The effect of endothelin-1, endothelin-2 and endothelin-3 in early cerulein-induced acute pancreatitis in rats

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Abstract

Purpose: To assess the effect of endothelins: ET-1, ET-2 and ET-3 on trypsinogen activation, lipase activity and histological changes in the pancreas in early (4 hrs) cerulein acute pancreatitis (AP) in rats.

Material and methods: In 45 Wistar rats with cerulein induced AP (2x40 μg/kg i.p. at 1 hour interval, the effect of endothelins at the dose 2x0.5 or 2x1.0 nmol/kg i.p. was assessed vs untreated AP; 6 healthy rats were control (C). Free active trypsin (FAT), total potential trypsin after activation with enterokinase (TPT), lipase in 12 000 xg supernatants of pancreatic homogenates and the plasma α-amylase were assayed. The %FAT/TPT was an index of trypsinogen activation.

Results: %FAT/TPT increased from 3.0±0.6 in C to 16.2±3.1 in AP ($p < 0.01$). ET-1 decreased this index to 4.8±1.1 after higher dose ($p < 0.01$); the effect of lower dose was insignificant. Attenuating effect of ET-2 was significant: 7.3±1.7 after higher dose ($p < 0.05$) and 6.1±0.9 after lower dose ($p < 0.01$). ET-3 diminished this index to 4.5±1.5 ($p < 0.01$) and to 6.3±2.2 ($p < 0.05$) respectively. Lipase activity in supernatant increased from 4.1±0.6 in C to 6.3±0.7 U/mg protein in untreated AP ($p < 0.05$) and plasma α-amylase from 7.0±0.6 in C to 25.9±4.3 U/ml in AP ($p < 0.001$), without essential changes in treated groups vs untreated AP. Higher doses of endothelins decreased inflammatory cell infiltration score in AP.

Conclusions: The exogenous endothelins, especially ET-2 and ET-3 and to lesser extent ET-1 exerted some protective effect in early, edematous acute pancreatitis by the attenuation of trypsinogen activation and inflammatory cell infiltration in the pancreas.

Key words: cerulein acute pancreatitis, endothelins, trypsin, lipase, α-amylase, histology.

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Introduction

The derangement of pancreatic microcirculation plays an important role in the pathogenesis of acute pancreatitis (AP). The edematous AP is characterized by almost double increase of pancreatic capillary flow (PCF) within 6 hrs, whereas in severe, necrotic form of AP, PCF decreases by half at this time [1]. Early, premature activation of trypsinogen in pancreatic tissue is thought to be a key factor in the pathogenesis of AP [2]. The interrelationships between alterations of PCF and pancreatic zymogen activation have been suggested [3,4].

Endothelin, a 21-residue polypeptide, isolated by Yanagisawa et al. [5] from the culture of porcine epithelial cells has been shown to be the most potent vasoconstrictor known to date. Later, three distinct human endothelins (ET) – related genes were identified and three corresponding familiar polypeptides (ET-1, ET-2 and ET-3) have been synthesized [6]. In the dog pancreas, ET-1 and ET-3 reduced pancreatic blood flow by half, whereas ET-2 only by 20% [7]. All forms of endothelins reduced pancreatic blood flow in healthy rats, however ET-1 was much more effective than ET-2 or ET-3 on a molecular basis [8].

ET-1 is thought to exert its effects by binding to two different receptors, classified as ET_A and ET_B. ET_A receptor has been found to be responsible for vasoconstriction, whereas ET_B was considered as a "vasodilator" receptor, mediating the release of vasodilating factors [9]. Recently, two subtypes of ET_B receptor: ET_{B1}, mediating vasodilatation, and ET_{B2}, contributing to the vasoconstrictor effects, have been identified [10]. In the rat

pancreas ET-1 and ET-3 mRNA and two classes of ET receptors have been found. ET_A receptor had a high affinity for ET-1, but a low affinity for ET-3, and ET_B receptor with equally high affinities for ET-1 and ET-3. No specific receptor for ET-2 was identified in this study [11].

In recent years a broad range of ET-1 receptor antagonists, less or more specific for the receptors ET_A or ET_B have been synthesized, enabling the studies of endothelin-1 action in different severe pathological entities [12-14]. The data on the effects of ET-1 and antagonists of its receptors in AP considerably differs, depending on the disease model, doses and the time of application [15-19]. Kogire et al. [20] found a protective effect of ET-1 infused simultaneously with cerulein on histological changes of the pancreas, whereas selective ETA receptor antagonist, BQ 123 increased pancreatic edema and inflammatory cell infiltration. On the contrary, in the studies of Liu et al. [21,22], the exogenous ET-1 has been shown to reduce pancreatic perfusion and aggravate mild cerulein-induced AP to a severe, necrotic form, whereas BQ 123 attenuated these changes.

