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European Journal of Pharmacology 473 (2003) 207–216
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Ischemic preconditioning reduces the severity of ischemia/reperfusion-induced pancreatitis

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Received 14 November 2002; received in revised form 3 June 2003; accepted 13 June 2003

Abstract

In various organs, including heart, kidneys, brain, liver and stomach, preconditioning by brief exposure to ischemia protects the organ against damage evoked by subsequent severe ischemia. This study has been undertaken to check whether two brief ischemic periods protect the pancreas against severe ischemia/reperfusion-induced pancreatitis and, if so, what is the role of sensory and vagal nerves in this phenomenon. In male Wistar rats, the ischemic preconditioning of the pancreas was performed by clamping of celiac artery (2 × 5 min with 5 min interval). Thirty minutes after preconditioning or sham operation, the ischemia/reperfusion-induced pancreatitis was evoked by clamping of inferior splenic artery for 30 min using microvascular clips, followed by 1 h reperfusion. Sensory nerves ablation was induced 10 days before final experiments by capsaicin. Truncal vagotomy was performed 1 week before the experiment. Exposure to regular 30-min pancreatic ischemia followed by 1 h reperfusion led to the development of acute hemorrhagic pancreatitis. Ischemic preconditioning, applied prior to induction of pancreatitis, caused the reduction in plasma lipase, plasma interleukin-1β and histological signs of pancreatic damage, as well as attenuated the reduction in pancreatic blood flow and DNA synthesis. Ablation of sensory nerves by capsaicin caused an aggravation of ischemia/reperfusion-induced pancreatic damage and attenuated a protective effect of ischemic preconditioning. Noxious effect of sensory nerves ablation on the pancreas was accompanied by the reduction in pancreatic blood flow and an increase in plasma interleukin-1β. Similar but less pronounced deleterious effect on the pancreas was observed after vagotomy. We conclude that: (1) pancreatic ischemic preconditioning reduces the severity of ischemia/reperfusion-induced pancreatitis; (2) this effect seems to be related, at least in part, to the improvement of pancreatic blood flow and the reduction in the release of proinflammatory interleukin-1β; (3) sensory and vagal nerves are involved in protective effect of ischemic preconditioning against pancreatic damage.

Keywords: Pancreatitis; Ischemic preconditioning; Blood flow, pancreatic; Sensory nerve; Vagal nerve, interleukin-1β

1. Introduction

Acute pancreatitis is a pathological process dependent on autodigestion caused by premature activation of zymogens to active enzymes, but a disturbance of pancreatic blood flow is involved in pathophysiology of acute pancreatitis (Waldner, 1992). There is the growing evidence that pancreatic ischemia plays an important role in the initiation of pancreatitis, or the progression to necrotizing pancreatitis (Klar et al., 1990; Gullo et al., 1996; Lonardo et al., 1999; Menger and Vollmar, 1999). Necrosis of pancreatic and peripancreatic tissue is recognized as a key factor in the evolution of the disease from mild to severe pancreatitis. Microvascular perfusion failure is essential for the development of clinical pancreatitis after cardiac (Lonardo et al., 1999) or aortic (Gullo et al., 1996; Sakorafas et al., 1998) surgery, hypovolemic shock (Warshaw and O'Hara, 1978), hypothermia (McLean et al., 1973) and transplantation of the pancreas (Fernandez-Cruz et al., 1993). Also, experimental studies show that ischemia alone may initiate pancreatitis and always aggravates pancreatic damage (Waldner, 1992; Klar et al., 1990; Menger and Vollmar, 1999; Dembiński et al., 2001), whereas vasodilatation and improvement of pancreatic blood flow inhibit the development of acute pancreatitis (Warzecha et al., 1997a,b). Moreover, in acute pancreatitis caused by other, primary nonvascular factors,

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doi:10.1016/S0014-2999(03)01994-0

the early disturbance of pancreatic circulation is observed (Gress et al., 1990; Kusterer et al., 1991). It is well known that disturbance of pancreatic microcirculation leads to formation of thrombi in capillaries, activation of leukocytes, release of proteolytic enzymes, formation of oxygen-derived free radicals and proinflammatory cytokines (Menger and Vollmar, 1999).

Various organs including heart (Murry et al., 1986), brain (Kato et al., 1994), kidney (Turman and Bates, 1997), liver (Kume et al., 1996), skeletal muscle (Mounsey et al., 1992) and stomach (Pajdo et al., 2001) respond to brief exposure to ischemia with an increase in resistance to severe ischemia, and this phenomenon is called ischemic preconditioning. Moreover, previous studies have shown that brief ischemia in organs other than heart is able to protect the heart against ischemic damage (Takaoka et al., 1999; Schoemaker and van Heijningen, 2000). This phenomenon is called as remote preconditioning.

Our present study was designed to determine whether ischemic preconditioning exhibits any protective effect on the pancreas and, if so, to elucidate the contribution of sensory and vagal nerves in this phenomenon.

2. Materials and methods

2.1. Animals and treatment

Studies were performed on 160 male Wistar rats weighing 200–220 g and were conducted following the experimental protocol approved by the Committee for Research and Animal Ethics of Jagiellonian University. Rats were fasted 18 h before final experiment but they had free access to the drinking water.

