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Short communication

The blood flow in pancreatico-duodenal grafts in rats: inhibition of nitric oxide synthase preferentially decreases islet blood flow

Annika M. Svensson, Stellan Sandler, Leif Jansson *

Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

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Abstract

In this study normoglycemic inbred Wistar-Furth rats were implanted with a syngeneic pancreatico-duodenal graft, i.e. a denervated pancreas. The blood flow to the intact native pancreas and to the transplanted gland was measured with a microsphere technique in anesthetized rats 2 weeks after transplantation. The animals were given an intravenous injection with saline alone, N^G-nitro-L-arginine (25 mg/kg body weight) or sodium nitroprusside (10 μg/kg body weight) 10 min before blood flow measurements. Administration of N^G-nitro-L-arginine increased mean arterial blood pressure and caused a pronounced decrease in whole pancreatic blood flow in both the native and transplanted gland. The islet blood flow was more markedly decreased by N^G-nitro-L-arginine in both the native and transplanted pancreas, and constituted about 4% of whole pancreatic blood flow compared with 10% in the control animals. Sodium nitroprusside markedly decreased mean arterial blood pressure, but did not affect pancreatic or islet blood flow in any of the glands. It is concluded that inhibition of nitric oxide synthase causes a preferential decrease in islet blood flow both in the native pancreas and in the transplanted pancreas. This suggests that nitric oxide which affects islet blood flow is mainly endothelial-derived, and does not emanate from external nervous fibers.

Keywords: Pancreatic islet; Pancreas transplantation; Nitric oxide (NO) synthase; Nitric oxide (NO); Blood flow regulation

1. Introduction

Previous studies have demonstrated that the pancreatic islets possess a blood flow regulation which is independent of that to the exocrine parts of the gland (Meyer et al., 1982; Jansson and Hellerström, 1983). Nervous (Jansson and Sandler, 1985; Jansson and Hellerström, 1986) and hormonal influences (Carlsson and Jansson, 1994) seem to affect these two vascular compartments differently. It has recently been shown that inhibition of nitric oxide (NO) synthase induces a preferential decrease in pancreatic islet blood flow, when compared with that to the whole gland (Svensson et al., 1994). NO is synthesized from arginine (Moncada, 1992), in endothelial cells, macrophages and neural cells (Moncada et al., 1991). There is now general agreement that NO is the most important physiological endothelial-derived relaxing factor (Feelisch et al., 1994), and in view of the finding referred to above it seems likely that NO is a regulator of islet blood flow also.

The islets are known to receive a rich autonomous innervation (Sundler and Böttcher, 1992), and the activity in these nerves can influence islet blood flow (Jansson and Hellerström, 1986). The aim of the present study was to evaluate, in the same animal, the effects of inhibition of nitric oxide synthase in a denervated transplanted pancreas when compared with the intact, native pancreas. This would enable an evaluation of whether NO that affects islet blood flow is mainly endothelial or nerve-derived. If NO is of endothelial origin, an effect on both the native and transplanted pancreas by inhibition of NO synthesis would be expected, whereas a predominantly neuronal origin of NO would be anticipated to cause effects on the native and not on the denervated transplanted gland.

* Corresponding author. Department of Medical Cell Biology, Biomedical Centre, P.O. Box 571, S-751 23 Uppsala, Sweden. Tel. +46-18-174396, fax +46-18-556401.

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2. Materials and methods

2.1. Animals

Inbred male Wistar-Furth rats (Anticimex, Sollentuna, Sweden) weighing approximately 300 g were used in all experiments. The animals were housed one per cage after transplantation and had free access to pelleted food (Type R36; AnalyCen, Lidköping, Sweden) and tap water.