The role of ET-2 and ET-3 in acute pancreatitis has not been studied as of yet. If endothelins could attenuate important pathways of early edematous pancreatitis, they could be useful in the prevention of AP escalation. If not, perhaps further studies on different antagonists of endothelin receptors might offer such an advantage.

Therefore, the purpose of the present study was to assess and to compare the effect of different endothelins: ET-1, ET-2 and ET-3 on the activation of trypsinogen, activities of other pancreatic enzymes and histological changes in the early course of edematous acute pancreatitis in rats.

Materials and methods

Animals

The experiments were carried out on 51 male Wistar rats, weighing 240-300 g, housed individually in wire bottomed cages in a room temperature of 21 ± 1°C using a 12 hours light – dark cycle. The animals were given a standard rat chow diet and fasted overnight before the experiment with free access to water. The care was provided in accordance with the current procedures for the care and use of laboratory animals. The protocol has been approved by the local Bioethical Commission.

Induction of acute pancreatitis: Acute cerulein pancreatitis was induced according to the method of Yamaguchi et al. [23]. The rats were injected with cerulein (Sigma Chemical Co., St. Louis, MO, U.S.A.) at a dose of 40 μg/kg of body weight (b.w.) intraperitoneally (i.p.) twice in 1 hour interval. In control rats, only solvent of cerulein (saline solution) was given i.p. In the treated rats, the solution of respective endothelins in NaCl 0.9% was given i.p. twice, simultaneously with cerulein.

Experimental design

Rats were subdivided into 8 groups as follows:

Group I. Control group (C), healthy rats, receiving only saline solution (NaCl 0.9%) i.p. at time 0 and 1 hour later (n=6).

Group II. Rats with cerulein-induced acute pancreatitis (AP) untreated. The solution of cerulein in equivalent volume of saline was given i.p. at 0 time and 1 hour later (n=8).

Group III. Rats with cerulein-induced AP, treated with ET-1 at a dose of 0.5 nmol/kg b.w i.p. twice, in 1 hr interval, simultaneously with cerulein (n=6).

Group IV. Rats with cerulein-induced AP treated with ET-1, at a dose of 1.0 nmol/kg b.w. i.p. twice, as in the group III (n=6).

Group V. Rats with cerulein AP treated with ET-2, at a dose of 0.5 nmol/kg b.w. i.p. twice, in 1 hr interval, simultaneously with cerulein (n=7).

Group VI. Rats with cerulein AP treated with ET-2, at a dose of 1.0 nmol/kg b.w. i.p. twice, as in the group III (n=6).

Group VII. Rats with cerulein AP treated with ET-3, at a dose of 0.5 nmol/kg b.w. i.p. twice, in 1 hr interval, simultaneously with cerulein (n=6).

Group VIII. Rats with cerulein AP treated ET-3, at a dose of 1.0 nmol/kg b.w. i.p. twice, as in the group III (n=6).

The volume of NaCl 0.9% as a solvent was equilibrated in all groups to 2x2 ml/kg b.w.

Preparation of pancreatic homogenate and the plasma: Four hours after the first cerulein injection (or saline in C group) a general anesthesia was induced with intraperitoneal ketamine at a dose of 40 mg/kg b.w., supported by pentobarbital at a dose of 20 mg/kg b.w. The blood samples was taken to the heparinized syringe by cardiac puncture and the rats were sacrificed by decapitation. The pancreases were quickly excised, freed from the peripancreatic tissues and weighed. For light microscopy, representative specimens of the pancreas were fixed and the sections were stained with hematoxylin and eosin.

The remaining portion of the pancreas was processed according to Yamaguchi et al. [23], meaning it was homogenized in ice-cold four volumes of 50 mmol/l Tris-HCl buffer (pH 8.0), containing nonorganic detergent Triton X-100, 0.5% v/v during 1 min by 3 full up-and down strokes using a motor driven glass-Teflon homogenizer (Thomas Scientific, New Jersey, U.S.A.) cooled with ice. The resulting homogenate was sonified for 20 seconds in an ice bath using Vibra cell, model VC 50, Sonics and Materials Inc., Danbury, CT, U.S.A. (frequency 20 kHz and amplitude 70). The volumes were then adjusted giving 10% homogenates, placed on ice for 20 min for further extraction of the enzymes, and then centrifuged at 12000 x g for 20 min at 4°C. The supernatants were used for the assays of trypsin activity performed within 6 hrs. The remaining portions of the supernatant were frozen at -80°C, for the assay of lipase activity and protein concentration.

