Endothelin-1 and pancreatic islet vasculature: studies in vivo and on isolated, vascularly perfused pancreatic islets

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Submitted 23 November 2006; accepted in final form 31 January 2007

Endothelin (ET) is one endothelium-derived substance that mediates its effect mainly through ETA receptors, which mediate constrictive responses on vascular smooth muscle cells (VSMC), and ETB receptors, which mediate vasodilation when present on endothelial cells through release of NO but vasoconstriction on VSMC (8, 45). There is general agreement that ET-1, which binds to both of these receptors, induces a pronounced splanchic vasoconstriction through ETA receptors (13), whereas other vascular beds where ETB receptors dominate may react with dilation (13). Thus the functional response to ET-1 varies throughout tissues and vascular beds depending on differences in distribution and expression of ETA and ETB receptors (20, 45).

In vitro studies have demonstrated that ET-1 dose-dependently potentiates glucose-stimulated insulin release in mice (16) and that both ET-1 and ET-3 stimulate insulin release at normal glucose concentrations in rats (9). It has been suggested that these effects are caused by an induced glucagon release from the α-cells rather than a direct effect on β-cells (4). Furthermore, ET-1 is known to have marked effects on the pancreatic circulation in several species (39, 40, 48) and has been suggested to mediate some of the adverse vascular effects during acute pancreatitis (22, 41, 50). In view of these considerations, the aim of the present study was to evaluate whether, and to what extent, exogenously administered ET-1 affected pancreatic islet blood flow in rats and, if so, to determine which ET-receptor subtype was involved in such a response.

MATERIALS AND METHODS

Animals. A total of 62 adult male Sprague-Dawley rats from a local breeding colony (Biomedical Center, Uppsala University, Uppsala, Sweden) weighing ~325 g were used in the in vivo experiments. In the perfusions of isolated islets, we used a total of 35 male C57BL/6 mice weighing ~25 g (Scabur, Sollentuna, Sweden). All animals had free access to standardized food (Type R3; Ewos, Stockholm, Sweden) and tap water and were housed in a room with a 12:12-h light-dark cycle, a temperature of 23°C, and humidity of 70%. All experiments were approved by the local animal ethics committee and were conducted in accordance with accepted standards of humane animal care.

Drug administration. The rats were randomly allocated to groups, and some were treated with bolus intravenous injections according to one of the protocols given in Table 1. Briefly, the rats were injected 15 min before blood flow measurements with 0.5 ml of the vehicle [1% polyoxyethylene-hydrogenated castor oil 60 (HCO 60; Nikkol Chemicals, Tokyo, Japan) in saline] alone or with the following
blood flow (ml/min), $Q_{\text{ref}}$

organisms by applying the formula $Q_{\text{org}}$

with both bright- and dark-field illumination. Blood flow was calcu-

lating from previous evaluations of their effects on blood

pressure (32).

A separate group of animals (not included in Table 1) was instead
given an intravenous injection of 0.5 ml saline or ET-1 (5 nmol/kg) dissolved in saline 1 min before the blood flow measurements.

Blood flow measurements in rats. Blood flow was estimated with a

microsphere technique using nonradioactive microspheres with a
diameter of 10 μm (E-Z Trac, San Diego, CA), as previously de-

scribed in detail (25). Briefly, rats were anesthetized by an intraperi-
toneal injection of thiobutabarbital sodium (120 mg/kg; Inactin; Re-

search Biochemicals International, Natick, MA), placed on a heated

operating table, and tracheotomized. Polyethylene catheters were

inserted into the ascending aorta (via the right carotid artery), the left

femoral artery, and left femoral vein. The carotid catheter was con-

nected to a pressure transducer (PDCR 75/1; Druck, Groby, UK) to

monitor the mean arterial blood pressure throughout the experiment.

This catheter was also used for microsphere injections. The femoral

arterial catheter was used to obtain an arterial reference blood sample
during the microsphere injections, whereas the venous catheter was

used for saline infusions and to administer drugs. After animals were

prepared, we allowed 15–30 min for blood flow to stabilize to

minimize influence of surgical stress.