Experiments were carried out on the following experimental groups (10 animals in each group): (1) sham-operated control rats; (2) rats exposed to ischemic preconditioning; (3) rats with ischemia/reperfusion-induced pancreatitis; (4) rats with ischemic preconditioning prior to ischemia/reperfusion-induced pancreatitis; (5) rats with ablation of sensory nerves and sham-operated; (6) rats with ablation of sensory nerves and exposed to ischemic preconditioning; (7) rats with ablation of sensory nerves and ischemia/reperfusion-induced pancreatitis; (8) rats with ablation of sensory nerves combined with ischemic preconditioning and induction of acute pancreatitis by ischemia followed by reperfusion; (9) rats with vagotomy and sham-operated; (10) rats with vagotomy and exposed to ischemic preconditioning; (11) rats with vagotomy and ischemia/reperfusion-induced pancreatitis; (12) rats with vagotomy combined with ischemic preconditioning and induction of acute pancreatitis by ischemia followed by reperfusion; (13) rats with ablation of sensory nerves combined with vagotomy and sham operation; (14) rats with ablation of sensory nerves and vagotomy, and exposed to ischemic preconditioning; (15) rats with ablation of sensory nerves and vagotomy combined with ischemia/reperfusion-induced pancreatitis; (16) rats with ablation of sensory nerves and vagotomy combined ischemic preconditioning and ischemia/reperfusion-induced pancreatitis.

Sensory nerves ablation was induced by capsaicin (Capsaicin, Sigma, St. Louis, MO, USA) injected subcutaneously at the total dose of 100 mg/kg over three consecutive days as described previously (Dembiński et al., 1996a). Injections were performed under ether anesthesia to prevent the pain reaction and respiratory impairment associated with capsaicin injection. After the last capsaicin injection, a recovery period of 10 days was allowed before the final experiments. To assess the effectiveness of sensory nerves ablation, 1 day before the final experiment, a drop of 0.33 mM solution of capsaicin was installed into the eye of each rat and the presence of wiping movements were examined. All animals pretreated with capsaicin showed negative wiping movement test, thus confirming functional deactivation of capsaicin sensitive nerves.

Truncal vagotomy was performed 1 week before final experiment. A longitudinal laparotomy was made in rats anesthetized with ketamine (50 mg/kg i.p., Bioketan, Biowet, Gorzów, Poland). Vagal trunks were freed along subdiaphragmatic esophagus, ligated and sectioned. Except hydratation with saline not any other treatment was used.

Ischemic preconditioning of pancreata were performed under ketamine anesthesia. After longitudinal laparotomy, the celiac artery was clamped two times for 5 min with 5-min interval. In sham-operated control rats, longitudinal laparotomy and mobilization of pancreas without clamping any arteries was performed.

Acute hemorrhagic pancreatitis was induced by ischemia followed by reperfusion as described previously (Dembiński et al., 2001). Briefly, 30 min after ischemic preconditioning or sham operation, rats were reanesthetized with ketamine and the ischemia of the splenic region of the pancreas was induced by clamping of splenic inferior artery using microvascular clips. Thirty minutes later, microvascular clips were removed to obtain 1 h reperfusion. For reperfusion time, the abdomen was closed by suture.

2.2. Determination of pancreatic blood flow

After 1 h reperfusion or 2 h after sham operation or ischemic preconditioning, the animals were anesthetized again with ketamine and the abdomen was opened. The pancreata were exposed for the measurement of the pancreatic blood flow by laser Doppler flowmeter using Laserflo, model BPM 402 A Blood Perfusion monitor (Vasamedics, St. Paul, MN), as described previously (Konturek et al., 1994). Pancreatic blood flow was measured in five different portions of the pancreas and the area of laser emission of the probe was about 1 mm^2, while the depth of measurement reached about 3 mm. It was recorded and presented as millilitre of blood flow per 100 g of tissue per minute (ml/100 g/min).

2.3. Determination of plasma lipase activity, and plasma interleukin-1β and interleukin-10 concentration

Immediately after measurement of pancreatic blood flow, the abdominal aorta was exposed and blood was taken for determination of plasma lipase and interleukin-1β. Plasma lipase activity was determined with a Kodak Ectachem DT II System analyzer (Eastman Kodak Company, Rochester, NY, USA) using Lipa DT Slides (Vitros DT Chemistry System, Johnson & Johnson Clinical Diagnostic, Rochester, NY, USA). The value of plasma lipase activity was expressed as units/litre (U/l). Plasma interleukin-1β and interleukin-10 were measured in duplicate using appropriate BioSource Cytoscreen rat kits based on a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (BioSource International, Camarillo, CA, USA). Concentration of interleukins was determined from standard curves of recombinant interleukin-1β or interleukin-10, respectively. The values of plasma interleukins concentration were expressed as picogram per millilitre (pg/ml).

2.4. Determination of pancreatic DNA synthesis

After the blood withdrawal, the pancreas was carefully dissected out from its attachment to the stomach, the duodenum and the spleen. Fat and excess tissue was trimmed away. Samples of pancreatic tissue were taken for study of DNA synthesis and morphological examination. The rate of DNA synthesis was determined as described previously (Warzecha et al., 1999). Briefly, the minced pancreatic tissue was incubated 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, Czech Republic). DNA content concentration was determined by Giles and Myers (1965) procedure. The incorporation of [^{3}H]thymidine into DNA was measured by counting DNA-containing solution in a liquid scintillation system. DNA synthesis was expressed as [^{3}H]thymidine disintegrations per minute per microgram DNA (dpm/μg DNA).

