Gender-specific regulation of pancreatic islet blood flow, insulin levels and glycaemia in spontaneously diabetic Goto–Kakizaki rats

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ABSTRACT

Patients with diabetes are often treated with a statin for hyperlipidaemia and an ACE (angiotensin-converting enzyme) inhibitor or angiotensin receptor antagonist for hypertension or albuminuria. These drugs may also exert beneficial metabolic effects, causing improved glucose tolerance in patients. Gender-related differences have also been observed in the clinical responsiveness to these drugs, but the mechanism behind this is unclear. In the present study, we have investigated whether these drugs and the fatty acid palmitate influence the pancreatic microcirculation, thereby having an impact on insulin secretion and glycaemia in vivo, in spontaneously diabetic male and female Goto–Kakizaki rats. In male rats, pancreatic IBF (islet blood flow) and total PBF (pancreatic blood flow) were increased significantly by pravastatin, captopril and irbesartan. Serum insulin levels were increased by pravastatin and captopril. Palmitate suppressed pancreatic IBF and increased blood glucose. In female animals, pancreatic IBF was stimulated by captopril, candesartan and irbesartan. Total PBF was increased by captopril, candesartan and irbesartan, and by pravastatin. Palmitate suppressed pancreatic IBF and serum insulin secretion. In conclusion, the present study lends support to the view that a local pancreatic RAS (renin–angiotensin system) and pravastatin may be selectively influencing the pancreatic microcirculation and therefore affecting insulin secretion and glycaemia. NEFAs (non-esterified fatty acids) impaired pancreatic IBF, suppressed insulin secretion and increased blood glucose. Substantial gender-related differences in the vascular and metabolic responses to these drugs prevail in this animal model of diabetes.

INTRODUCTION

The RAS (renin–angiotensin system) has been extensively studied since the first identification of renin by Tigerstedt and Bergmann in 1898 [1] and plays an important role in regulating blood volume and systemic vascular resistance [2]. It has become increasingly clear that a local RAS exists in the pancreas and some other tissues [3–6]. AngII (angiotensin II), considered the main effective peptide of the RAS, has been shown to adversely influence total PBF (pancreatic blood flow) and pancreatic IBF (islet blood flow) through vasocontractive effects, thereby suppressing insulin secretion [7,8]. ACE (angiotensin-converting enzyme), another component of the RAS, has been detected in endothelial cells of the vascular wall in humans [9–11] and rats [12–15]. This may be of particular importance in patients with diabetes, as many of them are treated with ACE inhibitors or angiotensin receptor inhibitors.

Key words: angiotensin-converting enzyme, blood flow, diabetes, gender, pancreatic islet, statin.

Abbreviations: ABF, adrenal blood flow; ACE, angiotensin-converting enzyme; AngII, angiotensin II; BP, blood pressure; GK, Goto–Kakizaki; i.v., intravenously; IBF, islet blood flow; KBF, kidney blood flow; NEFA, non-esterified fatty acid; PBF, pancreatic blood flow; RAS, renin–angiotensin system.

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antagonists for hypertension or as part of a renal protection effort. Another salient feature in patients with diabetes is hyperlipidaemia and elevated serum levels of NEFAs (non-esterified fatty acids; ‘free’ fatty acids); therefore many patients with diabetes use lipid-lowering drugs, most notably statins. Interestingly, gender differences were observed in large clinical trials, with more pronounced negative effects of diabetes on the lipid profile and BP (blood pressure) in women compared with men [16,17], and also the vascular responses to statins and RAS-interfering drugs appear to differ between men and women [18,19]. Recently, we have shown the stimulatory effects of such drugs on pancreatic IBF in normal male [20] and female [21] Wistar rats. However, it is not known how these drugs influence islet microcirculation, insulin secretion and glucose tolerance in diabetic animals. In the present study, we investigated the gender-specific effects of ACE inhibition, AngII receptor antagonism, statin treatment and palmitate administration on pancreatic IBF, insulin levels and glycaemia in GK (Goto–Kakizaki) rats, a widely used animal model of human Type 2 diabetes [22].

**MATERIALS AND METHODS**

**Animals and drugs**

Male (300–350 g) and female (200–250 g) GK rats were obtained from our KISÖS breeding colony (Animal Department, Stockholm South Hospital, Stockholm, Sweden). The Stockholm GK rat colony was established at KISÖS in 2004 from breeding pairs generously donated by Professor Robert V. Farese (Gladstone Institute of Cardiovascular Disease, San Francisco, CA, U.S.A.). All animals had free access to pelleted food and tap water and were kept under standard conditions. The experiments were approved by the local ethics committee.

