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Epidermal growth factor accelerates pancreatic recovery after caerulein-induced pancreatitis

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Abstract

We examined the influence of endogenous and exogenous epidermal growth factor (EGF) on pancreatic repair after acute pancreatitis. Caerulein-induced pancreatitis was evoked in rats with intact or removed salivary glands and EGF (10 μg/kg) was administered starting 24 h after cessation of caerulein infusion. The dose of EGF 10 μg/kg was chosen because it was the most effective in preliminary experiments when 1, 10 or 50 μg/kg of EGF was used. Caerulein administration caused acute edematous pancreatitis with biochemical and histological manifestation of pancreatic damage, followed by spontaneous regeneration. The effect of salivectomy on the course of acute pancreatitis was slight, resulting in additional reduction in pancreatic blood flow, DNA synthesis and in an increase in plasma interleukin 1β level. Treatment with EGF accelerated the healing of pancreatic damage, causing an increase in pancreatic blood flow and DNA synthesis. EGF caused faster normalization of plasma amylase and lipase activity and plasma interleukin 1β concentration, as well as, this peptide accelerated the restoration of pancreatic amylase activity. On histological examination, EGF caused reduction of pancreatic damage and acceleration of tissue repair. We conclude that EGF reduces the severity of pancreatic damage evoked by caerulein-induced pancreatitis-related pancreatic damage and accelerates tissue repair. The beneficial effects of EGF appear to depend, at least in part, on the improvement of pancreatic blood flow, as well as on an increase of pancreatic cell growth and limitation of the activation cytokine release.

Keywords: Pancreatitis; EGF (epidermal growth factor); Pancreatic regeneration; Interleukin 1β

1. Introduction

Epidermal growth factor (EGF), a 53 amino acid single-chain polypeptide, was originally isolated from male mouse submandibular glands (Cohen, 1962). It has also been found in duodenal Brunner's glands, kidneys and in several biological fluids such as saliva, milk, urine, gastric and duodenal juice (Gregory et al., 1979; Kasselberg et al., 1985). A significant amount of EGF was found in human (Hirata and Orth, 1979) and animal (Jaworek et al., 1992) pancreas and pancreatic juice. This peptide is a powerful mitogen and an inhibitor of gastric acid secretion (Dembiński et al., 1982; Bower et al., 1975). Previous studies have demonstrated that EGF participates in the maintenance of gastroduodenal mucosal integrity, protects the gastric mucosa against the damage caused by various noxious agents (Konturek et al., 1981; Konturek et al., 1989; Skov Olsen et al., 1984) and promotes healing of chronic gastric and duodenal ulceration in rats (Skov Olsen et al., 1986; Konturek et al., 1988). The excision of salivary glands (to remove the major source of endogenous EGF) is accompanied by an increase in the susceptibility of gastric mucosa to damage (Skinner et al., 1984) and by delay in peptic ulcer healing (Konturek et al., 1988). EGF modulates exocrine pancreatic secretion in vitro (Stryjek-Kamińska et al., 1995) and in vivo (Konturek et al., 1984) as well as stimulates pancreatic growth (Dembiński et al., 1982; Logsdon, 1986), acting directly on pancreatic acinar cells. It also enhances the survival of pancreatic cells in serum-free culture (Brannon et al., 1985).

Acute pancreatitis is an inflammatory disease associated with autodigestion of the pancreas due to intrapancreatic

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activation and release of digestive enzymes (Steer, 1993; Glasbrenner and Adler, 1993). During pancreatitis there occurs necrosis, edema, vascular injury, leukocytic infiltration (Gorelick et al., 1993) as well as an increase in overexpression of epidermal growth factor (EGF) receptor family (Ebert et al., 1996) and in EGF binding to acini during pancreatic regeneration (Brockenbrough et al., 1988). In our previous study, we found that EGF given before and during the induction of caerulein-induced pancreatitis protects against caerulein-evoked pancreatic damage (Warzecha et al., 1999). Another study (Liu et al., 1997) demonstrated that treatment with EGF may prevent septic complication in acute necrotizing pancreatitis. There is a lack of information as to whether EGF treatment after induction of pancreatitis can affect pancreatic tissue regeneration. An answer to this question is essential if EGF is to be used in the therapy of acute pancreatitis. Therefore, this study was designed to assess the influence of EGF administration after induction of caerulein-induced pancreatitis on pancreatic tissue repair and integrity.

2. Materials and methods

Studies were performed on male Wistar rats weighing 180–200 g. The animals were housed in cages with wire mesh bottoms, with normal room temperature and a 12-hour light–dark cycle. Drinking water and food were available ad libitum. The study was conducted following the experimental protocol approved by the Committee for Research and Animal Ethics of Jagiellonian University.

The effect of EGF on the course of caerulein-induced pancreatitis was investigated using rats with intact salivary glands or with resected salivary gland complex (salivectomy). Salivectomy was performed 10 days before the induction of pancreatitis to remove the major endogenous source of EGF. All control animals were sham-operated, including the transection of skin at the neck and handling of salivary glands without their removal.

Pancreatitis was induced by caerulein infusion in rats kept in individual cages. Caerulein (Takus, Pharmacia and Upjohn, Erlangen, Germany) was diluted in saline and infused s.c. for 5 h at 10 μg/kg/h and at a rate of 1.0 ml/h.

Experiments were carried out in two separate series. The first series was performed to choose the appropriate dose of EGF for further study. The following groups of animals were used: (1) rats with intact salivary glands infused with saline s.c. to serve as a control group; (2) rats with intact salivary glands and treated with EGF (human recombinant EGF, Pharma Biotechnologie Hannover, Hannover, Germany) at doses 1, 10 or 50 μg/kg (three s.c. injection daily, first injection 60 min after the termination of saline infusion); (3) rats with intact salivary glands and caerulein induced pancreatitis; (4) rats with intact salivary glands infused with caerulein and treated with EGF as in second group. The animals from the first series were killed 2 and 7 days after caerulein infusion.

