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The influence of nifedypine (calcium channel blocker) and Bay-K-8644 (calcium channel agonist) on the development of experimental acute pancreatitis

Calcium ions play an important role in stimulus-secretion coupling in the exocrine pancreas. The increasing evidence exists that Ca^{2+} functions as a second messenger for cholecystokinin. The exact mechanism by which calcium triggers the secretory response is not exactly known, probably calmodulin is involved in the pancreatic enzyme secretion. Calmodulin is capable of forming a reversible complex with calcium and this complex can activate many enzymes e.g. guanylate cyclases and phospholipase A2.

In the pancreatic acinar cells there are no indications of electrical excitability and secretion cannot be evoked by membrane depolarization *per se*. In electrically inexcitable cells calcium signalling typically is a biphasic process. Neurotransmitters and hormones stimulate an intracellular organelle to release the stored calcium into the cytoplasm, and this is followed by the entry of calcium into the cytoplasm from the extracellular space. The first phase is attributable to IP3 (inositol-1,4,5-triphosphate), the second is not known. Calcium-storing organelle probably produces a retrograde signal that activates calcium influx across the plasma membrane. The amylase release is independent of the extracellular calcium during the first minutes of secretion, but later the sustained pancreatic acinar secretion becomes dependent on the extracellular calcium .

The available literature presents contradictory data concerning the effect of agonists and antagonists of calcium channel on the development of AP. Thus it seems interesting to examine this problem.

MATERIAL AND METHODS

The experiments were carried out in male Wistar rats weighing 200-250g. The AP was induced according to the L a m p e l and K e r n method (7) by intravenous continuous infusion of cerulein in the doses of $5x10^{-6}$ g/kg/h for 12 hours by means of the infusion pump. Two days prior to the cerulein or physiological saline infusion (in control groups) the rats received nifedipine or Bay-K-8644 intraperitoneally in the increasing doses in each group. Each animal group consisted of 9 rats.

The rats were divided into eight groups: 1st group: control, treated with 12 hours of physiological saline infusion; 2nd group: rats treated with 12 hours of cerulein (Takus-Farmitalia) infusion at the dose of 5 ug/kg/h; 3rd group: rats treated intraperitoneally with nifedypine (Polfa, Poland) 2 times a day for 2 days in the total doses of 0.84mg/kg and then infusion of cerulein as in 2nd group; 4th group: rats treated intraperitoneally with nifedypine in total doses of 4.2mg/kg as in 3rd group and cerulein as in 2nd group; 5th group: rats treated intraperitoneally with nifedypine in total doses of 21.0mg/kg as in 3rd group and cerulein as in 2nd group; 6th group: rats treated intraperitoneally with nifedypine in total doses of 21.0mg/kg as in 3rd group and physiological saline as in 1st group; 7th group: rats treated intraperitoneally with Bay-K-8644 (RBI, USA) 2 times a day for 2 days in total doses of 2mg/kg and then infusion of cerulein as in 2nd group; 8th group: rats treated intraperitoneally with Bay-K-8644 like in 7th group and physiological saline as in 1st group.

Directly after the infusion the animals were killed, blood samples were collected and the amylase activity assayed in international units (j.U.). Also the pancreas was removed and weighed. The specimens collected for histopathological examinations were fixed in 10% formalin buffered to pH 7.4 with phosphatic buffer, embedded in paraffin and cut into 4 mm thick pieces which were stained with hematoxylin and eosin. The obtained results were compiled as the mean values and statistically analysed by means of t-Student test.

