

Original articles

Z. WARZECHA, A. DEMBIŃSKI, J. JAWOREK, P. CERANOWICZ, A. SZLACHCIC,
J. WALOCHA, S.J. KONTUREK

ROLE OF SENSORY NERVES IN PANCREATIC SECRETION AND CAERULEIN-INDUCED PANCREATITIS

Institute of Physiology, *Collegium Medicum*, Jagiellonian University, Cracow, Poland.

Sensory nerves are implicated in gastroprotection and regulation of visceral circulation but their role in exocrine secretion and pancreatic circulation in intact pancreas and in acute pancreatitis has not been established. We investigated the role of sensory fibers in pancreatic secretion *in vivo* and amylase release from pancreatic slices (containing nerve fibers) or isolated pancreatic acini, and in caerulein-induced pancreatitis. In conscious rats, the stimulation of sensory nerves by low dose of capsaicin given intraduodenally (0.25–0.5 mg/kg) reduced basal pancreatic secretion, whereas dose of 1 mg/kg increased this secretion. Deactivation of sensory nerves by neurotoxic dose of capsaicin (100 mg/kg over 3 days s.c.) 10 days before tests failed to affect basal secretion but diminished the secretion induced by feeding or the diversion of pancreatic juice. In pancreatic slices, capsaicin (10^{-10} – 10^{-6} M) increased enzyme secretion and this response was abolished by atropine (10^{-6} M) or previous deactivation of sensory nerves. In pancreatic acini, capsaicin failed to affect basal and stimulated amylase secretion in response to caerulein or urecholine. In intact rats, stimulatory dose of capsaicin (0.5 mg/kg i.g.) caused about 32% increase of pancreatic blood flow and it was without any effect on the pancreatic DNA synthesis, weight, RNA, DNA and protein content. In contrast, neurotoxic dose of capsaicin caused a reduction (by 27%) in pancreatic blood flow followed by a significant decrease in RNA content and DNA synthesis in pancreatic tissue. Infusion of caerulein (10 g/kg-h) for 5 h produced acute edematous pancreatitis accompanied by over 60% decrease in DNA synthesis, nearly 50% inhibition of pancreatic blood flow, and a significant increase in pancreatic weight, protein content and plasma amylase concentration. Stimulatory dose of capsaicin attenuated the pancreatic tissue damage in caerulein induced pancreatitis, as manifested by a significant reversal of pancreatic blood flow and DNA synthesis decrease. Capsaicin induced inactivation of sensory nerves prior to pancreatitis caused an increase of all parameters of pancreatic damage; pancreatic blood flow dropped by 68%, DNA synthesis decreased by 70%; pancreatic weight, protein content and plasma amylase were also significantly enhanced. We conclude that sensory neurons are involved in the regulation of pancreatic secretion by an indirect mechanism and exhibit a beneficial effect on the pancreatic integrity, mainly due to improving the pancreatic blood flow.

Key words: capsaicin, pancreatic secretion, pancreatic blood flow, pancreatitis, DNA, RNA.

INTRODUCTION

During the last decade, an increasing interest has been focused on sensory innervation in the gastrointestinal tract (1, 2) and a large amount of informations has been accumulated which indicate that sensory neurons play an important role in the stomach (3, 4) and duodenum (5, 6). Capsaicin represents a selective probe for the stimulation or ablation of the primary afferent neurons (1, 7). Stimulation of sensory neurons by intragastric administration of low dose of capsaicin was found to induce protection against the damage caused by ethanol and aspirin (3, 4), whereas the ablation of capsaicin-sensitive neurons led to an aggravation of gastric mucosal lesions (7—9) and prolonged the healing of these lesions (10) induced by a variety of ulcerogenes. These effects have been attributed, at least in part, to circulatory effects of capsaicin (2, 10, 11).

In the pancreas, vascular mechanism has been shown to play an important role in exocrine pancreatic secretion (12, 13) and seems to be responsible, at least in part, for pathogenesis of acute pancreatitis in animals (14, 15) and in humans (16, 17). Disturbance of microcirculation induces an acinar cell hypoxia with subcellular damage followed by the release and activation of lysosomal and secretory enzymes. It can lead to autodigestion and induction or aggravation of pancreatitis (18).

Capsaicin-sensitive sensory nerves are distributed to pancreatic islets, acini, ducts and vasculature (19). Previous study has shown that stimulation of these sensory nerves can increase an exocrine pancreatic secretion whereas their deactivation abolished the pancreatic response to intraduodenal capsaicin (20). However, the role of sensory nerves in the pancreas has not been sufficiently investigated.

In this study, using stimulatory and ablatory dose of capsaicin, we attempted to determine the influence of sensory nerves on pancreatic exocrine secretion and caerulein induced pancreatitis.

MATERIALS AND METHODS

Male Wistar rats, weighing 250—300 g, were used to examine the pancreatic secretion *in vivo* and *in vitro*. Studies with caerulein-induced pancreatitis were performed on male Wistar rats weighing 160—190g.

Ablation of afferent sensory nerves

Sensory deactivation was induced by pretreatment with capsaicin (Fluka, Buchs, Switzerland) in a total dose of 100 mg/kg, which was given in six injections [2.5 + 10 + 12.5 + 25 + 25 + 25 mg/kg subcutaneously (s.c.)] over 3 consecutive days. Two injections of capsaicin per day were given to rats under ether anesthesia, and a recovery period of 10 days was allowed before further

experiments. To assess the effectiveness of sensory denervation, the day before the pancreatitis induction or secretory studies a drop of 0.33 mM capsaicin solution was installed into rat eye. Capsaicin-pretreated animals showing any wiping movements were excluded from study.

Examination of pancreatic secretion in vivo

Secretory studies *in vivo* were performed in rats with chronic pancreatic fistula prepared as modification of technique described by Ormai *et al.* (21). Briefly, one polyethylene cannula was inserted into the common bile-pancreatic duct to serve as the pancreatic fistula. This cannula was connected to another polyethylene cannula placed in the duodenum to permit the circulation of the pancreatic juice into the duodenum. Both cannulas were brought to exterior and protected by a steel thimble allowing for the free movement of rats in their usual cages. During the experiment rats were placed in modified Bollman-type cages to maintain the minimum restraint necessary. The steel thimble was removed, the cannulas were disconnected, the pancreatic cannula was used to collect the pancreatic juice, and the duodenal cannula was employed for reinfusion of the juice (after dilution with saline 1:2) into the duodenum. The secretory studies usually started after six to eight days of recovery from the surgery and after 12 hours of food but not water deprivation. The pancreatic secretion was collected from pancreatic fistula in small preweighed vials in 15 min aliquots to measure the volume, protein and amylase outputs.