The second series was performed using EGF at a standard dose, 10 μg/kg. This series of studies was carried out with the following experimental groups: (1) sham-operated rats with intact salivary glands infused with saline s.c. to serve as control group; (2) sham-operated rats with intact salivary glands and caerulein-induced pancreatitis; (3) sham-operated rats with caerulein-induced pancreatitis and EGF administration (three s.c. injection daily, first injection 24 h after the termination of caerulein infusion); (4) salivectomized rats infused with saline s.c.; (5) salivectomized rats with caerulein-induced pancreatitis; (6) salivectomized rats with caerulein-induced pancreatitis treated with EGF as third group of animals. Groups of animals with caerulein-induced pancreatitis without EGF treatment were killed at 12, 24, 48, 72 h and 5, 7 or 10 days after caerulein infusion. EGF administration was started 24 h after cessation of caerulein infusion because at this time maximal pancreatic tissue damage was observed. Animals treated with EGF were killed 48, 72 h and 5, 7 or 10 days after caerulein infusion. At the end of the experiment animals were anesthetized with ether, weighed and the abdomen was opened. The pancreas was exposed for the measurement of the pancreatic blood flow. Pancreatic blood flow was measured using the H_2-gas clearance technique (Biotechnical Science, Model RBF-2, Osaka, Japan) as described previously (Konturek et al., 1987). The clearance curve of tissue H_2 was used to calculate the absolute flow rate (ml/min/100 g) in the pancreatic tissue and measurements were made in three areas of the pancreas. The mean values of these measurements were calculated and expressed as percentages of the flow rate recorded in the control pancreas in healthy rats treated with vehicle.

Immediately after the measurement of pancreatic blood flow the abdominal aorta was exposed and blood was taken for determination of plasma amylase, lipase and interleukin 1β. Plasma and pancreatic amylase activity were determined by an enzymatic method (amylase reagent, Dialab Diagnostic, Wien, Austria) and values were expressed as units/liter (U/l). Plasma interleukin 1β was measured in duplicate using the BioSource Cytoscreen rat Il-1β kit based on a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) (BioSource International, Camarillo, CA, USA). Interleukin 1β concentration was determined from a standard curve for recombinant interleukin 1β and concentration was expressed as picograms per milliliter. The ELISA detection limit of interleukin 1β was 3 pg/ml.

Plasma lipase activity was determined with a Kodak Ectachem DT II System analyzer (Eastman Kodak, Rochester, NY, USA) using Lipa DT Slides (Vitros DT Chemistry System, Johnson & Johnson Clinical Diagnostic, Rochester, NY, USA).

After blood withdrawal, the pancreas was carefully dissected out from its attachment to the stomach, the

duodenum, and the spleen. The rate of DNA synthesis in a portion of minced pancreatic tissue was determined by incubating the tissue at 37°C for 45 min in 2 ml of medium containing 8 μCi/ml of [^{3}H]thymidine ([6-^{3}H]thymidine, 20–30 Ci/mmol; Institute for Research, Production and Application of Radiosotopes, Prague, the Czech Republic). RNA and DNA were measured using methods as described earlier (Dembiński et al., 1982). The incorporation of [^{3}H]thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a liquid scintillation system. RNA and DNA were expressed as milligrams per total pancreas weight. DNA synthesis was expressed as disintegrations [^{3}H]thymidine per minute per microgram DNA (d.p.m./μg DNA).

Samples of pancreatic tissue excised for morphological examination were fixed in 10% formalin, embedded in paraffin and sections were stained with hematoxylin–eosin. The slides were examined histologically by two experienced pathologists without the knowledge of the treatment. Edema was graded histologically on a scale ranging from 0 to 3: 0 = no edema, 1 = interlobular edema, 2 = interlobular and moderate intralobular edema, and 3 = interlobular edema and severe intralobular edema. Leukocytic infiltration was also graded from 0 (absent) to 3 (maximal diffuse infiltration in the entire pancreatic gland) Grading of vacuolization was based on the percentage of cell involved: 0 = absent, 1 = less than 25%, 2 = 25–50% and 3 = more than 50%

2.1. Statistical analysis

The differences between mean values from various groups of experiments were compared by analysis of variance and Student's *t*-test for unpaired data. A difference with a P value of less than 0.05 was considered statistically significant. Results are expressed as means (±S.E.M.).

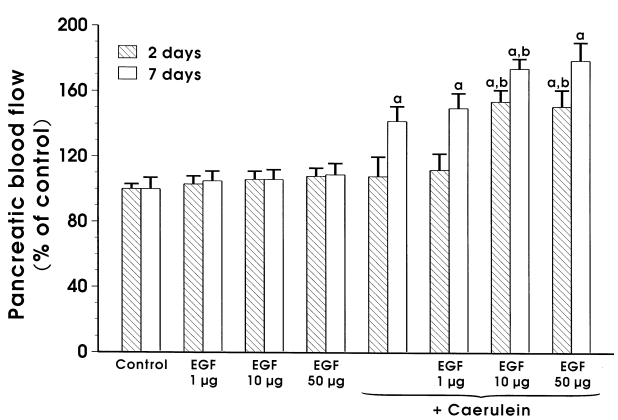

Fig. 1. Pancreatic blood flow in rats without or with caerulein-induced pancreatitis (caerulein 10 μg/kg/h for 5 h), treated with placebo, or EGF (given three times daily at doses of 1, 10 or 50 μg/kg). Mean ± S.E.M. of 6–10 observations. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein-induced pancreatitis.