Pravastatin and captopril were kindly given by Bristol Myers Squibb. Irbesartan was generously given by Sanoﬁ-Aventis, and candesartan was generously donated by Astra Zeneca. Sodium palmitate was from Sigma–Aldrich and dissolved in 10 % (v/v) ethanol.

**Surgical preparation**

Rats were anaesthetized with an intraperitoneal injection of sodium thiobutabarbitonal (120 mg/kg of body weight; Inactin™; Research Biochemicals International), and then placed on a heated operating table in order to maintain body temperature. Catheters were inserted into the ascending aorta via the right 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) in order to constantly monitor mean arterial BP. After the mean arterial BP had remained stable for at least 20 min, the animals were injected i.v. (intravenously) with 1 ml of saline, 1 ml of pravastatin (0.5 mg/kg of body weight), 1 ml of captopril (3 mg/kg of body weight), 1 ml of candesartan (10 mg/kg of body weight) or 1 ml of irbesartan (3 mg/kg of body weight). Irbesartan was tested separately due to delayed delivery. All substances were dissolved in saline.

The palmitate experiments were done in a separate series because the fatty acid had to be dissolved in 10 % ethanol, and thus controls were given equal amounts of this solvent only. To this end, 1 ml of palmitate (60 mg/kg of body weight) or solvent was injected i.v. exactly as described above for the other agents.

**Blood flow measurements**

The procedure was performed according to a protocol described in detail previously [23]. Briefly, 10 min after the injection of test substances, approx. (1.5–2.0) × 10⁵ non-radioactive microspheres (IMT; Stason Labs), with a mean diameter of 10 μm, were injected into the ascending aorta over 10 s. Starting 5 s before microsphere injection and continuing for a total 60 s, an arterial blood reference sample was collected from the catheter in the femoral artery. The amount of blood was confirmed by weighing each reference sample. Additional arterial blood samples were collected at the end of the experiment (approx. 30 min after the surgical procedure was done) for measurement of blood glucose and serum insulin concentrations.

The animals were then 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 removed, blotted and weighed. The microsphere contents of the adrenals, part of the kidney, pancreatic islets and exocrine parenchyma were determined separately in frozen–thawed preparations [24]. The blood flow values were calculated according to the formula: \( Q_{\text{org}} = Q_{\text{ref}} \times N_{\text{org}}/N_{\text{ref}} \), where \( Q_{\text{org}} \) is organ blood flow (ml/min), \( Q_{\text{ref}} \) is the withdrawal rate of the reference sample (ml/min), \( N_{\text{org}} \) is the number of microspheres in the organ and \( N_{\text{ref}} \) is the number of microspheres in the reference sample. A difference of <10 % in microsphere content between the two adrenal glands was taken to confirm an even distribution of the microspheres in the arterial blood stream.

**Determination of blood glucose and insulin concentrations**

The blood glucose concentration was analysed with test strips based on the glucose oxidase method (Medisense), and serum insulin concentrations were measured with an ELISA kit (rat insulin ELISA; Mercodia).

**Statistical calculations**

All values are given as the means ± S.E.M. \( P \) values between experimental groups were analysed with
Figure 1 Enhancement of total PBF by pravastatin, captopril, candesartan and irbesartan in male and female GK rats
Following injection i.v. of pravastatin (0.5 mg/kg of body weight), captopril (3 mg/kg of body weight) or candesartan (10 mg/kg of body weight) into GK rats, rates of blood perfusion in the whole pancreas (a) were measured using a microsphere technique. Separate experiments with irbesartan (3 mg/kg of body weight) are shown in (b). Bars represent means ± S.E.M. for five independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with controls, as determined by ANOVA.

Figure 2 Enhancement of pancreatic IBF by pravastatin, captopril, candesartan and irbesartan in male and female GK rats
Following injection i.v. of pravastatin (0.5 mg/kg of body weight), captopril (3 mg/kg of body weight) or candesartan (10 mg/kg of body weight) into GK rats, rates of blood perfusion in pancreatic islets (a) were measured using a microsphere technique. Separate experiments with irbesartan (3 mg/kg of body weight) are shown in (b). Bars represent means ± S.E.M. for five independent experiments. *P < 0.05 and **P < 0.01 compared with controls, as determined by ANOVA.