2.5. Histological examination

Samples of pancreatic tissue were excised, fixed in 10% formalin, embedded in paraffin and sections were stained with hematoxylin and eosin. Slides were examined histologically by two experienced pathologists without knowledge of treatment given (four slides per animal). The histological grading of edema was made using own scale ranging from 0 to 3; 0 = no edema, 1 = interlobular edema, 2 = interlobular and moderate intralobular edema and 3 = severe interlobular and intralobular edema. Grading of hemorrhages: 0 = absent, 1 = from one to two foci per slide, 2 = from three to five foci per slide, 3 = more than five foci per slide. Leukocyte infiltration was graded: 0 = absent, 1 = scarce perivascular infiltration, 2 = moderate perivascular and scarce diffuse infiltration, 3 = abundant diffuse infiltration. Findings of acinar necrosis were graded: 0 = absent, 1 = less than 15% of cells involved, 2 = from 15% to 35% of cells involved, 3 = more than 35% of cells involved. Grading of vacuolization was based on the percentage of cells involved: 0 = absent, 1 = less than 25%, 2 = 25–50% and 3 = more than 50%.

2.6. Statistical analysis

Comparison of the differences between the mean values of various groups of experiments was made by ANOVA (Analysis Of Variance) and the Tukey test for multiple comparisons (Statistica 6.0). A difference with a P value of less than 0.05 was considered statistically significant. Results are expressed as means (± S.E.M.).

3. Results

3.1. Morphological features

Pancreata of sham-operated animals with intact sensory and vagal nerves showed macroscopically and at light microscopic level no tissue alteration (Fig. 1A) (Table 1). Exposure to ischemic preconditioning alone caused only mild interlobular edema in half of cases. Rest of animals exposed to ischemic preconditioning applied alone did not show any histological alterations. Pancreatic ischemia followed by 1 h reperfusion produced acute hemorrhagic pancreatitis in all tested rats (Fig. 1B) (Table 1). Microscopic examination showed moderate inter- and intralobular edema accompanied with one to two foci of hemorrhages per slide. Inflammatory leukocyte infiltration was absent or limited to scarce perivascular. Necrosis was absent or involved less than 15% of acinar cells. Vacuolization was not observed in animals with ischemia/reperfusion-induced pancreatitis. Ischemic preconditioning applied prior to ischemia/reperfusion-induced pancreatitis strongly reduced histological signs of pancreatic damage (Fig. 1C) (Table 1). Edema was limited to interlobular space. Hemorrhages, leukocyte inflammatory infiltration, necrosis and vacuolization were not observed.

Pancreata of sham-operated animals with ablation of sensory nerves showed macroscopically and microscopically no tissue alteration, except mild interlobular edema in the part of cases (Table 1). Ablation of sensory nerves aggravated ischemia/reperfusion-induced pancreatic damage. Microscopic examination showed moderate or severe inter- and intralobular edema accompanied with one to five foci of hemorrhages per slide. Inflammatory leukocyte infiltration was scarce perivascular or absent. Necrosis was observed in all cases of ischemia/reperfusion-induced pancreatitis but involved less than 15% of acinar cells. Vacuolization was absent or observed in less than 15% of acinar cells. Ischemic

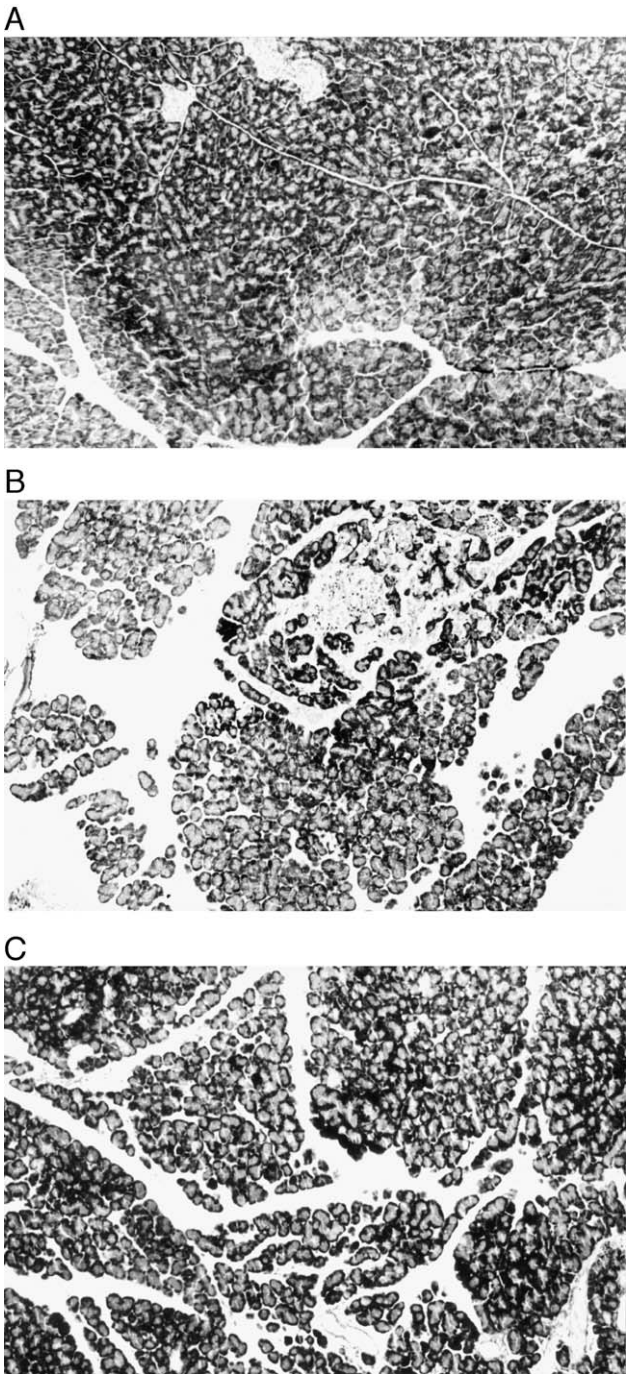

Fig. 1. Histological appearance of intact pancreas from sham-operated rats (control) (panel A), rats with ischemia/reperfusion-induced pancreatitis (panel B) and rats with ischemic preconditioning followed by ischemia/reperfusion-induced pancreatitis (panel C). Hematoxylin–eosine stain, magnification × 85.

preconditioning applied prior to ischemia/reperfusion-induced pancreatitis was without effect on pancreatic histology in animals with ablation of sensory nerves, except slight reduction of pancreatic necrosis (Table 1).