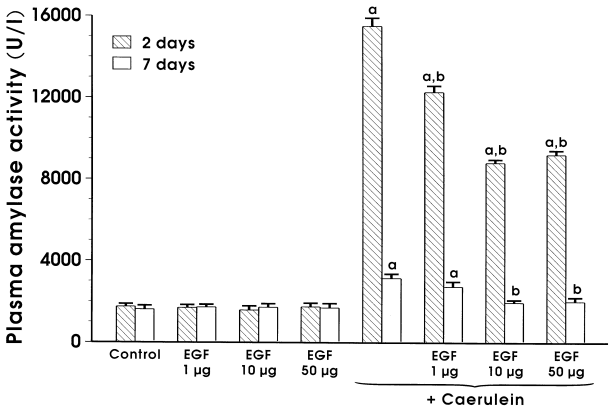

Fig. 2. Plasma amylase activity in rats in groups as in Fig. 1. Mean ± S.E.M. of 6–10 observations. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein-induced pancreatitis.

3. Results

3.1. First series of experiments

Pancreatic blood flow was not affected by EGF administered alone at 1, 10 and 50 μg/kg (Fig. 1) at any time of observation. Pancreatic blood flow 24 h after caerulein infusion (10 μg/kg/h s.c. for 5 h) was the same as the control. An increase of pancreatic blood flow was observed 5 days later. Exogenous EGF administered at 1 μg/kg in rats with acute pancreatitis did not affect significantly the changes in pancreatic blood flow evoked by caerulein. EGF, 10 μg/kg, caused a significant increase in pancreatic blood flow at 2 and 7 days in rats with pancreatitis. A similar effect was observed with 50 μg/kg of EGF.

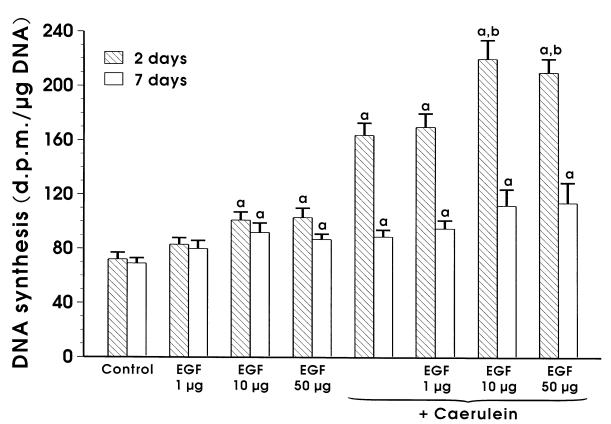

Fig. 3. DNA synthesis in rats as in groups in Fig. 1. Mean ± S.E.M. of 6–10 observations. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein-induced pancreatitis.

Plasma amylase activity in rats injected with various doses of EGF remained unchanged when compared to the control (Fig. 2). Caerulein-induced pancreatitis caused a ninefold increase in plasma amylase activity 2 days after pancreatitis development with a subsequent decrease at the seventh day. All doses of EGF tested decreased plasma amylase activity in acute pancreatitis. The strongest effect was observed with 10 μg/kg EGF, with a similar effect 50 μg/kg.

EGF given alone at 10 or 50 μg/kg significantly increased DNA synthesis at all times tested (Fig. 3). DNA synthesis 2 days after cessation of caerulein infusion was significantly increased, with a subsequent decrease at the seventh day. Administration of EGF, 1 μg/kg did not affect the changes in DNA synthesis evoked by caerulein infusion. EGF at both 10 and 50 μg/kg caused a significant and equal increase in DNA synthesis in the course of caerulein-induced pancreatitis.

EGF alone did not affect plasma interleukin 1β concentration at any doses used (Fig. 4). The induction of pancreatitis resulted in a significant increase in plasma interleukin 1β concentration. This effect was significantly reduced to the same extent at all times tested when EGF was administered at 10 or 50 μg/kg.

3.2. Pancreatic blood flow

As shown in Fig. 5, caerulein infusion (10 μg/kg/h s.c. for 5 h) induced a significant decrease in pancreatic blood flow, reaching 75 ± 6.0% of the control value 12 h after cessation of the infusion and this was followed by a significant rise above the control at the 7th and 10th days of observation. Salivectomy combined with caerulein infusion led to a decrease in pancreatic blood flow greater than that after caerulein alone and prolonged this decrease up to the second day. Pancreatic blood flow was markedly increased above the only 10 days after induction of pancreatitis control. Pancreatic blood flow in each group during regeneration increased progressively reaching its highest

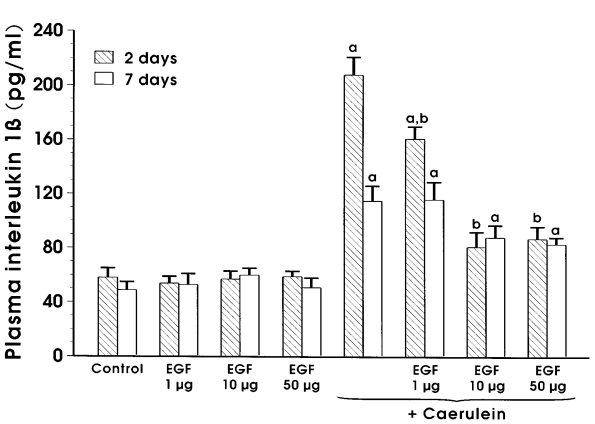

Fig. 4. Plasma interleukin 1β concentration in rats as in Fig. 1. Mean ± S.E.M. of 6–10 observations. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein-induced pancreatitis.