2.2. Pancreatico-duodenal transplantation

This procedure has been described in detail elsewhere (Jansson et al., 1992). Briefly, the donor rat was anesthetized with an intraperitoneal injection of chloral hydrate (360 mg/kg body weight), and placed on an operating table maintained at body temperature. The whole pancreas, together with approximately 3–4 cm of the adjacent small intestine, was dissected free from surrounding tissues. The preparation was flushed with 5–7 ml of cold (4°C) UW solution (Belzer UW-CSS; Du Pont Pharmaceuticals, Wilmington, DE, USA) via a catheter in the aorta at a pressure of approximately 100 cm H_2O. The warm ischemia time was < 2 min. The graft was then removed from the animal together with approximately 1 cm of the aorta, which contained the two arterial vessels to the gland, and stored at 4°C for 1.5–2 h (cold ischemia time) before being implanted into the recipient. The recipients were also anesthetized with chloral hydrate. The left kidney was removed, and the pancreatico-duodenal graft was anastomosed to the renal vessels by a non-suturing cuff technique as previously described in detail (Olausson et al., 1984; Jansson et al., 1992). The transplanted small intestine was sutured end-to-side to a loop of the colon of the recipient by ≈ 10 sutures with 7-0 silk. After closure of the abdominal wound, the animals were injected subcutaneously with 10 mg doxycycline (Idocyklin; Leo, Malmö, Sweden) and were observed until fully recovered from anesthesia.

2.3. Blood flow measurements

This procedure has been described in detail elsewhere (Jansson and Hellerström, 1983; Jansson et al., 1992). Briefly, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg body weight; Mebumal vet; Nordvacc, Solna, Sweden), and heparinized. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the femoral artery. The former catheter was connected to a pressure transducer to allow continuous monitoring of the mean arterial blood pressure. When the blood pressure had remained stable for at least 20 min either saline alone (2 ml/kg body weight), N^G-nitro-L-arginine (25 mg/kg body weight; Sigma Chemicals, St. Louis, MO, USA) or sodium nitroprusside (10 μg/kg body weight; Sigma) was given intravenously. Ten minutes later, $1.5–2.0 \times 10^5$ non-radioactive microspheres (NEN-Trac; DuPont Pharmaceuticals) with a diameter of 11 μm were injected over 10 s via the catheter with its tip in the ascending aorta. Starting 5 s before the microsphere injection, and continuing for a total of 60 s, an arterial blood sample was collected by free flow from the catheter in the femoral artery at a rate of approximately 0.5 ml/min. The animals were then killed and the adrenal glands, the transplanted pancreas and the native gland were removed, blotted, weighed and treated with a freeze-thawing technique to visualize the pancreatic islets and the microspheres. The microsphere contents and the blood flows were then determined. Blood flow values based on the microsphere content of the adrenal glands were used to confirm that the microspheres were adequately mixed with the circulation. A difference of $< 10\%$ in blood flow values between the glands was taken to indicate sufficient mixing, and all animals in Table 2 fulfilled this requirement.

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

2.4. Statistical calculations

All values are given as means $\pm$S.E.M. Groups of data were compared using Student's two-tailed unpaired t-test. When multiple comparisons were performed the values were compared by analysis of variance (ANOVA) in conjunction with Fisher's PLSD test.

3. Results

A total of six out of 23 transplanted animals were excluded from the study. Three of these had intra-abdominal abscesses precluding any graft blood flow measurements, whereas the microsphere blood flow measurement failed in three animals. All rats were normoglycemic before transplantation (data not shown) and at the time of blood flow measurement (Table 1). The weights of the transplanted pancreases were consistently lower than those of the native glands (Table 1). No macroscopic signs of inflammation or rejection could be discerned (data not shown). Administration of N^G-nitro-L-arginine or sodium nitroprusside did not affect the blood glucose concentration (Table 1). The mean arterial blood pressure was increased by 30% 10 min after N^G-nitro-L-arginine injection, but decreased

Table 1
Body weight, pancreatic weight, blood glucose and mean arterial blood pressure of inbred Wistar-Furth rats 2 weeks after a pancreatico-duodenal transplantation

