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Role of superoxide anion in pancreatic islet blood flow regulation in anesthetized rats

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Abstract

The aim of the investigation was to study the influence of the superoxide anion on pancreatic islet blood flow in rats. For this purpose, blood flow measurements were conducted with a microsphere technique 10 min after intravenous administration of different doses of superoxide dismutase (5, 15, 50, 100 or 1000 kU/kg body weight). In separate experiments, diethyldithiodicarbamate, an inhibitor of endogenous superoxide dismutase, was given to nontreated control rats or to rats subjected to a bilateral abdominal vagotomy before the injection. Only the highest dose of superoxide dismutase increased both whole pancreatic and islet blood flow. A 50% augmentation of fractional islet blood flow was seen. Administration of diethyldithiocarbamate induced marked hyperglycemia, which was partly prevented by vagotomy. Diethyldithiocarbamate decreased the whole pancreatic blood flow, while islet blood flow was maintained in both control and vagotomized rats. Consequently, a pronounced increase in fractional islet blood flow was noted in both these groups. We conclude that administration of superoxide dismutase and its inhibitor diethyldithiocarbamate influences pancreatic blood perfusion. In particular, superoxide dismutase causes a general increase in the whole pancreatic and islet blood flow, and an augmented fractional islet blood flow, presumably by a decrease in the local concentration of $O_2^{\cdot -}$, leading to increased concentration of NO. Diethyldithiocarbamate, on the other hand, by increasing the levels of $O_2^{\cdot -}$, decreases the whole pancreatic blood flow, whereas islet blood flow remains unaffected.

Keywords: Pancreatic islet; Superoxide; Superoxide dismutase; Blood flow; Nitric oxide (NO)

1. Introduction

The regulation of pancreatic islet blood flow depends on complex interactions between the nervous system, paracrine and endocrine effects of gastrointestinal hormones and, in particular, locally produced vascular constrictors and dilators (Jansson, 1994; Brunicardi et al., 1996). Nitric oxide (NO) is believed to constitute the main endothelium-derived relaxing factor (EDRF) (Palmer et al., 1987; Moncada et al., 1994). NO synthesis is, thus, essential for the maintenance of systemic blood pressure, as well as for regulation of regional blood flow throughout the body (Moncada et al., 1994). We have previously shown that both the whole pancreatic and islet blood perfusion is highly sensitive to inhibition of local NO production by a nonselective nitric oxide synthase (NOS) inhibitor (Svensson et al., 1994). Importantly, this effect is far more pronounced in the islets, leading to a marked decrease in fractional islet blood flow, i.e. the fraction of whole pancreatic blood flow that is diverted through the islets.

It has been proposed that oxygen radicals play a role in the regulation of blood perfusion by promoting vasoconstriction (e.g. Wolin, 1996; Spolarics, 1998). Previous studies have demonstrated that the concentrations of endogenous superoxide dismutase and superoxide anion $O_2^{\cdot}$– influence the vascular response during both normal and pathological conditions (Stewart et al., 1988; Hattori et al., 1991; Mugge et al., 1991, 1994; Laurindo et al., 1991; Meng et al., 1995; Wolin, 1996). $O_2^{\cdot -}$ has a potential to influence the levels of NO in the vasculature, since it can react with NO to form peroxynitrite, which by itself is a potent vaso-dilator and oxidant (Beckman et al., 1990; Pryor and Squadrito, 1995). An alternative pathway for $O_2^{\cdot -}$ is dismutation by $Cu^{2+}Zn^{2+}$ superoxide dismutase resulting in the formation of hydrogen peroxide.