RESULTS

Gender-specific effects of captopril, candesartan, pravastatin and irbesartan on blood flow
In male rats, captopril (3 mg/kg of body weight), pravastatin (0.5 mg/kg of body weight) and irbesartan (3 mg/kg of body weight) all significantly enhanced total PBF (Figure 1) and pancreatic IBF (Figure 2), whereas candesartan had no discernable effects (Figures 1a and 2a). KBF (kidney blood flow) was enhanced markedly by pravastatin (P < 0.01), candesartan (P < 0.05) and irbesartan (P < 0.05), but not significantly by captopril (Table 1). Pravastatin increased ABF (adrenal blood flow) significantly (P < 0.01), whereas captopril, candesartan and irbesartan failed to do so (Table 1).

In female rats, all test substances augmented total PBF markedly (Figure 1). Administration of captopril, candesartan and irbesartan induced an increase in pancreatic IBF (Figure 2), whereas pravastatin did not. KBF was markedly increased by pravastatin (P < 0.001), captopril (P < 0.001), candesartan (P < 0.001) and irbesartan (P < 0.01) (Table 1). Captopril and candesartan increased ABF significantly (both P < 0.01), whereas pravastatin and irbesartan had no such effects (Table 1).

Blood glucose concentrations, serum insulin levels and mean arterial BP
In male GK rats, pravastatin and captopril enhanced serum insulin levels (Table 2), whereas irbesartan and candesartan did not. The blood glucose concentration was unexpectedly increased by captopril (Table 2).

There were no significant differences in serum insulin levels in the female animals between any of the groups (Table 3). Surprisingly, however, pravastatin and captopril raised blood glucose slightly, which might be
Table 1: Effect of pravastatin, captopril, candesartan, irbesartan and palmitate on KBF and ABF in male and female GK rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Male rats</th>
<th>Female rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KBF (ml·min⁻¹·g⁻¹)</td>
<td>ABF (ml·min⁻¹·g⁻¹)</td>
</tr>
<tr>
<td>Control</td>
<td>9.4 ± 1.1</td>
<td>18.5 ± 1.7</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>30.5 ± 4.6**</td>
<td>39.1 ± 6.6**</td>
</tr>
<tr>
<td>Captopril</td>
<td>16.6 ± 4.8</td>
<td>19.9 ± 5.9</td>
</tr>
<tr>
<td>Candesartan</td>
<td>22.7 ± 6.7*</td>
<td>20.7 ± 6.8</td>
</tr>
<tr>
<td>Ethanol</td>
<td>7.75 ± 2.5</td>
<td>7.8 ± 1.9</td>
</tr>
<tr>
<td>Palmitate</td>
<td>6.7 ± 1.4</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>Control</td>
<td>9.1 ± 0.8</td>
<td>20.8 ± 0.9</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>25.3 ± 3.1*</td>
<td>36.9 ± 1.3</td>
</tr>
</tbody>
</table>

Table 2: Effect of pravastatin, captopril, candesartan, irbesartan and palmitate on blood glucose, serum insulin and BP in male GK rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood glucose (mmol/l)</th>
<th>Serum insulin (ng/ml)</th>
<th>BP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.7 ± 0.4</td>
<td>4.46 ± 0.4</td>
<td>101.6 ± 3.4</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>7.6 ± 0.6</td>
<td>7.19 ± 1.2*</td>
<td>101.6 ± 3.4</td>
</tr>
<tr>
<td>Captopril</td>
<td>10.9 ± 1.1**</td>
<td>8.89 ± 1.3**</td>
<td>108 ± 2.4</td>
</tr>
<tr>
<td>Candesartan</td>
<td>9.4 ± 1.4</td>
<td>5.35 ± 0.8</td>
<td>107 ± 2.7</td>
</tr>
<tr>
<td>Ethanol</td>
<td>8.2 ± 0.6</td>
<td>8.19 ± 1.9</td>
<td>110 ± 2.9</td>
</tr>
<tr>
<td>Palmitate</td>
<td>12.6 ± 1.9*</td>
<td>5.49 ± 0.9</td>
<td>115 ± 4.7</td>
</tr>
<tr>
<td>Control</td>
<td>8.26 ± 0.3</td>
<td>4.08 ± 0.1</td>
<td>95.6 ± 1.9</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>7.58 ± 0.6</td>
<td>4.22 ± 0.4</td>
<td>103.7 ± 3.7</td>
</tr>
</tbody>
</table>

