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THE EFFECT OF PROSTACYCLIN ANALOGUE—ILOPROST ON THE PANCREAS REGENERATION AFTER CAERULEIN-INDUCED ACUTE PANCREATITIS IN RATS

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Prostacyclin (PGI_2) and its stable analogue iloprost (I) exert beneficial effect in acute pancreatitis (AP). The aim of the study was to evaluate the role of iloprost in pancreas regeneration after AP in rats. Methods: AP was induced in male Wistar rats by s.c. injections of caerulein 12 μg/kg t.i.d. for 2 days. Rats were divided into four groups: control + saline (C); control + iloprost (C/I); AP + saline (AP); AP + I (AP/I). Rats were treated for 7 or 14 days. I (Schering AG) was given at the dose 1 μg/kg b.w., i.p., t.i.d. After the rats were killed, the pancreata were weighed and their protein, DNA, RNA, chymotrypsin, α-amylase contents were evaluated. Light microscopic examination of representative pieces of pancreas was performed. Results: Acute pancreatitis resulted in pancreas destruction observed even 7 days after the onset of the disease. The significant decrease of pancreatic weight, RNA and chymotrypsin contents were observed in AP rats when compared to C. The improvement of pancreatic histology and significant increase of DNA content were found in I treated (during 7 days) AP rats in comparison to untreated AP group. Two weeks after pancreatitis induction the pancreas regeneration occurred in both pancreatitis groups and it was connected with pancreas hypertrophy. Treatment with I resulted in slight not significant increase of some of cellular hypertrophy indices when compared to AP untreated animals. Healthy rats injected with I during 7 days showed significant elevation of DNA content in comparison to C. When treatment with I was prolonged up to 14 days such hyperplastic effect was not observed. Our results suggest, that treatment with iloprost exerts temporary hyperplastic influence on the pancreas of healthy rats and pancreas regenerating after caerulein-induced pancreatitis.

Key words: *acute pancreatitis, regeneration, nucleic acids, prostaglandins, iloprost.*

INTRODUCTION

Pancreas regeneration after partial resection, duct ligation injury, toxic injury and acute pancreatitis has been described (1). The time course of the regenerative response has been studied extensively but mechanisms and factors involved in this process are still poorly clarified (2).

The prostanoid imbalane has been found to be an important factor in the pathogenesis of acute pancreatitis (3). The protective effect of different natural

prostaglandins and their analogues on the course of acute pancreatitis has been shown (4—6). The mechanisms responsible for their beneficial influence involve: the stabilization of lysosomal membranes within acinar cells (7, 8), inhibition of intracellular activation of pancreatic digestive enzymes (9), improvement of blood supply (10). Prostacyclin and its analogue iloprost has been also shown to exert platelet antiggregatory influence. Beside that, both PGI_2 and iloprost inhibit the leukocytes chemotaxis and decrease the release of lysosomal hydrolases and the generation of oxygen free radicals from inflammatory cells, agents potentially noxious to the endothelial and acinar cells (11). Prostaglandins have been also shown to exert the trophic effect on gastrointestinal mucosa (12, 13) and liver (14). Contrary, the inhibitors of prostaglandin production exert some inhibiting action both on DNA synthesis and mitotic activity in the regenerative response of the rat liver after partial hepatectomy (15).

The goal of present study was to investigate the role of chemically stable carbacyclin analogue of PGI_2 — iloprost on the pancreas regeneration after caerulein induced acute pancreatitis in rats.

MATERIAL AND METHODS

Animals

Male Wistar rats (n = 52) weighing 240—260 g were used in the study. The animals were given a standard chow diet. Care was provided in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals (NIH publication # 85—23, 1985).

Experimental design

Acute pancreatitis was induced by s.c. injections of caerulein 12 $\mu g \cdot kg$ body weight^{-1} every 8 h for 2 days. This analogue of cholecystokinin (CCK) was dissolved in gelatin (16% w/v) to prolong its absorption (2). The control animals were injected with saline in gelatin s.c. Beginning from day 3, rats were allocated into four treatment groups (6—8 rats in each):

— Control rats injected with saline (C).
— Control animals treated with PGI_2 stable analog iloprost (C/I).
— Rats with AP injected with saline (AP).
— Rats with AP treated with iloprost (AP/I).

Rats were treated for 7 or 14 days. Iloprost (I) (Schering AG, Germany) was given i.p. at a dose of 1 μg per kg b.w. three times per day (t.i.d.) The animals were fed *ad libitum* with a standard diet during whole experiment with a 12 hour light-dark cycle. After an overnight fast with free access to water rats were decapitated. The pancreas was dissected as quickly as possible, free of connective tissue, fat, lymph nodes and weighed. The representative fragments of pancreatic tissue from each rat were fixed in 10% formaline, embedded in paraffin, sectioned at 5-μm slices and stained with hematoxylin and eosin for light microscopic examinations.