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Table 1
Effects of intravenous administration of saline (0.2 ml) or different doses of superoxide dismutase on anaesthetised rats

Substance given	Saline	Superoxide dismutase (kU/kg)				
		5	15	50	100	1000
No. of animals	8	6	7	6	6	5
Body weight (g)	358 ± 8	345 ± 9	344 ± 4	337 ± 9	351 ± 11	343 ± 7
Blood glucose (mmol/l)	4.0 ± 0.1	4.1 ± 0.2	4.2 ± 0.2	4.1 ± 0.2	4.2 ± 0.2	4.5 ± 0.7
Mean arterial blood pressure (mm Hg)	101 ± 3	99 ± 6	107 ± 6	98 ± 6	104 ± 8	105 ± 12
Islet blood flow (% of pancreatic blood flow)	11.3 ± 1.4	10.2 ± 1.5	10.4 ± 1.6	10.1 ± 1.3	9.7 ± 1.0	15.2 ± 1.1*

The substances were injected intravenously 10 min before the measurements. All values are means ± S.E.M.
*$P < 0.05$ and when compared with the saline-treated rats by Student's unpaired *t*-test.

Changes in superoxide dismutase activity, could, therefore be envisaged to diminish or enhance the biological activity of NO (Gryglewski et al., 1986; Rubanyi, 1988; Pryor and Squadrito, 1995; Oury et al., 1996).

In view of the importance of NO for islet blood flow regulation, we deemed it of interest to further elucidate the mechanisms by which locally produced free radicals influence islet blood perfusion. We, therefore, investigated the effects of alterations in the concentrations of $O_2^{\cdot -}$, achieved by administration of superoxide dismutase or the superoxide dismutase inhibitor diethyldithiocarbamate (Heikkila et al., 1976; Liu et al., 1996), on pancreatic blood perfusion in rats.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing approximately 350 g were obtained from a local breeding colony (Biomedical Centre, Uppsala University, Uppsala, Sweden). The animals had free access to tap water and pelleted food throughout the experiments. All experiments were approved by the local animal ethics committee.

2.2. Surgical preparation

The rats were anaesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg body weight). Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery and into the femoral artery and vein. Through the intravenous catheter, a continuous infusion of Ringer solution was given (5 ml/kg body weight/min) to substitute for fluid losses. The mean arterial blood pressure was recorded with a pressure transducer (PDCR 75/1; Druck, Groby, UK) connected to the catheter in the carotid artery. A bilateral abdominal vagotomy at the level of the esophagus was performed in some of the animals (Carlsson et al., 2000) before administration of any of the test substances referred to below.

2.3. Drugs

When the blood pressure had remained stable for at least 20 min, an intravenous injection of 0.2 ml of 0.5% (wt./vol.) bovine serum albumin dissolved in saline (hereafter referred to as vehicle) or superoxide dismutase derived from bovine erythrocytes (EC 1.15.1.1; Sigma-Aldrich Chemicals, St. Louis, MO, USA) dissolved in saline (5, 15, 50, 100 or 1000 kU/kg body weight) was given in the right femoral vein 10 min before the blood flow measurements.

Other animals were instead given an intraperitoneal injection of saline alone (2 ml/kg body weight) or diethyldithiocarbamate (Sigma; 1 g/kg body weight) dissolved in saline 20 min before the blood flow measurements. Some of the animals had been vagotomized (see above) before the administration of diethyldithiocarbamate.

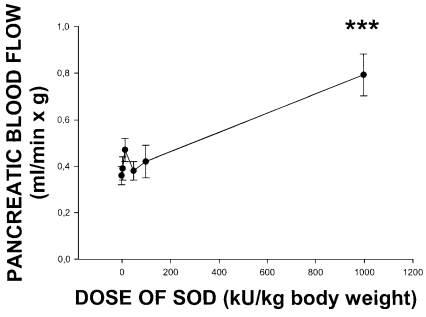

Fig. 1. Total pancreatic blood flow 10 min after an intravenous injection of vehicle (lowest marked dose) and different doses of superoxide dismutase (SOD). Values are means ± S.E.M. for five to eight observations (see Table 1). ***$P < 0.001$ when compared to the vehicle-treated rats.

Fig. 2. Pancreatic islet blood flow 10 min after an intravenous injection of vehicle (lowest marked dose) and different doses of superoxide dismutase (SOD). Values are means ± S.E.M. for five to eight observations (see Table 1). ***$P < 0.001$ when compared to the vehicle-treated rats.