RESULTS

In our studies the intensity of the inflammatory process in pancreas was determined by three parameters: the activity of amylase, the weight of pancreas (expressed as the percentage of the total rats' weight) and the histopathological changes under the light microscope. In the control groups (I, VI, VIII) all parameters were normal. In all the rats from 2nd group an AP development was observed coupled with a 15fold increase in amylase activity. In this group the increase in pancreas weight and histopathological features of the inflammatory process was also observed. In the 4th and 5th group of animals receiving nifedypine in higher doses before the infusion of cerulein a decrease in amylase activity in blood serum, the weight of the pancreas and a decrease in interstitial edema and inflammatory cell infiltration was obtained when compared with the group of animals receiving only cerulein (Fig. 1, Fig. 2, Fig. 3). The opposite effects were observed in 7th group of animals (receiving Bay-K-8644), which means the increase in amylase activity serum and an increase in the pancreas weight. An intensification of the morphological changes in the pancreas was also observed in this group during histological examination (Fig. 4).

DISCUSSION

The synthetic decapeptide cerulein has a strong and stimulating effect on exocrine pancreatic secretion. Cerulein infused intravenously in large doses evokes the characteristic microscopic changes, the significant increase in pancreatic mass and an increase in amylase activity. This method is used to induce an oedematous acute pancreatitis (7).

It is suggested that part of the inner surface of membranes from secretory granules in the pancreatic acinar cells acts as a calcium-buffering system that works in synergy with other protective mechanisms to stabilize the zymogen granule population. In pancreatitis due to a markedly reduced Ca^{2+} affinity of membranes from the secretory granule there occurs a disturbance in the ratio of membrane-bound

versus free Ca^{2+}. It could be one of the factors to be invoked to account for the instability of zymogen granules, an uncontrolled proenzyme activation seen in cerulein pancreatitis.

Pancreatic acini do not have voltage-dependent Ca^{2+}-channels. The activation of Ca^{2+} influx is required for the secretory response to cholecystokinin or carbachol in pancreatic acinar cells. The mechanisms by which these agonists cause Ca^{2+} influx is unknown. Three main mechanisms of voltage-independent calcium influx are considered: receptor-operated calcium channel, second messenger-operated calcium channel and depletion-operated calcium current (DOCC). Activation of DOCC provides a source of calcium for refilling of the intracellular calcium stores and is regulated by the concentration of calcium within the store. Several mechanisms have been suggested for DOCC regulation, but the exact mechanism is still not known. In pancreatic acinar cells cyclic GMP or GTP-hydrolyzing protein were shown to modulate DOCCs and it could be the mechanism of depletion-activated Ca^{2+} entry. G u k o v s k a y a and P a n d o l (4) provided convincing evidence that NO (nitric oxide) produced by NO synthase mediated the stimulation by carbachol of cyclic GMP formation, which in turn evoked Ca^{2+} influx in the pancreatic acinar cells of rats. X u et al. (15) showed that depletion of intracellular Ca^{2+} stores activated NO synthase to generate cyclic GMP and regulate Ca^{2+} influx in the rat pancreatic acinar cells. During carbachol stimulation Ca^{2+} released from the internal stores activates a Ca^{2+}-dependent constitutive NO synthase to generate NO from L-arginine. The activity of NO synthase is regulated by Ca^{2+} content present in the internal stores.

Table 1. The influence of nifedypine and Bay-K-8644 on amylase activity in blood serum and on weight of pancreas in percentage of rat's mass

Experimental groups	Amylase activity in international units j.U. mean ± SD	Weight of pancreas in percent of total rat's mass mean ± SD
Group I infusion of physiological saline	2000 ± 800	0.43 ± 0.06
Group II infusion of cerulein	31000 ± 3000 * II/I	1.20 ± 0.20 * II/I
Group III nifedypine 0.84mg/kg and cerulein	30900 ± 2800 ** III/II	1.18 ± 0.19 ** III/II
Group IV nifedypine 4.2mg/kg and cerulein	27590 ± 1900 **** IV/II	0.86 ± 0.19***IV/II
Group V nifedypine 210mg/kg and cerulein	24500 ± 2800 * V/II	0.80 ± 0.20 * V/II
Group VI nifedypine 21.0mg/kg and physiological saline	2100 ± 700 ** VI/I	0.40 ± 0.04 ** VI/I
Group VII Bay-K-8644 2 mg/kg and cerulein	35400 ± 2900 *** VII/II	1.41 ± 0.18***** VII/II
Group VIII Bay-K-8644 2mg/kg and physiological saline	1950 ± 800 ** VIII/I	0.41 ± 0.04** VIII/I