The rats were then injected with drugs according to one of the

protocols referred to above. A total of 0.3 ml of microspheres

(1.5–2.0 × 10⁵) was injected 1 or 15 min after administration of test

substances into the ascending aorta during 10 s. An arterial reference

blood sample (~0.4 ml) was collected from the femoral artery by free

draw flow during 1 min, starting 5 s before the microsphere injection. The

pancreas, adrenals, proximal part of duodenum, and distal part of

colon were removed, carefully dissected free from fat and lymph

nodes, weighed, and prepared for visualization of microspheres

with a technique described in detail elsewhere (24). Microspheres

in both the blood sample and organs were counted under a microscope

with both bright- and dark-field illumination. Blood flow was calcu-
lated on the basis of microsphere contents of reference sample and

organs by applying the formula $Q_{\text{org}} = Q_{\text{ref}} \times N_{\text{org}}/N_{\text{ref}}$, where

$Q_{\text{org}}$ = organ blood flow (ml/min), $Q_{\text{ref}}$ = flow of the reference sample (ml/min), $N_{\text{org}}$ = number of microspheres present in the organ, $N_{\text{ref}}$ = number of microspheres present in the reference sample. The number of microspheres present in the adrenal glands was counted to confirm adequate mixing of the microspheres with arterial blood. A difference of <10% was taken to indicate sufficient mixing.

Blood glucose and serum insulin concentrations in rats. Blood

samples for analysis of serum insulin and blood glucose concentra-
tions were obtained from the femoral artery, after collection of the

reference sample, and analyzed with ELISA (rat insulin ELISA;

Mercodia, Uppsala, Sweden) and test reagent strips (MeditSense,

Stockholm, Sweden), respectively.

Isolation and preparation of mouse islets for single islet perfusion.

Animals were killed by cervical dislocation, and the pancreas was
quickly removed and placed in cold (4°C) albumin-enriched (1%)
DMEM (Sigma-Aldrich, Stockholm, Sweden). Islets were dissected
with their arterioles intact (18) using a modification (30) of a previ-
ously described technique for renal glomeruli (37, 38). The time for
dissection was limited to 60 min, and most of the obtained islets were
large (diameters of 400–600 μm). The islets, with their attached
arterioles, were cut with miniblades and transferred into a chamber
on a stage of an inverted microscope. This experimental set-up allows
movement and adjustment of concentric holding and perfusion pip-
ettes (Luigs & Neumann, Ratingen, Germany). The perfusion system
(Vestavia Scientific, Vestavia Hills, AL) used manually produced pipettes from custom glass tubes (Drummond Scientific, Broomall, PA). A holding pipette was used to keep the islet in place while
another holding pipette, into which the ends of the arterioles were
aspirated, was put in place. The latter had an aperture of ~30 μm,
whereas the inner perfusion pipette, with an aperture of 5 μm, was
advanced into the lumen of the blood vessel.

Perfusion of single mouse islets. The technique used for perfusion
of single islets was adapted from that used for renal glomeruli (30). The perfusion pipette was connected to a manometer, and a reservoir containing the perfusion solution (Krebs-Ringer bicarbonate buffer with 10% HEPES and 1% BSA), 5.5 mM d-glucose, and additions as
given below. The flow was adjusted by pressure measurements,
aiming at 40 mmHg throughout the perfusion period, which corre-
sponds to a flow of ~40 nl/min. The end magnification (~300 times) and
analysis of data have been previously described in detail (30). The experimental set-up allowed us to measure the diameter of the blood vessels continuously and to record changes at a resolution of <0.2 μm.

Krebs-Ringer bicarbonate buffer with 10% HEPES and 1% BSA,
with pH adjusted to 7.4, was also used for the chamber in which the
islets were located. However, the concentration of BSA was only
0.1%. All buffers were exposed to air throughout the experiments.
Criteria for using an islet arteriole were remaining basal tone, no
pronounced vasodilation, and a fast and complete constriction in
response to administration of KCl solution (100 mM). Arterioles were
allowed to recover for 10 min after the KCl test.

In all series of experiments, the images from the last 10 s of each
control or treatment period were used for statistical analyses. Only one
concentration-response curve, with or without pretreatment with one
other drug, was obtained in each of the perfused islets.

