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ADAPTATION OF PANCREAS TO REPEATED CAERULEIN-INDUCED PANCREATITIS IN RATS

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Induction by caerulein of acute pancreatitis with tissue damage and acinar cells loss is followed by recovery. We studied biochemical, histological and functional regeneration of pancreatic tissue after repeated acute pancreatitis. Pancreatitis was evoked in rats by s.c. infusion of caerulein (10 μg/kg/h) for 5 h. After infusion, rats were divided into three groups. First group was infused with caerulein one time, in the second group infusion of caerulein was repeated 10 days later. The third groups was infused with caerulein for the 3rd time 10 days after the 2nd infusion. Rats were sacrificed at time sequence of 0, 12, 24, 48, 72 hours and at 5th, and 10th day after last infusion of caerulein. Pancreatic blood flow (PBF) was measured using laser Doppler flowmeter. Plasma and pancreatic amylase, pancreatic weight, RNA and DNA contents, and histological changes were determined. We found that DNA and RNA content, as well, as histological changes in 1st group showed progressive regeneration after 3 days. Regeneration after 1st time caerulein-induced pancreatitis was almost completed within 10 days and amylase content in the tissue and plasma amylase level returned to normal values. Each subsequent infusion of caerulein caused significantly less pronounced destruction of the pancreatic tissue, however, the regeneration occurred progressively later than after the 1st or 2nd infusion. Tissue repair after the 2nd infusion reached peak at 5th day while after 3rd infusion at 10th day. PBF dropped after 1st caerulein induced pancreatitis by about 50% but with repeated caerulein induced pancreatitis lower decreases in PBF were observed and they returned in shorter time back to control value. These results indicate that the pancreas is able to adapt to repeated injury and this is manifested by cumulative decrease of pancreatic damage after each repetition of induction of acute pancreatitis and correlated with the preservation of PBF, however, the pancreatic tissue regeneration is significantly delayed.

Key words: *caerulein, acute pancreatitis, DNA synthesis, pancreatic regeneration, adaptation.*

INTRODUCTION

Acute pancreatitis is a severe disease with significant morbidity and mortality for which does not exist sufficient specific treatment. It is generally accepted, that in most of cases the pancreas needs about one month to recover

completely from acute experimental pancreatitis. Several experimental models to produce acute pancreatitis have been developed but most frequently used model involves an excessive stimulation of pancreatic secretion by the administration of submaximal dose of caerulein (1, 2). High doses of caerulein cause acute interstitial pancreatitis with depletion of digestive enzymes from acinar cells, decrease of pancreatic blood flow and total DNA, and histological evidence of pancreatic damage (3, 4). Pancreatic regeneration occurs after caerulein-induced pancreatitis but little is known about the rate and degree of pancreatic regeneration occurring spontaneously after acute pancreatitis; also the mechanisms and factors involved in these processes are still poorly understood. The regenerative response of the damaged pancreas is probably triggered by a complex mechanism involving gastrointestinal hormonal peptides (5, 6). Some role plays, also, tumor necrosis factor-α (TNF-α) which is produced excessively in rats with caerulein induced pancreatitis (7). Production of TNF-α by the peritoneal macrophages is suggested to be an essential factor in the evolution of this disease.

On the other hand clinical observations show that recovery of the pancreas after acute pancreatitis is not always complete (8). Almost nothing is known about the ability of pancreas to adapt to repeated acute inflammation and what mechanisms are involved in these processes. The objective of this study was to identify the degree of the recovery of the pancreas from the hormone-induced pancreatitis and above all, to evaluate the ability of pancreas to respond to repeated caerulein challenge.

MATERIALS AND METHODS

Studies were performed on male Wistar rats, weighing 160—190 g. Animals were housed in cages with wire mesh bottoms in room temperature with a 12 hour light dark cycle. Drinking water and food were available ad libitum.

Experiments were performed in three groups; the first group of animals was infused with caerulein one time only, in the second group a caerulein-induced pancreatitis was repeated after 10 days of recovery from the first induction of pancreatitis, and in the 3rd group, the caerulein-induced pancreatitis was developed after 10 days recovery from the second infusion of caerulein. In all groups of experiments animals were sacrificed at the time sequence as follows: 0, 12, 24, 48 or 72 h and 5 or 10 days after caerulein infusion. Ten rats were infused with saline (0.9% NaCl) and were used as control group.

Pancreatitis was induced by caerulein that was diluted in saline and infused s.c. for 5 h in conscious animals at a dose of $10\ \mu g \times kg^{-1} \times h^{-1}$ and at a rate of 1 ml/h. The animals were held in the individual Bollman cages through the time of infusion.