Biochemical assays

The pieces of pancreatic tissue were homogenized using motor-driven ground-glass homogenizator either in 0.6 mol/L perchloric acid for nucleic acids determinations or in the ice-cold 0.1 mol/L Tris-HCl buffer (pH = 8.0) for protein, α-amylase and chymotropsin assays.

DNA and RNA were extracted according to Mainz et al. (16). DNA was assayed according to Volkin and Cohn (17) using calf thymus DNA as a standard. RNA was measured after overnight hydrolysis in 0.3 mol/L KOH according to Munro and Fleck (18). Tissue α-amylase and chymotrypsin activities were determined according to the Bernfeld (19) and Hummel (20), respectively. Protein content was measured according to Lowry et al. (21).

All reagents except soluble starch (Merck, Darmstadt) were purchased from Sigma Chemical Co.

Statistical analysis

All values were expressed as means ± SEM. Statistical significance of the difference between the groups was assessed using t test for unpaired observations and Fischer test. $P < 0.05$ was considered statistically significant.

RESULTS

Biochemical assays

Pancreatic weight (PW) decreased significantly in untreated rats with acute pancreatitis (AP) when compared to C (by 11%) 7 days after the pancreatitis induction (*Table 1*). Such decrease of PW was not observed after the treatment with iloprost in AP/I rats comparing to C, however PW in this group was significantly lower than in the control rats injected with I (C/I) ($p < 0.05$). Healthy animals injected with I during 7 days had higher PW than C rats (increase, by 8.8%, ns).

No significant differences of PW between healthy and pancreatitis rats were found 14 days after the onset of pancreatitis.

Pancreatic protein contents in both groups with AP (untreated or treated with I) tended to decrease 7 days after the induction of acute pancreatitis when compared to control (by 16% and 12% respectively), however differences were not significant (*Table 1*). Treatment with I during two weeks resulted in significantly higher protein content in AP rats as compared to C injected with I (when data were expressed per 100 g of b.w., the protein content increased also in comparison to C animals, data not shown).

Table 1. Pancreatic weight, total protein, enzymes and nucleic acids contents in caerulein-induced acute pancreatitis in rats treated with iloprost [#]. Means ± SE are reported.

Parameter	Control (C)	Control-Iloprost (C/I)	Acute Pancreatitis (AP)	AP-Iloprost (AP/I)
P. weight (mg)				
7 days	847 ± 44	930 ± 38	747 ± 29 *	823 ± 34 °
14 days	1036 ± 85	826 ± 32	940 ± 44	962 ± 77
Protein (mg)				
7 days	87.8 ± 8.8	94.3 ± 10.4	73.7 ± 7.8	77.1 ± 8.9
14 days	90.2 ± 18.1	88.0 ± 9.8	108.6 ± 7.3	119.5 ± 11.1 °
Amylase (U)				
7 days	26769 ± 3349	31963 ± 3473	22386 ± 4672	23690 ± 3692
14 days	15663 ± 2347	10760 ± 1468	16238 ± 2211	20933 ± 3489 °
Chymotrypsin (U)				
7 days	362.7 ± 52.8	425.2 ± 115.5	126.0 ± 25.2 *	242.4 ± 35.3 */**
14 days	159.8 ± 27.8	159.4 ± 27.2	199.0 ± 24.7	232.9 ± 56.6
RNA (mg)				
7 days	24.3 ± 1.4	24.2 ± 2.2	18.7 ± 1.2 *	18.9 ± 1.2 */°
14 days	24.5 ± 1.7	21.8 ± 1.3	27.2 ± 0.9	23.5 ± 1.9
DNA (mg)				
7 days	4.49 ± 0.22	6.12 ± 0.40 *	4.85 ± 0.08	5.4 ± 0.17 */**
14 days	4.65 ± 0.24	4.67 ± 0.35	4.07 ± 0.15	4.65 ± 0.28
RNA/DNA				
7 days	5.4 ± 0.19	4.09 ± 0.50 *	3.87 ± 0.29 *	3.53 ± 0.31 *
14 days	5.3 ± 0.43	4.69 ± 0.12	6.70 ± 0.37 *	5.23 ± 0.61

[#] Iloprost (I) was injected at a dose of 1 μg/kg b.w. tid i.p. for 7 or 14 days.

* values significantly different from control ($p < 0.05$).

** values significantly different from acute pancreatitis untreated rats ($p < 0.05$).

° values significantly different from control rats treated with I ($p < 0.05$).

Healthy animals injected with iloprost showed higher α-amylase content in comparison to C group (the difference was significant when data were expressed per 100 g of b.w., data not shown). After 14 days of treatment with I, α-amylase content increased significantly in AP rats vs. their control (C/I) (*Table 1*).