The samples of heparinized blood were centrifuged at 4000 rpm with cooling to 4°C, the resulting plasma was collected and frozen at -80°C for the assay of α-amylase activity.

Biochemical assays

1. Trypsin activity: Free active trypsin (FAT) and total potential trypsin (TPT) in the supernatants of pancreatic homogenates were estimated according to Yamaguchi et al. [23] with this exception that Nα-p-tosyl-L-arginine methyl ester hydrochloride (TAME) 1 mmol/l was used as a substrate and the absorbance of released product was estimated at 247 nm wave length in an automatic spectrophotometer Pye Unicam SP 505 (Cambridge, U.K.) as in our previous studies [24,25].

Table 1. **Free active trypsin (FAT), total potential trypsin (TPT) and the index of trypsinogen activation (% FAT/TPT) in the supernatants of pancreatic homogenates in early cerulein-induced acute pancreatitis (AP) untreated and treated with different endothelins (ET-1, ET-2 and ET-3) vs control group(C) in rats. Means ± S.E.M**

Nº	Group	FAT μg/mg protein	TPT μg/protein	% FAT/TPT
I	Control (C) (n=6)	0.366 ±0.083	11.95 ±1.20	3.0 ±0.6
II	AP untreated (n=8)	1.254 ±0.145	8.70 ±1.50	16.2 ±3.1
III	AP + ET-1 (n=6) 2 x 0.5 nmol/kg	1.670 ±0.260	11.46 ±0.92	15.3 ±2.9
IV	AP + ET-1 (n=6) 2 x 1.0 nmol/kg	0.607 ±0.120	14.17 ±1.66	4.8 ±1.1
V	AP + ET-2 (n=7) 2 x 0.5 nmol/kg	1.102 ±0.189	16.14 ±1.34	7.2 ±1.4
VI	AP + ET-2 (n=6) 2 x 1.0 nmol/kg	1.097 ±0.156	18.11 ±1.52	6.1 ±0.9
VII	AP + ET-3 (n=6) 2 x 0.5 nmol/kg	0.661 ±0.153	16.14 ±1.11	4.5 ±1.5
VIII	AP + ET-3 (n=6) 2 x 1.0 nmol/kg	1.073 ±0.377	17.03 ±0.53	6.3 ±2.2

Important statistical significance of differences between groups:
FAT: I/II $p<0.001$, II/IV $p<0.01$, II/VII $p<0.02$;
TPT: I/II n.s., II/III $p<0.02$, II/IV $p<0.05$, II/V $p<0.001$, II/VI $p<0.01$, II/VII $p<0.001$, III/VIII $p<0.001$;
% FAT/TPT: I/II $p<0.01$, II/IV $p<0.01$, II/V $p<0.05$, II/VI $p<0.01$, II/VII $p<0.001$, II/VIII $p<0.05$, III/IV $p<0.01$

Total potential trypsin (TPT) in the supernatants was estimated after activation of trypsinogen with enterokinase in 1:1 dilution in 50 mmol Tris-HCl buffer, pH 8.0 for 30 min at 37ºC. The freshly prepared working solution of enterokinase contained 2 mg of enzyme/ml of the same buffer [23]. The time of activation proved to be sufficient for maximal activation.

The activity was expressed in μg of trypsin/mg of protein by comparison with the calibration curve of increasing concentrations of bovine trypsin, type I. The % FAT/TPT ratio served as an index of trypsinogen activation [23].

2. Lipase activity in the supernatants of pancreatic homogenates was assayed with tributyrin (1,2,3 – tributylglycerol) as a substrate and with the pehametric method using autotitrator (Radiometer, Copenhagen, Denmark) and 0.2 mol/l NaOH as in our previous studies [24,25].

3. a-Amylase activity in the plasma was assayed with colorimetric method with soluble starch as substrate as in our previous studies [26,27].

All reagents, with the exception of soluble starch were purchased from Sigma Chemicals Co., St Louis, MO, U.S.A.