Determination of pancreatic blood flow

At the time sequence as described above the animals were anesthetized with ether, weighed and the abdominal cavity was opened. The pancreas was exposed for the measurement of the blood flow in the pancreatic tissue by laser Doppler flowmeter (LDF) using Laserflo, model BPM_2 Blood

perfusions monitor (Vasamedics Inc., St. Paul, MN). Blood flow was measured in five different portions of pancreas and the area of laser emission of the probe was 1 mm^2. Since the depth of measurement by LDF was about 3 mm. The technique used allowed to determine the pancreatic blood flow which was recorded and expressed as ml/100 g tissue/min. In this study the pancreatic blood flow was presented as percent of control value obtained in rats infused with saline (ml/h s.c. for 5 h).

Determination of plasma amylase concentration

Immediately after measurement of pancreatic blood flow, the abdominal aorta was exposed and blood was taken for plasma amylase determination. Plasma amylase was determined by an enzymatic method (Amylase reagent, Dialab Diagnostic Ges. MBH, Wien, Austria). The values were eypressed as units/litre (U/L).

Histological examination

Pancreas specimens were excised from the body portion, fixed in 10% formalin, embedded in paraffin and sections were stained with hematoxylin and eosin. The slides were examined histologically without the knowledge of the treatment given. The histologic grading of edema was made using a scale ranging from 0 to 3; 0 = no edema, 1 = interlobular edema, 2 = interlobular edema and moderate intralobular edema, 3 = interlobular edema and severe intralobular edema. Leukocytes infiltration was graded from 0 (absent) to 3 for maximal alterations (diffuse infiltration in the entire pancreatic gland). Grading of vacuolization was based on the appropriate percentage of cell involved; 0 = absent, 1 = less than 25%, 2 = 25—50%, and 3 = more than 50%.

Determination of DNA synthesis and RNA, DNA, protein content

After withdrawal of blood, the pancreas was carefully dissected out from its attachment to the stomach, the duodenum, and the spleen. Fat and excess tissue were trimmed away. The pancreas was rinsed with saline, blotted on paper, weighed and minced. The rate of DNA synthesis in the 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 Radioisotopes, Prague, Bohemia). The reaction was stooped with 0.4 N perchloric acid containing carrier thymidine (5 mM). Tissue samples were centrifuged and the precipitate was washed twice in cold 0.2 N perchloric acid and then recentrifuged. RNA was hydrolyzed in 0.3 M KOH incubated for 90 min at 37°C. DNA and protein were reprecipitated with 10% perchloric acid. After standing for 10 min on ice, the tubes were centrifuged and RNA content of the supernatant was measured using orcinol reaction (9). DNA in the residual pellet was solubized in 10% perchloric acid by heating at 70°C for 20 min. Denaturated protein was removed by centrifugation for 20 min. Using calf thymus as a standard, the DNA content of the samples was determined by Giles and Myers procedure (10). The final pellet was solubilized in 1 M NaOH and its protein content was determined by the method of Lowry et al (11). The incorporation of [^{3}H]thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a liquid scintillation system. RNA, DNA, protein contents were expressed as milligrams (mg) per total pancreas weight. DNA syntheis was expressed as disintegrations per minute (DPM) [^{3}H]thymidine per microgram (μg) DNA.

Statistical analysis

Comparison of the difference between the mean values of various groups of experiments was made by analysis of variance and the Wilcoxon's rank-sum test. A difference with a p value of less than 0.05 was considered statistically significant. Results are expressed as means (±SEM).

RESULTS

Subcutaneous infusion of caerulein at a dose of 10 µg/kg/h for 5 hours consistently produced pancreatitis in all tested rats. The pancreas appeared grossly swollen and enlarged with visible collections of edematous fluid. Peritoneal fluid was present in all animals. First infusion of caerulein decreased the DNA synthesis nearly by 50% when compared to control (*Fig. 1*). It was