Pancreatic chymotrypsin content, 7 days after the pancreatitis induction was significantly lower in both groups with AP in comparison to C ($p < 0.05$), (*Table 1*). When I was injected to AP rats the chymotrypsin content significantly increased (by 48%) when compared to untreated animals ($p < 0.05$). PGI_2 analogue tended to increase pancreatic chymotrypsin content in healty animals by 14.7% in comparison to C (ns). No significant differences were found in this parameter between all groups 14 days after the onset of pancreatitis.

Acute pancreatitis resulted in the significant decrease of pancreatic RNA content (in both groups) when compared to C, even 7 days after the induction of inflammation ($p < 0.01$), (*Table 1*). Iloprost did not change RNA content in healthy rats after 7 days of treatment (in comparison to C). No significant differences were found between treated and untreated groups 14 days after the pancreatitis induction.

Highly significant increase ($p < 0.005$) of pancreatic DNA content was observed after 7 days in healthy animals treated with I when compared to C and in AP rats injected with I when compared to the control group or untreated rats with AP (*Table 1*). After 14 days of experiment DNA contents in all groups were similar.

RNA/DNA ratio decreased in AP group 7 days after induction of the disease when compared to the control group. Treatment with I both healthy and AP rats was followed by proportional lowering of this ratio in both treated groups. Two weeks after the pancreatitis induction RNA/DNA ratio increased in AP rats in comparison to C ($p < 0.05$).

Using total DNA content as a measure of the cell number, it can be seen that iloprost treatment during 7 days induces pancreatic growth both in healthy and in AP animals, causing significant hyperplasia (*Table 2*). This influence of iloprost has not been observed after 14 days of treatment (*Table 2*). The cellular hypertrophy was established by dividing the total contents of RNA, protein, amylase and chymotrypsin by the total content of DNA. Pancreas regeneration after acute pancreatitis was connected with cellular hypertrophy 14 days after the pancreatitis induction. The indices of cellular hypertrophy (protein/DNA, amylase/DNA, chymotrypsin/DNA) in AP rats were slightly augmented by I treatment, but at this interval of time the difference did not achieved the level of statistical significance (*Table 2*).

Table 2. The effect of iloprost treatment on indices of pancreatic tissue hyperplasia and cellular hypertrophy in the rats with caerulein-induced acute pancreatitis #. Means ± SEM are reported.

Parameter	Control-Iloprost (C/I)	Acute pancreatitis (AP)	AP-Iloprost (AP/I)
Hyperplasia			
DNA 7 days	1.36 *	1.08	1.20 */**
14 days	1.00	0.87	1.00
Hypertrophy			
RNA/DNA			
7 days	0.75	0.71	0.65
14 days	0.88	1.26 *	0.97
Protein/DNA			
7 days	0.79	0.77	0.72
14 days	0.94	1.38 *	1.49
Amylase/DNA			
7 days	0.87	0.75	0.71
14 days	0.71	1.19	1.38
Chymotrypsin/DNA			
7 days	0.93	0.32	0.55
14 days	1.05	1.46 *	1.53

Values are the ratios of experimental groups to control group after 7 and 14 days of treatment with iloprost (I) at a dose of 1 μg/kg b.w. tid i.p. for 7 or 14 days. Total DNA content represents the pancreas hyperplasia. Cellular hypertrophy was established by dividing total contents of RNA, protein, amylase and chymotrypsin by the total content of DNA. Values significantly greater than 1 (in comparison to control) were accepted as a indices of cellular hypertrophy.

* Values significantly greater than 1, were significantly different from controls ($p < 0.05$).

** Values significantly different in comparison with acute pancreatitis rats ($p < 0.05$).

Light microscopic examination

Fig. 1 shows the normal pancreas appearance in control rats. The iloprost treatment of healthy rats resulted in little increase of eosinofilic granules within acinar cells (to similar extent after 7 and 14 days) when compared to control untreated rats (not shown).

Seven days after the induction of pancreatitis the oedema and small inflammatory infiltration in untreated rats were present. The acinar cells showed some signs of lesion (cytoplasmic degeneration and vacuolization) and a few dead acinar cells were also found (*Fig. 2*). The treatment with I resulted in the improvement of histological changes, however vacuolization of acinar cells and small inflammatory infiltration with polymorphonuclear cells were still observed (*Fig. 3*).