Vagotomy did not affect pancreatic histology in sham-operated rats and in animals exposed to ischemic preconditioning applied alone (Table 1). Vagotomy aggravated ischemia/reperfusion-induced pancreatic damage but deleterious effect of vagotomy was weaker than effect of sensory nerves ablation. In contrast to animals with ablation of sensory nerves, hemorrhages were limited to one or two foci per slide, necrosis was absent or involved less than 15% of acinar cells and no vacuolization was observed (Table 1). On the other hand, moderate or severe inter- and intralobular edema was observed in all cases. Ischemic preconditioning applied prior to ischemia/reperfusion-induced pancreatitis slightly reduced pancreatic damage in vagotomized rats. Edema was limited to interlobular space. Hemorrhages, leukocyte inflammatory infiltration, necrosis and vacuolization were not observed (Table 1).

In sham-operated rats, vagotomy combined with ablation of sensory nerves did not affect pancreatic tissue morphology (Table 1). Ischemic preconditioning led to the development of mild interlobular edema and one to two foci of hemorrhages per slide in half cases. Rest of animals exposed to ischemic preconditioning alone did not show any histological alteration (Table 1). Vagotomy combined with ablation of sensory nerves aggravated ischemia/reperfusion-induced pancreatic damage leading to the maximal tissue alteration. A severe inter- and intralobular edema was accompanied with three to five foci of hemorrhages. Vacuolization was observed in less than 25% of acinar cells. Ischemic preconditioning applied prior to ischemia/reperfusion-induced pancreatitis was without any protective effect on pancreatic histology in animals with vagotomy combined with ablation of sensory nerves (Table 1).

3.2. Pancreatic blood flow

Pancreatic blood flow in sham-operated rats with intact nerves reached 39.9 ± 0.7 ml/100 g tissue/min (Fig. 2). In these rats, ischemia/reperfusion-induced pancreatitis caused a significant reduction in pancreatic blood flow by 20.0 ml/100 g tissue/min ($p = 0.000029$). Ischemic preconditioning applied prior to induction of pancreatitis strongly and significantly reversed the pancreatitis-induced fall of pancreatic blood flow ($p = 0.000029$). In this group of animals, pancreatic blood flow reached 31.8 ml/100 g tissue/min.

In sham-operated rats, ablation of sensory nerves caused a decrease of pancreatic blood flow by 9.5 ml/100 g tissue/min and ischemic preconditioning had no significant effect on this parameter. In animals with ablation of sensory nerves, ischemia/reperfusion-induced pancreatitis caused a maximal reduction in pancreatic blood flow, which reached 7.4 ± 0.4 ml/100 g tissue/min. In this group of animals, ischemic preconditioning partly and significantly reversed a pancreatitis-evoked decrease in pancreatic blood flow ($p = 0.000364$), but value obtained was still lower than pancreatic blood flow in animals with intact sensory nerves without exposure to ischemic preconditioning.

Vagotomy reduced pancreatic blood flow in sham-operated rats by 5.5 ml/100 g tissue/min ($P = 0.020107$), but the effect of vagotomy on pancreatic blood flow was

Table 1
Effect of sham operation (SO), ischemic preconditioning (IP) and ischemia/reperfusion-induced pancreatitis (I/R) applied alone or their combination on morphological features of pancreatic tissue

		Histology				
		Edema	Hemorrhages	Inflammatory infiltration	Necrosis	Vacuolization
Sham-operated (SO)—control		0	0	0	0	0
Ischemic preconditioning (IP)		0/1	0	0	0	0
Ischemia/reperfusion (I/R)		2	1	0/1	0/1	0
IP + I/R		1	0	0	0	0
Capsaicin denervation	SO	0/1	0	0	0	0
	IP	0/1	0/1	0	0	0
	I/R	2/3	1/2	0/1	1	0/1
	IP + I/R	2/3	1/2	0/1	0/1	0/1
Vagotomy	SO	0	0	0	0	0
	IP	0/1	0	0	0	0
	I/R	2/3	1	0/1	0/1	0
	IP + I/R	2	1	0	0	0
Capsaicin denervation + vagotomy	SO	0	0	0	0	0
	IP	0/1	0/1	0	0	0
	I/R	3	2	1	1/2	1
	IP + I/R	3	2	1	1/2	1

Ten animals in each group, four slides per animal. Numbers represent the predominant histological grading in each group.

weaker than effect of sensory nerves ablation. Ischemic preconditioning increased pancreatic blood flow in vagotomized rat by 4.8 ml/100 g tissue/min but this effect was insignificant ($P = 0.089883$). Induction of pancreatitis reduced pancreatic blood flow to similar value as in animals with pancreatitis and intact vagal nerves. In vagotomized rats, ischemic preconditioning prior to induction of acute pancreatitis partly reversed a pancreatitis-evoked reduction in pancreatic blood flow ($P = 0.012810$), but this effect was lower than in animals with intact sensory and vagal nerves.