Table 2
Effects of saline or diethyldithiocarbamate (DEDTC; 1 g/kg body weight) on pancreatic and islet blood flows

Substance given	Saline	DEDTC	Saline	DEDTC
Vagotomy	–	–	+	+
No. of animals	8	7	8	7
Body weight (g)	347 ± 6	345 ± 9	339 ± 7	353 ± 6
Blood glucose (mM)				
Before injection	3.6 ± 0.2	3.7 ± 0.2	5.5 ± 0.4	5.6 ± 0.2
20 min after injection	3.5 ± 0.2	17.6 ± 0.5***	5.7 ± 0.4	8.9 ± 0.5***
Mean arterial blood pressure (mm Hg)	118 ± 6	99 ± 4*	104 ± 6	90 ± 3*
Islet blood flow (% of pancreatic blood flow)	6.7 ± 0.8	20.8 ± 2.1**	10.9 ± 1.0	23.7 ± 4.0**

The substances were injected intraperitoneally 20 min before the measurements. In some animals, a bilateral vagotomy was performed before injections of saline or DEDTC. All values are means ± S.E.M.

* $P < 0.05$, when compared with corresponding saline treated rats by Student's unpaired *t*-test.

** $P < 0.01$, when compared with corresponding saline treated rats by Student's unpaired *t*-test.

*** $P < 0.001$, when compared with corresponding saline treated rats by Student's unpaired *t*-test.

2.4. Blood flow measurements

The procedure for blood flow measurements has been described in detail elsewhere (Jansson and Hellerström, 1983). Briefly, approximately $1.5–2 \times 10^5$ nonradioactive microspheres (NEN-Trac®; DuPont Pharmaceuticals, Wilmington, DE, USA), with a diameter of 11 μm, were injected during 10 s through the catheter with its tip in the ascending aorta. Starting from 5 s before the microsphere injection, and continuing for a total of 60 s, an arterial blood reference sample was collected by free flow (approximately 0.40 ml/min) from the catheter placed in the femoral artery. The exact withdrawal rate in each experiment was estimated by weighing the sample. The animals were then killed and the pancreas and adrenal glands were removed in toto, blotted and weighed. The organs were treated with a freeze–thawing technique, which visualizes the microspheres (Jansson and Hellerström, 1981). This technique also allows distinguishing between microspheres in the endocrine and exocrine parts of the pancreas. The blood flow values were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$, where Q_{org} is organ blood flow (ml/min), Q_{ref} withdrawal rate of the reference sample (ml/min), N_{org} number of microspheres in the organ and N_{ref} the number of microspheres in the reference sample. The blood flow to each of the adrenal glands was calculated in each experimental animal. A difference in adrenal blood flow <10% between the two glands confirmed adequate mixing of the microspheres into the circulation.

2.5. Blood glucose measurements

Blood samples were taken from the femoral arterial catheter immediately after the blood flow measurements. The samples were analyzed for blood glucose concentration with test reagent strips (ExacTech®; Baxter Travenol, Deerfield, IL, USA).

2.6. Statistical calculations

All values were expressed as means ± S.E.M. Calculations of statistical significance were performed with single factor factorial analysis of variance in conjunction with Bonferroni's test by use of Sigmastat® (SSPD; Erfart, Frankfurt, Germany). $P < 0.05$ was considered to be statistically significant.

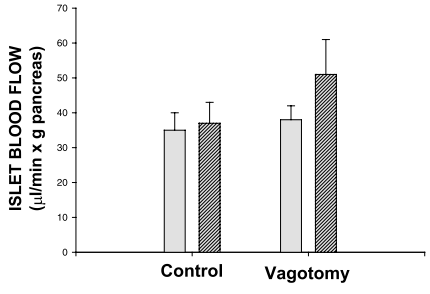

Fig. 3. Islet blood flow 20 min after an intraperitoneal injection of saline (open bars) or diethyldithiocarbamate (hatched bars; DEDTC; 1 g/kg body weight). Some of the animals were controls; others were subjected to bilateral, abdominal vagotomy before administration of saline or DEDTC. Values are means ± S.E.M. for seven to eight experiments.

