0.01$.

Discussion

In man, acute pancreatitis occurs in two forms, the interstitial oedematous form and the necrotizing haemorrhagic. The majority of the patients show the former course of the disease (Büchler, 1991). Experimental models exist for both types of acute pancreatitis (see Adler *et al.*, 1986). Hyperstimulation of the exocrine function of the pancreas with caerulein, a decapeptide analogue of cholecystokinin, is a widely used method of inducing morphological and biochemical changes in the gland, that are similar to those found in oedematous pancreatitis in man (Willemer *et al.*, 1990). The involvement of endogenous kinins in clinical pancreatitis was already suspected soon after the discovery of the kallikrein-kinin system (Werle *et al.*, 1958). Several enzymes found in the pancreas are able to release kinins from their precursors, the kininogens. Kallikrein, which is present in the pancreas in large amounts, is a specific kininogenase. Trypsin and cathepsin B are also capable of releasing kinins. Both enzymes are activated in the pancreas during experimental pancreatitis (Yamaguchi *et al.*, 1989). The intracellular fusion of zymogen granules with lysosomes, and subsequent release of their contents into the interstitial space, observed after hyperstimulation of the exocrine pancreas (Watanabe *et al.*, 1984;

Saluja *et al.*, 1987), suggests a mechanism for the activation of such enzymes. Previously, we have shown that Hoe 140, a recently developed, potent and long-acting BK antagonist (Lembeck *et al.*, 1991; Hock *et al.*, 1991; Wirth *et al.*, 1991; Griesbacher & Lembeck, 1992b), is effective in preventing the formation of the pancreatic oedema and of the second phase of hypotension observed during experimental acute pancreatitis (Griesbacher & Lembeck, 1992a). However, it was observed that the caerulein-induced increases in the activities of amylase and lipase in serum were augmented by Hoe 140. These results suggested that the kinin-mediated pancreatic oedema partly traps activated enzymes, such as amylase or lipase, within the pancreas.

Pancreatic oedema, hypovolaemia and haemoconcentration

The concept that endogenous kinins are responsible for some of the symptoms of pancreatitis, in particular the pronounced hypotension which can extend to circulatory shock, was based mainly on the fact that the blood or ascitic fluid of patients or experimental animals with pancreatitis contained a factor that lowered blood pressure when injected into recipient animals (Nugent & Atendido, 1966), had a BK-like effect on smooth muscles (Amundsen *et al.*, 1968; Ofstad, 1970), and had physicochemical properties similar to BK (Amundsen *et al.*, 1968; Ofstad, 1970; Ellison *et al.*, 1981). Intravenous injection of BK lowers blood pressure mainly by vasodilatation. This might lead to the assumption that the kinins, which are released endogenously during acute pancreatitis, produce hypotension by this mechanism. However, kinins are extremely shortlived *in vivo* (Ward, 1991) and a continuous release of kinins over a prolonged period of time would be necessary to produce the hypotension which occurs during acute pancreatitis by this mechanism. On the other hand, it has also been demonstrated that a considerable amount of fluid, presumably blood plasma, accumulates in the pancreas and in the peritoneal cavity (Ryan *et al.*, 1964). This suggests that hypovolaemia, due to plasma extravasation, may be the main cause for the circulatory shock in acute pancreatitis.

In our previous study with Hoe 140 (Griesbacher & Lembeck, 1992a) we showed that this compound prevented or reduced the development of pancreatic oedema in rats when it was injected before, or up to 25 min after, the start of an infusion of caerulein. Hoe 140, injected s.c. in the dose used (100 nmol kg⁻¹), effectively blocks the actions of i.v. BK within 5 min (personal observations). Therefore, it was concluded that endogenous kinins increase vascular permeability in the pancreas only during the first 30–45 min of the infusion of caerulein. The time course of the development of the pancreatic oedema shows that the oedema formation reaches a maximum within about 45 min (see Figure 1). Plasma proteins no longer leave the circulation during the 2nd h of the caerulein infusion. This indicates that vascular permeability apparently has returned to a normal value by this time. In addition, reduced blood flow through the pancreas, due to the collapse of a considerable proportion of the capillaries (Gress *et al.*, 1990), might also be partially responsible for the fact that no further extravasation takes place. The water content of the pancreas showed a slight decline during this period (Figure 1a); the amount of Evans blue-stained plasma proteins in the pancreas remained constant (Figure 1b). The continuous increase in the total protein concentration in the oedema fluid (Figure 1c) could, thus, be explained by a concentrating effect due to the onset of reabsorption of fluid, but not of proteins, from the tissue to the vasculature.

The results described above show that the vascular permeability in the pancreas is increased only during the first 30–45 min of the caerulein infusion. This increase in vascular permeability can be entirely attributed to the actions of endogenous kinins because Hoe 140 completely prevented the

formation of the oedema. On the other hand, the pronounced hypotension is present throughout the 2 h of the experiment (see Griesbacher & Lembeck, 1992a). Thus, a kinin-mediated hypovolaemia seems to be the most likely explanation for the fall in blood pressure. Provided that the total volume of the corpuscular elements of the blood remains constant, changes in the haematocrit allow the estimation of changes in total blood volume and plasma volume. By measuring the haematocrit repeatedly during the experiment, we were able to demonstrate a 48% fall in plasma volume and a reduction of total blood volume by 28%. Thus, the kinin-mediated pancreatic and retroperitoneal oedema can be seen as the main, if not the sole, cause of the hypotension. The fact that the time-course of the changes in the haematocrit (Figure 2) runs in parallel with the time-course of the hypotension (compare Figure 2 in Griesbacher & Lembeck, 1992a) and with the time-course of the oedema formation in the pancreas (see Figure 1) further supports this view. Whether there are additional factors contributing to hypotension is currently being investigated. The haemoconcentration observed during acute pancreatitis, together with the pronounced hypotension, will certainly have a severe effect on the microcirculation of the pancreas. Hoe 140 completely prevents haemoconcentration and hypotension and, therefore, might prevent tissue damage due to impaired tissue oxygenation.