Several series of tests, each performed on 6—8 animals with chronic pancreatic fistula were carried out, including:

1. Tests with pancreatic juice returned to the duodenum throughout the experiment to determine basal pancreatic secretion in rats with intact or capsaicin deactivated sensory nerves.
2. Tests with pancreatic juice returned to the duodenum and intraduodenal capsaicin in low doses (0.25—1.0 g/kg) to stimulate of afferent sensory nerves.
3. Tests with the pancreatic juice returned to the duodenum and feeding of liquid mixed meal (6 g of white bread mixed in proportion 1:1 with milk) to examine the postprandial secretion in rats with intact or capsaicin deactivated sensory nerves.
4. Tests with the diversion of pancreatic juice to the exterior (DPJ) throughout the experiment to examine the postdivertional pancreatic secretion in rats with intact or capsaicin deactivated sensory nerves.

Pancreatic protein secretion in the study *in vivo* was measured by the method of Lowry (22) with bovine plasma albumin as a standard and expressed as mg per 15 min.

Examination of the pancreatic secretion in vitro

Isolated pancreatic acini and mechanically isolated pancreatic slices (containing fragments of nerves and vessels) were prepared according to the method of Amsterdam *et al.* (23). Pancreatic acini were obtained by digestion of pancreata by highly purified collagenase (CLSPA 600 U/ml — Worthington Biochemical Co., Freehold, U.S.A.). Pancreatic slices were obtained by mechanical separation of pancreatic fragments. Dispersed pancreatic acini and pancreatic slices were suspended in fresh incubation medium (containing 1% of essential and nonessential amino acid mixture, 1% of serum albumin and 0.01% of trypsin inhibitor), saturated with oxygen and incubated for 30 min at 37°C in shaking bath in the presence of tested agents. Following series of experiments were carried out using dispersed acini or pancreatic slices obtained from rats with intact or capsaicin deactivated sensory nerves:

1. Study of basal amylase secretion by incubation of dispersed acini or pancreatic slices in the medium alone.
2. Study of amylase secretion from pancreatic acini stimulated by caerulein (10^{-9} — 10^{-7} M) or urecholine (10^{-7} — 10^{-3} M)

3. Tests with pancreatic acini incubated in the presence of capsaicin (10^{-7} — 10^{-4} M) alone or in the combination with a constant dose of urecholine (10^{-5} M) or caerulein (10^{-12} M).
4. Tests with pancreatic slices incubated in the presence of capsaicin (10^{-7} — 10^{-4} M) alone or in the combination with a constant concentration of atropine (10^{-6} M).

After incubation, the suspensions of pancreatic acini or pancreatic slices were centrifuged and the supernatants were separated. The amylase content in the supernatants, pancreatic acini or pancreatic slices was determined as described by Bernfeld (24) and an amylase release was expressed as percent of total amylase.

Study with caerulein-induced pancreatitis

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

Several series of experiments were carried out including: (1) control (0.9% NaCl s.c.); (2) capsaicin 2×0.25 mg/kg given intragastrically (i.g.) (to stimulate the sensory nerves); (3) capsaicin 100 mg/kg s.c. to deactivate sensory nerves; (4) caerulein induced pancreatitis; (5) caerulein induced pancreatitis plus stimulatory dose of capsaicin (0.5 mg/kg of capsaicin given i.g. in divided two equal doses: first 30 min prior to caerulein infusion, second 3 h later); (6) capsaicin in sensory nerves ablatory dose (100 mg/kg) followed by caerulein to induce pancreatitis.

Determination of pancreatic blood flow

After infusion of caerulein for 5 h, the animals were anesthetized with ether, weighed and the abdominal cavity was opened. The pancreatic blood flow was measured by the laser Doppler flowmeter technique, using Laserflow model BPM 403A (Blood Perfusion Monitor, Vasamedics Inc., St. Paul, MN, U.S.A.). 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 is about 3 mm, the technique used determined pancreatic blood flow per 100 g tissue per minute. The pancreatic blood flow was recorded in the pancreatic tissue as displayed on the digital panel meter and presented as percent change from control value obtained in rats infused with saline.

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 by an enzymatic method (Amylase reagent, Dialab Diagnostic Ges. MBH, Wien, Austria). The values were expressed as units/litre (U/L).

Determination of DNA synthesis and RNA, DNA, protein content

After blood withdrawn 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 incubation of the tissue at 37°C for 45 min in 2 ml of medium containing $8 \mu\text{Ci/ml}$ of [^3H]thymidine ([^3H]-thymidine, 20—30 Ci/mmol, Institute for Research, Production and Application of Radioisotopes, Prague, Bohemia). The reaction was stopped with 0.4 N perchloric acid containing carrier thymidine (5 mM). Tissue samples were centrifuged and the precipitate 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 (25). DNA in the residual pellets was

solubilized 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 (26). The final pellet was solubilized in 1 M NaOH and its protein content was determined by the method of Lowry (22). The incorporation of [³H]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 per total pancreas weight. DNA synthesis was expressed as disintegrations per minute (dpm) [³H]thymidine per microgram 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 (\pm S.E.M.).

RESULTS

Pancreatic secretion *in vivo*

In conscious rats with chronic pancreatic fistula, the basal pancreatic protein secretion was well sustained at a level of about 12.5 ± 0.8 mg/15 min (Fig. 1). Capsaicin given intraduodenally at a dose 0.25 and 0.5 mg/kg tended to reduced basal pancreatic secretion by about 7% and 17%, respectively,