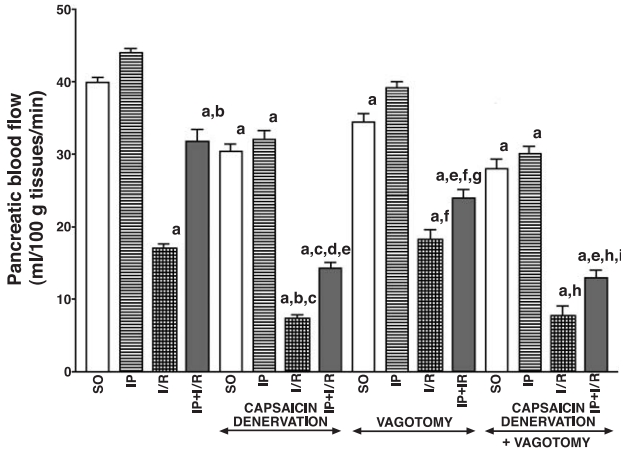

Fig. 2. Effect of sham operation (SO), ischemic preconditioning (IP), ischemia/reperfusion-induced pancreatitis (I/R) and combination of ischemic preconditioning with ischemia/reperfusion-induced pancreatitis (IP + I/R) on pancreatic blood flow in animals with intact nerves or animals with ablation of sensory nerves (capsaicin denervation) or vagotomy or ablation of sensory nerves combined with vagotomy. Mean ± S.E.M. $N = 10$ in each group of animals. [a] $P < 0.05$ compared to SO control rats with intact nerves; [b] $P < 0.05$ compared to I/R with intact nerves; [c] $P < 0.05$ compared to SO rats with capsaicin denervation; [d] $P < 0.05$ compared to rats with ablation of sensory nerves + I/R; [e] $P < 0.05$ compared to rats with intact nerves and exposed to IP + I/R; [f] $P < 0.05$ compared to SO vagotomized rats; [g] $P < 0.05$ compared to vagitomized rats + I/R; [h] $P < 0.05$ compared to SO rats with ablation of sensory nerves + vagotomy; [i] $P < 0.05$ compared to rats with ablation of sensory nerves + vagotomy + I/R.

Vagotomy combined with ablation of sensory nerves led to a reduction in pancreatic blood flow in sham-operated animals to 28.0 ± 1.3 ml/100 g tissue/min. Ischemic preconditioning did not significantly affect pancreatic blood flow in sham-operated rats with vagotomy combined with ablation of sensory nerves. In rats with vagotomy and ablation of sensory nerves, ischemia/reperfusion-induced pancreatitis caused a fall of pancreatic blood flow to the value similar as in animals with pancreatitis and ablation of sensory nerves. Ischemic preconditioning applied prior to induction of acute pancreatitis significantly reversed this fall of pancreatic blood flow ($P = 0.042915$), but this effect was weaker than changes observed in animals with intact sensory nerves.

3.3. Pancreatic DNA synthesis

In sham-operated control rats with intact sensory and vagal nerves, pancreatic DNA synthesis reached value of 57.8 ± 1.6 dpm/μg DNA (Fig. 3). Ischemic preconditioning applied alone was without significant effect on pancreatic DNA synthesis in animals with intact sensory and vagal nerves, as well as, in animals with vagotomy, ablation of sensory nerves or vagotomy combined with ablation of sensory nerves. Ischemia/reperfusion-induced pancreatitis caused a fall of pancreatic DNA synthesis in animals with intact sensory and vagal nerves to the value 39.0 ± 3.0 dpm/μg DNA; in animals with ablation of sensory nerves to

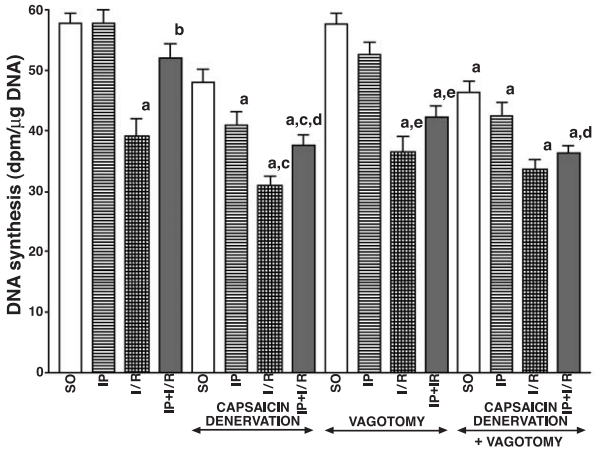

Fig. 3. Effect of sham operation (SO), ischemic preconditioning (IP), ischemia/reperfusion-induced pancreatitis (I/R) and combination of ischemic preconditioning with ischemia/reperfusion-induced pancreatitis (IP + I/R) on pancreatic DNA synthesis in animals with intact nerves or animals with ablation of sensory nerves (capsaicin denervation) or vagotomy or ablation of sensory nerves combined with vagotomy. Mean ± S.E.M. $N = 10$ in each group of animals. [a]$P < 0.05$ compared to SO control rats with intact nerves; [b]$P < 0.05$ compared to I/R with intact nerves; [c]$P < 0.05$ compared to SO rats with capsaicin denervation; [d]$P < 0.05$ compared to rats with intact nerves and exposed to IP + I/R; [e]$P < 0.05$ compared to SO vagotomized rats.

the value 31.0 ± 1.5 dpm/μg DNA; in animals with vagotomy to the value 36.6 ± 2.4 dpm/μg DNA; and in animals with ablation of sensory nerves combined with vagotomy to the value 30.4 ± 1.8 dpm/μg DNA. Ischemic preconditioning applied prior to induction of acute pancreatitis significantly reversed the fall of pancreatic DNA synthesis in animals with intact sensory and vagal nerves ($P = 0.000776$). In animals with ablation of sensory nerves or vagotomy, or combination of ablation of sensory nerves with vagotomy, effect of ischemic preconditioning applied before I/R on pancreatic blood flow was weak and statistically insignificant.