Histological examination: Ten slides from 5 rats from each group (50 slides per group) stained with hematoxyline and eosine (H&E) were evaluated at a magnification of 200x in light microscopy by an expert pathologist (A. A.), who was not familiar with the experimental code at this time. The edema, inflammatory infiltrate, necrosis and vacuolization were scored from 0 to 3 degrees of severity according to Kyogoku et al. [28]. Generally, the interstitial edema was scored as follows: 0 = absent; 1 = expansion of interlobular septa; 2 = expansion of intralobular septa; 3 = separation of acini. The inflammatory infiltrate: 0 = absent; 1 = less than 20 neutrophils per field; 2 = 20-50 neutrophils; 3 = more than 50 neutrophils per field. Parenchymal necrosis: 0 = absent; 1 = less than approximately 5%; 2 = 5-20%; 3 = more than 20% of the involved area. The vacuolization: 0 = absent; 1 = less than 20% of acinar cells with vacuoles per field; 2 = 20-50%; 3 = more than 50%.

Statistical analysis. The results of biochemical assays are reported as means ± S.E.M. and after performing an F test for the equality of variances, the means were compared using the t-test for unpaired data. Histologic data were expressed as range of the scores and means ± S.E.M. and compared using Mann-Whitney's test for two groups. The differences with $p < 0.05$ were considered statistically significant.

Results

Tab. 1 illustrates the activities of trypsin and the index of trypsinogen activation in the supernatants containing enzymes extracted using organic detergent Triton X-100. FAT in cerulein AP group is markedly higher (about 3 times) than in the control group ($p < 0.001$), whereas a decrease of TPT activity was insignificant. In the groups with AP treated with endothelins, the increase of FAT was attenuated by half only after higher dose of ET-1 ($p < 0.01$) and after lower dose of ET-3 ($p < 0.02$). In all groups with AP treated with endothelins, TPT was higher than in untreated group with AP, but not significantly different in comparison to control group. The degree of trypsinogen activation (%FAT/TPT) in the untreated group was evidently higher (5.4x) than in the control group ($p < 0.01$). It was attenuated 2.25-3.60 times by the treatment with higher dose of ET-1 and with both doses of ET-2 and ET-3. However, lower dose of ET-1 was ineffective in this respect. Nevertheless, the values of %FAT/TPT in treated groups with AP, after effective attenuation of the trypsinogen activation remained 50%-140% higher than in the control group.

Tab. 2 shows the activity of lipase in the supernatant of pancreatic homogenate from untreated AP, which was ca 50% higher than in control group ($p < 0.05$). The effect of the treatment with endothelins on this activity was insignificant, with the exception of some diminishing influence of higher dose of ET-3 ($p < 0.02$). The plasma α-amylase activity was 3.7 times elevated in cerulein AP ($p < 0.001$) and none treatment affected significantly this activity.

The histological scores of edema, inflammatory cell infiltration, necrosis and vacuolization were significantly increased in the groups with AP, supporting the development of cerulein-induced pancreatitis. The mean score of edema was slightly

Table 2. **Lipase activity in the supernatants of pancreatic homogenates and plasma α-amylase in cerulein-induced acute pancreatitis (AP) untreated and treated with different endothelins (ET-1, ET-2 and ET-3) vs control group (C) in rats. Means ± S.E.M. are reported**

Nº.	Group	Lipase U/mg protein	α-Amylase U/ml
I	Control (C) (n=6)	4.11 ±0.58	7.0 ±0.63
II	AP untreated (n =8)	6.34 ±0.69	25.9 ±4.28
III	AP + ET-1 2 x 0.5 nmol/kg (n=6)	7.67 ±1.36	19.2 ±4.66
IV	AP + ET-1 2 x 1.0 nmol/kg (n=6)	5.53 ±0.84	18.7 ±3.28
V	AP + ET-2 2 x 0.5 nmol/kg (n=7)	6.68 ±0.77	27.2 ±2.98
VI	AP + ET-2 2 x 1.0 nmol/kg (n=6)	7.00 ±0.87	21.6 ±5.54
VII	AP + ET-3 2 x 0.5 nmol/kg (n =6)	4.53 ±0.86	26.4 ±2.62
VIII	AP + ET-3 2 x 1.0 nmol/kg (n=6)	3.87 ±0.42	29.8 ±3.63

Important statistical significance of differences:
Lipase: I/II $p<0.05$, II/VIII $p<0.02$;
α-Amylase: I/II $p<0.001$