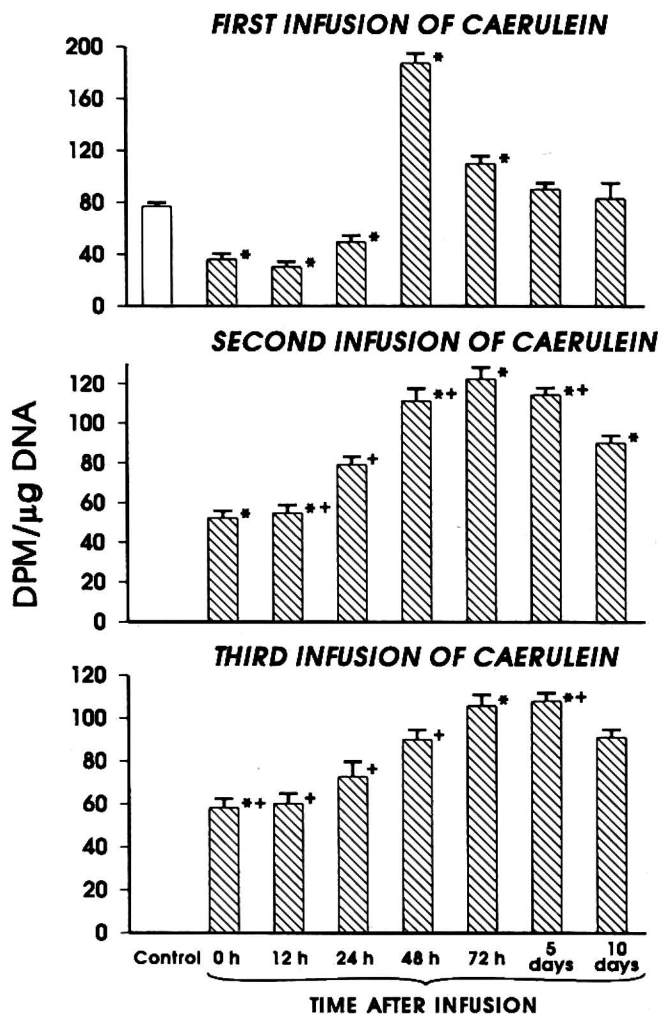

Fig. 1. DNA synthesis in the pancreas after 1st, 2nd and 3rd induction of pancreatitis. Each column represents mean±SEM. N = 8—10 rats. $*p<0.05$ compared to control. $^{+}p<0.05$ compared to value observed after 1st infusion of caerulein.

followed by significant increase of DNA synthesis 48 h later (nearly by 140% of control) and this parameter returned almost to control value at fifth day from the pancreatitis development. In this group of experiments plasma amylase concentration showed 6 fold increase (*Fig. 2*) immediately after cessation of caerulein infusion and was back to control value five days later. The pancreatic amylase content dropped by 57% (*Fig. 3*) at time 0 h after first induction of

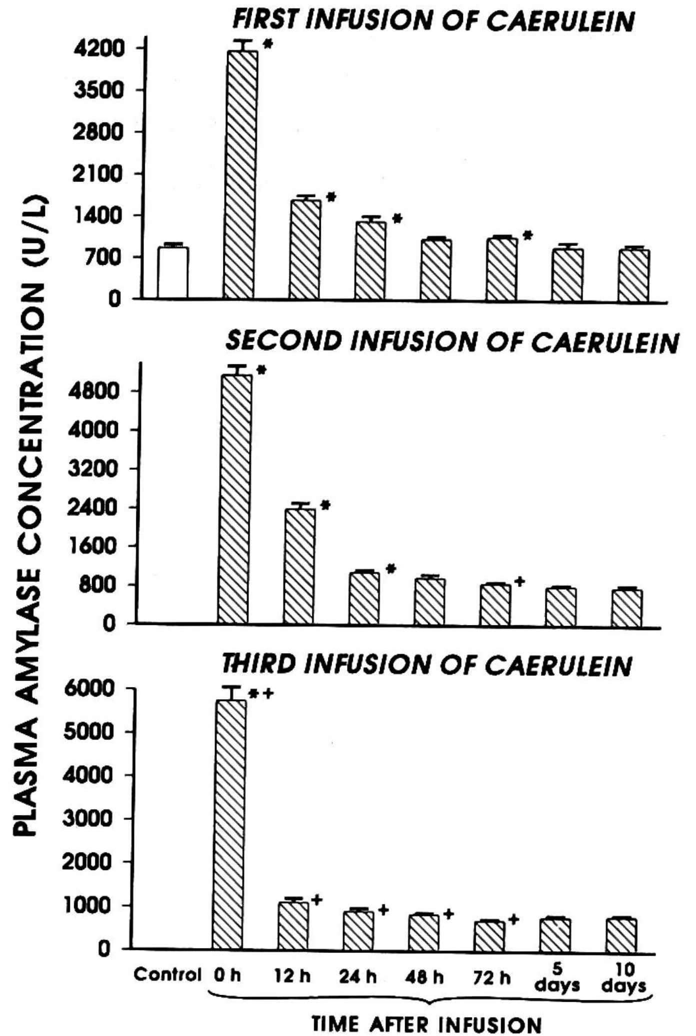

Fig. 2. Plasma amylase concentration in the pancreas after single and repeated caerulein-induced pancreatitis as on *Fig. 1.* N = 8—10. The data are expressed as mean ± SEM. *$p < 0.05$ compared to control. $^+p < 0.05$ compared to value observed after first infusion of caerulein at the same time of examination.