Perfusion protocols for islets. Each experiment began with a
15-min equilibrium period with buffer containing 5.5 mM glucose in
both the bath and perfusion solution. The islets were then exposed to
ET-1 for a total of 14 min. Each concentration (10⁻⁷ to 10⁻¹² M) was
applied for 2 min, beginning with the lowest, either alone or in
combination with one of the ET-receptor blockers (BQ-123 or BQ-
RESULTS

The rats tolerated the surgical procedures well, with the exception of three animals that were excluded due to low mean arterial blood pressure (<80 mmHg). A further two animals were excluded from the study because the difference between left and right adrenal microsphere content was >10%.

**Blood glucose and insulin.** Blood glucose and insulin results are shown in Tables 2 and 3. ET-1 in itself caused a slight decrease ($P < 0.05$) in blood glucose concentration 1 min after administration ($5.3 \pm 0.2$ vs. $5.9 \pm 0.9$ mmol/l in ET-1 treated and control rats, respectively), but this decrement was gone after 5 min. Administration of BQ-123, either alone or in combination with BQ-788, caused a slight increase in blood glucose concentrations at the time of the blood flow measurements 15 min later. However, serum insulin concentrations were not influenced at any of the time points tested.

**Blood pressure.** Blood pressure results are shown in Tables 2 and 3. Neither ET-1 nor the receptor antagonists affected the hematocrit values of the treated animals (data not shown). Mean arterial blood pressure decreased 1 min after administration of ET-1. However, 5 min after administration, ET-1 caused a slight increase in mean arterial blood pressure. BQ-788, either alone or given together with BQ-123, caused a slight increase in mean arterial blood pressure when given in conjunction with ET-1. BQ-123 alone had no such effect in animals given ET-1. None of the receptor antagonists, by themselves or in combination, affected the blood pressure in saline-treated control rats.

Pancreatic and islet blood flow. Pancreatic and islet blood flow results are shown in Figs. 1–3. ET-1 did not influence the blood flow to any of the organs tested 1 min after administration (Table 3). However, 5 min after ET-1 administration, both total pancreatic and islet blood flow values were decreased irrespective of whether any of the ET-receptor antagonists were administered or not at the same time. Administration of BQ-788 alone or in combination with BQ-123 to saline-treated rats decreased both total pancreatic and islet blood flow. BQ-123 given alone to saline-treated rats had no effect ($P > 0.10$) on either pancreatic or islet blood perfusion. The effects on blood perfusion referred to above were more marked on the islet circulation as manifested by a more pronounced decrease in fractional islet blood flow after ET-1 administration. This was not influenced by either BQ-123 or BQ-788 (Fig. 3).