Statistically significant difference

* $p<0.001$, ** $p> 0.05$, *** $p<0.01$, **** $p<0.02$, *****$p< 0.05$

I - compared to group I, II - compared to group II

B a h n s o n et al. (1) using patch-clamp recordings demonstrated that the intracellular injections of cyclic GMP activated the same Ca^{2+} entry pathway that is activated during agonist stimulation. These results suggest a model in which the intracellular Ca^{2+} release leads to the formation of cyclic GMP which, in turn, activates a plasma membrane Ca^{2+} influx transport.

In our study the smallest dose of nifedipine applied before induction of acute pancreatitis did not have an effect on the development of AP. We obtained a statistically important decrease in intensity of the inflammatory signs in pancreas expressed by amylase activity, weight of pancreas and microscopic signs when greater doses of nifedipine were applied in comparison with rats treated only with cerulein. We found under the light microscope the decrease in the interstitial edema and the inflammatory cell infiltrations.

Probably the lack of any inhibition of either CCK- or carbachol-stimulated secretion at low concentrations of Ca^{2+}-channel blockers is connected with the lack of voltage dependent Ca^{2+}-channels in pancreatic acini (9). It is suggested that verapamil acts on muscarinic receptors in noncompetitive manner (9) or may cause many nonspecific membrane effects because of their high concentration in the membranes (2). It also has been reported that Ca-antagonistic dihydropyridines in high concentrations block the calmodulin-dependent enzymes activation (12) .

The beneficial effect of verapamil on the development of AP observed L e a h y et al. (8) after the retrograde injection of sodium taurocholate in rats. Calcium blockade with verapamil succesfully ameliorated AP but delayed treatment was not effective. S h e n (13) observed significantly increased pancreatic blood flow and pancreatic tissue perfusion in rats in the early phase of AP. The results of the study performed by R e g a l a d o et al. (11) also suggest a beneficial effect of verapamil on AP induced by ligation of the bile duct in the rat. The verapamil treatment decreased significantly tissue activity of trypsin and chemotrypsin, but there was no statistical difference in serum amylase activity. W a n g et al. (14) reported that verapamil counteract endothelial barrier injury in pancreatic tissue and cell organella induced by AP in rats. L a k e et al. (6) found some limited tissue impairment in mice after treatment with verapamil.

However, the effect of verapamil on canine pancreatic secretion stimulated by cholecystokinin has shown contradictory results. B u r n s et al. (3) obtained an inhibition of amylase secretion, but not of lipase or bicarbonate.

N i e d e r a u et al. (10) studied effects of verapamil on exocrine pancreatic secretion in man. The pancreatic secretion stimulated by cholecystokinin, secretin or by sham feeding were unaffected by intravenous verapamil in the doses which were the highest ones recommended in cardiological practice when compared to the saline control. At the same time in man lower or similar doses of verapamil as used in this study have been effective in inhibiting other calcium-mediated hormonal or secretory actions e.g. verapamil inhibits glucose-induced release of insulin.

In our study Bay-K-8644 applied before induction of acute pancreatitis caused a statistically important increase in intensity of the inflammatory signs in pancreas expressed by amylase activity, weight of pancreas and microscope signs.

According to H e i s l e r et al. (5) Bay-K-8644 exerts a biological effect on the tissue not known to contain voltage-sensitive calcium channels. Racemic Bay-K-8644 did not enhance the secretory response to either carbachol or cholecystokinin in isolated rat pancreatic acinar cells, however, when co-applied with vasoactive intestinal peptide or forskolin it potentiated the amylase secretion. The exact mechanism of secretogogue action of Bay-K-8644 remains still unresolved (5).

Our investigations suggest a beneficial effect of the high dose nifedypine and the opposite effect of Bay-K-8644 on the development of cerulein induced AP in rats.