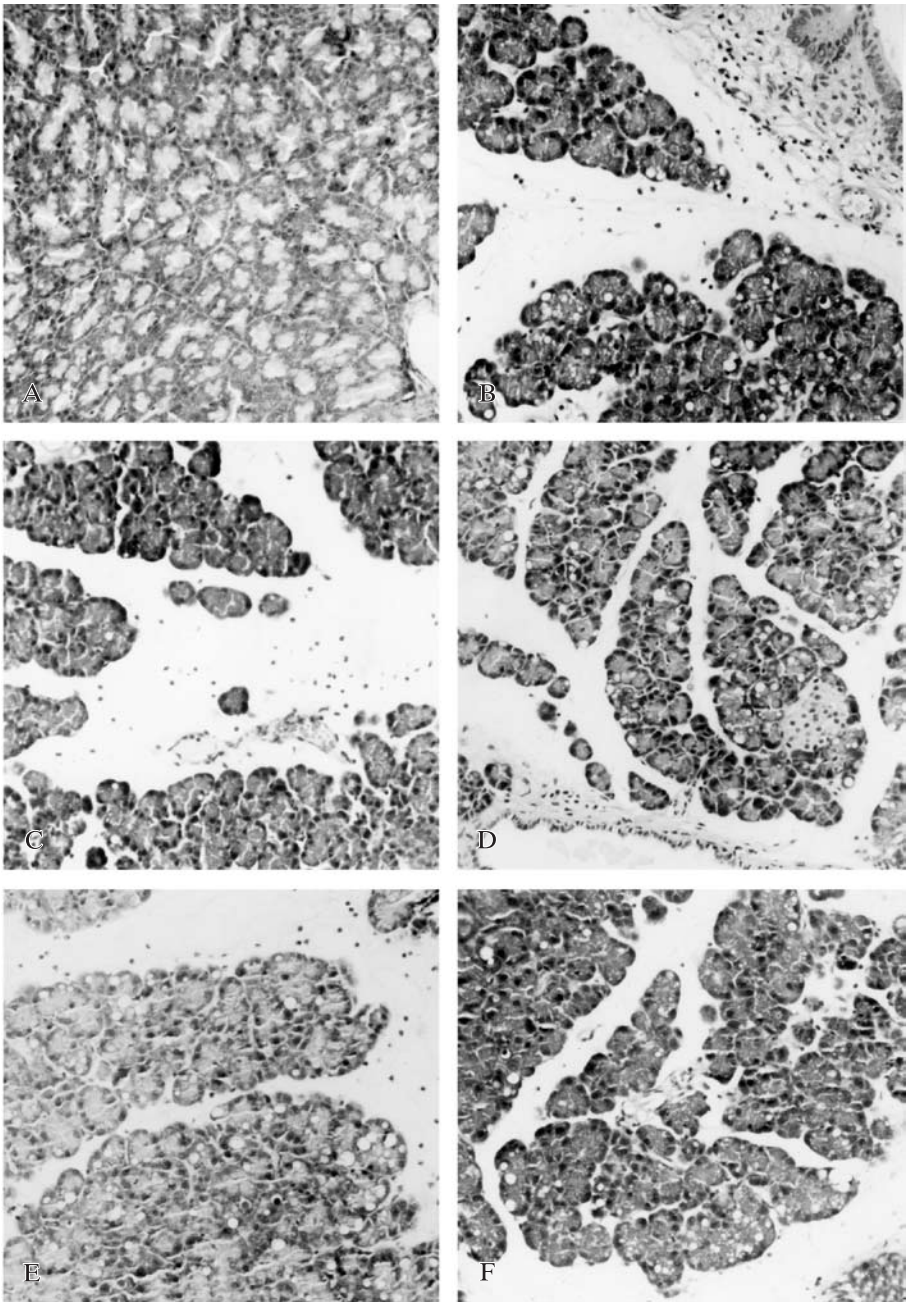
Table 3. **Morphologic changes in the pancreas in early caerulein-induced AP in rats in untread group with AP and in groups treated with endothelins (ET-1, ET-2 and ET-3)#**