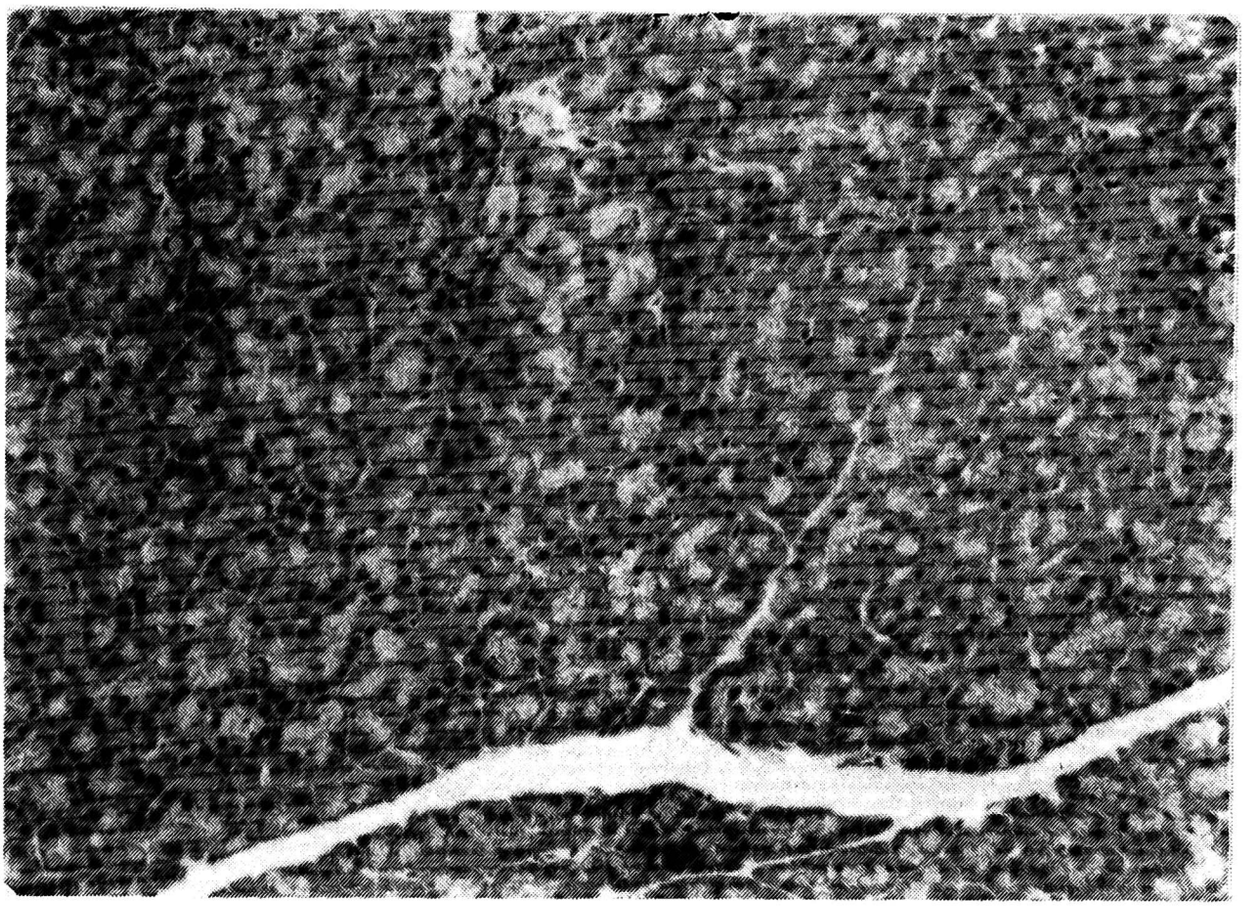

Fig. 1. Normal pancreas of rat from Control group (C 7 days). Mag. × 160.