REFERENCES

1. Bahnson T., Pandol S. et al.: Cyclic GMP modulates depletion-activated Ca entry in pancreatic acinar cells. J. Biol. Chem. 268, 10808, 1993.
2. Bechem M., Hebisch S. et al.: Ca^{2+} agonists: new, sensitive probes for Ca^{2+} channels. TiPS, 9, 257, 1988.
3. Burns G., Gersten M. et al.: The effect of verapamil on pancreatic exocrine secretion. Surgery, 90, 474, 1986.
4. Gukovskaya A., Pandol S.: Nitric oxide production regulates cGMP formation and calcium influx in pancreatic acinar cells. Am. J. Physiol., 266, G350, 1994.
5. Heisler S., Desjardins D. et al.: Bay-K-8644-stimulated amylase secretion from pancreatic acinar cells. Can. J. Physiol. Pharmacol. 66, 32, 1987.
6. Lake-Bakaar G., Lynbsky S. et al.: Dose-dependent effect of continuous subcutaneous verapamil infusion on experimental acute pancreatitis in mice. Dig. Dis. Sci. 40, 2349, 1995.
7. Lampel M., Kern H.: Acute interstitial pancreatitis in the rat induced by excessive doses of a pancreatic secretagogue. Vichows Arch. A. 373, 97, 1977.
8. Leahy A., Darzi A. et al.: Verapamil is beneficial in a model of acute pancreatitis after endoscopic retrograde cholangiopancreatography. Br. J. Surg. 79, 1241A, 1992.
9. Mossner J., Schwarz J. et al.: Influence of calcium channel blockers on exocrine pancreatic secretion by isolated rat acini. Res. Exp. Med., 188, 255, 1988.
10. Niederau C., Hellmann A. et al.: Effects of verapamil on exocrine pancreatic secretion in man. Dig. Dis. Sci. 30, 72, 1985.
11. Regalado P., Pardo C. et al.: The effects of verapamil in experimental acute pancreatitis in the rat. Rev. Esp. Enferm. Dig. 82, 35, 1992.
12. Schramm M., Towart R.: Modulation of calcium channel function by drugs. Life Sciences, 37, 1843, 1985.
13. Shen J.: Effects of verapamil on prostacyclin, thromboxane and pancreatic blood flow of rats with experimental acute pancreatitis. Chung-Hua-I-Hsueh-Tsa-Chih-Taipei, 71, 488, 1991.
14. Wang X., Deng X. et al.: Antioxidant and calcium channel blockers counteract endothelial barier injury induced by acute pancreatitis in rats. Scand. J. Gastroenterol. 30, 1129, 1995.
15. Xu X., Star R. et al.: Depletion of intracellular Ca stores activates nitric-oxide synthase to generate cGMP and regulate Ca influx. J. Biol. Chem. 269, 12645, 1994.

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EXPLANATION TO FIGURES

Fig.1. Control group. Normal texture of pancreas parenchyma with the presence of pancreatic islets and of intralobular ducts and pancreatic vessels. Magn. 400x. H + E staining.

Fig. 2. Rats treated with 12 hours intravenous infusion of cerulein (5 μg/kg/h). Pancreas parenchyma shows significant impairment with the features of acute pancreatitis (AP). Oedema, inflammatory infiltrations, congestion, erythrocytes present in interstitial tissue. The glandular epithelium

disintegration, parenchymatous and vacuolar degeneration of the cells, the blurring of the intercellular borders in the extrasecretory follicles. Magn. 400x. H + E staining.

Fig. 3. Rats treated intraperitoneally with nifedypine in the total doses of 21.0 mg/kg and cerulein as in 2nd group. (group V). In comparison with group II, the increase of AP features is observed. There are numerous normal follicles without parenchymatous and vacuolar degeneration of the cells. Normal texture of pancreas parenchyma with the presence of pancreatic islets and of intralobular ducts and pancreatic vessels. Magn. 400x. H + E staining.