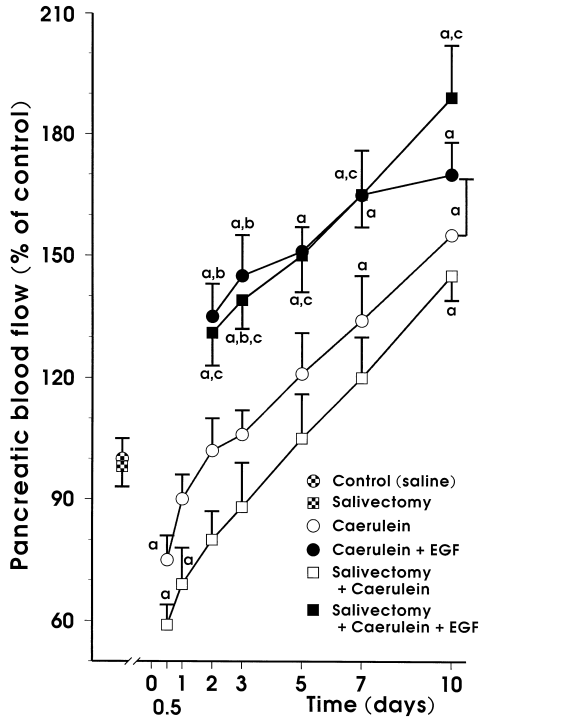

Fig. 5. Pancreatic blood flow in rats without or with caerulein-induced pancreatitis, sham-operated or salivectomized (caerulein 10 μg/kg/h for 5 h), treated with placebo, or EGF (10 μg/kg three times daily, starting 24 h after cessation of caerulein infusion). Mean ± S.E.M. of 6–14 observations. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein-induced pancreatitis, [c]$P < 0.05$ compared with caerulein-induced pancreatitis combined with salivectomy.

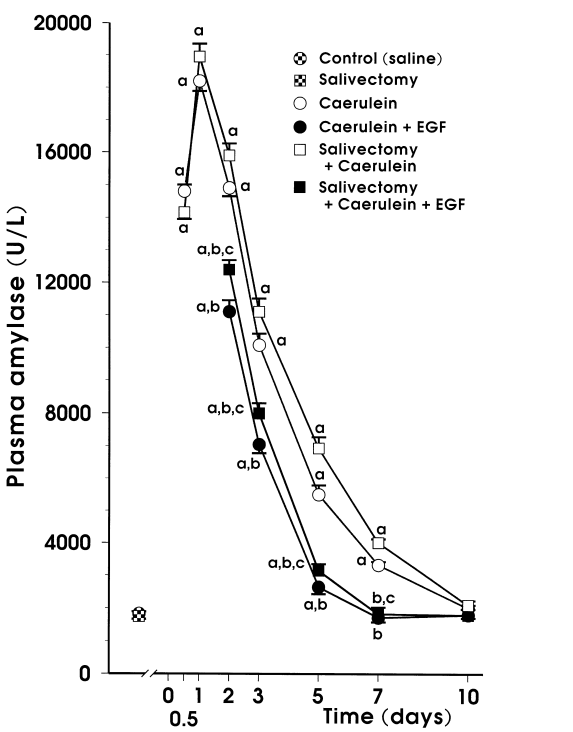

Fig. 6. Plasma amylase activity in rats in groups as in Fig. 5. Mean ± S.E.M. of 6–14 observations. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein-induced pancreatitis, [c]$P < 0.05$ compared with caerulein-induced pancreatitis combined with salivectomy.

values at the 10th day after induction of pancreatitis; administration of exogenous EGF caused an additional increase in pancreatic blood flow. This effect of EGF was significant at the second and third day.

3.3. Biochemical parameters

After induction of pancreatitis, plasma amylase activity (Fig. 6) was elevated, reaching maximal value 24 h after cessation of caerulein infusion (18 200 ± 310 U/l for animals with intact salivary glands, 18 950 ± 405 U/l for salivectomized animals). Ten days later plasma amylase activity returned to control value in all groups tested. EGF administration combined with pancreatitis in animals with intact or removed salivary glands caused a significant decrease in plasma amylase activity at the second, third, fifth and seventh day after caerulein infusion.

Plasma lipase activity curves during the course of caerulein-induced pancreatitis (Fig. 7) exhibited changes similar to those obtained with plasma amylase activity, however, maximal plasma lipase activity was reached 12 h after cessation of caerulein infusion. Administration of EGF caused a significant reduction in plasma lipase activity 48 h after the development of acute pancreatitis.

Pancreatic amylase activity (Fig. 8) was diminished following induction of pancreatitis by caerulein in all groups after 12 h and remained decreased to this level up to the third day, and then tended to return to its control

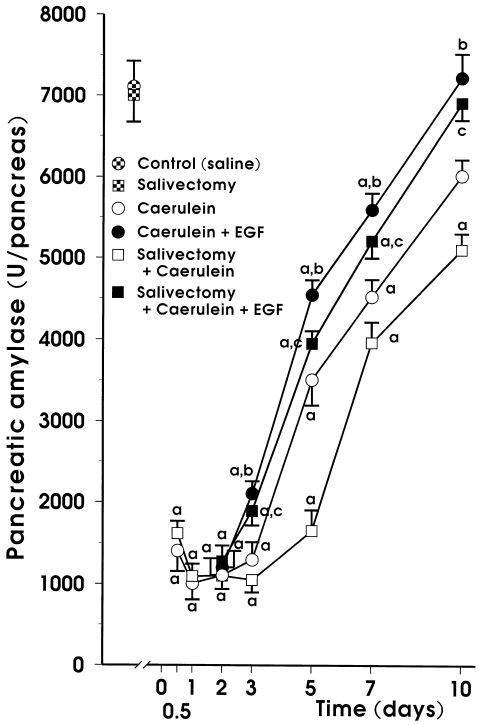

Fig. 8. Pancreatic amylase activity in rats as in Fig. 5. Mean ± S.E.M. of 6–14 observations. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein-induced pancreatitis, [c]$P < 0.05$ compared with caerulein-induced pancreatitis combined with salivectomy.