Substance given	Saline ($n = 9$)	NNA ($n = 5$)	Nitroprusside ($n = 6$)
Body weight (g)	297 ± 5	301 ± 7	312 ± 6
Pancreatic weight (mg)			
-native	1128 ± 39	1117 ± 42	1120 ± 46
-transplanted	895 ± 56 [a]	918 ± 34 [a]	944 ± 58 [a]
Blood glucose (mM)	5.2 ± 0.3	4.4 ± 0.4	5.5 ± 0.4
Mean arterial blood pressure (mm Hg)	81 ± 4	108 ± 9 [b]	65 ± 2 [c]

The animals were injected intravenously with either saline (2 ml/kg body weight), N^G-nitro-L-arginine (NNA; 25 mg/kg body weight) or nitroprusside (10 μg/kg body weight) dissolved in saline 10 min before recordings. Values are means ± S.E.M. n = number of animals. [a] $P < 0.01$ when compared with the corresponding value for the native gland. [b] $P < 0.02$ and [c] $P < 0.001$ when compared with saline-injected rats.

with 35% after sodium nitroprusside administration (Table 1).

The basal islet blood flow of the pancreatico-duodenal graft in saline-treated rats was higher than native organ blood flow (Table 2), whereas the corresponding value for whole pancreatic blood flow did not attain statistical significance ($P > 0.10$). A marked decrease in whole pancreatic and islet blood flow in both the native and transplanted gland was observed after administration of N^G-nitro-L-arginine (Table 2). The decrease was more pronounced in the islets as evidenced by the decrease in the fractional islet blood flow, i.e. the fraction of whole pancreatic blood flow diverted through the islets, from 10% to ≈ 4% (Table 2). Sodium nitroprusside did not affect whole pancreatic or islet blood flow in either the native or transplanted pancreas when compared with saline-injected rats (Table 2).

4. Discussion

The hyperperfusion with blood seen in the islets of the transplanted pancreas compared with that of the native gland confirms previous findings for pancreatico-duodenal grafts in rats (Jansson et al., 1992). Hyperperfusion has also been observed in other transplanted organs in several species, and is likely to be due to the denervation caused by the surgical procedure (Perry et al., 1986; Henderson et al., 1989). Since no functional re-innervation is likely to occur in heterotopically implanted organs, it is plausible that the blood flow increase remains after implantation.

Administration of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine, caused a reduction of pancreatic blood flow in both the native and transplanted gland. In particular the islet blood flow was decreased, to values around 10 μl/min × g pancreas, resulting in a 50% reduction of fractional islet blood flow. It is unlikely that the changes in blood flows in the native and transplanted pancreas observed after administration of N^G-nitro-L-arginine are caused by the moderate increase in arterial blood pressure, since previous studies have demonstrated that elevation of the mean arterial blood pressure up to 130 mm Hg has no effects on the blood flow of the rat pancreas (Jansson and Sandler, 1985). Furthermore, we also consider it unlikely that the effects of N^G-nitro-L-arginine are due to a direct constrictive effect of the drug on vascular smooth muscle, in view of the dose and time interval used in the present study.

Administration of sodium nitroprusside caused a marked decrease in mean arterial blood pressure, below the level required for autoregulation of pancreatic blood perfusion (Kvietys et al., 1982). In previous studies we found that a 50% reduction in blood pressure, induced by hemorrhagic hypotension or hemodilution, caused a decrease to the same degree in both the whole pancreatic and the islet blood flow (Jansson, 1992; Hindlycke and Jansson, 1993). However, when blood pressure was reduced to ≈ 50 mm Hg in normal rats by administration of the NO donor, sodium nitroprusside, at twice the concentration used in the present study, only whole pancreatic blood flow was decreased, whereas islet blood flow remained unchanged (Jansson, 1992). With a lower dose of sodium nitroprusside, as in the present study, no decrease in either whole pancre-

Table 2
Pancreatic and islet blood flow in the native and transplanted (Tx) glands in inbred Wistar-Furth rats 2 weeks after a pancreatico-duodenal transplantation