Fig. 4. Rats treated intraperitoneally with Bay-K-8644 2mg/kg and cerulein as in 2nd group (group VII). Compared with group II, the focal increase of the intensification of the destructive proceses is found. The follicles are damaged in various degree. The parenchymatous and vacuolar degeneration of the glandular tissue are observed. Oedema, inflammatory infiltrations, congestion, erythrocytes present in interstitial tissue. Magn. 400x. H + E staining.

SUMMARY

The aim of this paper was to evaluate the influence of nifedypine (calcium channel blocker) and Bay-K-8644 (calcium channel agonist) on cerulein acute pancreatitis (AP) in rats. AP was induced according to the Lampel and Kern method (1) by the continuous intravenous infusion of cerulein in the doses of $5x10^{-6}$g/kg/h for 12 hours.

There was obtained a statistically significant decrease in serum amylase activity and pancreatic weight in the groups treated with higher doses of nifedypine before infusion of the cerulein compared with rats treated only with cerulein. However, in the group treated with Bay-K-8644 before infusion of cerulein statistically significant increase was obtained in serum amylase activity and pancreatic weight compared with the group treated only with cerulein.

The investigations suggest a beneficial effect of higher doses of nifedypine on cerulein induced AP. The inflammatory changes in the pancreas in the groups treated with nifedypine observed under the light microscope were smaller than in the group treated only with cerulein.

Wpływ nifedypiny (antagonisty kanału wapniowego) oraz Bay-K-8644 (agonisty kanału wapniowego) na rozwój ostrego doświadczalnego zapalenia trzustki

Celem pracy była ocena wpływu nifedypiny (blokera kanału wapniowego) i Bay-K-8644 (agonisty kanału wapniowego) na ostre doświadczalne zapalenie trzustki (OZT), wywołane ceruleiną u szczurów. OZT wywoływano u szczurów metodą Lampela i Kerna przez ciągłą infuzję dożylną ceruleiny w dawce $5x10^{-6}$ g/kg/h przez 12 godzin. Uzyskano statystycznie istotne zmniejszenie aktywności amylazy i masy trzustki w grupach szczurów otrzymujących nifedypinę przed infuzją ceruleiny w porównaniu z grupą szczurów otrzymujących tylko ceruleinę. Natomiast w grupie szczurów otrzymujących Bay-K-8644 przed infuzją ceruleiny uzyskano statystycznie istotny wzrost aktywności amylazy i masy trzustki w porównaniu z grupą zwierząt otrzymujących tylko ceruleinę. Zmiany histopatologiczne stwierdzane w mikroskopie świetlnym w grupach zwierząt otrzymujących nifedypinę były mniejsze w porównaniu z grupą zwierząt otrzymujących ceruleinę. Nasze badania sugerują korzystny wpływ dużych dawek nifedypiny na rozwój OZT wywołanego ceruleiną. Natomiast w grupach zwierząt otrzymujących Bay-K-8644 nastąpiło pogorszenie badanych parametrów.