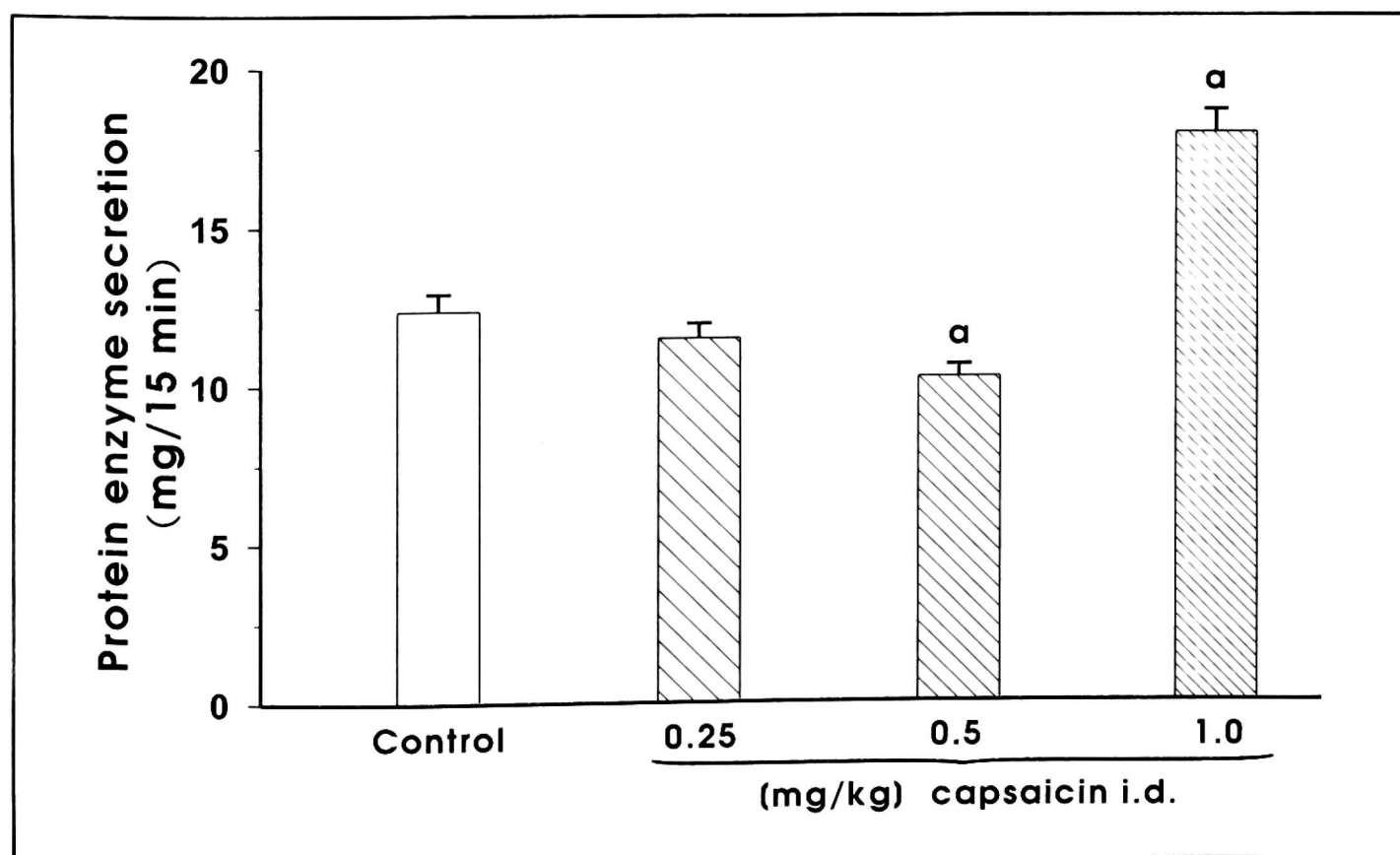


Fig. 1. Pancreatic protein enzyme secretion from the chronic pancreatic fistula under basal condition in conscious rats in tests with increasing doses of capsaicin given intraduodenally.

Mean \pm S.E.M. of 6 separate tests on 4–6 rats. $P < 0.05$ compared with control value.