Table 3: Effect of pravastatin, captopril, candesartan, irbesartan and palmitate on blood glucose, serum insulin and BP in female GK rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood glucose (mmol/l)</th>
<th>Serum insulin (ng/ml)</th>
<th>BP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.3 ± 0.6</td>
<td>4.05 ± 0.25</td>
<td>94.2 ± 3.5</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>11.2 ± 0.4**</td>
<td>3.09 ± 0.26</td>
<td>98.1 ± 1.3</td>
</tr>
<tr>
<td>Captopril</td>
<td>11.3 ± 0.6***</td>
<td>3.01 ± 0.09</td>
<td>102 ± 3.6</td>
</tr>
<tr>
<td>Candesartan</td>
<td>9.7 ± 11</td>
<td>3.14 ± 0.12</td>
<td>105 ± 2.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>9.3 ± 0.8</td>
<td>4.15 ± 0.43</td>
<td>109 ± 5.9</td>
</tr>
<tr>
<td>Palmitate</td>
<td>9.95 ± 0.5</td>
<td>3.1 ± 0.17*</td>
<td>110 ± 1.7</td>
</tr>
<tr>
<td>Control</td>
<td>9.16 ± 0.4</td>
<td>3.08 ± 0.1</td>
<td>95.1 ± 1</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>10.4 ± 0.6</td>
<td>3.1 ± 0.1</td>
<td>105.2 ± 6.1</td>
</tr>
</tbody>
</table>

Due to surgical stress, Irbesartan had no effects on serum insulin or blood glucose levels (Table 3). No effects were found on mean arterial BP by any of the treatments given (Tables 2 and 3).

Effects of palmitate on blood flow, insulin levels and glycaemia

Palmitate significantly decreased islet blood flow in both male and female rats (Figure 3), but had no significant effects on total PBF in either gender (results not shown). The reason for gender differences in basal pancreatic IBF (Figure 3) is unknown, but might be related to increased vascular sensitivity to ethanol in female rats (perhaps in part due to lower body weight than males). In male rats, palmitate markedly increased blood glucose, along with a non-significant decrease in serum insulin (Table 2). In female rats, palmitate significantly lowered the serum insulin concentration, but failed to have a significant impact on glycaemia in these animals (Table 3). In female rats only, palmitate evoked an increase in ABF, but did not influence KBF in either gender (Table 1).

DISCUSSION

Type 2 diabetes is increasing in prevalence globally and is seen in ever-younger age groups [25]. The disease...
is characterized not only by hyperglycaemia, but also by insulin resistance with attendant hypertension, dyslipidaemia and increased levels of circulating NEFAs. Most patients with diabetes are being treated with one or more antidiabetic drugs, a lipid-lowering statin and an ACE inhibitor or angiotensin receptor antagonist for hypertension and/or albuminuria. In clinical trials, it has been repeatedly observed that these kinds of drugs also influence the rate of incident diabetes [26], causing an improved glucose tolerance in patients. Consistent with this, we have shown previously that these drugs exert a substantial short-term impact on islet blood perfusion, insulin secretion and glucose tolerance in vivo in normal Wistar rats of both male and female gender [20,21]. To evaluate whether these drugs also influence islet microcirculation, insulin levels and glycaemia under diabetic conditions, in the present study we have investigated the effects of these drugs in spontaneously diabetic GK rats in both male and female groups.

Our findings reveal that irbesartan, an AngII receptor antagonist, and captopril, an ACE inhibitor, induced a robust increase in pancreatic IBF and total PBF in both male and female GK rats. This beneficial effect confirms our previous studies with the administration of these drugs to normal Wistar rats of both genders [20,21]. Captopril also significantly increased serum insulin concentrations in male GK rats. Previous studies have shown that locally produced AngII suppressed pancreatic IBF [4,27], lending support to the findings in our present study. Additionally, previous results also suggest that irbesartan has the capacity to enhance vasodilatation [4,27], which might be another mechanism contributing to our present findings.