3.4. Plasma lipase activity

Plasma lipase activity in sham-operated rats with intact sensory and vagal nerves (control group) reached 52.3 ± 4.9 U/l (Fig. 4). Ablation of sensory nerves and vagotomy applied alone or in their combination did not affect plasma lipase activity in sham-operated rats. Ischemic preconditioning, applied without induction of acute pancreatitis, did not significantly affect plasma lipase activity in any group of animals. Ischemia/reperfusion-induced pancreatitis caused an increase in plasma lipase activity in all groups of animals. The highest values were observed in animals with ablation of sensory nerves (331.2 ± 20.9 U/l). Ischemic preconditioning strongly and significantly reduced an ischemia/reperfusion-evoked increase in plasma lipase activity in animals with intact sensory and vagal nerves ($P = 0.000041$). Weaker, but still significant effect was also observed in animals with vagotomy without ablation of sensory nerves (0.025322). Ablation of sensory nerves alone and in combination with vagotomy abolished the effect of ischemic preconditioning on plasma lipase activity. In these experimental groups, plasma lipase activity reached a similar value in animals with ischemic preconditioning applied prior to ischemia/reperfusion-induced pancreatitis as in animals with induction of acute pancreatitis without ischemic preconditioning.

3.5. Plasma interleukin-1β and interleukin-10 concentration

In control sham-operated rats with intact sensory and vagal nerves, plasma interleukin-1β concentration was 73.0 ± 3.2 pg/ml (Fig. 5). In animals with intact nerves, ischemia/reperfusion-induced pancreatitis caused an increase in plasma interleukin-1β by 93% ($P = 0.000029$). Ischemic preconditioning did not significantly affect plasma interleukin-1β concentration in sham-operated animals with intact sensory and vagal nerves but reduced this parameter by 20% ($P = 0.00913$) in animals with ischemia/reperfusion-induced pancreatitis and intact nerves. Ablation of sensory nerves alone and in combination with vagotomy increased plasma interleukin-1β concentration by 22% and 35%, respectively, but only in the last case this effect was statistically significant (0.04504). Vagotomy alone did not affect plasma interleukin-1β concentration. In animals with ablation of sensory nerves combined with ischemia/reperfusion-induced pancreatitis, plasma interleukin-1β concentration reached significantly higher level

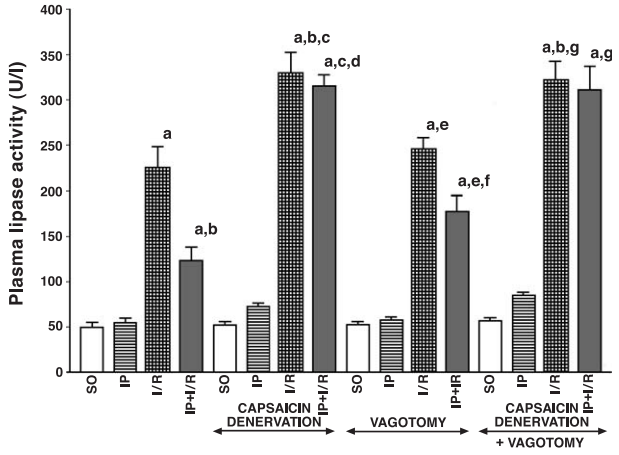

Fig. 4. Effect of sham operation (SO), ischemic preconditioning (IP), ischemia/reperfusion-induced pancreatitis (I/R) and combination of ischemic preconditioning with ischemia/reperfusion-induced pancreatitis (IP + I/R) on plasma lipase activity in animals with intact nerves or animals with ablation of sensory nerves (capsaicin denervation) or vagotomy or ablation of sensory nerves combined with vagotomy. Mean ± S.E.M. $N = 10$ in each group of animals. [a]$P < 0.05$ compared to SO control rats with intact nerves; [b]$P < 0.05$ compared to I/R with intact nerves; [c]$P < 0.05$ compared to SO rats with capsaicin denervation; [d]$P < 0.05$ compared to rats with intact nerves and exposed to IP + I/R; [e]$P < 0.05$ compared to SO vagotomized rats; [f]$P < 0.05$ compared to rats with vagotomy + I/R; [g]$P < 0.05$ compared to SO rats with capsaicin denervation + vagotomy.