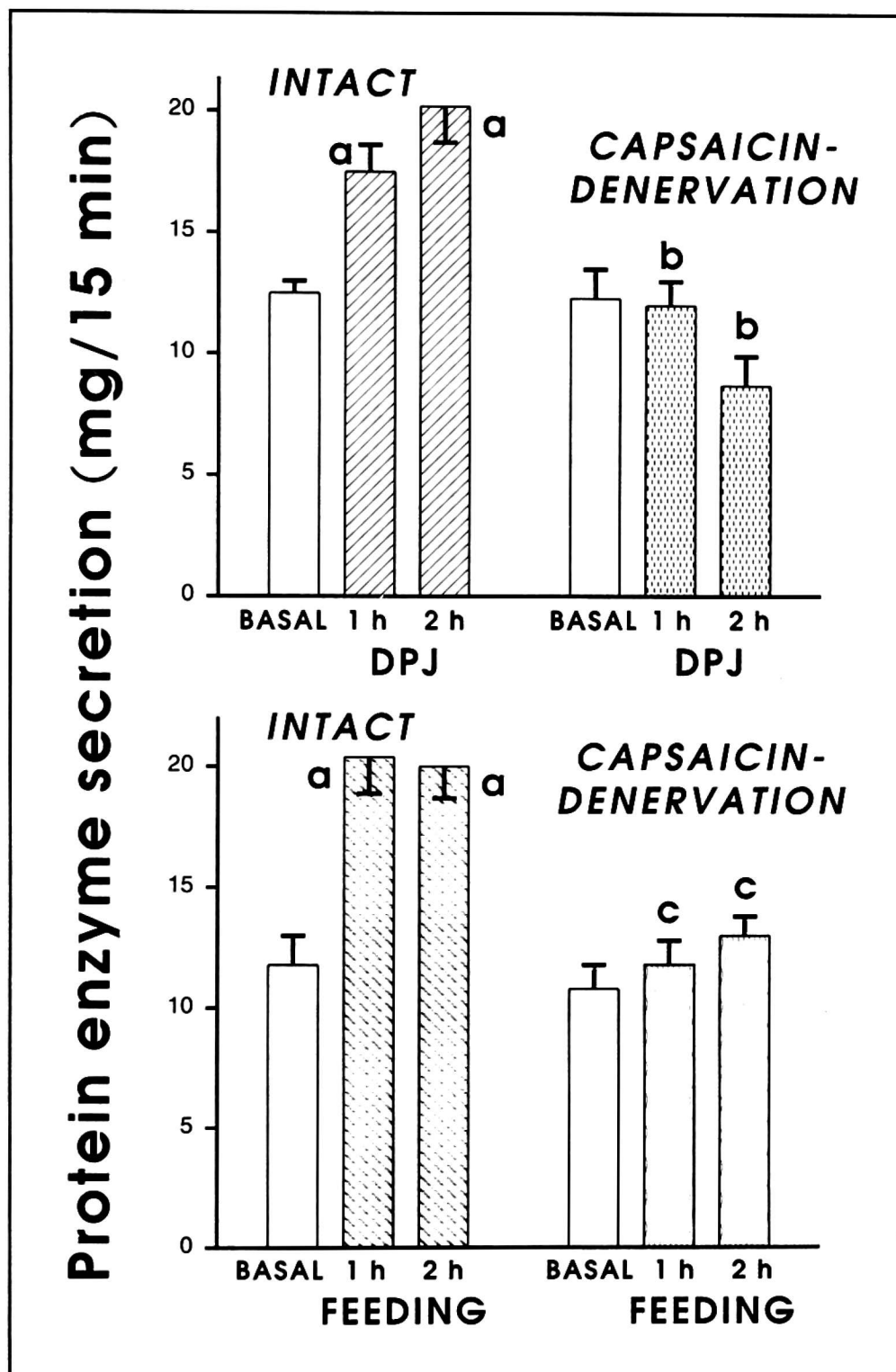


Fig. 2. Pancreatic protein enzyme secretion in response to diversion of pancreatic juice (DPJ) to the exterior or feeding in conscious rats with intact or capsaicin-deactivated sensory nerves. Mean \pm S.E.M. of 6 separate tests on 4–6 rats. ^aP < 0.05 compared with basal value in rats with intact innervation. ^bP < 0.05 compared with value in response to DPJ in intact rats at the same time of observation. ^cP < 0.05 compared with value in response to feeding in intact rats at the same time of observation.

whereas intraduodenal capsaicin at a dose 1.0 mg/kg significantly augmented basal protein output up to 145% of control value (Fig. 1). Deactivation of afferent sensory nerves was without any significant effect on pancreatic basal protein secretion (Fig. 2) but abolished effects of intraduodenal capsaicin on this secretion (data not shown).

Following diversion of pancreatic juice to the exterior protein enzyme secretion in conscious rats with intact sensory nerves reached 140% of basal value within first hour and 161% within second hour (Fig. 2). In rats with intact sensory nerves, feeding of mixed meal increased the basal protein enzyme secretion within the first and second hour to 173% and 170% respectively. Deactivation of afferent sensory nerves completely abolished stimulatory effect

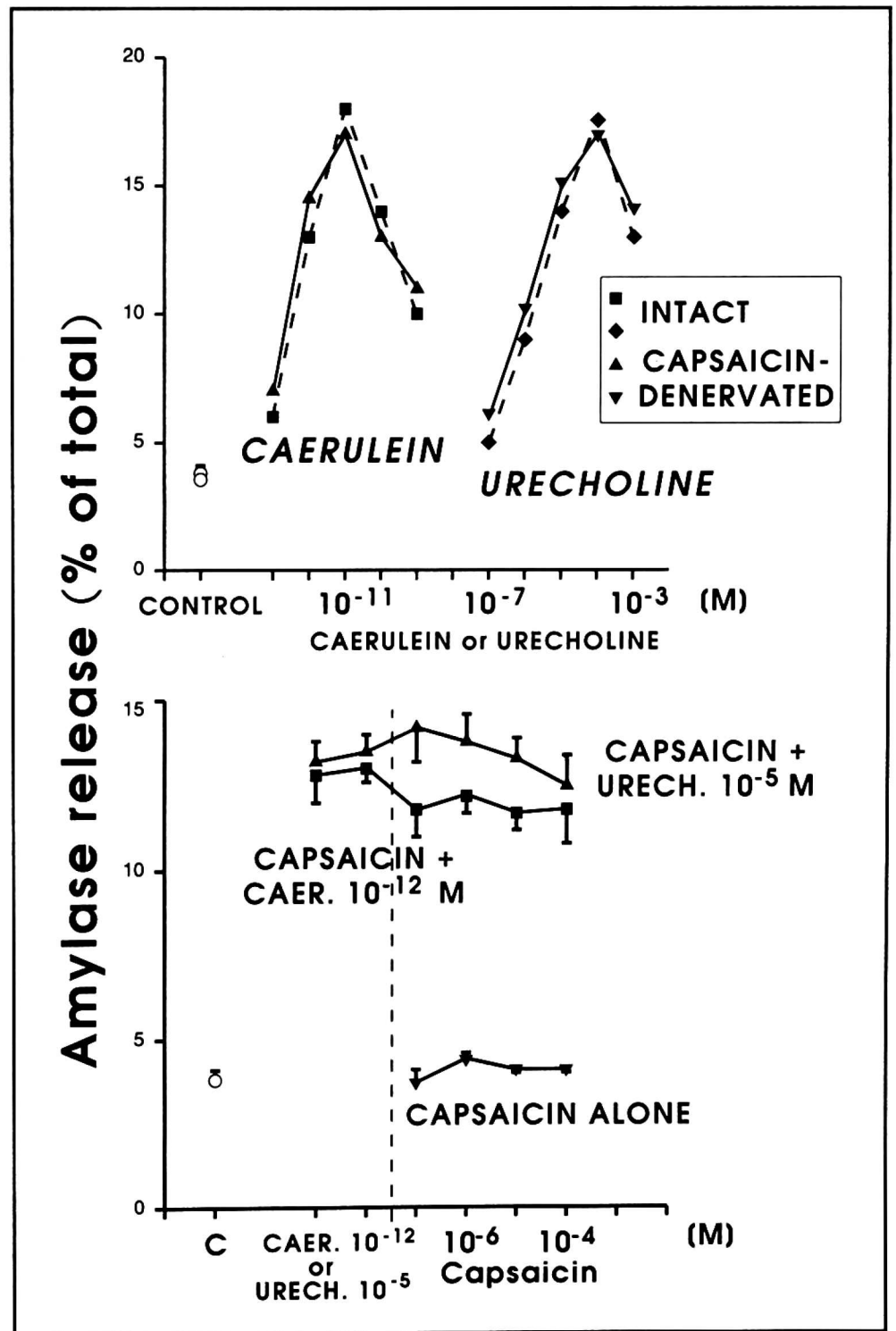


Fig. 3. Amylase release from resting and stimulated by caerulein, urecholine or capsaicin pancreatic acini obtained from intact or capsaicin denervated rats. Mean \pm S.E.M. of 6–8 experiments.

of diversion of pancreatic juice to exterior and strongly reduced secretory response to feeding (Fig. 2).