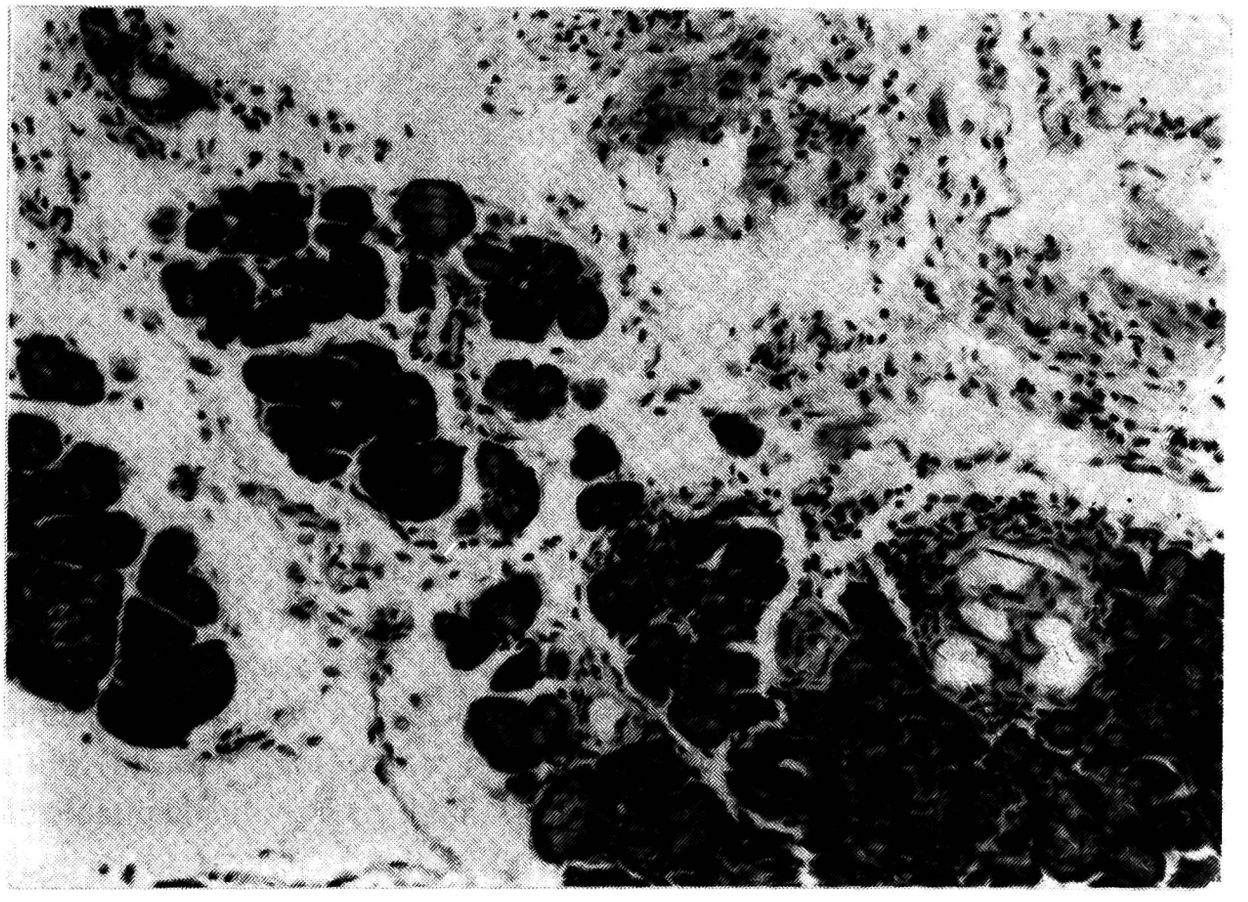

Fig. 2. Caerulein-induced pancreatitis — 7 days (AP 7 days). Oedema and inflammatory cell infiltration, signs of cytoplasmatic degeneration, vacuolization and sporadic necrosis of acinar cells. Mag. × 160.