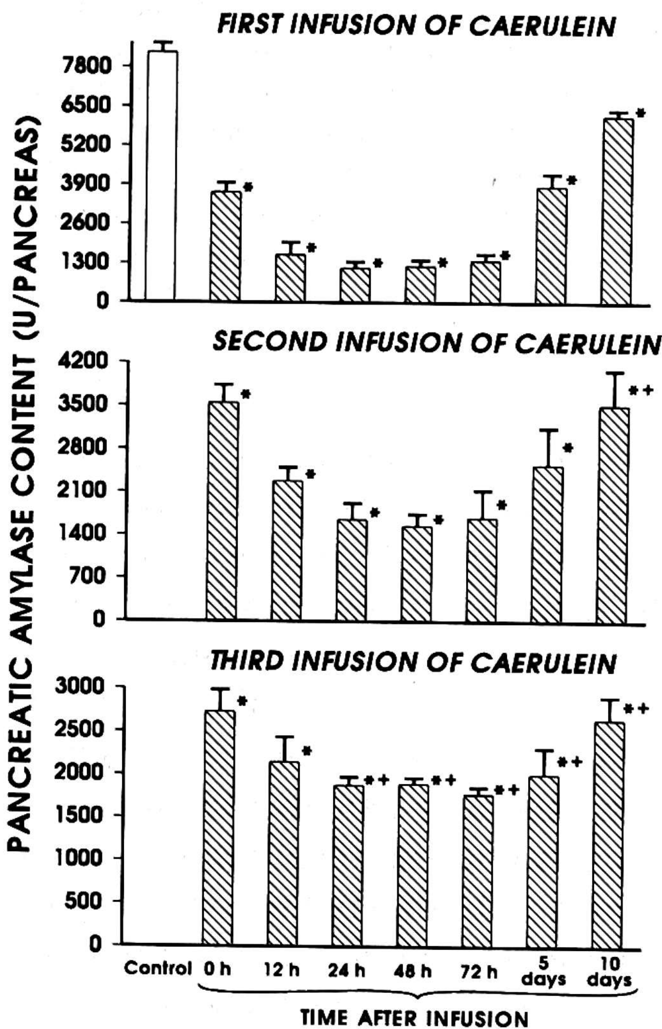

Fig. 3. Pancreatic amylase content after single and repeated caerulein induced pancreatitis. N = 8—10. The data are expressed as mean ± SEM. *$p < 0.05$ compared to control. $^+p < 0.05$ compared to value observed after 1st infusion of caerulein at the same time of examination.

pancreatitis reaching minimal value after 24 h and tended to return to control value after 10 days. Pancreatic blood flow, as measured by laser Doppler flowmetry at the termination of 5 h infusion of caerulein, was reduced by about 61% of the value recorded in rats infused with saline (*Fig. 4*) and it was

