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Pancreatic islet blood flow is selectively enhanced by captopril, irbesartan and pravastatin, and suppressed by palmitate ☆

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Abstract

Diabetic patients are often treated with a lipid lowering statin and an ACE inhibitor or angiotensin receptor antagonist against hypertension or albuminuria. These drugs may also improve glucose tolerance, but the mechanism for this remains elusive. We now studied whether these drugs and the fatty acid palmitate influence insulin secretion *in vivo* in rats through effects on islet blood perfusion. Whole pancreatic blood flow was markedly increased by captopril and irbesartan, and decreased by palmitate. Islet blood flow was significantly and preferentially enhanced by captopril, irbesartan, and pravastatin, and suppressed by palmitate. Both captopril and irbesartan raised serum insulin concentrations significantly. However, glycemia was not affected in any group. In conclusion, the present study suggests that a local pancreatic RAS and pravastatin may be selectively controlling pancreatic islet blood flow and thereby influencing insulin secretion. The antidiabetic actions of statins and RAS inhibitors might in part occur through the beneficial direct islet effects shown here. Conversely, free fatty acids that are elevated in type 2 diabetic patients may contribute to an impaired nutritive islet blood flow and thereby further aggravate the diabetic state by limiting the supply of insulin needed to curb hyperglycemia.

Keywords: Islet; Blood flow; Microcirculation; Renin-angiotensin system; Statin; ACE inhibitor; Angiotensin receptor antagonist; Insulin; Diabetes

The systemic renin-angiotensin system (RAS) plays a crucial role in the regulation of arterial blood pressure. In the past few years, it has become increasingly clear that local RAS also exist in various tissues, implying that high local levels of angiotensin II (Ang II) might exert paracrine influences on neighboring cells [1,4–7]. In the pancreas of several species, mRNA encoding angiotensinogen and renin, as well as substantial levels of angiotensin II, have been detected [1,4–7]. Ang II has been shown to adversely influence pancreatic and islet blood flow through vasoconstractive effects [8,9]. Also, high affinity binding sites for Ang II were recently localized specifically to islet β-cells by double immunostaining and Ang II was found to block glucose-stimulated insulin secretion, an event fully reversible by losartan [5]. It is thus conceivable that pancreatic Ang II, locally produced by intrinsic RAS, may adversely influence insulin secretion *in vivo*, either directly by suppressing β-cell insulin exocytosis or indirectly through inhibitory effects on islet blood perfusion [5]. This may be of particular importance in diabetic patients since hypertension is markedly overrepresented in these individuals [2,3], and angiotensinogen expression seems to be upregulated by hypertension [2,3]. Hence, many diabetic patients are treated with ACE inhibitors or angiotensin receptor antagonists against their hypertension or as part of a renal protection strategy. An additional hallmark of diabetic cardiovascular risk is hyperlipidemia and elevated serum levels of free fatty acids; consequently many diabetic patients use lipid lowering drugs, most notably statins. Interestingly, both ACE inhibitors or angiotensin receptor antagonists and certain statins have been reported to decrease the risk

☆ *Abbreviations:* ABF, adrenal blood flow; ACE, angiotensin-converting enzyme; Ang II, angiotensin II; ELISA, enzyme-linked immunosorbent assay; IBF, islet blood flow; KBF, kidney blood flow; NO, nitric oxide; PBF, pancreatic blood flow; RAS, renin-angiotensin system.

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of developing diabetes in large clinical trials [2]. However, the mechanisms behind these antidiabetic effects remain elusive. In this paper, we aimed at evaluating the influence of ACE inhibition, angiotensin receptor antagonism, pravastatin treatment, and palmitate administration on pancreatic and islet blood flow, as well as on blood glucose and insulin concentrations, in the rat. We show that these vasoactive drugs that are frequently given to diabetic patients may directly stimulate the insulin-secreting β-cell by preferentially increasing islet blood flow and that palmitate may exert opposite effects.

Materials and methods

Animals and drugs. Male Wistar rats (ScanBur, Sollentuna, Sweden), weighing 300–350 g, were used in all experiments. The animals had free access to pelleted food (Type R34; ScanBur, Sollentuna, Sweden) and tap water at all times. All experiments were approved by the local Animal Ethics Committee at Uppsala University. Captopril and pravastatin were graciously donated by Bristol-Myers Squibb Company (New York, NY). Irbesartan was generously given by Sanofi-Synthelabo (Paris, France), whereas sodium palmitate was bought from Sigma–Aldrich (St. Louis, MO). Palmitate was administered in a 10% ethanol solution.