Recently, another AngII receptor antagonist, candesartan, proved efficacious in reducing fibrosis in and around the islets and prevented the loss of endothelial cells in diabetic mouse islets by long-term treatment, suggesting that candesartan may partially prevent the deterioration of glucose tolerance by affording protection against progressive β-cell damage [28,29]. Our results reveal that candesartan increased pancreatic IBF and total PBF significantly only in female GK rats, but not in male animals. This gender difference was not observed for irbesartan or captopril. The underlying mechanism behind this finding remains elusive at this point, but gender-related differences in the responsiveness to RAS-interfering drugs have long been observed. A large body of evidence has been obtained in animals indicating that components of the RAS are significantly influenced by gender at the tissue level [30–37], whereas clinical studies in humans have predominantly evaluated gender effects on circulating, i.e. systemic, RAS [38–45]. The characterization of an oestrogen-responsive element in the 5′-flanking region of the angiotensinogen gene was an important early finding to prove an interaction with sex hormones and the RAS at the molecular level [34,46]. More recent studies indicate that renin is suppressed by oestrogen [44]. Collectively, these studies are in line with the observation that plasma renin levels are lower in women compared with men [44,47]. In addition, AT1 receptors (AngII type 1 receptors) may also be down-regulated by oestrogen, whereas oestrogen deficiency leads to the up-regulation of this receptor [36]. In female transgenic rats with an activated RAS, it was shown that oestrogen treatment may afford protection against hypertension, possibly by amplifying the vasodilator contributions of Ang-(1–7), while reducing the formation and vasoconstrictor actions of AngII [48]. Conceivably, any of these mechanisms (or other) could contribute to our present findings of gender-related differences in this system.

Pravastatin, an HMG-CoA (3-hydroxy-3-methyl-lutaryl-CoA) reductase inhibitor, is clinically used in order to reduce the cholesterol level and thereby prevent dyslipidaemia. Our present study shows a preferential increase in total PBF and pancreatic IBF, as well as a significantly increased serum insulin level, in male GK rats following pravastatin administration. In contrast, in female animals, total PBF was enhanced but not pancreatic IBF. In our previous studies in female non-diabetic Wistar rats, pravastatin actually increased both pancreatic IBF and total PBF [21]. The reason for this difference is currently unknown; however, with increasing age, persistent hyperglycaemia may induce islet hypoperfusion in GK rats [49]. Islet blood hypoperfusion is accompanied by islet capillary hypertension. Such shear stress changes are known to change the gene expression in surrounding cells [49], possibly having an impact on islet function. Dyslipidaemia, a salient feature in Type 2 diabetes, is known to impair endothelium-mediated vasodilatation [50,51]. Pravastatin has beneficial anti-inflammatory effects on endothelial function [52–55].
and, therefore, may significantly influence selective tissue perfusion.

When making comparisons with the diabetes-preventive actions of pravastatin and RAS-interfering drugs noted in clinical trials [56], it should be kept in mind that our present results reflect only very acute effects of the substances tested. Although studies on long-term effects of such drugs on pancreatic IBF are scarce [6,28], this interesting issue is worthy of further investigation. However, it is beyond the scope of the present study, whose aim was to monitor short-term actions.

Type 2 diabetes is commonly associated with elevated serum NEFAs, due to insulin resistance and increased lipolysis [25,27,56,57]. NEFAs are generally believed to be involved in endothelial dysfunction and vascular damage in Type 2 diabetes [25,27,56,57]. Our previous study [20] showed that palmitate preferentially suppressed pancreatic IBF in non-diabetic male Wistar rats. In the present study, palmitate decreased both total PBF and pancreatic IBF in both male and female GK rats. This might be one mechanism by which NEFAs may have a negative impact on β-cell function, i.e. through impeding nutritive pancreatic IBF and, thereby, worsening further the diabetic state by limiting the supply of insulin needed to curb hyperglycaemia. Our results in this respect are at odds with many previous findings that acute effects of saturated fatty acids on glucose-induced insulin secretion are normally positive [58], both in vitro and in vivo, and we have no obvious explanation for this apparent discrepancy which deserves further investigation in future work.

In summary, several vasoactive drugs that are frequently given to patients with diabetes and reportedly may improve their glucose tolerance, selectively enhanced pancreatic IBF in this animal model of Type 2 diabetes. Gender-related differences in the vascular responses to these drugs were also observed in this system. In addition, NEFAs may aggravate hyperglycaemia in diabetes by limiting pancreatic IBF.

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