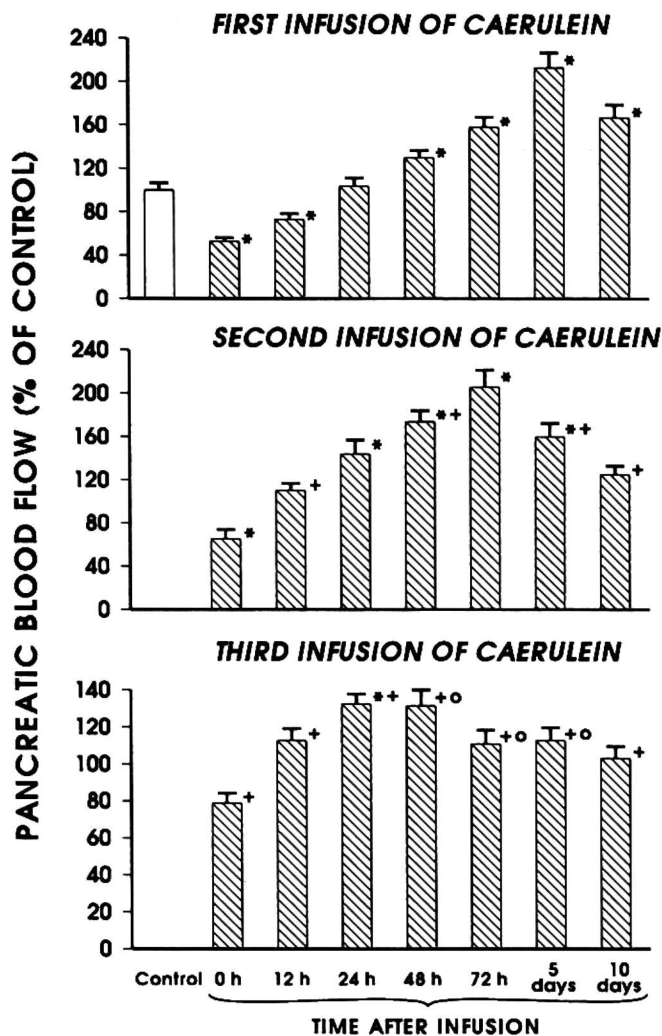

Fig. 4. Pancreatic blood flow after 1st, 2nd and 3rd induction of of pancreatitis. N = 8—10. The data are expressed as mean ± SEM. *$p<0.05$ compared to control. $^{+}p<0.05$ compared to value observed after first infusion of caerulein at the same time of observation. $^{\circ}p<0.05$ compared to value observed after second infusion of caerulein at the same time of observation.

followed by a significant increase 48 h later reaching peak at 5 th day after pancreatitis induction. Immediately after caerulein administration, the pancreatic weight (*Table 1*) was significantly increased and was close to control value 24 h later showing subsequent decrease below the control value two days after pancreatitis development. Pancreatic weight returned to control value at 10 th day. RNA content showed a significant decrease 24 h and 48 h after pancreatitis development, whereas DNA content was diminished in the pancreas between 24 h and 5 days. Both, RNA and DNA contents were back to control at 10th day. Protein content immediately after pancreatitis induction showed a significant increase above the control value and then declined below the control value throughout the 5 days to reach at 10 th day the control value. Histologically, in rats infused with caerulein, the interlobular and intralobular oedema was accompanied by a perivascular infiltration of leukocytes and the

Table I. Effect of saline (control), and single or repeated (10 days intervals) caerulein s.c. infusion ($10\ \mu g \times kg^{-1} \times h^{-1}$ for 5 h) on pancreatic weight, RNA, DNA and protein content.

	CONTROL	0 h	12 h	24 h	48 h	72 h	5 days	10 days
AFTER ONE INFUSION OF CAERULEIN								
Pancreatic weight (mg)	966±52	1482±63*	1295±60*	1086±25	703±35*	707±31*	800±34	900±40
RNA content (mg)	9.70±0.29	9.53±0.37	8.95±0.39	8.10±0.19*	8.25±0.25*	8.84±0.21	9.09±0.28	9.81±0.32
DNA content (mg)	5.31±0.08	5.16±0.12	5.08±0.09	4.08±0.08*	4.16±0.12*	4.32±0.13*	4.98±0.07*	5.28±0.07
Protein content (mg)	180±9.5	233±8.4*	210±10.2	180±8.7	105±9.3*	120±6.9*	133±6.9*	159±11.4
AFTER TWO INFUSIONS OF CAERULEIN								
		0 h	12 h	24 h	48 h	72 h	5 days	10 days
Pancreatic weight (mg)		1407±108*	1175±7.7*	1097±72	992±24	944±52	807±31*	905±32
RNA content (mg)		9.50±0.26	9.01±0.22	8.39±0.23*	8.55±0.39	8.61±0.21	9.83±0.23	9.32±0.31
DNA content (mg)		5.23±0.08	5.00±0.12	4.60±0.07*+	4.56±0.11*+	4.68±0.16	4.86±0.09*	5.01±0.10
Protein content (mg)		187±10.9	160±9.2+	144±8.2*+	120±7.9*	123±7.8*	131±9.8*	154±8.7
AFTER THREE INFUSIONS OF CAERULEIN								
		0 h	12 h	24 h	48 h	72 h	5 days	10 days
Pancreatic weight (mg)		1176±66	873±49	853±46	873±26	852±43	829±33	863±35
RNA content (mg)		9.28±0.35	8.99±0.29	8.89±0.18+	8.85±0.21	8.79±0.21	8.98±0.16	9.17±0.31
DNA content (mg)		5.06±0.07	5.10±0.13	4.92±0.11*+	4.88±0.15*+	4.91±0.08*+	4.83±0.11*	4.99±0.13
Protein content (mg)		165±7.4+	160±9.2+	145±8.3*+	146±7.2*+	159±8.6+	155±8.3	158±7.3

Mean±SEM of 8—10 rats. Asterisk indicates significant change ($p<0.05$) as compared to the control value. Cross indicates significant changes as compared to value after first caerulein infusion at the same time of observation.

presence of vacuolization in over half of acinar cells. Pancreatic edema and vacuolization reached maximum at the time 0 h, whereas leukocyte infiltration reached peak 24 h from the end of caerulein infusion, then tended to regress throughout the rest of the time of experiment showing only small changes at the tenth day of the experiment. Regeneration processes started 12 h after pancreatitis development reaching peak at the third day.