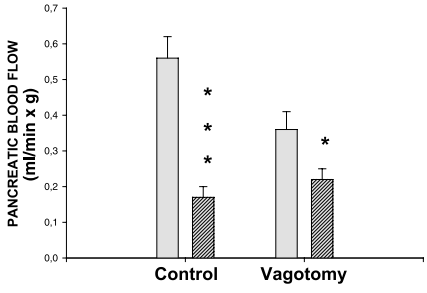

Fig. 4. Total pancreatic blood flow 20 min after an intraperitoneal injection of saline (open bars) or diethyldithiocarbamate (hatched bars; DEDTC; 1 g/kg body weight). Some of the animals were controls; others were subjected to bilateral, abdominal vagotomy before administration of saline or DEDTC. Values are means ± S.E.M. for seven to eight experiments. * $P < 0.05$ and *** $P < 0.001$ when compared to the corresponding saline-injected rat.

3. Results

3.1. Effects of superoxide dismutase

Administration of superoxide dismutase affected neither mean arterial blood pressure nor blood glucose concentrations in any of the groups in Table 1. Doses of superoxide dismutase ranging from 5 to 100 kU/kg body weight did not affect whole pancreatic (Fig. 1), islet (Fig. 2) or fractional islet blood flow (Table 1). However, when the highest dose of superoxide dismutase, 1000 kU/kg body weight, was administered, an increase in both whole pancreatic (Fig. 1) and islet blood flow (Fig. 2) was seen. The increase in islet blood perfusion was more pronounced, as evidenced by the augmented fractional islet blood flow (Table 1).

3.2. Effects of diethyldithiocarbamate

Administration of diethyldithiocarbamate produced marked hyperglycemia in nonvagotomized animals (Table 2). The effect of the substance was less pronounced in animals that had undergone vagotomy (Table 2). Vagotomy per se caused a small decrease in mean arterial blood pressure ($P<0.05$) when saline- and diethyldithiocarbamate-injected rats were compared (Table 2). Administration of diethyldithiocarbamate caused a slight decrease in blood pressure in both control and vagotomized animals (Table 2). The absolute values of islet blood flow were similar in all groups of animals (Fig. 3). Whole pancreatic blood flow was lower in vagotomized saline-injected rats compared to nonvagotomized saline treated animals ($P<0.05$; Fig. 4). Administration of diethyldithiocarbamate decreased pancreatic blood flow in both vagotomized and nonvagotomized rats (Fig. 2), leading to a marked increase in fractional islet blood flow in both groups (Fig. 5).

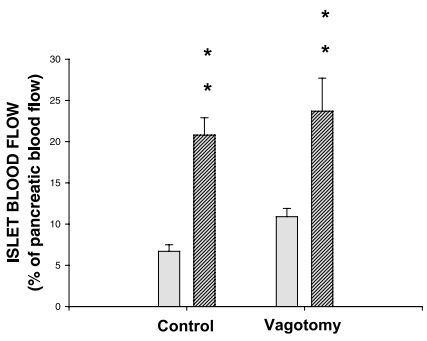

Fig. 5. Fractional islet blood flow 20 min after an intraperitoneal injection of saline (open bars) or diethyldithiocarbamate (hatched bars; DEDTC; 1 g/kg body weight). Some of the animals were controls; others were subjected to bilateral, abdominal vagotomy before administration of saline or DEDTC. Values are means ± S.E.M. for seven to eight experiments. **$P<0.01$ when compared to the corresponding saline-injected rat.