Egress of activated enzymes from the pancreas

It was noted previously, that pretreatment of rats with the BK antagonist, Hoe 140, despite its inhibitory actions on oedema formation and hypotension, augmented the increases in the serum activities of amylase and lipase at the end of the 2 h infusion of caerulein (Griesbacher & Lembeck, 1992a). It was hypothesized that the oedema of the pancreas partially traps activated enzymes in the tissue and that prevention of the oedema formation would improve the egress of the enzymes from the gland. In order to check this explanation we have now determined the enzyme activities in serum as well as in the pancreas. The augmentation by Hoe 140 of the enzyme activities in serum was again seen when the time-courses of the rises in amylase and lipase activities in serum were determined. In the pancreas, the activities of amylase and lipase increased throughout the course of the infusion of caerulein. If our hypothesis were correct, then the enzyme activities in the pancreas ought to be reduced by treatment with Hoe 140. As can be seen from Figure 3, pretreatment with Hoe 140 potentially attenuated the caerulein-induced rises in enzyme activities in the pancreas.

The present results verify the proposition that prevention by Hoe 140 of increased vascular permeability would greatly facilitate the egress of activated enzymes from the pancreas during acute pancreatitis. Since accumulation of active lipase in the tissue, in conjunction with other factors frequently implicated in the pathogenesis of acute pancreatitis, such as bile salts, is thought to be important for the development of pancreatic necroses (Creutzfeldt & Schmidt, 1970), the effects of Hoe 140 described here would appear to be of great importance for the treatment of acute pancreatitis in man.

The augmentation by Hoe 140 of the serum activities of amylase and lipase during caerulein-induced acute pancreatitis also indicated that Hoe 140 does not inhibit the caerulein-induced stimulation of the exocrine pancreas. *In vitro* experiments confirmed that Hoe 140, in concentrations which effectively abolish the effects of BK, do not affect caerulein-induced contractions of the guinea-pig ileum and, thus, has no effect on CCK receptors (data not shown).

Trypsin has been implicated in the pathogenesis of acute pancreatitis by Forrell (1955). Trypsin and other proteases have also been found to be increased in a model of experi-

mental pancreatitis similar to the one used in the present investigation (Yamaguchi *et al.*, 1989). In the present investigation, we have found that active trypsin might be slightly increased in serum and in the pancreas. However, the measured trypsin activities were near the limits of detection so that a definite statement as to the effects of Hoe 140 on trypsin activity cannot be made.

Comparison of the effects of Hoe 140 and protease inhibitors

In caerulein-induced pancreatitis oedema was found to be wholly due to the actions of endogenously released kinins since Hoe 140 prevented the oedema formation. In addition to kallikrein, the specific kininogenase, trypsin has the ability to release kinins from their precursor peptides (Rocha e Silva *et al.*, 1949). Both trypsin and kallikrein can be inhibited by protease inhibitors such as aprotinin or SBTI (see Vogel, 1979). Aprotinin has been used for the treatment of acute pancreatitis in experimental and in clinical trials. Neither aprotinin nor SBTI had a significant inhibitory action on oedema formation in our experiments regardless of whether they were administered before or after the start of the caerulein infusion (compare Figure 4). In contrast, our previous results with Hoe 140 (Griesbacher & Lembeck, 1992a) showed that it was effective even when injected up to 25 min after the induction of pancreatitis by caerulein.

All clinical studies with aprotinin, with one exception (Trapnell *et al.*, 1974), have failed to show a positive effect which contrasts with the results obtained in experimental investigations (see Steinberg & Schlesselman, 1987). This discrepancy has been explained by the fact that, in experimental studies, aprotinin was usually administered before the induction of pancreatitis, whereas this of course does not occur in clinical situations. On the other hand, recent clinical investigations have shown that positive results can be obtained when therapy with protease inhibitors is introduced within the first 24 h after the onset of the disease (Harada *et al.*, 1991). Bearing in mind that the time course of pathological events is much more rapid in experimental studies than in the clinical course of the human disease (see Steinberg & Schlesselman, 1987), the ability of Hoe 140 to reduce pathological events in the pancreas when administered after the induction of pancreatitis (Griesbacher & Lembeck, 1992a) adds to the possible therapeutic value of Hoe 140.

In summary, it has been demonstrated that in experimental acute pancreatitis the release of endogenous kinins in the pancreas leads to a massive extravasation of plasma into the pancreatic and adjacent retroperitoneal tissues. The loss of plasma from the circulation results in severe hypotension and haemoconcentration. These pathological events could be prevented by timely intervention with the potent BK antagonist, Hoe 140. Treatment with Hoe 140 also reduced the accumulation of enzymes which are released and activated in the pancreas during the course of the pancreatitis. The concomitant augmentation of the enzyme activities in serum necessitates further assessment of the prognostic consequences of treatment with Hoe 140. Thus, other factors, which, when released into the blood stream, cause tissue or organ damage, have to be studied. Since the severity of pancreatitis does not correlate with the serum levels of amylase and lipase, but rather with the extent of pancreatic oedema and its sequelae (Büchler, 1991), it is conceivable that prevention of oedema formation will also prevent the formation of such factors.

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