### Table 2. Endothelin effects 1 min after administration

<table>
<thead>
<tr>
<th>Substances given</th>
<th>Vehicle</th>
<th>BQ-123</th>
<th>BQ-788</th>
<th>BQ-123+BQ-788</th>
<th>Vehicle</th>
<th>BQ-123</th>
<th>BQ-788</th>
<th>BQ-123+BQ-788</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min before</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>328±8</td>
<td>316±14</td>
<td>334±6</td>
<td>337±7</td>
<td>314±9</td>
<td>326±6</td>
<td>324±2</td>
<td>329±10</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>6.2±0.1</td>
<td>6.3±0.3</td>
<td>6.6±0.2</td>
<td>6.4±0.3</td>
<td>5.7±0.3</td>
<td>7.4±0.9*</td>
<td>6.6±0.2</td>
<td>7.5±0.4*</td>
</tr>
<tr>
<td>Serum insulin, ng/ml</td>
<td>2.12±0.37</td>
<td>2.07±0.37</td>
<td>2.15±0.21</td>
<td>2.28±0.45</td>
<td>2.98±0.70</td>
<td>3.14±0.76</td>
<td>3.02±0.68</td>
<td>2.75±0.38</td>
</tr>
<tr>
<td>Mean arterial blood pressure before, mmHg</td>
<td>120±3</td>
<td>120±4</td>
<td>117±4</td>
<td>123±6</td>
<td>122±5</td>
<td>123±5</td>
<td>124±7</td>
<td>123±4</td>
</tr>
<tr>
<td>Mean arterial blood pressure after, mmHg</td>
<td>118±2</td>
<td>116±2</td>
<td>112±5</td>
<td>126±7</td>
<td>132±3§</td>
<td>126±7</td>
<td>135±2§</td>
<td>127±7</td>
</tr>
<tr>
<td>Duodenal blood flow, ml·min⁻¹·g⁻¹</td>
<td>1.58±0.18</td>
<td>1.05±0.08</td>
<td>0.86±0.13*</td>
<td>1.20±0.20</td>
<td>1.13±0.20</td>
<td>1.52±0.36</td>
<td>1.65±0.26§</td>
<td>1.40±0.31</td>
</tr>
<tr>
<td>Colonic blood flow, ml·min⁻¹·g⁻¹</td>
<td>0.44±0.10</td>
<td>0.63±0.13</td>
<td>0.49±0.11</td>
<td>0.47±0.09</td>
<td>0.64±0.09</td>
<td>0.51±0.21</td>
<td>1.21±0.25‡</td>
<td>0.65±0.19</td>
</tr>
<tr>
<td>Adrenal blood flow, ml·min⁻¹·g⁻¹</td>
<td>3.01±0.29</td>
<td>1.85±0.23*</td>
<td>1.94±0.19*</td>
<td>1.90±0.29*</td>
<td>1.00±0.11†</td>
<td>2.36±0.59</td>
<td>1.32±0.17</td>
<td>1.27±0.15‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Male Sprague-Dawley rats were injected intravenously with 0.5 ml of vehicle (1% oil in saline), BQ-123 or BQ-788 (0.1 mg/kg body wt) or BQ-123 and BQ-788 in combination 15 min before measurements. Animals were then given saline or ET-1 (5 nmol/kg) 10 min after antagonist administration, i.e., 5 min before blood flow measurements. *$P < 0.05$ and ‡$P < 0.01$ compared with animals treated with vehicle ± saline. §$P < 0.05$ compared with corresponding saline-treated group (ANOVA with Bonferroni’s correction). †$P < 0.05$ compared with value before administration of any of the test substances (Student’s paired $t$-test).

AJP-Endocrinol Metab • VOL 292 • JUNE 2007 • www.ajpendo.org
Perfusion of single mouse islets. A representative islet with its arteriole and supplying intralobular artery is shown in Fig. 4, A and B. When ET-1 was administered to single, isolated islets, a dose-dependent, very strong contraction of the islet arteriole was seen (Fig. 4, C and D, and Fig. 5B). The ET-1 receptor antagonists BQ-123 and BQ-788 when administered alone did not affect islet arteriolar diameter (Fig. 5A). When different concentrations of ET-1 were evaluated, we saw a significant islet arteriolar contraction at concentrations higher than $10^{-10}$ M (Fig. 5B). Simultaneous administration of BQ-788 changed the dose-response curve toward the right, and the responses to ET-1 at concentrations of $10^{-10}$ or $10^{-9}$ M were attenuated (Fig. 5B). However, when ET-1 was given at $10^{-8}$ or $10^{-7}$ M, no change in diameter was observed when the values were compared with those of islets given ET-1 alone (Fig. 5B). The ET-1 concentration of $10^{-7}$ M (Fig. 5B).

Other organ blood flow values. Duodenal blood flow was unaffected by ET-1 treatment. Administration of BQ-788 alone, but not in combination with BQ-123, decreased duodenal blood flow, whereas BQ-123 had no effects. BQ-788 given alone increased the colonic blood perfusion in rats given ET-1. The other two combinations had no effects.

ET-1 decreased adrenal blood flow in vehicle-treated rats, but this could be prevented by administration of BQ-123 alone, but not in combination with BQ-788. BQ-788 in itself had no effect on ET-1-treated rats. Both receptor antagonists, by themselves or in combination, decreased adrenal blood flow in saline-treated rats.