Blood flow measurements. The experiments were performed according to a protocol previously described in detail [16]. The animals were anesthetized with an intraperitoneal injection of thiobutabarbital sodium (120 mg/kg body weight; Inactin™, Research Biochemicals International, Natick, MA) and placed on a heated operating table to maintain body temperature. Polyethylene catheters were inserted into the ascending aorta, via the right common carotid artery, and into the left femoral artery. The catheter in the aorta was connected to a pressure transducer (model PDCR 75/1, Druck Ltd., Groby, Leicestershire, UK) to allow constant monitoring of the mean arterial blood pressure. After the blood pressure was stable, the animals were injected intravenously with 1 ml of saline, 1 ml of pravastatin (0.5 mg/kg), 1 ml of irbesartan (3 mg/kg) or 1 ml of captopril (3 mg/kg). All these substances were dissolved in saline. Ten minutes later, $1.5–2.0 \times 10^5$ non-radioactive microspheres (IMT, Stason Labs, Irvine, CA), with a mean diameter of 10 μm, were injected during 10 s via the catheter with its tip located in the ascending aorta. An arterial blood sample was collected from the catheter in the femoral arterial 5 s before the microsphere injection, and this process continued for a total of 60 s.

In a separate set of experiments (Fig. 4a–c), the effects of sodium palmitate on blood flow, serum insulin levels, and glycemia were investigated. This was done in a separate series, since the fatty acid had to be dissolved in 10% ethanol, and thus controls were given this solvent only. To this end, 1 ml of palmitate (60 mg/kg BW) or solvent was injected i.v. exactly as described for the other drugs above.

The exact withdrawal rate in each experiment was determined by weighing the sample. Additional arterial blood samples were obtained and later analyzed for hematocrit, blood glucose, and serum insulin concentrations (see below). After the animals were killed by cervical dislocation, the whole pancreas and both adrenal glands, as well as a 100-mg slice of the left kidney (including both cortex and medulla), were collected. The microsphere contents in these organs were determined separately. The organs were treated with a freeze-thawing technique [17], which enabled the visualization and localization of the microspheres from either the endocrine or the exocrine parenchyma of the pancreas. This was achieved by applying a microscope (Zeiss MB6; Leica AB, Stockholm, Sweden) equipped with both bright and dark field illumination. The former type of illumination allowed us to count the microspheres, whereas the latter enabled localization of the microspheres from either the endocrine or exocrine parenchyma [17]. The number of microspheres in the islets and exocrine tissue was counted as previously described in detail [17]. The microsphere contents of the adrenal glands were used as a control to confirm an even distribution of the microspheres in the arterial circulation. The microsphere content of each of the arterial reference samples was determined by transferring the samples to glass microfiber filters and counting the microspheres in a stereomicroscope.

The blood flow rates were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$, where Q_{org} denotes organ blood flow (mL/min), Q_{ref} denotes withdrawal rate of the reference sample (mL/min), N_{org} denotes the number of microspheres in the organ, and N_{ref} denotes the number of microspheres in the reference sample.

Measurement of glucose and insulin concentrations. Blood glucose concentrations were measured with test reagent strips (Medisense, Solna, Sweden) and serum insulin concentrations with ELISA kit (Rat Insulin ELISA, Mercodia, Uppsala, Sweden).

Statistical analysis. All values are given as means ± SEM. Statistical comparisons were made with two-way analysis of variance (ANOVA) (SigmaStat; SSPD, Erfart, Germany). A value of $p < 0.05$ was deemed statistically significant.

Results

Effects of captopril, irbesartan, and pravastatin on blood flow

Intravenous injection of captopril (3 mg/kg BW) and irbesartan (3 mg/kg BW) significantly enhanced PBF, whereas pravastatin (0.5 mg/kg BW) had no such effects (Fig. 1a). IBF was significantly (Fig. 1b) and preferentially (Fig. 1c) augmented by all three substances.

Renal blood flow was markedly increased after administration of irbesartan and captopril, whereas pravastatin had no effects (Fig. 2a).

Only captopril augmented adrenal blood flow significantly, whereas irbesartan and pravastatin failed to influence adrenal blood perfusion (Fig. 2b).

Blood glucose concentrations, serum insulin levels, and mean arterial blood pressure

Captopril and irbesartan increased serum insulin levels, whereas pravastatin failed to do so (Fig. 3a). There were no discernable differences in blood glucose concentrations between any of the treatment groups (Fig. 3b). No effects on mean arterial blood pressure (averaging 110 mmHg) were detected by any of the treatments given (data not shown).

Effects of palmitate on blood flow

As shown in Fig. 4a, i.v. injection of palmitate (60 mg/kg BW) significantly decreased PBF. As are evident from Fig. 4b and c, IBF was significantly (Fig. 4b) and preferentially (Fig. 4c) suppressed by palmitate. The fatty acid also decreased KBF, but did not significantly influence ABF (not shown). Palmitate affected neither mean arterial blood pressure, blood glucose nor serum insulin concentrations (data not shown).

Discussion

Type 2 diabetes is increasing in the Western world and is seen in ever-younger age groups [10]. We can expect this to