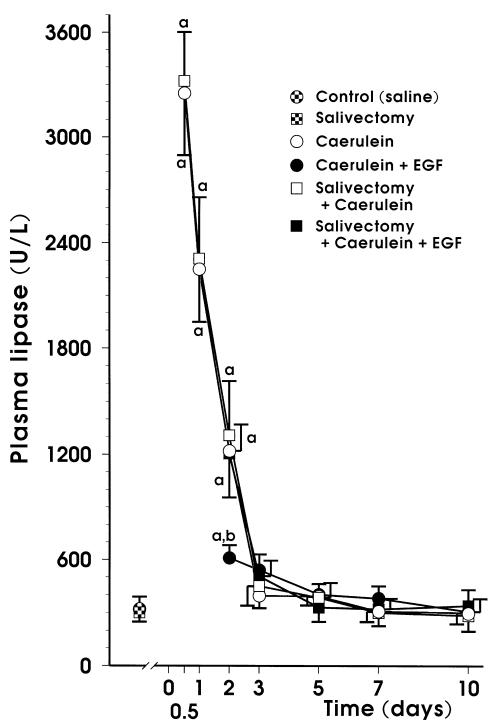

Fig. 7. Plasma lipase activity in rats in groups as in Fig. 5. Mean ± S.E.M. of 6–14 observations. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein-induced pancreatitis, [c]$P < 0.05$ compared with caerulein-induced pancreatitis combined with salivectomy.

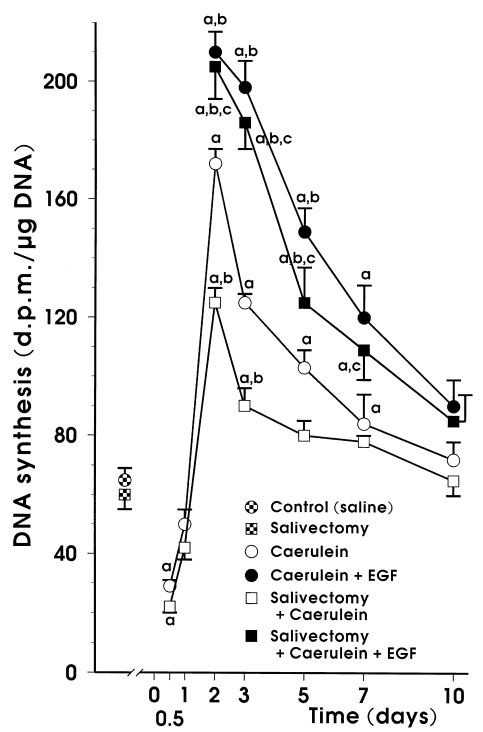

Fig. 9. DNA synthesis in rats as in groups in Fig. 5. Mean ± S.E.M. of 6–14 observations. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein-induced pancreatitis, [c]$P < 0.05$ compared with caerulein-induced pancreatitis combined with salivectomy.

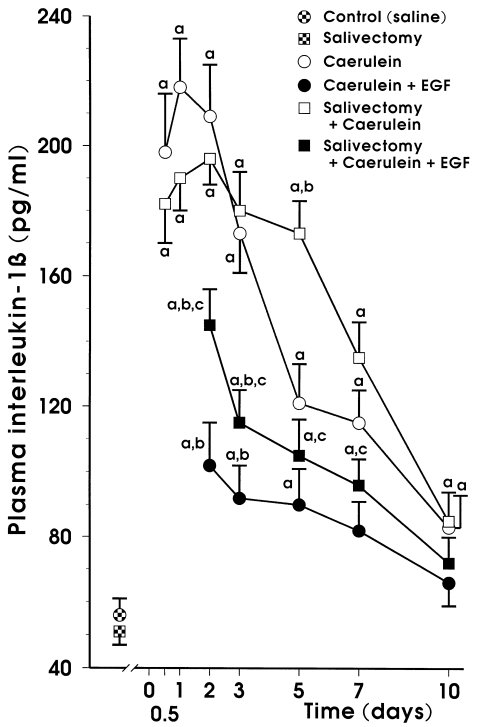

Fig. 10. Plasma interleukin 1β concentration in rats as in Fig. 5. Mean ± S.E.M. of 6–14 observations. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein-induced pancreatitis, [c]$P < 0.05$ compared with caerulein-induced pancreatitis combined with salivectomy.

value. The recovery of pancreatic amylase activity was significantly faster after addition of exogenous EGF and was manifested as a significant difference in this activity from the third to the 10th day. On the other hand, salivectomy tended to delay the recovery of pancreatic amylase activity in rats with caerulein-induced pancreatitis but this effect did not reach statistical significance at any periods tested.

DNA synthesis decreased significantly in all groups tested immediately after pancreatitis induction and this was followed by a significant rise with a peak at the second day (Fig. 9). This increase was significantly higher in groups of rats which, additionally, were injected with EGF (caerulein alone 172 ± 5 d.p.m./μg DNA vs. Caerulein + EGF 210 ± 7 d.p.m/μg DNA and caerulein + salivectomy 125 ± 5 d.p.m./μg DNA vs. caerulein + salivectomy + EGF 205 ± 11 d.p.m./μg DNA). Moreover, DNA synthesis in salivectomized rats with pancreatitis was significantly lower 2 and 3 days after the induction of pancreatitis than in the sham-operated animals with acute pancreatitis.