**DISCUSSION**

No changes were seen in blood flow values 1 min after ET-1 administration. Thus the initial reactions to ET-1, encompassing a slight decrease in mean arterial blood pressure (52), is not associated with any changes in blood perfusion in any of the studied splanchnic organs. The decrease in total pancreatic blood flow seen 5 min after ET-1 administration confirms several previous studies (39, 40, 48). Most of the ET-1-induced vasoconstrictive effects in the pancreas, and in the splanchnic circulation in general, have been suggested to be due to ET$_A$-receptor activation, presumably on VSMC (1, 3, 12, 41). Indeed, interference with ET-1 signaling has been shown to ameliorate some of the vascular consequences of experimental acute pancreatitis (22, 41, 50). Also, the blood perfusion of the islets was decreased by ET-1 administration, although more markedly than that of the remaining pancreas as evidenced by...
a decrease in fractional islet blood flow. Thus the islet vascular system seems to be more sensitive to the constrictive effects of exogenous ET-1 than the blood vessels in the exocrine pancreas. It should be noted that β-cells have an abundant expression of endothelin-converting enzyme (47) and are likely to produce ET-1 (36). Furthermore, ET<sub>A</sub> receptors are present on α-cells (4) and ET<sub>B</sub>-receptors on β- and δ-cells (51). Thus it seems likely that ET-1 may affect islet endocrine function through paracrine interactions. In line with this, ET-1 is known to stimulate insulin secretion (4, 9, 16), possibly through glucagonotropic effects (4). Whether endothelin-converting enzyme or ET receptors are present in islet endothelial cells or islet arteriolar VSMC is unknown, but the presently observed reduction in blood flow suggests that this is likely. It should be noted that the ET-1-induced islet blood flow decrease is very pronounced. The present findings are in line with previous observations that endothelium-derived vasoactive factors, such as ANG II (6) and NO (46), have profound effects on islet blood perfusion. Thus the islet blood perfusion seems to be very sensitive to the effects of locally produced vasoactive factors, and a very interesting field for future studies would be the interaction between the metabolic status of endocrine cells and the production of such factors (7, 35, 46).

To decide which ET receptors were responsible for the drastic changes in pancreatic and islet blood perfusion, we administered the selective ET<sub>A</sub>- and ET<sub>B</sub>-receptor inhibitors BQ-123 and BQ-788, respectively (8). In saline-treated control rats, BQ-123 affected neither total pancreatic nor islet blood flow, suggesting that locally produced endogenous ET-1 does not affect pancreatic circulation through ET<sub>A</sub> receptors during basal conditions in this model. However, the ET<sub>B</sub>-receptor inhibitor BQ-788 decreased both total pancreatic and islet blood flow in saline-treated control rats, when both given alone and in combination with BQ-123. This is likely to reflect the fact that ET<sub>B</sub> receptors are present mainly on endothelial cells and only to a limited extent on VSMC, in both the endocrine and exocrine compartments of the pancreas, where they participate in the formation of the vasodilators NO and prostacyclin (10, 45, 49). If so, partial inhibition of any of these substances induced by normal production of ET-1 could lead to a decrease in both flow values (35, 46). Another possibility is that ET<sub>B</sub> receptors also may function as a clearance receptor for ET-1 (45). This means that inhibition of this receptor may increase the availability of ET-1 to ET<sub>A</sub> receptors, which could facilitate a vasoconstriction.

A more surprising finding was that we were unable to block the vasoconstrictive effects of exogenous ET-1 in vivo with any of the inhibitors alone or in combination with one another. This would suggest that the vasoconstrictive effects in the pancreas and islets are unrelated to any of the known ET receptors. A third type of receptor with greater affinity for ET-3, so-called ET<sub>C</sub> receptors, has been cloned from Xenopus laevis (27). However, to date, no mammalian counterpart has been found (8), and it seems unlikely that it is involved in the present response. The blockers used are considered to be specific for their receptors (8) and do not interfere with one another when given simultaneously (28, 53). The presence of “atypical” ET-receptor subtypes have been reported, and it has been suggested that they may reflect the presence of ET<sub>A</sub>-ET<sub>B</sub> heterodimerism (45). The response of such receptors to the present blockers is as yet uncharacterized.

A more likely possibility for the observed in vivo findings is that exogenous ET-1 may directly interfere with other vasoactive substances, such as NO, prostaglandins, or ANG II. It is known that NO can displace ET-1 from its receptors on VSMC (14), to terminate the ET-1 response. Furthermore, interactions between ET-1 and the peptides of the angiotensin system have been described (43), although not to an extent that it can explain the findings of the present study. In view of the extreme sensitivity of the islet circulation to NO (35, 46) and ANG II (6), it may be that rather minor interactions induced by ET-1 in the pancreas may tip the balance over in favor of a vasoconstriction.