After second infusion of caerulein, the reduction in DNA synthesis in the pancreatic tissue was less pronounced when compared to that after first infusion (*Fig. 1*). The synthesis of DNA reached the lowest value at time 0 h and this was followed by a significant increase which started earlier (14 h after caerulein infusion) and reached the highest value at time 72 h.

An increase of plasma amylase concentration at the time 0 h and 12 h was slightly higher after second infusion of caerulein but after that this parameter returned to the control value three days earlier than after first induction of pancreatitis (*Fig. 2*). The pancreatic amylase content at time 0 h was not significantly different from that after the first infusion, but subsequent decrease of pancreatic amylase content was less pronounced than after first infusion (*Fig. 3*) Pancreatic blood flow after second induction of pancreatitis was reduced at the time 0 h to smaller degree than after the first one and showed earlier recovery with the blood flow peaking at 72 h after caerulein infusion. Pancreatic weight, after second induction of pancreatitis increased significantly at time 0 h above the control value in the same manner as after first caerulein infusion (*Table 1*) but was back to control after 48 h and did not show such an intensive reduction in the pancreatic weight as that observed after first infusion of caerulein. RNA content showed the significant decrease 24 h after second pancreatitis induction, whereas DNA content was dominished in the pancreas between 24 h and 5 day.

Protein content was decreased significantly between 24 h and 5 days after induction of pancreatitis. Histologically, in rats infused with caerulein for the second time, the edema was somewhat less pronounced and did not differ significantly from control value 5 days later; also leukocytes infiltration and vacuolization were smaller than after first induction of pancreatitis. The regeneration processes showed delay and reached peak at the 5 th day of observation.

The third caerulein infusion a caused decrease of the DNA synthesis which was significant only immediately after pancreatitis induction (*Fig. 1*). The DNA synthesis was subsequently restored and reached maximum 5 days later. At the time 0 h plasma amylase concentration (*Fig. 2*) was elevated after third infusion of caerulein to the extend significantly higher than after first and second infusion but 12 h later plasma amylase returned back to control value showing significantly lower level than after first and second pancreatitis induction. Pancreatic amylase content dropped after third pancreatitis induction to the lesser extend than after first or second one and remained below the control value till the last observation 10 days later (*Fig. 3*). Pancreatic blood flow after third infusion of caerulein did not show any significant changes at the time 0 h when compared to control value and exhibited a subsequent increase reaching maximum 24 h later (*Fig. 4*) The following increase of the blood flow was significantly lower than after first or second caerulein infusion throughout

Table II. Time course of histological changes such as edema, leukocyte infiltration, vacuolization, and regeneration in pancreatic tissue induced by s.c. repeated infusion of caerulein 10 μg/kg/h for 5 h.

	HISTOLOGY			
	Edema (0—3)	Infiltration (0—3)	Vacuolization (0—3)	Regeneration (0—3)
Saline infusion (control)	0.30 ± 0.15	0	0	0
Time after first caerulein infusion				
0 h	2.40 ± 0.16^{a}	1.90 ± 0.10^{a}	2.30 ± 0.15^{a}	0
12 h	1.89 ± 0.11^{a}	$2.55 \pm 0.17^{a,b}$	$1.11 \pm 0.11^{a,b}$	$0.44 \pm 0.17^{a,b}$
24 h	2.22 ± 0.15^{a}	$2.66 \pm 0.17^{a,b}$	$1.33 \pm 0.23^{a,b}$	$0.55 \pm 0.17^{a,b}$
48 h	2.00 ± 0.15^{a}	$2.50 \pm 0.16^{a,b}$	$0.60 \pm 0.22^{a,b}$	$0.80 \pm 0.13^{a,b}$
76 h	$1.50 \pm 0.18^{a,b}$	$1.25 \pm 0.16^{a,b}$	$0.50 \pm 0.19^{a,b}$	$2.25 \pm 0.16^{a,b}$
5 days	$1.00 \pm 0.15^{a,b}$	$1.10 \pm 0.10^{a,b}$	0.20 ± 0.13^{b}	$1.77 \pm 0.22^{a,b}$
10 days	0.80 ± 0.13^{b}	$0.60 \pm 0.16^{a,b}$	0.10 ± 0.10^{b}	0.80 ± 0.20^{a}
	Edema (0—3)	Infiltration (0—3)	Vacuolization (0—3)	Regeneration (0—3)
Time after second caerulein infusion				
0 h	2.11 ± 0.11^{a}	1.66 ± 0.16^{a}	$1.66 \pm 0.16^{a,c}$	0.22 ± 0.15
12 h	1.90 ± 0.10^{a}	2.10 ± 0.10^{a}	1.00 ± 0.15^{a}	0.22 ± 0.20
24 h	2.00 ± 0.00^{a}	$1.90 \pm 0.10^{a,c}$	1.00 ± 0.15^{a}	0.50 ± 0.17^{a}
48 h	1.80 ± 0.13^{a}	$1.80 \pm 0.13^{a,c}$	0.30 ± 0.15	0.30 ± 0.15^{c}
72 h	1.12 ± 0.12^{a}	1.12 ± 0.12^{a}	0.25 ± 0.25	0.25 ± 0.16^{c}
5 days	0.55 ± 0.17	1.00 ± 0.16^{a}	0.11 ± 0.11	2.11 ± 0.11^{a}
10 days	0.55 ± 0.17	0.66 ± 0.16^{a}	0.11 ± 0.11	0.11 ± 0.11^{c}
	Edema (0—3)	Infiltration (0—3)	Vacuolization (0—3)	Regeneration (0—3)
Time after third caerulein infusion				
0 h	$1.63 \pm 0.18^{a,c}$	$1.12 \pm 0.12^{a,c}$	$1.00 \pm 0.00^{a,c,d}$	0.12 ± 0.12
12 h	$1.33 \pm 0.16^{a,c,d}$	$1.77 \pm 0.15^{a,c}$	0.88 ± 0.11^{a}	0^{c}
24 h	$1.60 \pm 0.16^{a,c,d}$	$1.40 \pm 0.16^{a,c,d}$	$0.30 \pm 0.15^{c,d}$	$0^{c,d}$
48 h	1.66 ± 0.16^{a}	$1.11 \pm 0.11^{a,c,d}$	0.33 ± 0.23	0^{c}
72 h	$0.88 \pm 0.11^{a,c}$	0.88 ± 0.11^{a}	0^{c}	0.11 ± 0.11^{c}
5 days	0.20 ± 0.13^{c}	$0.60 \pm 0.16^{a,c,d}$	0	$0.30 \pm 0.21^{c,d}$
10 days	0.22 ± 0.11^{c}	0.66 ± 0.16^{a}	0	$2.11 \pm 0.11^{a,b,d}$