Plasma interleukin 1β levels (Fig. 10) were sharply increased 12 h after caerulein infusion in sham-operated rats and in salivectomized rats. Five days later both values tended to decrease but remained elevated above the control value to the 10th day. Addition of EGF to the infusion caused a significant reduction in the increase of interleukin

Table 1
Effect of EGF administration (10 μg/kg three times daily, starting 24 h after cessation of caerulein infusion) on pancreatic weight and nucleic acids content after caerulein-induced pancreatitis (caerulein s.c. 10 μg/kg/h for 5 h) in rats with intact or removed salivary glands. Observations at 12, 24, 48, 72 h and 5, 7 and 10 days after caerulein infusion
Mean ± S.E.M. for 6–14 rats.

	12 h	24 h	48 h	72 h	5 days	7 days	10 days
Pancreatic weight (mg)							
Saline (795 ± 69)							
Caerulein	1298 ± 80[a]	1310 ± 109[a]	1415 ± 84[a]	972 ± 87	911 ± 105	692 ± 54	689 ± 26
Salivectomy (792 ± 34)							
Salivectomy + caerulein	1263 ± 76[a]	1292 ± 99[a]	1479 ± 76[a]	947 ± 61	887 ± 72	673 ± 54	705 ± 41
Caerulein + EGF			1031 ± 99[b]	862 ± 63	832 ± 54	732 ± 41	781 ± 565
Salivectomy + caerulein + EGF			1115 ± 80[c]	987 ± 96	851 ± 42	626 ± 48	752 ± 49
RNA content mg / pancreas							
Saline (8.89 ± 0.16)							
Caerulein	8.36 ± 0.13	8.03 ± 0.10[a]	8.09 ± 0.14[a]	8.34 ± 0.14	8.54 ± 0.19	8.76 ± 0.17	8.99 ± 0.19
Salivectomy (7.93 ± 0.28)							
Salivectomy + caerulein	8.13 ± 0.10[a]	7.96 ± 0.09[a]	8.10 ± 0.15[a]	8.21 ± 0.19	8.35 ± 0.18	8.81 ± 0.20	9.76 ± 0.18
Caerulein + EGF			8.74 ± 0.12[b,c]	8.80 ± 0.19	8.99 ± 0.19	9.07 ± 0.13	9.15 ± 0.18
Salivectomy + caerulein + EGF			8.69 ± 0.11[c]	8.73 ± 0.19	8.79 ± 0.16	8.91 ± 0.16	9.20 ± 0.18
DNA content (mg / pancreas)							
Saline (4.82 ± 0.17)							
Caerulein	4.21 ± 0.15	3.92 ± 0.08[a]	4.17 ± 0.09[a]	4.49 ± 0.15	4.62 ± 0.16	4.79 ± 0.19	5.08 ± 0.17
Salivectomy (4.49 ± 0.22)							
Salivectomy + caerulein	4.08 ± 0.13[a]	3.86 ± 0.08[a]	4.17 ± 0.12[a]	4.30 ± 0.14	4.47 ± 0.19	4.62 ± 0.18	4.97 ± 0.07
Caerulein + EGF			4.56 ± 0.13	4.76 ± 0.22	4.81 ± 0.11	5.21 ± 0.19	5.32 ± 0.21
Salivectomy + caerulein + EGF			4.43 ± 0.13	4.52 ± 0.19	4.59 ± 0.11	5.03 ± 0.12	5.22 ± 0.22

[a]$P < 0.05$ compared to the control value with intact salivary glands.
[b]$P < 0.05$ compared to value after caerulein alone at the same observation time.
[c]$P < 0.05$ compared to salivectomized rats infused with caerulein at the same observation time.

1β concentration. In contrast, salivectomy delayed the decrease of interleukin 1β when compared to that in sham-operated animals. This effect was significant at the fifth day of observation.

Pancreatic weight in caerulein-induced pancreatitis (Table 1) was significantly increased, reaching at 12 h after the end of caerulein infusion 1298 ± 80 mg vs. 795 ± 69 mg. Pancreatic weight returned to its control value during subsequent days after caerulein infusion. Salivectomy combined with pancreatitis did not affect significantly the rise in pancreatic tissue weight after caerulein infusion or the return of this weight to the control value. Exogenous EGF diminished the caerulein infusion-evoked increase of pancreatic weight in both intact and salivectomized rats and this effect was significant 48 h after pancreatitis induction.

The RNA content was significantly decreased 24 and 48 h after pancreatitis induction when compared to the control (8.03 ± 0.10 mg and 8.09 ± 0.14 vs. 8.89 ± 0.16 mg). Salivectomy in rats with caerulein-induced pancreatitis did not affect significantly the RNA content in the tissue when compared to that in sham-operated animals with pancreatitis. Exogenous EGF prevented the drop in RNA content in both sham-operated and salivectomized group.

The DNA content was decreased significantly 24 and 48 h after caerulein-induced pancreatitis in sham-operated rats. In salivectomized animals this decrease occurred earlier, at 12 h after pancreatitis development. Exogenous EGF prevented these decreases of DNA content in both sham-operated and salivectomized rats.

3.4. Histological findings

Infusion of caerulein consistently produced morphological features of caerulein-induced pancreatitis in all rats tested (Table 2). In sham-operated rats without EGF treatment the edema and the infiltration were significantly increased up to 3 days of observation, whereas the leukocytic infiltration was increased up to the fifth day. Vacuolization was resolved in all groups after 2 days of pancreatitis. Salivectomy did not affect significantly the histological signs of pancreatitis. Exogenous EGF caused a decrease of tissue edema that was significant at the third day of observation (caerulein + sham-operated 2.0 ± 0.0 vs. caerulein + EGF 1.2 ± 0.2 or salivectomy + caerulein + EGF 1.4 ± 0.2). Vacuolization was significantly reduced 2 days after caerulein-induced pancreatitis in the groups of salivectomized animals receiving exogenous EGF. Tissue infiltration after pancreatitis was less pronounced in groups of rats receiving EGF than in animals without EGF treatment and this effect was significant at the third day of observation (caerulein 1.0 ± 0.0 vs.