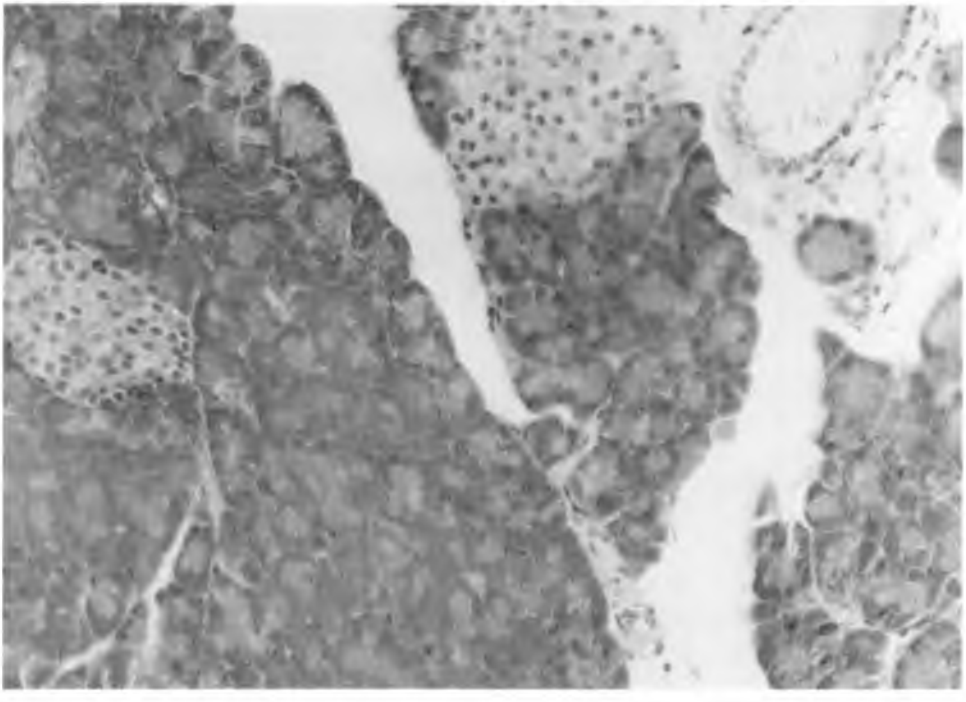

Fig. 1

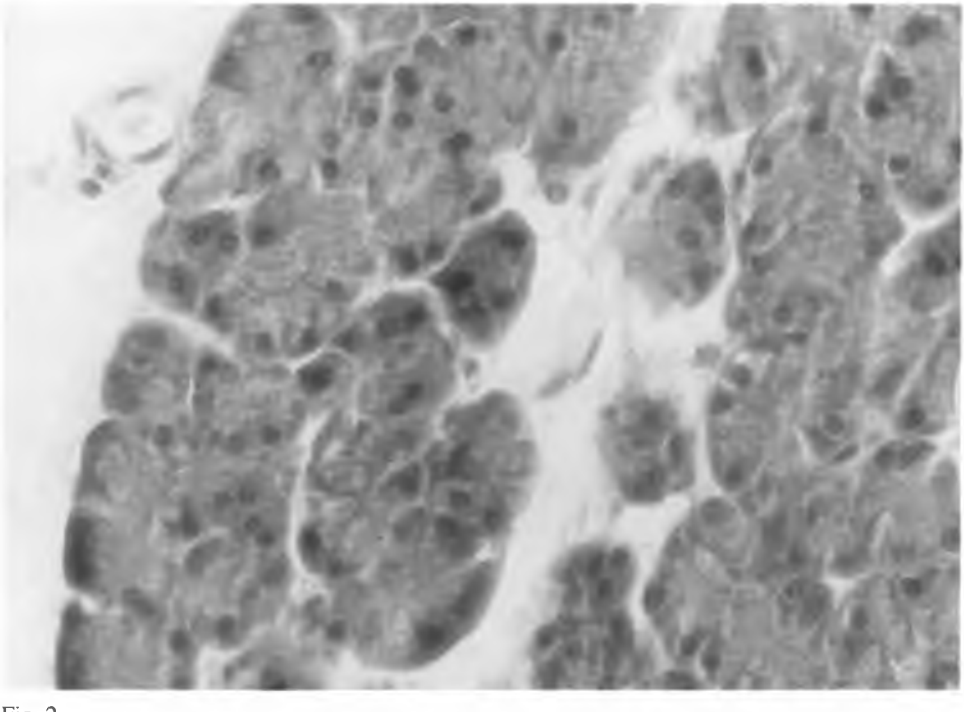

Fig. 2