Pancreatic secretion in vitro

Dispersed pancreatic acini obtained from rats with intact sensory nerves showed spontaneous amylase release under basal condition (control) reaching $3.6 \pm 0.5\%$ of total amylase content (Fig. 3). Addition of caerulein in gradually increased concentration 10^{-13} – 10^{-9} M or urecholine in concentration 10^{-7} – 10^{-3} M produced a dose dependent increase of amylase secretion. The maximal secretory effect occurred at 10^{-11} M of caerulein or 10^{-4} M of urecholine. Deactivation of sensory nerves did not change the secretory response of

pancreatic acini to caerulein or urecholine (*Fig. 3*, top). Incubation of isolated pancreatic acini in the presence of increasing concentration of capsaicin (10^{-9} — 10^{-4}) did not affect significantly amylase release under basal and stimulated conditions (*Fig. 3*, bottom).

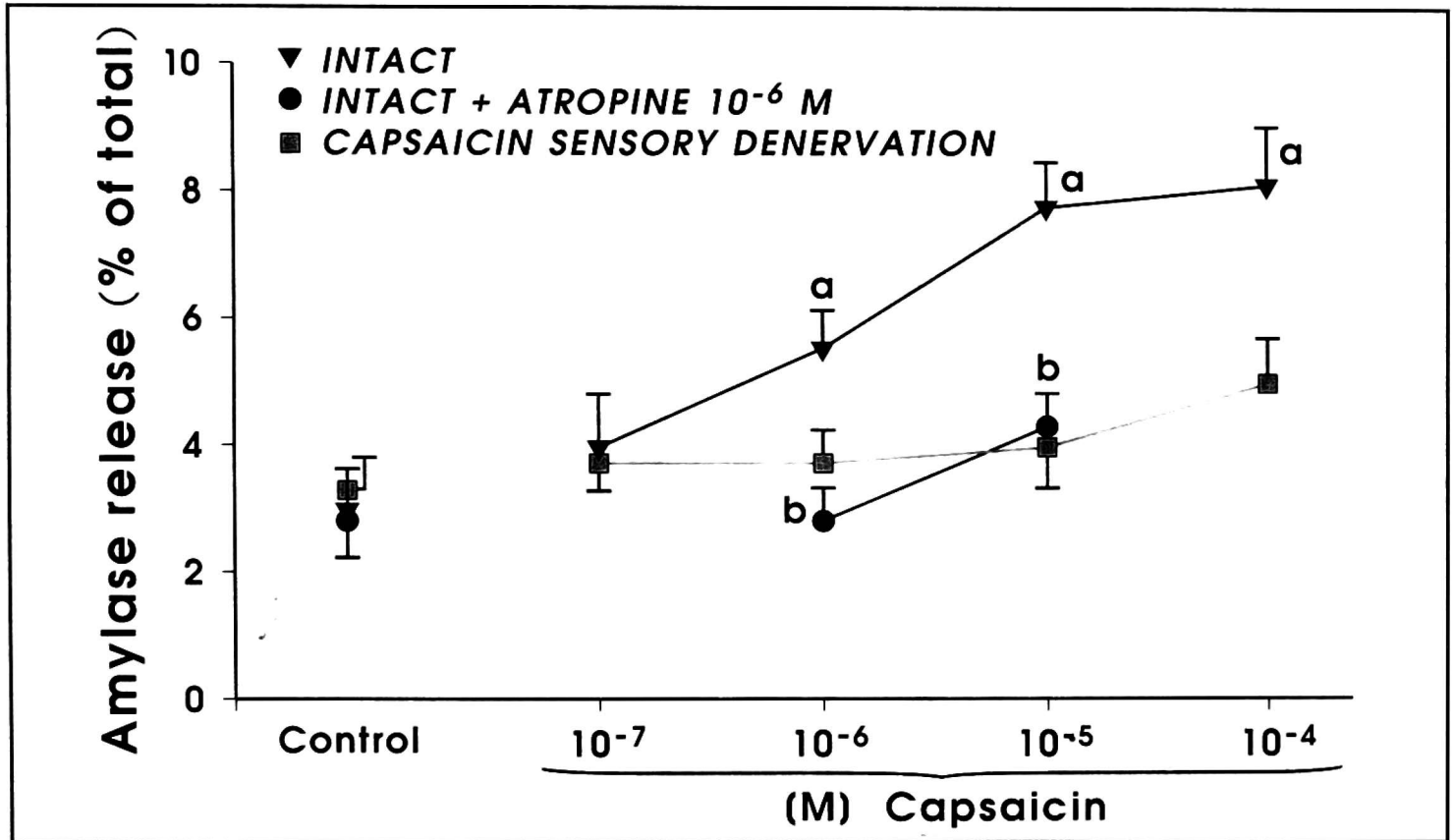


Fig. 4. Amylase release from mechanically isolated slices in response to increasing concentration of capsaicin in intact and capsaicin denervated pancreas. Mean \pm S.E.M. of 6—8 experiments. ^a $P < 0.05$ compared with unstimulated control value. ^b $P < 0.05$ compared with value without atropine.

Basal secretion of amylase from pancreatic slices averaged $3.0 \pm 0.7\%$ of total amylase content (*Fig. 4*). Addition of capsaicin (10^{-7} — 10^{-4} M) into the incubation medium with pancreatic slices caused dose dependent increase of amylase release. The maximum capsaicin-induced amylase secretion was achieved at 10^{-4} M reaching $8.4 \pm 1.3\%$ of total amylase release. This capsaicin-induced increase of amylase secretion from pancreatic slices was abolished by 10^{-6} M of atropine. Deactivation of sensory nerves in rats from which the pancreatic slices were prepared completely eliminated stimulatory effect of capsaicin.

Biochemical and circulatory alterations in caerulein-induced pancreatitis

Subcutaneous infusion of caerulein at a dose $10 \mu\text{g} \times \text{kg}^{-1} \times \text{h}^{-1}$ for 5 h consistently produced edematous pancreatitis in all tested rats. Pancreas was grossly swollen and enlarged with visible collection of edematous fluid. The weight of pancreas was almost doubled (*Table 1*).

Table 1. Effect of saline (control), stimulatory doses of capsaicin (0.5 mg/kg) and ablatory doses of capsaicin (100 mg/kg) given alone or in combination with caerulein (10 µg/kg/h for 5 h) on pancreatic weight, DNA synthesis, and protein, RNA, DNA contents.