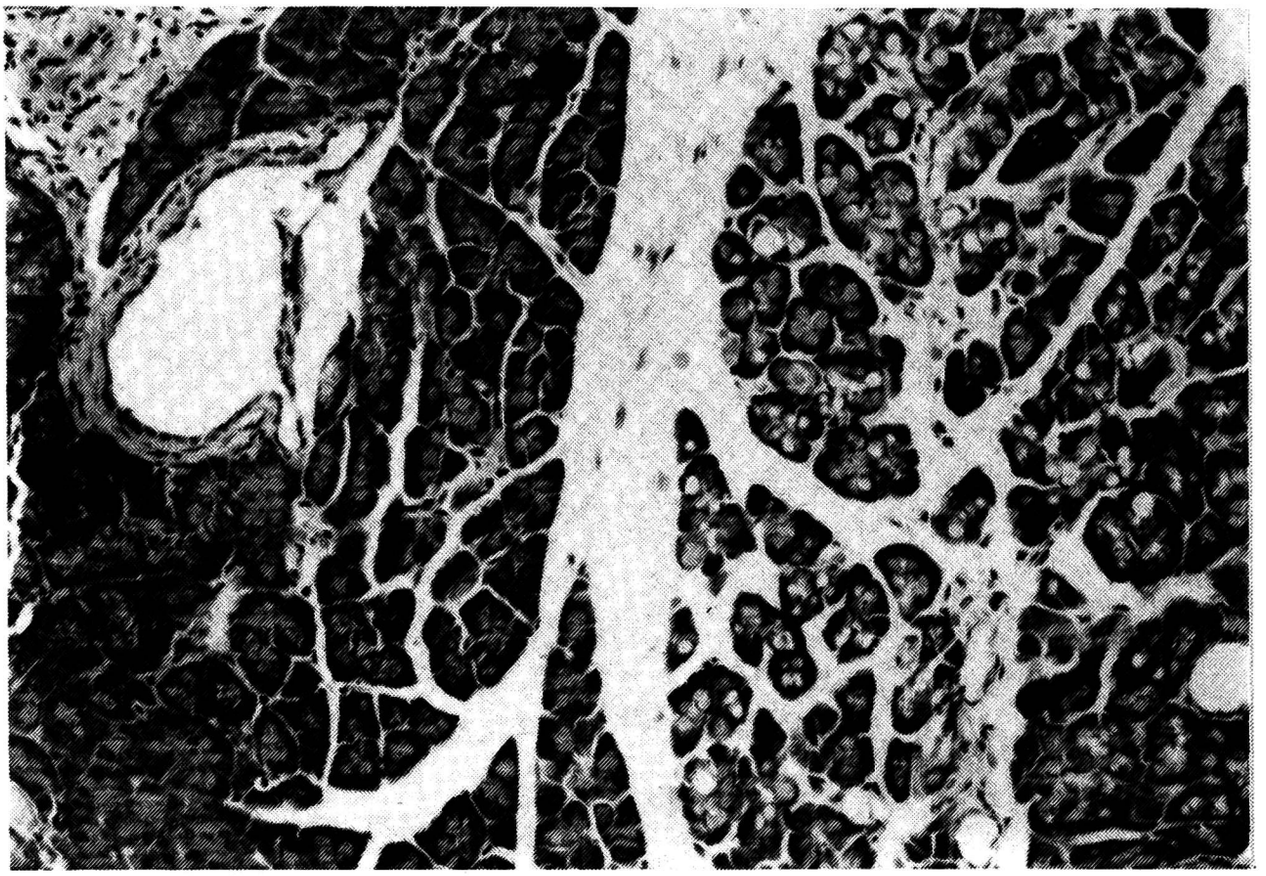

Fig. 3. Caerulein-induced pancreatitis — 7 days, treated with iloprost 1 μg/kg b.w. t.i.d. (AP/I 7 days). Intersititial oedema and leucocyte infiltration less advanced than in untreated group. The vacuolization and some degenerative changes of acinar cells can be observed but cell necrosis is really scarce. Mag. × 160.