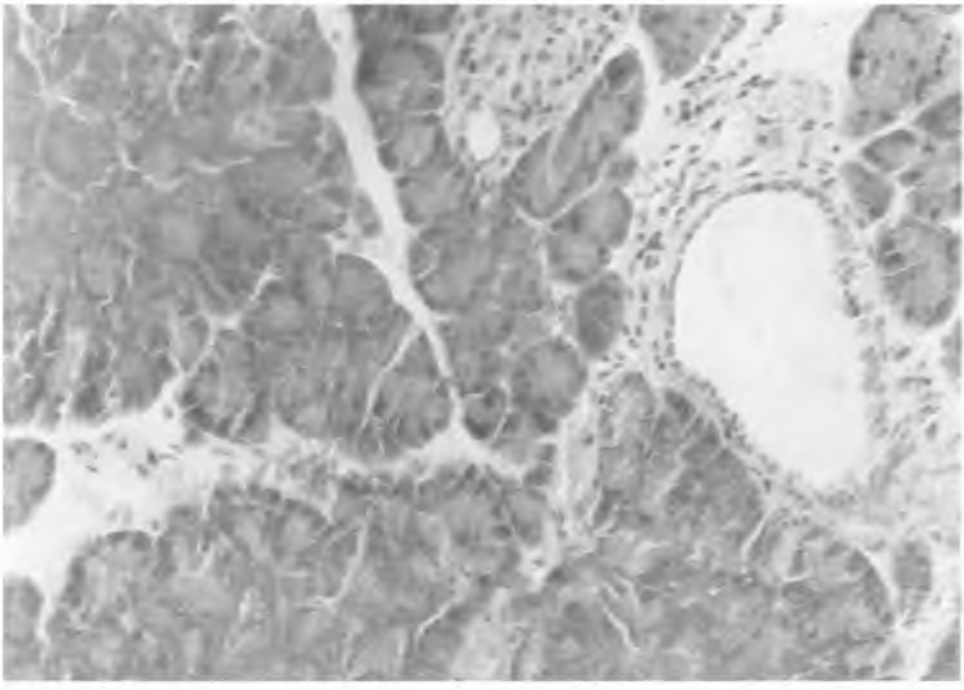

Fig. 3

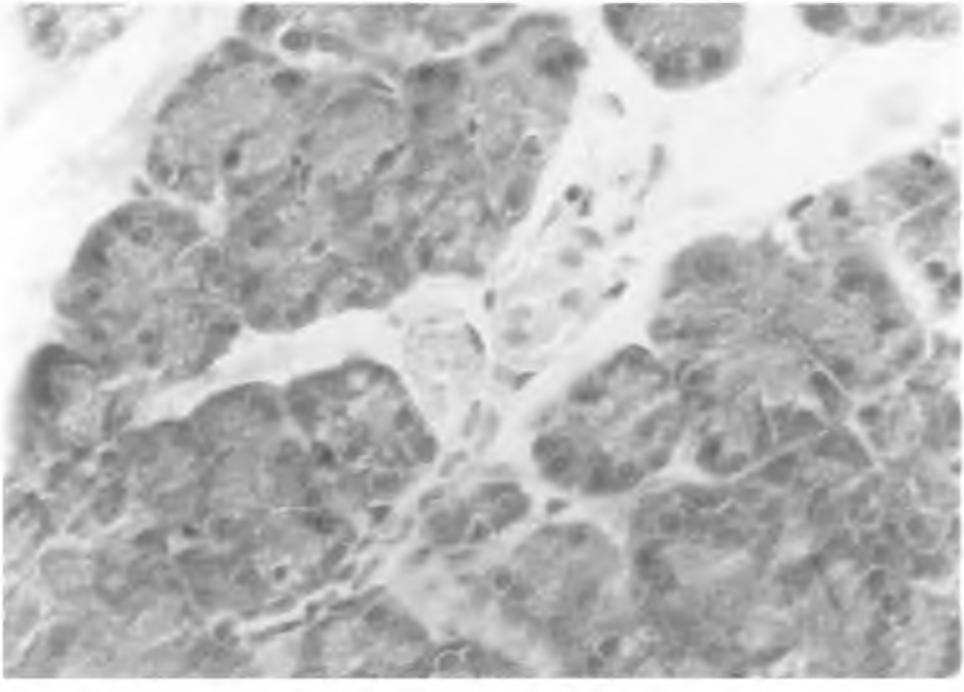

Fig. 4