GROUP	PANCREATIC WEIGHT (mg)	DNA SYNTHESIS (dpm/µg DNA)	PROTEIN CONTENT (mg)	RNA CONTENT (mg)	DNA CONTENT (mg)
Control	730 ± 48	68.3 ± 2.9	150.0 ± 4.7	7.8 ± 0.4	2.81 ± 0.21
Capsaicin 0.5 mg/kg	754 ± 34	72.2 ± 3.7	152.7 ± 7.4	7.9 ± 0.5	2.82 ± 0.11
Capsaicin 100 mg/kg	728 ± 25	48.0 ± 2.0 ^a	146.3 ± 7.3	5.3 ± 0.5 ^a	2.77 ± 0.12
Caerulein 10 µg/kg/h	1392 ± 91 ^a	28.8 ± 7.3 ^a	200.0 ± 7.3 ^a	6.8 ± 0.4	2.78 ± 0.38
Caerulein + Capsaicin 0.5 mg/kg	1153 ± 54 ^{a,b}	42.0 ± 3.6 ^{a,b}	173.1 ± 6.7 ^a	7.4 ± 0.4	2.85 ± 0.2
Caerulein + Capsaicin 100 mg/kg	1637 ± 70 ^a	18.3 ± 1.8 ^{a,b}	205.0 ± 6.0 ^a	4.9 ± 0.4 ^a	2.83 ± 0.17

Mean ± S.E.M. of 8—10 rats. ^aP < 0.05 compared with control value. ^bP < 0.05 compared with value obtained in test with caerulein alone.

DNA synthesis was deeply decreased to 42% of control value whereas total RNA and DNA contents were not changed. The pancreatic protein content increased by about 33% (Table 1) and plasma amylase concentration was almost tripled (Fig. 5). Pancreatic blood flow measured at the end of a five hour infusion of caerulein was reduced nearly 48% as compared to the rats infused s.c. with saline for the same period of time (Fig. 6).

Stimulatory dose of capsaicin (0.5 mg/kg) given alone was without any significant effect on the pancreatic weight, DNA synthesis and total RNA, DNA and protein content (Table 1). The plasma amylase concentrations was not changed, and pancreatic blood flow was increased by 33% (Fig. 6).

Pretreatment with ablatory dose of capsaicin given alone caused significant reduction in DNA synthesis by 30%, RNA content by about 30% (Table 1) and pancreatic blood flow by about 27%. Other measured biochemical parameters of pancreatic integrity such as DNA and protein contents and plasma amylase concentration were not significantly affected.

Addition of stimulatory dose of capsaicin to caerulein infusion significantly reduced the severity of pancreatitis. The increase in pancreatic weight was less

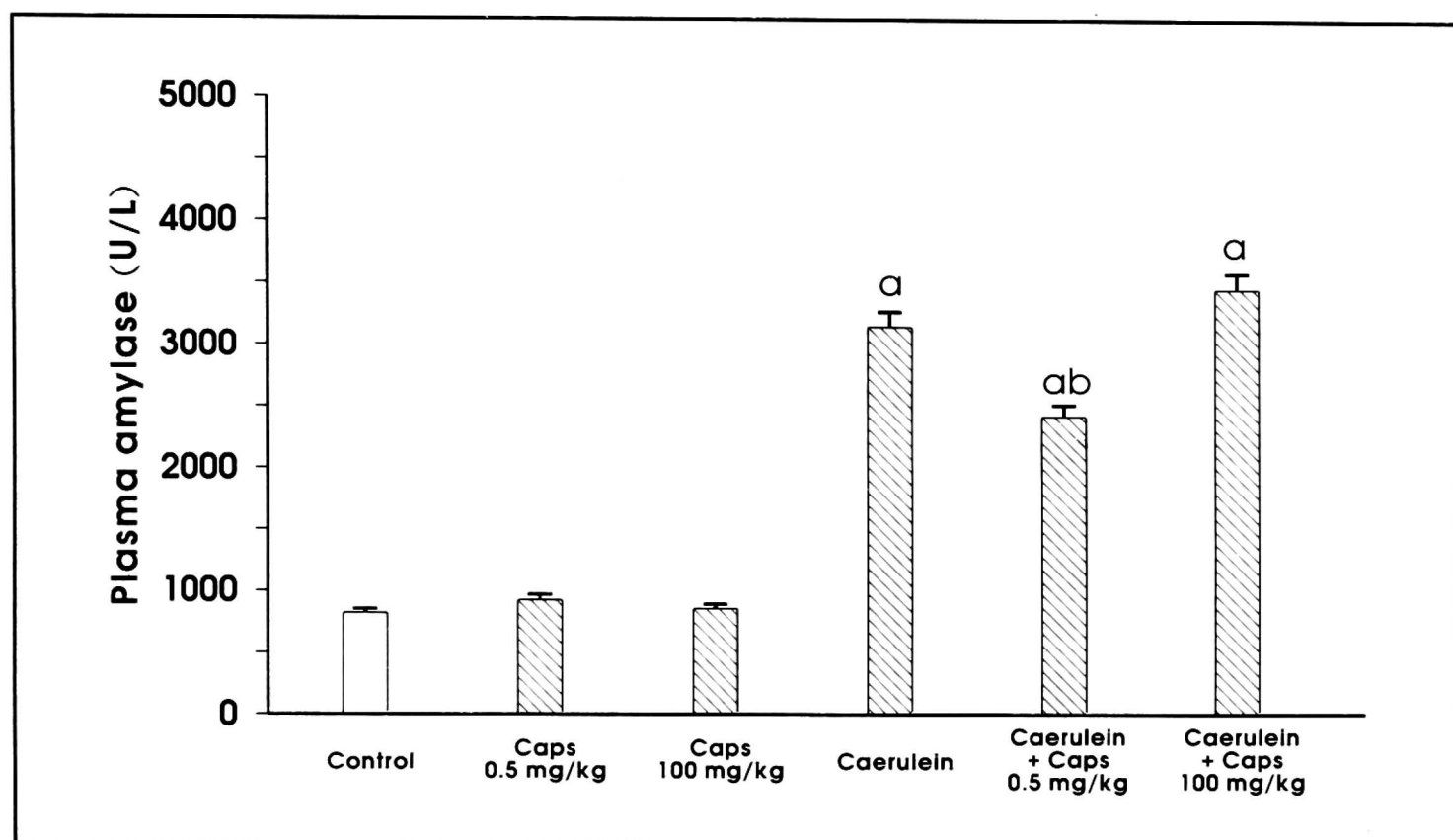


Fig. 5. Effect of stimulatory or neurotoxic doses of capsaicin given alone or in combination with caerulein on plasma amylase concentration. Mean \pm S.E.M. of 8–10 rats. ^aP < 0.05 compared with control value. ^bP < 0.05 compared with caerulein alone.