Table 2
Effect of EGF administration (10 μg/kg three times daily, starting 24 h after cessation of caerulein infusion) on histological features in the pancreas after caerulein-induced pancreatitis (caerulein s.c. 10 μg/kg/h for 5 h) in rats with intact or removed salivary glands. Observations at 12, 24, 48, 72 h and 5, 7 and 10 days after caerulein infusion
Mean ± S.E.M. for 6–14 rats.

	12 h	24 h	48 h	72 h	5 days	7 days	10 days
Edema (0–3)							
Saline (0.8 ± 0.2)							
Caerulein	2.5 ± 0.3[a]	1.8 ± 0.2[a]	1.8 ± 0.2	2.0 ± 0.0[a]	1.0 ± 0.2	1.0 ± 0.0	1.0 ± 0.0
Salivectomy (0.8 ± 0.2)							
Salivectomy + caerulein	2.0 ± 0.0[a]	2.2 ± 0.4[a]	1.8 ± 0.2[a]	1.6 ± 0.4	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.0
Caerulein + EGF			1.2 ± 0.2	1.2 ± 0.2[b]	1.2 ± 0.2	1.0 ± 0.2	0.6 ± 0.2
Salivectomy + caerulein + EGF			1.4 ± 0.2	1.4 ± 0.2[b]	1.2 ± 0.2	1.2 ± 0.2	0.6 ± 0.2
Infiltration (0–3)							
Saline (0)							
Caerulein	1.2 ± 0.2[a]	1.4 ± 0.2[a]	1.4 ± 0.2[a]	1.0 ± 0.0[a]	0.6 ± 0.2[a]	0	0
Salivectomy (0)							
Salivectomy + caerulein	2.0 ± 0.0[a]	1.4 ± 0.4[a]	1.4 ± 0.3[a]	1.0 ± 0.2[a]	0.8 ± 0.2[a]	0	0
Caerulein + EGF			1.0 ± 0.2	0.2 ± 0.2[b]	0.2 ± 0.2	0	0
Salivectomy + caerulein + EGF			1.2 ± 0.2	0.2 ± 0.2[b]	0.2 ± 0.2	0.2 ± 0.2	0
Vacuolization (0–3)							
Saline (0)							
Caerulein	2.0 ± 0.3[a]	1.0 ± 0.1[a]	1.0 ± 0.2[a]	0	0	0	0
Salivectomy (0)							
Salivectomy + caerulein	1.8 ± 0.2[a]	1.2 ± 0.2[a]	1.2 ± 0.1[a]	0	0	0	0
Caerulein + EGF			0.6 ± 0.1[a]	0	0	0	0
Salivectomy + caerulein + EGF			0.6 ± 0.1[a,c]	0	0	0	0

[a] $P < 0.05$ compared to the control value with intact salivary glands.
[b] $P < 0.05$ compared to value after caerulein alone at the same observation time.
[c] $P < 0.05$ compared to salivectomized rats infused with caerulein at the same observation time.

caerulein + EGF 0.2 ± 0.2 or salivectomy + caerulein + EGF 0.2 ± 0.2).

4. Discussion

Several lines of evidence indicate that EGF participates in the regulation of pancreatic exocrine function (Stryjek-Kamiska et al., 1995; Konturek et al., 1984) and modulates the proliferation of acinar and ductal cells (Brannon et al., 1985). Like other polypeptide growth factors, EGF interacts with a membrane-bound receptor at the surface of target cells (Korc et al., 1994). EGF handling by the pancreatic acinar cell is altered during the proliferative response to partial pancreatectomy (Brockenbrough et al., 1988). Also, an enhanced expression of EGF in rats with pancreatitis was demonstrated by immunochemistry and the detection of EGF mRNA in pancreatic tissue (Konturek et al., 1998). In an earlier study we demonstrated that exogenous EGF has a protective effect against the damage caused by acute pancreatitis (Warzecha et al., 1999). In that study as well as in our present study we observed that EGF, 10 μg/kg, had beneficial effects similar to those of higher doses of EGF in the course of acute pancreatitis. On the other hand, treatment with higher doses of EGF was reported to lead to some side effects (Vinter-Jensen et al., 1997). For these reasons the 10-μg/kg dose of EGF was chosen as standard dose. In the present study, EGF administration was started 24 h after the end of caerulein infusion because the maximal tissue damage was observed at this time. This protocol eliminated the protective action of EGF on the pancreas so that the influence of EGF on the healing processes in the pancreas could be studied.

This study provided evidence that the EGF accelerates pancreatic recovery after caerulein-induced pancreatitis. This effect is due, at least in part, to the improvement of pancreatic blood flow, decrease of cytokine generation and increase in pancreatic tissue DNA synthesis. These conclusions are supported by the following findings; (a) extirpation of salivary glands, one of the main sources of EGF, delayed the recovery of the pancreas and this was accompanied by a prolonged reduction of pancreatic blood flow, reduction in DNA synthesis and increase of interleukin 1β concentration and (b) exogenous EGF, given parentally, enhanced pancreatic tissue recovery in rats with intact salivary glands and in salivectomized animals.