Mean ± SEM of 8—10 observations. ${}^{a}P < 0.05$ compared with saline infused rats. ${}^{b}P < 0.05$ compared with time 0 h after first caerulein infusion. ${}^{c}P < 0.05$ compared with value obtained at the same time of observation after first infusion of caerulein. ${}^{d}P < 0.05$ compared with value obtained at the same time of observation after second caerulein infusion.

the remaining time of experiment. The RNA content was not significantly affected by the third caerulein infusion (*Table I*) and 24 h later showed significantly higher value when compared to first pancreatitis induction. The

DNA content was decreased between 24 h and 5 days but to a lesser extend than after the first caerulein infusion. The protein content in this part of experiments was significantly decreased from 24 h to 48 h after pancreatitis induction but was significantly higher in remaining time of experiment when compared to the first caerulein infusion. Histological signs of pancreatic damage (*Table 2*) such as edema, leukocytes infiltration or vacuolization were less pronounced when compared to the first or second administration of caerulein, and regeneration processes were significantly smaller reaching the highest value 10 days after the last caerulein infusion.

DISCUSSION

The present study confirms and extends previous findings from various laboratories which indicated that supramaximal hormonal stimulation of the rat exocrine pancreas leads to reversible self-destruction of the gland (2, 13) followed by spontaneous regression of the inflammation accompanied by subsequent regeneration processes (13). The major finding of this study was to demonstrate that the pancreas is able to adapt to repeated insults of acute inflammation induced by caerulein infusion and exhibits spontaneous regeneration. Our data present for the first time such evidence, based on the observation that repeated infusions of caerulein lead to gradual limitation of pancreatic damage.

This acute caerulein-induced pancreatitis model, initially described by Lambel and Kern (1) and later modified by Wood et al. (14), consists of gross edema, leukocyte infiltration and cell vacuolization. The other characteristic symptoms, such as, elevated serum amylase and the decrease of total RNA and DNA contents were also seen. It is of interest that pancreatic blood flow was dramatically depressed immediately after first induction of pancreatitis and this preceded the drop of DNA synthesis, indirectly suggesting an important role of the fall in the tissue blood flow in the induction of pancreatic damage. This notion is supported by the information that application of hyperbaric oxygen attenuated in isolated pancreatic acinar cells the biochemical evidences of caerulein-induced injury (15). Moreover, it was clearly shown that the inhibition of nitric oxide synthesis aggravates the course of acute pancreatitis (4), however, it was also shown that the overdose of NO participates in oxidative injury during pancreatitis by binding the SH group (16).