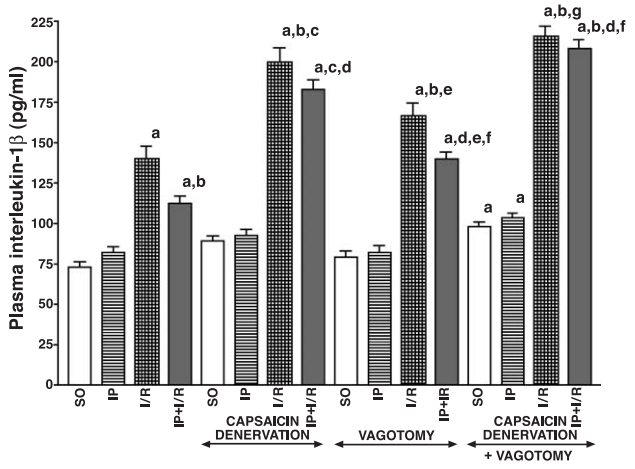

Fig. 5. Effect of sham operation (SO), ischemic preconditioning (IP), ischemia/reperfusion-induced pancreatitis (I/R) and combination of ischemic preconditioning with ischemia/reperfusion-induced pancreatitis (IP + I/R) on plasma interleukin-1β concentration in animals with intact nerves or animals with ablation of sensory nerves (capsaicin denervation) or vagotomy or ablation of sensory nerves combined with vagotomy. Mean ± S.E.M. $N = 10$ in each group of animals. $^{a}P < 0.05$ compared to SO control rats with intact nerves; $^{b}P < 0.05$ compared to I/R with intact nerves; $^{c}P < 0.05$ compared to SO rats with capsaicin denervation; $^{d}P < 0.05$ compared to rats with intact nerves and exposed to IP + I/R; $^{e}P < 0.05$ compared to SO vagotomized rats; $^{f}P < 0.05$ compared to vagotomized rats and exposed to I/R; $^{g}P < 0.05$ compared to SO rats with capsaicin denervation + vagotomy.

than that in animals with intact sensory nerves (199.8 ± 9.2 vs. 140.8 ± 6.8 pg/ml; $P = 0.000029$). A maximal increase in plasma interleukin-1β was observed in animals with induction of pancreatitis combined with ablation of sensory nerves and vagotomy. In this group of animals, plasma interleukin-1β reached 215.3 ± 6.8 pg/ml. Ischemic preconditioning strongly and significantly reduced an ischemia/reperfusion-evoked increase in plasma interleukin-1β concentration in animals with intact sensory and vagal nerves ($P = 0.00913$). Also, plasma interleukin-1β concentration was reduced by ischemic preconditioning in animals with vagotomy combined with ischemia/reperfusion-induced pancreatitis ($P = 0.019254$). Ischemic preconditioning was without significant effect on plasma interleukin-1β concentration in animals with induction of pancreatitis combined with ablation of sensory nerves alone, as well as, with ablation of sensory nerves and vagotomy.

In control sham-operated rats, plasma interleukin-10 reached value of 63.2 ± 7.9 pg/ml. Neither ablation of sensory nerves nor vagotomy, nor ischemia/reperfusion-induced pancreatitis applied alone nor in their combination significantly affect plasma interleukin-10 concentration.

4. Discussion

The protective effect of ischemic preconditioning against lesions caused by subsequent severe ischemia was primary described in the heart by Murry et al. (1986). However, to our best knowledge, nobody has studied this phenomenon in the pancreas. The present study is the first demonstration that ischemic preconditioning applied prior to ischemia/reperfusion-induced pancreatitis strongly reduces the severity of acute pancreatitis. The beneficial effect of ischemic preconditioning in the pancreas was manifested by a reduction in plasma lipase activity, a decrease in plasma concentration of proinflammatory interleukin-1β and an increase in pancreatic DNA synthesis. There was found close relationship between evoked by ischemic preconditioning, a decrease in biochemical signs of pancreatitis and the improvement of pancreatic blood flow, as well as a reduction in histological score of pancreatic damage.

Inflammatory infiltration plays an important role in development of pancreatic damage in the course of acute pancreatitis. Leukocytes adhere to the vascular endothelium forming plagues and contribute to the injury by reducing blood flow via occlusion of microvessels and release of mediators of tissue damage (Kusterer et al., 1993). Leukocytes infiltrate pancreatic tissue and cause a release of proinflammatory cytokines such as interleukin-1β, interleukin-6 and tumor necrosis factor-α within the pancreas and systematically (Norman et al., 1994; Fink and Norman, 1996). Interleukin-1β plays a crucial role in the release of other members of the proinflammatory cytokine cascade and activates the systemic acute phase of inflammation (Dinarello, 1991). The study performed by Norman et al. (1995) has shown that blockade of interleukin-1β prevents the rise in serum interleukin-6 and tumor necrosis factor-α level, and protects the pancreas against damage in the course of acute pancreatitis. Similar effect was observed after using of antileukocyte antibody. Leukocyte depletion by this antibody decreases the production of proinflammatory cytokines and reduces the severity of pancreatic damage in the course of experimental pancreatitis (Inoue et al., 1995; Fink and Norman, 1996). These observations are in agreement with our present data and partly elucidate the mechanism of protective effect after ischemic preconditioning. In our present study, ischemic preconditioning has reduced the leukocyte infiltration of pancreatic tissue and inhibited the production of interleukin-1β leading to the reduction of pancreatic damage.

In contrast to interleukin-1β, interleukin-10 has been found to be a major antiinflammatory cytokine (Moore et al., 1993). It reduces activation of macrophages and inhibits the production of reactive oxygen species (Moore et al., 1993) and proinflammatory cytokines (de Waal et al., 1991). Van Laethem et al. (1995) have shown that administration of interleukin-10 before and during induction of acute pancreatitis decreases the severity of pancreatitis. Also, administration of some growth factor such as hepatocyte growth factor (Warzecha et al., 2001b) or insulin-like growth factor-1 (Dembiński et al., 2002) increases a plasma interleukin-10 concentration and inhibits the development of acute pan-

creatitis. In our present study, we have not observed any alteration of plasma interleukin-10 concentration after ischemia/reperfusion-induced pancreatitis or ischemic preconditioning applied alone or in combination with induction of acute pancreatitis. This result indicates that protective effect of ischemic preconditioning on the pancreas is not dependent on the release of interleukin-10.