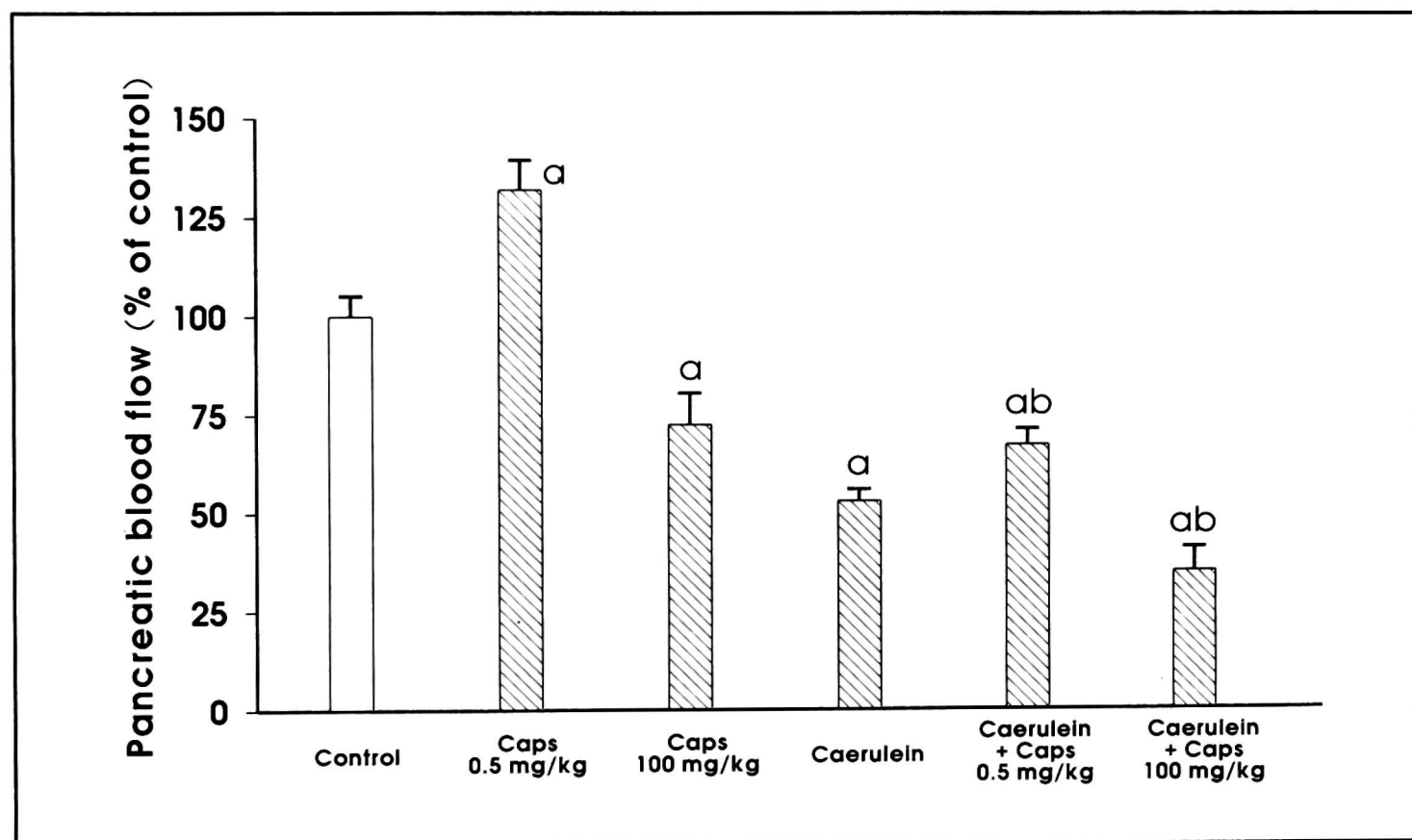


Fig. 6. Effect of stimulatory or neurotoxic doses of capsaicin given alone or in combination with caerulein on pancreatic blood flow. Mean \pm S.E.M. of 8–10 rats. ^aP < 0.05 compared with control value. ^bP < 0.05 compared with caerulein alone.

pronounced ($158 \pm 7\%$ vs $191 \pm 12\%$ of control value), DNA synthesis was higher (42.0 ± 3.6 dpm/ μ g DNA vs 28.8 ± 3.6 dpm/ μ g DNA) and an increase in protein content was smaller then compared to caerulein infusion alone ($115 \pm 4\%$ vs $133 \pm 5\%$). Moreover, the increase in plasma amylase concentration and the fall in pancreatic blood flow caused by caerulein were partially reversed by stimulatory dose of capsaicin.

Pretreatment with ablatory doses of capsaicin prior to the administration of caerulein-induced pancreatitis significantly augmented the reduction in DNA synthesis and pancreatic blood flow caused by caerulein. Plasma amylase concentration and pancreatic protein content were increased, but these effects were not statistically significant.

DISCUSSION

The role of afferent sensory nerves in pancreatic secretion was assessed in this study using two experimental models, one *in vivo* and another *in vitro*, either in intact or in capsaicin-denervated rats. Capsaicin, the pungent ingredient found in red pepper (7), represents a selective tool for the stimulation or ablation of unmyelinated primary afferent sensory nerves (1, 7). Low doses of capsaicin are able to stimulate the activity of these nerves, whereas high, systemic doses of capsaicin produce a long lasting depolarization and functional deactivation of sensory nerves (27).

In our study, in rats equipped with chronic pancreatic fistulas the intraduodenal administration of capsaicin at dose 0.25 and 0.5 mg/kg inhibited basal pancreatic secretion, whereas at higher dose (1.0 mg/kg) it increased this secretion. This biphasic effects of intraduodenal capsaicin on basal pancreatic secretion can be explained by heterogeneity of afferent sensory fibers or different activity of these fibers according to the grade of stimulation. It has been demonstrated previously, that low dose of capsaicin leads to the release of neuropeptides such as calcitonin gene-related peptide (CGRP) or substance P (SP) from afferent sensory fibers (28, 29). The exogenous CGRP given intracerebrally or peripherally inhibited exocrine pancreatic secretion (30) and Debas *et al.* (31) suggested that the inhibition of pancreatic secretion by CGRP is neurally mediated and depends on release of somatostatin. On the other hand, an increase of basal pancreatic secretion induced in our study by duodenal administration of capsaicin at a dose of 1.0 mg/kg can be explained by findings of Li and Owyang (32). They showed, that a physiological increase of CCK plasma level acts *via* stimulation of afferent vagal pathway to mediate the stimulation of pancreatic enzyme secretion. In that case it is possible that capsaicin at dose 1 mg/kg works by activation of sensory nerves.