4. Discussion

The presence of constitutive NOS, as well as expression of inducible NOS after cytokine exposure, has previously been demonstrated in islet endothelial cells in culture (Suschek et al., 1994). In the same series of experiments, the synthesis of NO in islet endothelial cells was shown to be glucose-dependent, in contrast to endothelial cells from the aorta (Suschek et al., 1994). We previously observed that nonselective inhibition with different inhibitors of NOS caused a pronounced decrease in islet and whole pancreatic blood flow in normal and Type 2 diabetic rats (Svensson et al., 1994; Svensson et al., 1995). Islet blood flow normally amounts to about 7–10% of the whole pancreatic blood flow, although the islet volume constitutes only 1–2% of the total pancreatic volume (Jansson, 1994; Brunicardi et al., 1996). Inhibition of NOS reduced the fractional islet blood flow in normal rats to about 4–5%, i.e. values similar to the lowest islet blood flows previously recorded (Jansson, 1994). This suggests that the continuous formation of NO is crucial for maintenance of the relatively high basal blood perfusion in the islets. In other experiments, we had demonstrated that administration of the β-cell toxin alloxan, which is known to generate free oxygen radicals (Grankvist, 1981; Malaisse, 1982), led to decreased whole pancreatic blood flow, while islet blood flow markedly increased (Jansson and Sandler, 1992). Administration of superoxide dismutase together with alloxan augmented whole pancreatic blood flow and a further increase in islet blood flow was seen (Jansson and Sandler, 1992). Interestingly, administration of superoxide dismutase was shown to reduce islet microvascular injury induced by streptozotocin, another β-cell toxin, in rats (Enghofer et al., 1997).

The present study shows a net increase in whole pancreatic blood flow and a marked increase in islet blood flow, resulting in a moderately increased fractional islet blood flow, after administration of superoxide dismutase, which agrees with previous investigations (Jansson and Sandler, 1992; Enghofer et al., 1997). However, it should be noted that in both this and our previous study, administration of a high dose of superoxide dismutase (1000 kU/kg body weight) was required to achieve any effects on pancreatic circulation. It can be envisaged that the effect of superoxide dismutase is linked to its capacity to dismute $O_2^{\cdot -}$, thus, delaying and impairing the rate of breakdown of NO by its reaction with $O_2^{\cdot -}$ (Pryor and Squadrito, 1995; Oury et al., 1996). However, it should be pointed out that the observed effects on the pancreatic vasculature after administration of superoxide dismutase is likely to be the net result of complex stimulatory and inhibitory processes. The metabolism of NO and $O_2^{\cdot -}$ involves several potentially vasoactive intermediates. Hydrogen peroxide is produced in vascular endothelial cells as a by-product of several oxidative pathways (Wolin et al., 1987; Rubanyi, 1988) and, in particular, by the superoxide dismutase catalyzed dismutation of $O_2^{\cdot -}$ (see

below). NO and $O_2^{\cdot -}$ also react to form peroxynitrite (Beckman et al., 1990; Pryor and Squadrito, 1995), which is likely to be of crucial importance for the vaso-dilation seen during inflammation and endotoxemic shock (Wizemann et al., 1994; Szabo et al., 1995). $O_2^{\cdot -}$ may oxidize norepinephrine, and administration of superoxide dismutase was shown to inhibit the action of norepinephrine (Rubanyi, 1988). Thus, the action on norepinephrine may be another mechanism whereby $O_2^{\cdot -}$ could affect islet blood perfusion.

In the second series of experiments, the effects of the endogenous superoxide dismutase inhibitor diethyldithiocarbamate were examined. This compound is a potent copper and iron chelating agent (Liu et al., 1996), which directly inhibits $Cu^{2+}Zn^{2+}$ superoxide dismutase by copper chelation (Heikkila et al., 1976; Mugge et al., 1994; Liu et al., 1996). Administration of diethyldithiocarbamate resulted, as expected, in decreased pancreatic blood flow. However, pancreatic islet blood flow was preserved, leading to increased fractional islet blood flow. In vitro, diethyldithiocarbamate has been shown to scavenge $O_2^{\cdot -}$, hydrogen peroxide (Rogers et al., 1979) and peroxynitrite, with formation of several reactive intermediates (Liu et al., 1996). In addition, diethyldithiocarbamate can alter catecholamine metabolism by inhibiting β-hydroxylase, leading to a decrease in the levels of norepinephrine (Rogers et al., 1979). Whether or not any of these possible side effects confound the results of the present study is unclear. The notion that the net effect of diethyldithiocarbamate is a shift towards vasoconstriction was supported by a study where diethyldithiocarbamate reversed the general vaso-dilation during septic shock (Broner et al., 1993). Diethyldithiocarbamate is also known to inhibit the action of EDRF on endothelial cells in tissue culture during intact NO production (Mugge et al., 1991).