No	Group	Edema	PMN infiltration	Necrosis	Vacuolization
I	Control (C) (n=6)	0-1 (0.12±0.03)	0-1 (0.06±0.02)	0-0 (0.00±0.00)	0-1 0.08±0.03
II	AP untreated (n=8)	1-3 (2.04±0.07)	0-3 (1.61±0.08)	0-2 (0.58±0.06)	1-3 (1.88±0.08)
III	AP + ET-1 2x0.5 nmol/kg (n=6)	2-3 (2.62±0.05)	0-3 (1.47±0.09)	0-2 (0.57±0.06)	1-3 (1.96±0.07)
IV	AP + ET-1 2x1.0 nmol/kg (n=6)	1-3 (2.12±0.08)	0-2 (1.04±0.05)	0-2 (0.54±0.06)	1-3 (1.92±0.08)
V	AP + ET-2 2x0.5 nmol/kg (n=7)	1-3 (2.61±0.05)	1-3 (1.59±0.05)	0-2 (0.81±0.07)	1-3 (2.30±0.07)
VI	AP + ET-2 2x1.0 nmol/kg (n=6)	0-3 (2.30±0.08)	0-3 (1.29±0.08)	0-2 (0.63±0.06)	1-3 (1.89±0.08)
VII	AP + ET-3 2x0.5 nmol/kg (n=6)	1-3 (2.32±0.07)	0-3 (1.55±0.08)	0-2 (0.82±0.06)	1-3 (2.18±0.08)
VIII	AP + ET-3 2x1.0 nmol/kg (n=6)	1-3 (2.04±0.08)	0-3 (1.36±0.06)	0-2 (0.70±0.06)	1-3 (1.94±0.08)

Values are expressed as range of scores, with means ±S.E.M. in parentheses.
Important statistical significance of differences:
Edema: I/II $p<0.001$, II/III,V $p<0.001$, II/VI,VII $p<0.01$; III/IV $p<0.001$;
PMN infiltration: I/II $p<0.001$, II/IV $p<0.001$, II/VI $p<0.02$, II/VIII $p<0.05$;
Necrosis: I/II $p<0.001$, II/V $p<0.05$, II/VII $p<0.02$;
Vacuolization: I/II $p<0.001$, II/V $p<0.01$, II/VII $p<0.05$

increased in the group treated with lower doses of ET-1 and ET-2, and even less elevated after higher dose of ET-2 and lower dose of ET-3. The infiltration with polymorphonuclear (PMN) cells was markedly decreased after higher dose of ET-1 and to less extent after higher dose of ET-2 and ET-3. The mean scores of necrosis and vacuolization were comparable in untreated and treated groups with AP, with the exception of the groups after lower dose of ET-2 and ET-3, where some slight elevation of this score was observed (*Tab. 3*). The representative features of histological changes in AP groups in comparison to control group are depicted on *Fig. 1.*

Figure 1. **The representative histological features of pancreatic tissue in early (4 hrs) cerulein-induced acute pancreatitis (AP) untreated and treated with different endothelins. (*A*). Normal appearance of the rat pancreas. (B). Acute cerulein-induced (2 x 40 μg/kg b.w. i.p. at 1 hour interval), edematous pancreatitis. (*C*). Cerulein-induced AP treated with lower dose (2 x 0.5 nmol/kg b.w. i.p.) of endothelin-1, given simultaneously with cerulein. (*D*). Cerulein-induced AP treated with higher dose (2 x 1.0 nmol/kg b.w. i.p.) of endothelin-1. (*E*). Cerulein-induced AP treated with higher dose (2 x 1.0 nmol/kg b.w. i.p.) of endothelin-2. (*F*). Cerulein induced AP treated with higher dose (2 x 1.0 nmol/kg b.w. i.p.) of endothelin-3. Magnification x160. Staining: Hematoxyline and eosine (H&E)**



Discussion

Our results indicate the significant, 2.25-3.60 times attenuation of augmented trypsinogen activation in the pancreatic tissue of the rats with early (4 hrs) cerulein induced pancreatitis (AP) after treatment with endothelins: ET-1, ET-2, and ET-3 at a dose of 1 nmol/kg b.w., and ET-2 or ET-3 at a dose of 0.5 nmol/kg b.w., given twice introperitoneally at 1 hour interval simultaneously with cerulein. The treatment with endothelins generally did not affect the elevation of pancreatic lipase and plasma α-amylase activities with the exception of higher dose of ET-3, which attenuated to some degree the increase of lipase activity. Parallelly, the higher doses of endothelins studied (especially ET-1) decreased a PMN infiltration of pancreatic tissue in

histological examination. The scores of necrosis and vacuolization were not diminished by endothelins, and even they were slightly increased after lower doses of ET-2 and ET-3. Similarly, the mean scores of edema were slightly increased after lower doses of ET-1, ET-2 and ET-3 and after higher dose of ET-2. Consequently, our results suggest that the endothelins at chosen doses, given at the beginning of cerulein-induced AP could be effective in the attenuation of trypsinogen activation and inflammatory reaction in the early course of edematous pancreatitis. The effect of ET-1 on trypsinogen activation at higher dose was evidently more expressed than insignificant effect of lower dose. On the contrary, similar effects of both doses of ET-2 and ET-3, could suggest that the maximal effect could be achieved even with lower doses of these endothelins.