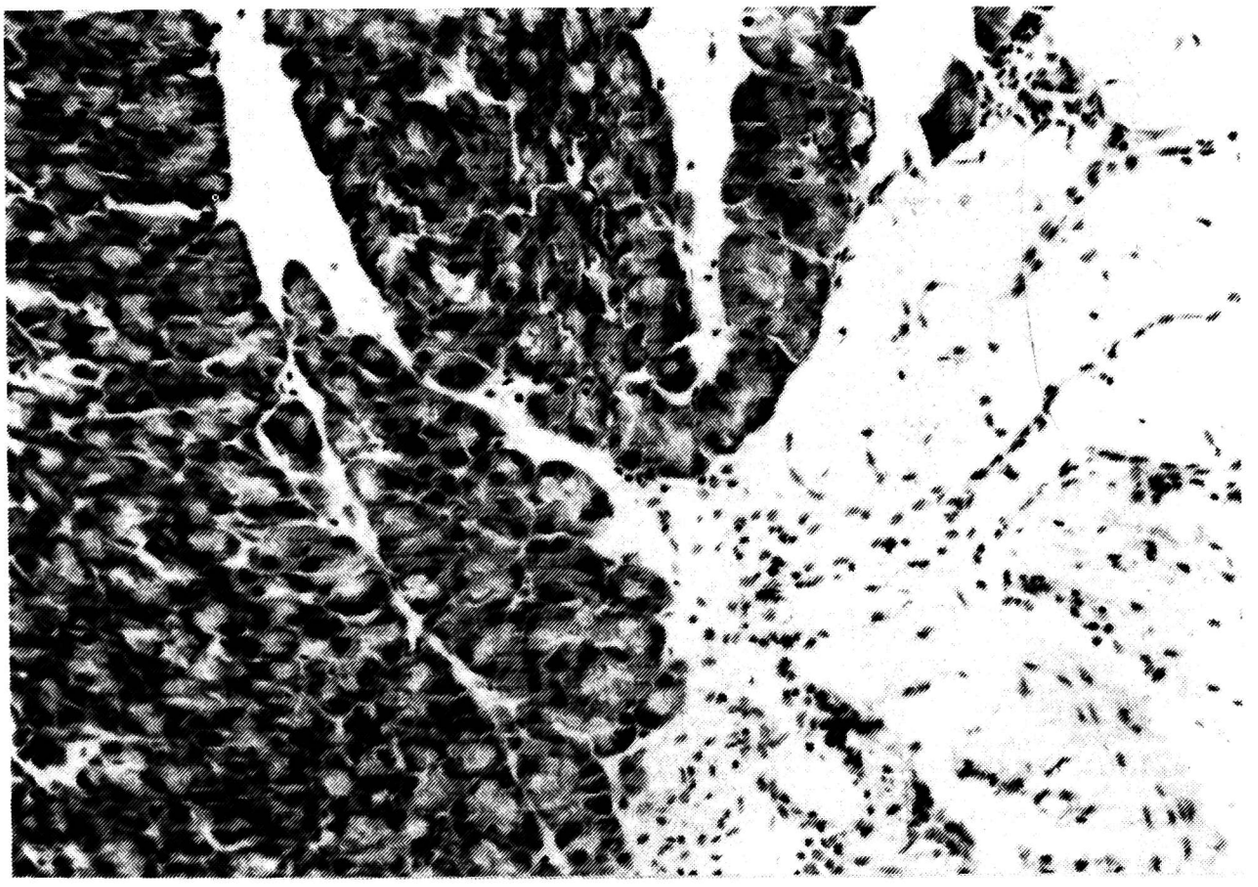

Fig. 4. Caerulein-induced pancreatitis — 14 days (AP 14 days). Traces of oedema and inflammatory cell infiltration. Small degree disarrangement of acinar cell morphology. Discrete degenerative changes of acinar cells. Evident improvement in comparison to AP lasting 7 days. Mag. × 160.

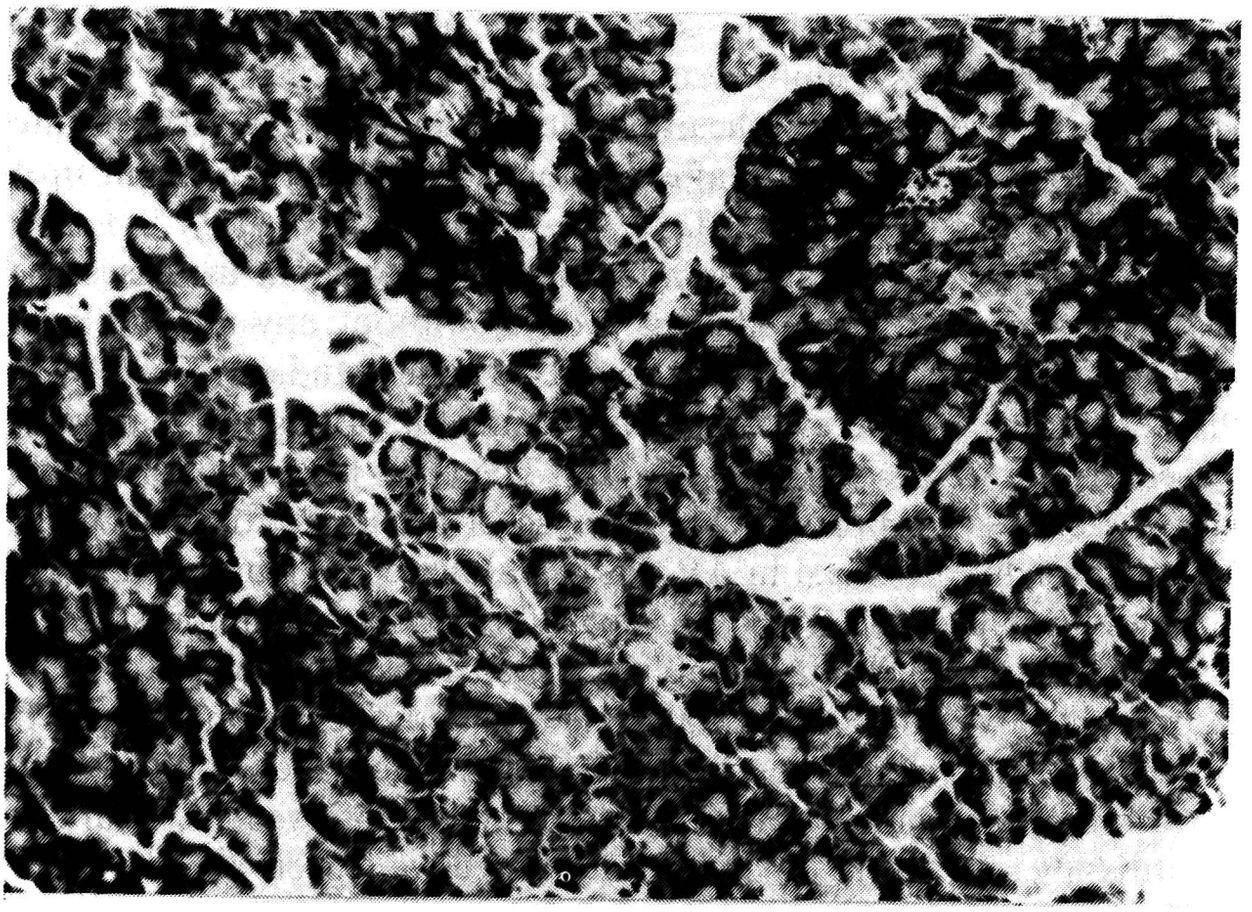

Fig. 5. Caerulein-induced pancreatitis — 14 days, treated with iloprost 1 μg/kg b.w. t.i.d. (AP/I 14 days). Inflammatory infiltration and oedema is virtualy absent. Minimal degenerative changes of acinar cells. Evident improvement in comparison to AP rats treated with I during 7 days (AP/I 7 days) as well as to 14-days AP group without treatment (AP 14 days). Mag. × 160.