In the present study, administration of diethyldithiocarbamate resulted in a moderate decrease in mean arterial blood pressure in both vagotomized and nonvagotomized rats. The decrease in mean arterial pressure was, as such, not sufficient to affect pancreatic blood flow, since the exocrine pancreas exhibits autoregulation of the blood flow at this level of arterial blood pressure (Kvietys et al., 1982).

Administration of glucose stimulates islet blood flow selectively (Jansson and Hellerström, 1983), leading to an increase in fractional islet blood flow. In the present experiments, administration of diethyldithiocarbamate caused an increase in blood glucose concentration, which was largely, but not completely, overcome by vagotomy. This degree of hyperglycemia could potentially confound the islet blood flow measurements. We have previously demonstrated that the effect of hyperglycemia on islet blood flow is counteracted by vagotomy (Jansson and Hellerström, 1986). However, it should be noted that independent induction of local production of vasoactive substances like adenosine may shift the balance towards increased islet blood flow during hyperglycemia (Carlsson et al., 2002).

The preferential increase in islet blood flow after administration of superoxide dismutase, and the augmented fractional islet blood flow after diethyldithiocarbamate administration, is intriguing. Indeed, our previous studies have indicated that the blood flow to the islets is regulated independently from that of the whole pancreatic blood perfusion (Jansson, 1994). A difference in sensitivity to the action of NO between the smooth muscular cells of the endocrine and the exocrine vascular beds would provide a possible explanation. Increased local concentrations of $O_2^{\cdot -}$ would increase the degradation of NO, and thereby potentially affect pancreatic blood perfusion. It should be noted in this context that islet endothelial cells contain more NOS than those of the exocrine parenchyma (Suschek et al., 1994) and thereby possess an increased potential for NO production. Therefore, the net effect of $O_2^{\cdot -}$ on the endocrine pancreas may be less pronounced.

An alternative explanation for the vaso-dilation within the pancreas after administration of high doses of superoxide dismutase may be that the enzyme prevents a direct vasoconstrictive effect of $O_2^{\cdot -}$ on intrapancreatic resistance vessels. Previously it has been reported that $O_2^{\cdot -}$ may mediate endothelium-dependent vasoconstriction in the cerebral circulation (Katusic and Vanhoutte, 1989). A third alternative is that superoxide dismutase may participate in the generation of hydrogen peroxide, a substance which has been considered a candidate for being an endothelium-derived hyperpolarizing factor (Campbell and Gauthier, 2002). Administration of the hydrogen peroxide scavenging enzyme catalase in rats have suggested that hydrogen peroxide may be a mediator of intestinal hyperaemia during inflammation (Ruh et al., 2000). We are at present further examining this notion in our experimental system.

In conclusion, superoxide dismutase administration caused a general increase in the whole pancreatic and islet blood flow, and an augmented fractional islet blood flow, presumably by a decrease in the local concentration of $O_2^{\cdot -}$ leading to increased concentration of NO. Inhibition of superoxide dismutase by diethyldithiocarbamate decreased pancreatic blood flow, but not islet blood flow, resulting in increased fractional islet blood flow. The fact that islet blood flow was preserved in the face of decreased whole pancreatic blood flow after administration of diethyldithiocarbamate adds to an increasing body of evidence for independent regulation of islet and exocrine pancreatic blood flow. In particular, it points at the ability of the body to preserve pancreatic islet blood flow under conditions of decreased whole pancreatic blood flow.

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