Our previous study has shown that that the pancreas is able to adapt to the repeated episodes of acute caerulein-induced pancreatitis (Dembiński et al., 1996b). After each subsequent development of acute pancreatitis, the initial pancreatic damage, the fall of pancreatic blood flow and an inhibition of pancreatic DNA synthesis were smaller than after previous induction of acute pancreatitis. The similar effect was found in our present study when induction of acute pancreatitis by ischemia/reperfusion was preceded by ischemic preconditioning. Also, in this case, the pancreatic damage, the fall of pancreatic blood flow and the drop of pancreatic were reduced.

Pancreatic ischemia with intracellular damage of acinar cells is able to activate the lysosomal and digestive enzymes within the pancreas, leading to the pancreatic tissue damage, autodigestion and the induction of pancreatitis (Waldner, 1992). Clinical and experimental studies have shown that pancreatic ischemia may initiate the acute pancreatitis and always aggravates pancreatic damage (Gullo et al., 1996; Lonardo et al., 1999; Menger and Vollmar, 1999; Klar et al., 1990). The severity of such experimental pancreatitis is closely correlated with tissue ischemia. The moderate and severe pancreatitis was found to be accompanied by progressive decrease in pancreatic blood perfusion (Knoefel et al., 1994). In mild experimental pancreatitis, the additional reduction in the pancreatic blood flow by exposure of animals to stress leads to augmentation pancreatic damage and development of hemorrhagic pancreatitis (Furukawa et al., 1993). In our present study, pancreatic ischemia with reperfusion has induced acute hemorrhagic pancreatitis and reduced the pancreatic blood flow. Ischemic preconditioning alone has increased pancreatic blood flow, applied in combination with ischemia/reperfusion-induced pancreatitis has reversed an ischemia/reperfusion-induced fall of pancreatic blood flow. This observation suggests that the improvement of pancreatic microcirculation contributes to the protective effect of ischemic preconditioning.

Ischemic preconditioning alone did not affect pancreatic DNA synthesis. Induction of acute pancreatitis caused a reduction in the pancreatic DNA synthesis, what may be considered as an index of pancreatic damage. In rats with induction of pancreatitis, ischemic preconditioning attenuated the ischemia/reperfusion-evoked fall in pancreatic DNA synthesis. This observation is an additional evidence of protective effect of ischemic preconditioning on the pancreas.

Capsaicin-sensitive primary sensory nerves serve for conduction of sensory, mainly nociceptive information, to the central nervous system, but also they are able to release neuromediators, mainly calcitonin gene-related peptide, from the activated peripheral endings, and this process is a basic for local ‘‘axon reflex’’ (Holzer, 1991). Sensory nerves have been shown to play a role in the maintenance of tissue integrity. In the pancreas, an activation of sensory nerves (Dembiński et al., 1996a; Warzecha et al., 2001a) or treatment with calcitonin gene-related peptide (Warzecha et al., 1997b, 2001a) prior to induction of acute pancreatitis attenuates the pancreatic damage, whereas deactivation of sensory nerves contributes to the enhancement of acute pancreatitis severity (Dembiński et al., 1996a; Warzecha et al., 2001a). Protective effect of sensory nerves activation against pancreatic damage has been shown to be dependent on an increase in pancreatic blood flow, a reduction in production of proinflammatory cytokines and a decrease in plasma activity of pancreatic digestive enzymes (Warzecha et al., 1997a, 2001a). The same protective mechanisms have been observed in our present study after ischemic preconditioning. Ablation of sensory nerves has aggravated ischemia/reperfusion-induced pancreatic damage and attenuated a protective effect of ischemic preconditioning. These results indicate that sensory nerves are involved in the protective effect of ischemic preconditioning against pancreatic damage.

In our present study, vagotomy has attenuated a protective effect of ischemic preconditioning in ischemia/reperfusion-induced pancreatitis, but deleterious effect of vagotomy was less pronounced than effect of sensory nerves ablation by capsaicin. The difference between deleterious effects evoked by sensory nerves ablation and vagotomy seems to be dependent on distribution of capsaicin-sensitive afferent sensory fibers. The afferent sensory information from the gut is transmitted by vagal and spinal afferent fibers to the nodose and dorsal root ganglia, respectively (Holzer, 1991; Grundy, 2002), however, the information encoded by vagal and spinal afferents is different (Grundy, 2002). Vagal afferents convey predominantly physiological information, which is used as the basis of reflex mechanism to control motor and secretory function of the gut. The noxious events are mostly conveyed by spinal afferents, which form the main pathway for pain perception (Grundy, 2002). Calcitonin gene-related peptide (CGRP) is a major mediator of thin unmyelinated capsaicin-sensitive primary afferent nerves (Holzer, 1994). Study performed by Sternini and Anderson (1992) has shown that the vast majority of CGRP-containing afferent neurons supplying the pancreas are located in dorsal root ganglia. Only a minor component of afferent innervation of the pancreas derived from vagal CGRP-containing neurons (Sternini and Anderson, 1992).

In summary, our present data demonstrate that pancreatic ischemic preconditioning reduces the severity of ischemia/reperfusion-induced pancreatitis. Sensory and vagal nerves are involved in this protective effect of ischemic preconditioning in the pancreas.

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