In our studies, the DNA synthesis, an index of cell proliferation, dropped immediately after induction of pancreatitis to be followed by the increase 48 h later. This increase in DNA synthesis is consistent with the observation of Willemer at al. (2) who studied mitotic activities by labeling index of [^{3}H] thymidine and found that this index in acinar cells increased between second and fifth day after caerulein administration. Additionally, they observed

enhanced proliferative activity of fibroblasts 24 h after induction of pancreatitis. These reults are in keeping with the morphological changes observed by us and others (12, 18) who demonstrated that the regenerative process starts 48 h after single caerulein infusion and is almost completed 10 days later.

Acute pancreatitis can be developed on several ways (8, 18—20), including retrograde injection of taurocholate or ethionine supplemented, choline deficient diet, leading usually to full recovery at least within 21 to 28 days, depending of the methods used. Such functional and structural recovery was also observed in our study, however, as was reported by Seidensticker et al (8) the full restoration to normal values does not necessarily occur and evolution to chronic pancreatitis is possible (8).

In our study repeated induction of pancreatitis led to the development of adaptation processes as manifested by both biochemical and histological changes. The initial inhibition of the DNA synthesis after second and third caerulein infusion was smaller than after first infusion but was followed by similar changes in RNA and DNA contents. Histologically, after repeated induction of pancreatitis, an initial pancreatic damage, including edema, leukocyte infiltration and vacuolization, was decreased and disappeared earlier than after first caerulein infusion. These changes showed a constant progress after each next peptide infusion. It must be pointed out that one of the most accepted markers of the pancreatic tissue damage such as an elevation of plasma amylase concentration was unexpectedly increased with initial peak at the time 0 h after subsequent caerulein infusion. This inconsistency between pancreatic condition and plasma amylase concentration can be explained by smaller drop of pancreatic blood flow after each consecutive induction of pancreatitis. The improvement of pancreatic blood flow probably allowed to remove active digestive enzymes from the pancreatic tissue and protected pancreas against damage caused by these enzymes. For the same reason the initial peak of plasma amylase concentration was followed by statistically significant earlier reduction in this parameter after repeated induction of pancreatitis. The drop of the blood flow after each consecutive caerulein infusion was smaller and was followed by an earlier increase of this parameter. The initial increase of pancreatic weight and protein content after induction of pancreatitis was probably due to the edema of pancreatic tissue due to the leak of fluid and plasma proteins from blood vessels to pancreatic interstitial tissue. A later decrease of pancreatic weight and protein content was an effect of withdrawal of injured, or dead cells and exsudation from the tissue. The minimal pancreatic weight and protein content observed during regeneration indicates how much of the pancreatic tissue remained intact after each induction of pancreatitis. The changes of above parameters were less pronounced after repeated induction of pancreatitis, and this is an additional

evidence of improvement of pancreatic tissue condition, clearly showing the ability of the pancreas to adapt to repeated injury.

On the other hand, in our studies the increased delay in the start of regeneration processes in the pancreas after each consecutive pancreatitis induction was observed. This may be a consequence of less pronounced damage that was histologically determined after the second and the third caerulein infusion. It is possible that smaller tissue damage reduces the involvement of growth promoting factors in tissue repair and for these reasons the tissue regeneration is delayed.

Infusion of supramaximal doses of caerulein was shown to cause a marked reduction in normotypic discharge of zymogen granules at the luminal plasma membranes (2) and an ectopic discharge of individual granules and vacuoles through the lateral plasma membrane. Several alterations in capillary system were reported (2). It is, however, a question whether changes in pancreatic blood flow are a cause of pancreatitis or represent merely a secondary phenomenon occuring as a consequence of acinar cell damage, intracellular activation of digestive enzymes and activation of inflammatory mediators.

Overstimulation of the pancreas by caerulein results in edematous interstitial form of acute pancreatitis in which spontaneous regeneration can be strongly inhibited by specific CCK receptor antagonist (17) and accelerated by small doses of exogenous hormone. In contrast, it does not mach the treatment in diet-induced or necrotizing pancreatitis (21).

Lately, tyrosine kinase, phospholipase D activity (22) and the increase of silver staining nucleolar organizer regions (23) were shown to be related to the regeneration of the pancreas. The biochemical improvement of the regeneration processes in the pancreas after caerulein induced pancreatitis is correlated with the changes in ornithine decarboxylase (ODC) activity in the pancreas. Morriset at al (24) has shown that ODC — the key enzyme in the synthesis of polyamines, which are essential in cellular growth and differentiation processes — was significantly increased in the caerulein induced pancreatitis. Finally, these data suggest that pancreas is able to regenerate after hormonally stimulated pancreatitis and exhibits an adaptation to the repeated insults. The precise mechanism of this adaptation remains obscure but is strictly correlated with the changes of the blood flow in the pancreatic tissue.

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