Substance given	Saline ($n = 9$)		NNA ($n = 5$)		Nitroprusside ($n = 6$)	
Pancreas	Native	Tx	Native	Tx	Native	Tx
Pancreatic blood flow (μl/min × g)	370 ± 20	460 ± 70	170 ± 30 [c]	210 ± 30 [b]	380 ± 90	310 ± 70
Islet blood flow (μl/min × g pancreas)	28 ± 2 [d]	49 ± 7	8 ± 2 [c]	8 ± 2 [c]	40 ± 13	39 ± 11
Islet blood flow (% of pancreatic blood flow)	8.2 ± 0.8	10.3 ± 1.1	4.4 ± 1.4 [a]	4.2 ± 1.0 [b]	10.2 ± 1.0	11.7 ± 1.3

The animals were injected with either saline, N^G-nitro-L-arginine (NNA) or nitroprusside as described in Table 1. Values are means ± S.E.M. n = number of animals. [a] $P < 0.05$, [b] $P < 0.02$ and [c] $P < 0.001$, when compared with the corresponding value for saline-injected rats. [d] $P < 0.02$ when compared with the corresponding value for pancreatico-duodenal grafts.

atic or islet blood flow occurred. This can be assumed to be explained by a difference in sensitivity between endocrine and exocrine blood vessels to the excess amounts of NO generated by sodium nitroprusside (cf. below). Thus, when the mean arterial blood pressure is decreased barely below the level at which autoregulation of pancreatic and islet blood flow normally occurs (cf. Kvietys et al., 1982), as in the present study, the NO is able to overcome the constriction induced by the hypotension. However, when the decrease in mean arterial blood pressure is more marked (Jansson, 1992) only the islets, which might be more sensitive to NO, are able to maintain their blood perfusion.

The effects of N^G-nitro-L-arginine on whole pancreatic and islet blood flow in the native gland are in line with previous findings on pancreatic blood perfusion (Konturek et al., 1993; Svensson et al., 1994). It is conceivable that the regulation of islet blood flow takes place in afferent arterioles immediately before their entrance into the islets (Bonner-Weir and Orci, 1982). The present finding that N^G-nitro-L-arginine preferentially decreased islet blood flow, suggests that islets are more dependent on a continuous generation of NO to maintain the blood perfusion than is the exocrine pancreas, thereby supporting the findings with sodium nitroprusside (see above). The origin of the NO which may affect islet arterioles is presently not known, but it may be derived from an endothelial and/or a neuronal source. It is therefore of interest that N^G-nitro-L-arginine exerted the same effects in the native and transplanted rat pancreas, since the latter is likely to be deprived of external nerves. It can thus be assumed that, in the grafted pancreas, NO production is mainly inhibited at the endothelial cell level. The evidence for this notion is strengthened by the recent observation that islet endothelial cells contain large amounts of nitric oxide synthase (Suschek et al., 1994). It should be noted that N^G-nitro-L-arginine did not change the distribution of microspheres within the denervated perfused rat pancreas in an in vitro preparation (Jansson and Sandler, 1991). However, this preparation is perfused with buffers supplemented with dextran and albumin at a pressure < 50 mm Hg. It may be that the flow distribution in this low-pressure in vitro preparation is not as dependent on NO as the vasculature is in vivo.

It cannot be excluded that some intrinsic nervous elements containing NO, within the grafted pancreas and duodenum, which might have survived the transplantation procedure would influence the regulation of pancreatic blood flows. However, in preliminary immunocytochemical studies performed 2 weeks after transplantation, only a few remaining nerve fibers adjacent to the duodenum were found (Korsgren, Jansson, Ekblad and Sundler, unpublished observation). That other cells, e.g. macrophages or other leukocytes, are involved is less likely in view of their scarcity within the islets.

The present findings lend further support to the notion that the islet vasculature possesses mechanisms for blood flow regulation which are independent of that of the exocrine pancreas (cf. Jansson and Sandler, 1985, 1992; Jansson and Hellerström, 1986). Furthermore, the present results strongly argue for a role of the local, presumably endothelial, production of NO for the maintenance of islet blood flow.

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