After 14 days quite a large amount of eosinofilic granules within acinar cells were found in AP group. Small number of inflammatory cells was also present (*Fig. 4*). In AP group treated with I during 14 days the inflammatory infiltration was virtualy absent and the number of eosinofilic granules in acinar cells was increased as compared to AP untreated rats (*Fig. 5*).

DISCUSSION

In our previous study (2) it was found, that DNA content returned to control values 13 days after AP induction. Thus, at present study we have choosen 7 and 14 days as time points for trophic parameters investigation. Our results indicate that even 7 days after the onset of acute pancreatits the signs of pancreas destruction are still present. Iloprost treatment during seven days resulted in the significant increase of DNA content in AP rats. This hyperplastic effect seen in the first phase of regeneration was not observed after 14 days of treatment. Pancreatic trophic parameters of AP untreated rats 14 days after the onset of pancreatitis induction reached control values, supporting that spontaneously occurring regeneration after acute pancreatitis was completed. The prostacyclin analogue given during 14 days after the

induction of AP showed only slight tendency to increase the trophic parameters over values found in untreated AP rats.

The iloprost treatment of healthy rats during 7 days also resulted in pancreatic hyperplasia, however after prolongation of the treatment up to 14 days this effect was not observed.

We have not yet any explanation for such DNA content increases (in both groups of rats treated with I during 7 days) without any changes in RNA content but similar effect was also found in other studies (22, 23).

Prostaglandins, known cytoprotective agents for gastrointestinal tract have been used also in the treatment of AP (24). The most promising prostaglandin derivative seems to be prostacyclin, because of broad spectrum of biological actions, potentially beneficial in AP. PGI_2 stabilized the pancreatic lysosomal membranes in bile-induced AP in dogs (7). PGI_2 and iloprost increased survival rate of rats with taurocholate pancreatitis (25, 26).

Iloprost has five times longer half-time in blood circulation than PGI_2 (27) and possess a wide range of potentially beneficial activities in AP. It protects the lysosomal membrane integrity, as it was observed in taurocholate pancreatitis (9). It exerts a direct cytoprotective effect as was found in experimental ulcerogenesis, at a dose of 1 μg/kg b.w. (28). Iloprost also improves microcirculation and the treatment with this agent could counteract the imbalance of PGI_2/TXA_2 occurring in AP resulting in the improvement of histopathological changes in AP (25).

The trophic effect of different prostaglandins on gastrointestinal mucosa has been described. Fitzpatrick et al. (12) found that 16, 16-dimethyl-PGE_2 (ig) increased ornithine decarboxylase (ODC) activity by 2 hr, and chronic administration of PGE_2 up to one week increased RNA, DNA and protein content in the rat duodenal mucosa in association with the elevation of ODC activity. PGI_2 analogue (TRY-200) was also shown to stimulate ODC activity within duodenum, jejunum and ileum to similar extent as a refeeding (potent trophic factor), however endogenous prostaglandins seems to play no role in feeding-associated induction of ODC activity (29).

Short term prostacyclin treatment evoked significant elevation of DNA content in gastric fundic mucosa, while RNA and protein remained unchanged (22). Long term treatment (40 and 80 days) with PGI_2 (po) results in thickening of gastric fundic mucosa and was connected with the cellular hyperplasia as DNA content increased significantly in comparison to untreated rats (protein and RNA contents remained unchanged) (23). These results partly correspond to the effect of PGI_2 analogue treatment on pancreas of healthy and AP rats obtained by us.

In our study iloprost treatment during 7 days resulted in pancreas hyperplasia of healthy and AP rats. The lack of hyperplastic or evident trophic influence of iloprost after 14 days in both groups could result from the pancreas

„adaptation" to this treatment. This adaptation may result from the receptor desensitization due to often, prolonged drug application, analogically as has been shown for decreased platelet sensitivity to PGI_2 infusion during 7 days (decreased receptor affinity with increased number of binding sites) (30). Modesti et al. (31) have found that after short term of iloprost infusion (6 h) the reversible reduction of platelet PGI_2 sensitivity occurred, and was related to a contemporary down-regulation of PGI_2 receptors. We did not found in the literature the explanation for the exact mechanisms of PGI_2 influence on pancreas growth/regeneration.

In view of fact, that PGI_2 analogue exerts potentially beneficial effect in acute pancreatitis, the possible mechanisms of its influence on pancreas growth and regeneration require to be investigated.

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