The mode of EGF action on the pancreatic exocrine tissue remains unknown. We found that salivectomy combined with caerulein infusion produced a prolonged decrease of pancreatic blood flow when compared to that in intact rats with caerulein-induced pancreatitis. Exogenous EGF increased pancreatic blood flow. Adequate blood flow plays a crucial role in the physiological function of the pancreas and its disturbance is involved in the pathophysiology of this organ, leading to the intrapancreatic activation and release of lysosomal and exocrine enzymes causing pancreatic autodigestion, and production of reactive oxygen species (Waldner, 1992). Both moderate and severe pancreatitis were found to be accompanied by a progressive decrease in blood flow (Trudo Knoefel et al., 1994). A mild edematous pancreatitis, such as induced by caerulein infusion in our study, was found to be accompanied by initial hyperemia (Trudo Knoefel et al., 1994) followed by a severe reduction in pancreatic circulation (Furukawa et al., 1993). Additional reduction of the pancreatic blood flow by exposure of animals with pancreatitis to stress leads to augmentation of the edematous pancreatitis and hemorrhagic damage of pancreatic tissue (Furukawa et al., 1993). It was reported that excision of salivary glands aggravates experimental gastric ulcers (Skinner and Tepperman, 1981; Skinner et al., 1984), suggesting that EGF swallowed with saliva was acting directly on the gastric mucosa. The pancreas may be affected by EGF from saliva absorbed in substantial amounts by the mucosa of the oral cavity in adult rats (Purushotham et al., 1995). In our present study we observed the regulatory effect of endogeneous EGF on the pancreatic blood flow, the cytokine activation and DNA synthesis but it is of interest that no marked aggravation of pancreatitis occurred after salivectomy. The explanation for the weak effect of an endogenous source of EGF in our study could have been due to the involvement of EGF originating from other endogenous sources (Kasselberg et al., 1985), including the pancreas that was shown to produce EGF during pancreatitis (Konturek et al., 1998). Support for this hypothesis is that, in this and in our earlier studies (Warzecha et al., 1999), salivectomy alone in healthy rats without caerulein-induced pancreatitis failed to affect pancreatic blood flow, suggesting that endogenous EGF in intact rats does not contribute significantly to the maintenance of pancreatic blood flow.

The major finding of the present study was the demonstration that exogenous EGF accelerates the recovery of the pancreas after pancreatitis. This effect was closely correlated with the improvement in pancreatic blood flow. In our previous study (Dembiski et al., 1982), prolonged treatment of healthy rats with EGF resulted in increased weight of the pancreas and elevated DNA synthesis and increased DNA and RNA contents in the tissue. Moreover, in another study, this peptide increased the weight of the mucosa in the stomach and in the intestine as well as elevated the nucleic acid content in these organs (Konturek et al., 1988).

Overexpression of transforming growth factor-α (TGF-α), another peptide sharing the structural and biological properties of EGF, was detected in the first days after pancreatic duct ligation in rats (Wang et al., 1997). Moreover, chronic pancreatitis is associated with an increased concentration of TGF-α and epidermal growth factor receptor (Korc et al., 1994), and increased expression of EGF receptor was also demonstrated in human acute pan-

creatitis (Eber et al., 1995). The results of our and other studies are consistent with the conclusion that EGF is strongly involved in recovery processes taking place after acute pancreatitis.

Another growth factor such as TGF-β1 and cytokines, especially interleukin 1β, interleukin 6 and TNF-α, are rapidly produced during acute pancreatitis in the pancreas and are released into the circulation (Norman et al., 1995). It was shown earlier (Norman et al., 1994), that the pancreatic tissue level of interleukin 1β and interleukin 6 rose faster and reached a higher value than the serum level, indicating that the pancreas itself is a major source of induction of cytokine release during caerulein-induced pancreatitis. In our present study, the interleukin 1β serum level was sharply increased after induction of pancreatitis and remained elevated above the control up to the end of the observation period. After EGF treatment, the interleukin 1β concentration in the serum was lower and returned to the control value 10 days after caerulein-induced pancreatitis. Interleukin 1β is a well-known component of acute inflammation and plays a crucial role in the induction of the release of other members of the cytokine cascade (Kingsnorth, 1997). It was shown that interleukin 1β blockade by naturally occurring receptor antagonists almost completely attenuates the rise in serum interleukin 6 and TNF-α level and decreases the severity of experimental acute pancreatitis (Norman et al., 1995; Kingsnorth, 1997). This close correlation between serum cytokine level and severity of pancreatitis is in good agreement with observations of others (Heath et al., 1993). In such a case the mode of action of EGF after caerulein-induced pancreatitis on the acceleration of regeneration depends, at least in part, on the suppression of the cytokine cascade as evidenced by the reduced plasma interleukin 1β level.

On the other hand, it should be mentioned (Vinter-Jensen at al., 1997) that longer (4 weeks) treatment with EGF of mature Goetingen minipigs leads to enlargement and increase in height of epithelial interlobular ductal cells of the pancreas with accumulation of glycoconjugates in columnar cells, which is similar to changes observed in chronic pancreatitis. Also, overexpression of EGF receptor (Korc et al., 1992) in human pancreatic cancer is associated with a rise of the EGF and TGF-α level. For these reasons the dose and duration of EGF treatment must be carefully considered.

The biochemical parameters of this study showing a beneficial effect of EGF on caerulein-induced pancreatitis are consistent with our histological findings showing that treatment with EGF decreased tissue edema, shortened the time of vacuolization and reduced tissue infiltration. In our study, salivectomy remained without significant effect on the histological signs of pancreatitis resolution, which is in agreement with facts discussed above, related to other parameters tested. The data presented suggest that exogenous EGF reduces the severity of acute pancreatitis and that this beneficial effect depends, at least in part, on the limitation of cytokine release and improvement of pancreatic blood flow.

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