We have chosen the dosage of endothelins basing on the classical study of their effect on pressor responses in anesthetized rats. In the response to intravenous bolus injection (1 nmol/kg) of ET-1, ET-2 and ET-3, late pressor effect developed 10-20 min after the injection and lasted >1 hour. The mean time required for blood pressure 50% recovery to base-line levels was 90 min for ET-1, 120 min for ET-2 and 50 min for ET-3 [6]. For the prolongation of endothelin action we repeated the bolus after 1 hour, therefore the total higher dose amounted 2 nmol/kg b.w. within 4 hours. To disclose eventual dose response effect, we used also twice lower dosage 0.5 nmol/kg given twice at 1 hour interval. This dose was intermediate between low dose of ET-1 (0.1 nmol/kg) and high dose (1 nmol/kg) used by Filep et al [13] in the study of vascular permeability. This permeability in the pancreas was not affected significantly by ET-1 treatment in their study.

Cerulein-induced pancreatitis is a well established, experimental model of acute interstitial, edematous pancreatitis. It has been shown to involve the interaction of supramaximally stimulating doses of the cholecystokinin analog (cerulein) with low-affinity pancreatic acinar cell cholecystokinin (CCK)–A receptors. The consecutive co-localization of lysosomal cathepsin B with digestive enzyme zymogens in cytoplasmic vacuoles enables intracellular trypsinogen activation, thought to be a "trigger mechanism" of acute pancreatitis [29]. Simultaneous blockade of apical secretion is a prerequisite to bass-lateral output of partially activated zymogens into the interstitial space with a consecutive release of cytokines and vasoactive mediators, causing chemoattraction of inflammatory cells and activation of vascular endothelium [4]. In last years, the role of neutrophils in the intrapancreatic trypsin activation by NADPH oxidase (hydrogen peroxide and superoxide) in cerulein-induced pancreatitis in rodents has been documented [30].

In fact, in our study, the attenuation of trypsinogen activation after higher doses of endothelins was associated with decreased score of inflammatory cell infiltration. Nevertheless, lower doses of ET-2 and ET-3 did not decrease the score of inflammatory infiltration and edema, despite of attenuation of trypsinogen activation and even slight increase of necrosis and vacuolization scores was noted. Moreover, lower dosage of ET-1 affected neither trypsinogen activation nor PMN infiltration or necrosis and vacuolization scores, and even slightly increased the edema score. It could suggest that not all histological aspects of local inflammatory reaction depends on trypsinogen activation. The possible explanation could be independent activation of intracellular trypsinogen and nuclear factor-κB (NF-κB) in cerulein-induced pancreatitis, which was found to be required for the production of chemokines by pancreatic acinar cells, initiating the inflammatory cascade [31].

The mechanism of attenuating effect of endothelins on the trypsinogen activation in early, cerulein-induced AP is not easy to explain. In human acute pancreatitis, the level of endothelin-1 was found to be elevated [32]. It is not known whether it is a component of defensive mechanism or a mediator of injuring effects of AP on other organs. The only known effect of endothelins in healthy pancreas is vasoconstriction, with consequent reducing the pancreatic blood flow, the most prominent after ET-1 on molecular basis [7,8]. Two types of endothelin receptors: ET_A with high affinity for ET-1 and 175 times lower affinity for ET-3 and ET_B, with equally high affinities for ET-1 and ET-3 in the rat pancreatic acini have been found, but endothelins neither stimulated the enzyme secretion nor do they alter intracellular Ca^{+2} or cAMP [11]. Therefore the direct role of endothelins in the physiology of pancreas remains to be elucidated.

Cerulein-induced acute pancreatitis is a mild self-limited form of the disease. However, after additional stress it could progress into severe necro-hemorrhagic form, probably by decreases of pancreatic microperfusion [28]. The early microcirculatory changes in the pancreas with acute cerulein-induced pancreatitis include the increase of pancreatic capillary blood flow, increase permeability of the endothelium and accumulation of extravasated fluid in the perilobular space, which were aggravated after additional cold stress. The permeability changes are followed by a decrease in flow velocity and early delayed leukocyte adherence in the pancreatic microcirculation [33]. The most important difference between mild edematous pancreatitis and severe, necrotic form is evident in the early, 6 hrs period of observation. Pancreatic capillary flow in edematous, cerulein-induced AP rapidly increases to 188% of baseline and remains elevated up to 6 hrs of experiment, whereas in severe necrotic AP it decreases to 47% of baseline during 6 hrs. Complete capillary stasis develops in 38% of capillaries in severe AP and is absent in edematous AP [1]. These differences could be crucial for the explanation of controversial role of endothelin-1 and endothelin-1 receptors in AP reported in the literature.