The effects of intraduodenal capsaicin are partly in disagreement with data of Gicquel *et al.* (20), who found, that intraduodenal capsaicin stimulates the basal pancreatic secretion in doses above and also below 1 mg/kg. This discrepancy is probably caused by different models of experiment. Gicquel *et al.* used rats under general anesthesia with acute pancreatic fistula, whereas we used conscious rats with chronic fistulas. Basal pancreatic protein secretion in conscious rats is much higher than in anaesthetized animals (21). Probably, the low basal secretion and short time period between preparation of acute fistula and secretory study unabled Gicquel *et al.* to observe that the low dose of capsaicin can inhibit basal pancreatic secretion.

Deactivation of sensory nerves by high subcutaneous dose of capsaicin did not alter basal pancreatic secretion in our study and this is consistent with the observation of Gicquel *et al.* (20) who concluded that basal pancreatic secretion is not related to tonic activity of sensory nerves. Capsaicin-induced functional deactivation of sensory nerves abolished also the pancreatic response to intraduodenal capsaicin, providing an evidence that intraduodenal capsaicin acts by the activation of afferent sensory nerves and this effect disappears after the deactivation of these nerves by high dose of systemic capsaicin.

Pancreatic secretory response to feeding or to diversion of pancreatic juice was completely eliminated by deactivation of sensory nerves. This result provided an evidence that activation of pancreatic secretion by food or diversion of pancreatic juice involves afferent sensory capsaicin-sensitive nerves. These findings confirm the observation of Li and Owyang (32) who demonstrated that perivagal application of capsaicin abolished stimulatory effects of food administration or diversion of pancreatic juice on pancreatic enzyme secretion.

In pancreatic acini, the capsaicin did not affect enzyme secretion in basal condition and in response to pancreatic secretagogues. The same effect was observed when pancreatic acini were obtained either from intact or capsaicin-denervated rats. These results provide convincing evidence that effect of capsaicin on pancreatic secretion is indirect, neurally mediated, and involves sensory nerves. In pancreatic slides (containing fragments of extrinsic nerves, vessels and intrinsic nerves), the administration of capsaicin stimulated pancreatic enzyme secretion. This secretory response to capsaicin was abolished by previous deactivation of sensory nerves or addition of atropine. This observation leads us to speculation that secretory effect of capsaicin can also depend on activity of intrinsic pancreatic nerves and this action involves a stimulation of muscarinic receptors. It is supported by previous findings in dogs (33) and rats (34, 35) that the pancreas contains extensive intrinsic nerves, which, with the exception of substance P- and NPY-nerves, are independent of the integrity of the extrinsic nerves. Moreover, Niebel *et al.* (36) and Singer *et al.* (37) found in dogs that atropine still suppresses the basal pancreatic

secretion after truncal vagotomy, and after truncal vagotomy plus celiac and superior mesenteric ganglionectomy. It allowed them to conclude that the basal cholinergic tone of pancreas is not solely dependent on the integrity of the vagus and splanchnic nerves but also intrinsic pancreatic nerves.

In our present study the stimulation of afferent sensory fibers by capsaicin under normal conditions increased the pancreatic blood flow but did not affect significantly the DNA synthesis, DNA, RNA, protein contents, and pancreatic weight. In rats with the ablation of afferent sensory nerves, the pancreatic weight, DNA content and plasma amylase concentration were not altered but we found a significant inhibition of DNA synthesis (which is an index of cell proliferation) and a decrease in RNA content. These effects can be explained by a prolonged reduction of pancreatic blood flow and a decrease in stimulation of the pancreas due to sensory denervation.

According to our previous study (38) the pancreatic blood flow, measured by laser Doppler flowmetry at the end of caerulein infusion in caerulein induced pancreatitis, was dramatically reduced to about half of the normal flow in the intact pancreas and this alteration preceded the drop in DNA synthesis by 58%. The changes in pancreatic blood flow closely correlated with the increase in the pancreatic weight and protein content. Caerulein infusion almost doubled the pancreatic weight and increased the pancreatic protein content by 33%, whereas plasma amylase concentration, which is one of the most accepted markers of the pancreatic tissue damage, was almost tripled. The increase of pancreatic weight and protein content after induction of pancreatitis seems to be due to the edema of pancreatic tissue and the leak of fluids and plasma proteins from blood vessels to pancreatic interstitial tissue.

The major finding of present study is the observation that capsaicin sensitive nerves affect the course of caerulein-induced pancreatitis. The stimulation of afferent sensory neurons by low dose of capsaicin attenuated the severity of caerulein-induced pancreatitis as evidenced by the improvement of the biochemical parameters of pancreatic conditions: the increase in pancreatic weight, pancreatic protein content and plasma amylase concentration was less pronounced, whereas a pancreatic blood flow and DNA synthesis reached higher value than in group with caerulein infusion alone. Deactivation of afferent sensory neurons by high, systemic dose of capsaicin aggravated the caerulein induced pancreatic damage, and this was manifested by additional reduction in DNA synthesis and pancreatic blood flow.

The effects of stimulation or deactivation of sensory nerves on caerulein-induced pancreatitis seem to depend predominantly on changes of pancreatic blood flow. These findings confirm and extend previous reports of various investigators which indicated the important role of vascular mechanism in pathogenesis of acute pancreatitis (14—17). The observed improvement of pancreatic blood flow after stimulation of sensory nerves could

reduce the hypoxia in pancreatic tissue and limit tissue damage caused by disturbance of local circulation. As was suggested by Waldner (18) the cell hypoxia with subcellular damage is followed by intrapancreatic release and activation of lysosomal and secretory enzymes, leading to pancreatic autodigestion and induction or aggravation of pancreatitis.

In summary, the results of present investigation demonstrate that sensory neurons play an important role in the regulation of pancreatic secretion by an indirect mechanism and are involved in the protection of pancreas against damage, mainly by enhancement of nutritive blood supply to the pancreas and an increase of pancreatic cell proliferation.

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Author's address: Z. Warzecha, Institute of Physiology Jagiellonian University School of Medicine, 16 Grzegórzecka str., 31-531 Cracow, Poland