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Fig. 4. A: isolated islet with an afferent arteriole connected to an intralobular artery. B: section of the arteriole with side branches cut before start of perfusion. An arteriole is shown at higher magnification, both when perfused with buffer (C) and when perfused with buffer with ET-1 added at 10<sup>-7</sup> M (D).
which would be manifested as an increased perfusion pressure, thereby making the notion referred to above on impaired flow due to a permeability increase less likely. Somewhat more surprising, blocking of ETB receptors with BQ-788 induced a right shift of the dose-response curve for ET-1. Thus a partial prevention of vasoconstriction is provided at ET-1 concentrations of $10^{-9}$ and especially $10^{-10}$ M. ETB receptors are normally found on both endothelial cells, where they induce vasodilation via NO and prostaglandins, and VSMC, where they induce vasoconstriction (10, 45, 49). The present findings suggest that ETB receptors are mainly confined to arteriolar VSMC and not the endothelial cells, since vasoconstriction was prevented. If ETB receptors dominated on endothelial cells, a more pronounced constriction would be seen after administration of BQ-788, due to decreased formation of NO and/or prostacyclin.

In contrast to the findings in the pancreas, both duodenal and colonic blood flows were less affected by exogenous administration of ET-1. The most surprising finding was that ET-1 given in conjunction with inhibition of ETB receptors, either alone or in combination with ETA receptor inhibition, increased colonic blood flow. We are unable to explain this finding, but it may be due to a redistribution of blood within the splanchic vascular bed induced by the unopposed vascular constriction caused by ET-1 on ETA receptors.

The adrenal glands were sensitive to both BQ-123 and BQ-788, both of which decreased their blood flow. ET-1 markedly diminished adrenal blood flow, and this could be prevented by inhibition of ETA receptors with BQ-123. This is in accordance with previous findings where ET-1 has been suggested to participate in the regulation of adrenal blood flow (19).

The doses of ET-1, BQ-123, and BQ-788 used in vivo were chosen from previous evaluations in our laboratory (32) and induce only minor changes in systemic blood pressure or hematocrit and to minimize systemic effects. Furthermore, a recent study has demonstrated that these doses of BQ-123 and BQ-788 produce complete inhibition of the receptors (26), without any confounding effects of anesthesia (42). It is unlikely that any of the observed effects in the present study are caused by the minor changes in mean arterial blood pressure. In addition, there were no effects on serum insulin concentrations seen in any of the animals. The blood glucose concentrations, on the other hand, were slightly increased when ET-1 was given together with the ETA-receptor antagonist BQ-123. The reasons are unknown. The degree of hyperglycemia was such that it does not affect blood perfusion (23).

The present study demonstrated that the pancreatic islet vasculature was more sensitive to the vasoconstrictive effects of exogenous ET-1 than the exocrine pancreatic blood vessels. Furthermore, the islet blood flow seen after administration of ET-1 was extremely low, comparable only to those seen after total inhibition of NO formation (46). Separate antagonists against ETA or ETB receptors were not able to prevent the decrease in any of these ET-1-induced blood flow responses in vivo, neither alone nor in combination. However, in ex vivo perfusions of isolated islets, inhibition of ETA receptors prevented ET-1-induced vasoconstriction except at very high ET-1 concentrations. Furthermore, an ETB-receptor inhibitor induced a right shift in the dose-response curve to ET-1. This shows that islet arterioles possess both ETA and ETB receptors, which induce vasoconstriction (10, 45, 49). The present findings suggest that ETA receptors are mainly confined to arteriolar VSMC and not the endothelial cells, since vasoconstriction was prevented. If ETB receptors dominated on endothelial cells, a more pronounced constriction would be seen after administration of BQ-788, due to decreased formation of NO and/or prostacyclin.

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receptors and suggests that exogenously administered ET-1 interferes with other systems of vasoactive mediators in the pancreas in vivo.

ACKNOWLEDGMENTS

The skilled technical assistance of Astrid Nordin is gratefully acknowledged.

GRANTS

The study was supported by a generous Innovative Grant from the Juvenile Diabetes Research Foundation International and partially by grants from the Swedish Research Council (72X-109, 04-03522-32), the EFSD/MSD Diabetes Research Foundation International, and the Swedish Heart and Lung Foundation (20040645), the Wallenberg Foundation, the Family Ernors Fund, and the Ingabritt and Arne Lundberg Fund.

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