Liu et al. [21] have observed that ET-1 administered at a dose of 250, 500 or 750 pmol/kg b.w. as intraaortal bolus 4 times at 1 hr intervals immediately after the second cerulein (2 x 10 μg/kg intraperitoneally at 1 hr interval) injection, evoking edematous AP, caused a dose dependent increase in pancreatic damage. The scores of vacuolization and necrosis of acinar cells were the most elevated with the highest dose of endothelin-1. Eibl et al. [17] have compared the effect of exogenous endothelin-1 and selective antagonist of it receptor A, LU-135 252 both in edematous, cerulein-induced AP and in severe, bile salt + cerulein AP. LU-135 252 injected i.v. at a dose of 50 mg/kg, 6 hrs after induction of AP decreased capillary permeability by 69% in the pancreas in edematous (cerulein-induced) pancreatitis and by 63% in severe (bile salt + cerulein-induced pancreatitis), where the increase of permeability in the untreated group was higher. Exogenous ET-1 at a dose of 1.25 μg/kg/hr (it means 0.5 nmol/kg/h, because the m.w. of ET-1 = 2492) i.v. during

6 hours, increased capillary permeability by 104% in the sham operated rats, by 40% in the cerulein-induced pancreatitis but did not further augment the extremal increase of permeability in necrotic pancreatitis. The authors suggest that the increase of capillary permeability by ET-1 is an unfavorable effect in edematous AP, which could be counteracted by selective antagonist of ET_A receptor. On the other hand, Kogire et al. [20], in the rat pancreatitis induced by continuous i.v. infusion of supramaximal dose of cerulein (5 μg/kg/hr) for 3.5 hr, found that exogenous endothelin-1 (infused throughout the cerulein infusion at a dose of 100 pmol/kg/hr) exerted a protective effect. It was associated with less advanced inflammatory cell infiltration, acinar cell vacuolization and a decrease in the pancreatic edema index. The potent ET_A receptor antagonist BQ-123 at a dose of 3 mg/kg/h infused concomitantly with cerulein, further augmented pancreatic edema and the extent of inflammatory cell infiltration was greater than with cerulein alone. According to the authors opinion some protective effect of endothelin-1 in their study could be dependent on the secondary increased production of endogenous prostagladins. In fact, endothelin-1 is known to release prostacyclin [34] and a protective effect of prostacyclin analogs in taurocholate pancreatitis has been found in our previous studies [25,27].

It would be reasonable to assume that ET-1 given in properly chosen dose at the beginning of edematous pancreatitis, when pancreatic blood flow is increased, could exert rather beneficial than injuring effect by attenuating trypsinogen activation and inflammatory infiltration in the pancreas. The same could be pertinent also for ET-2 and ET-3, as their potentially suppressing effect on pancreatic microcirculation may be less important than the effect of ET-1. However, it should be kept in mind that the lower dosage of ET-2 and ET-3 increased slightly the necrosis and vacuolization scores, despite attenuation of trypsinogen activation. Therefore, more studies are needed to resolve their action in different models of AP.

Lipase is a recognized factor in the damage to isolated acinar cells in a degree similar to the action of chymotrypsin [35]. The increase of its activity in pancreatic tissue in our study could support its role in the cellular injury in cerulein-induced AP as reflected by the necrosis and vacuolization scores. Its activity was not depressed by the endothelins treatment (with one exception, which we could not explain). It is tempted to assume, that even after attenuation of trypsinogen activation, the inflammatory process in the pancreas could be perpetuated by lipolytic enzymes, with lipase as a representative in present work. This notion could be supported by an increased activity of the plasma α-amylase in the groups with cerulein AP, despite of the application of endothelins.

In summary our results support the positive effect of endothelin-1, -2, and -3, given simultaneously with cerulein in the course of mild, edematous acute pancreatitis in rats, as evidenced by the attenuation of trypsinogen activation and limitation of inflammatory cell infiltration in the pancreatic tissue. Such effects could be useful against progression of the disease into more severe forms and in the prevention of so called "post-ERCP pancreatitis". However, not all biochemical and histological aspects of AP, studied in this work, are favorable influenced by endothelins in chosen doses. Therefore this issue requires further studies on the mechanism of endothelins action in different models of acute pancreatitis.

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