Vasoactive drugs enhance pancreatic islet blood flow, augment insulin secretion and improve glucose tolerance in female rats

Zhen HUANG*, Leif JANSSON† and Åke SJÖHOLM*

*Department of Internal Medicine, Karolinska Institutet, Stockholm South Hospital, SE 118 83 Stockholm, Sweden, and †Department of Medical Cell Biology, University of Uppsala, SE-751 23 Uppsala, Sweden

ABSTRACT

Pravastatin, irbesartan and captopril are frequently used in the treatment of patients with Type 2 diabetes. These drugs also exert beneficial metabolic effects, causing an improved glucose tolerance in patients, but the precise mechanisms by which this is achieved remain elusive. To this end, we have studied whether these drugs influence insulin secretion in vivo through effects on islet blood perfusion. Captopril (3 mg/kg of body weight), irbesartan (3 mg/kg of body weight) and pravastatin (0.5 mg/kg of body weight) were injected intravenously into anaesthetized female Wistar rats. Blood flow rates were determined by a microsphere technique. Blood glucose concentrations were measured with test reagent strips and serum insulin concentrations were measured by ELISA. Pancreatic blood flow was markedly increased by pravastatin (P < 0.001), captopril (P < 0.05) and irbesartan (P < 0.01). Pancreatic islet blood flow was significantly and preferentially enhanced after the administration of captopril (P < 0.01), irbesartan (P < 0.01) and pravastatin (P < 0.001). Kidney blood flow was enhanced significantly by pravastatin (P < 0.01), irbesartan (P < 0.05) and captopril (P < 0.01). Captopril and pravastatin also enhanced late-phase insulin secretion and positively influenced glycaemia in intraperitoneal glucose tolerance tests. In conclusion, the present study suggests that a local pancreatic renin–angiotensin system and pravastatin treatment may be selectively controlling pancreatic islet blood flow, augmenting insulin secretion and thereby improving glucose tolerance. Our findings indicate significant gender-related differences in the vascular response to these agents. Since statins and renin–angiotensin system inhibitors are frequently used by diabetic patients, the antidiabetic actions of these drugs reported previously might occur, in part, through the beneficial direct islet effects shown in the present study.

INTRODUCTION

Type 2 diabetes is increasing in the Western world and is seen in ever-younger age groups. The disease is characterized not only by hyperglycaemia, but also by insulin resistance with attendant dyslipidaemia, hypertension and endothelial dysfunction. These aberrations may result in cardiovascular and renal disease, and have an adverse impact on the prognosis of diabetic patients in terms of longevity and quality of life. Gender differences also exist, with more pronounced negative effects of diabetes on the lipid profile and BP (blood pressure) in women compared with men [1,2]. The diabetic state may also impede the cardioprotective actions normally
conferred by oestrogen [3]. To improve the prognosis of diabetic patients, several risk factors other than glycaemia (e.g. hypertension, albuminuria, dyslipidaemia etc.) need to be treated as well. Diabetic patients whose risk factor profile is well controlled are thus commonly being treated with one, or more, antidiabetic drug, a lipid-lowering statin and an ACE (angiotensin-converting enzyme) inhibitor or Ang II (angiotensin II) receptor antagonist against hypertension and albuminuria.

The systemic RAS (renin–angiotensin system) plays a crucial role in the regulation of arterial BP. Over the past few years, it has become increasingly clear that a local RAS also exists in various tissues, implying that high local levels of Ang II might exert paracrine influences on neighbouring cells [4]. In the pancreas of several species, mRNA encoding angiotensigen and renin, as well as substantial levels of Ang II, have been detected [4]. Ang II has been shown to adversely influence PBF and IBF (pancreatic and islet blood flow respectively) through vasoconstrictive effects [4].

Interestingly, both ACE inhibitors or Ang II receptor antagonists and certain statins have been reported to decrease the risk of developing diabetes in large clinical trials [5]. However, the mechanisms behind these antidiabetic effects remain elusive. We have shown recently the stimulatory effects of these drugs on pancreatic IBF in male rats [6]. In the present study, we have investigated the influence of ACE inhibition (captopril), Ang II receptor antagonism (irbesartan) and statin (pravastatin) treatment on PBF and IBF, as well as on glycaemia and insulin concentrations in normal female rats.

MATERIALS AND METHODS

Animals and drugs

Female Wistar rats (ScanBur), weighing 300–500 g, were used in all experiments. The animals had access to pelleted food (Type R34; ScanBur) and tap water ad libitum. All experiments were approved by the local Animal Ethics Committee at Uppsala University. Captopril and pravastatin were generously given by Bristol Myers Squibb. Irbesartan was generously given by Sanofi-Synthelabo.

Blood flow measurements

The experiments were performed according to a protocol described in detail previously [7]. Animals were anaesthetized with an intraperitoneal injection of sodium thiobutabarbital (120 mg/kg of body weight; Inactin™; Research Biochemicals International), 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) to allow constant monitoring of the MABP (mean arterial BP). After BP was stable, the animals were injected intravenously with 1 ml of saline, 1 ml of pravastatin (0.5 mg/kg of body weight), 1 ml of irbesartan (3 mg/kg of body weight) or 1 ml of captopril (3 mg/kg of body weight). All of these substances were dissolved in saline. After 10 min, (1.5–2.0) × 10^5 non-radioactive microspheres (IMT; Stason Labs), 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.

The exact withdrawal rate in each experiment was determined by weighing the sample. Additional arterial blood samples were obtained and analysed later for haematocrit, 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 [8], which enabled the visualization and localization of the microspheres from either the endocrine or the exocrine parenchyma of the pancreas. This was achieved by using a microscope (Zeiss MB6; Leica) equipped with both bright- and dark-field illumination. Bright-field illumination allowed the microspheres to be counted, whereas the dark-field illumination enabled localization of the microspheres from either the endocrine or exocrine parenchyma [8]. The number of microspheres in the islets and exocrine tissue was counted as described in detail previously [8]. 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 microfibre filters and counting the microspheres on a stereomicroscope.

The blood flow rates 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.

IPGTT (intraperitoneal glucose tolerance test)

An IPGTT was performed simultaneously with the blood flow measurements. The animals were injected intraperitoneally with a 30% (w/v) d-glucose (2 g of glucose/kg of body weight). Blood samples were drawn from the tail vein immediately before and at 10, 30, 60,
and 120 min after glucose administration. Area under the curve for the IPGTT was determined by computerized image analysis.

**Measurement of glucose and insulin concentrations**

Blood glucose concentrations were measured with test reagent strips (Medisense; Solna) based on the glucose oxidase method, and serum insulin concentrations were monitored with an ELISA kit (rat insulin ELISA; Mercodia). The insulin ELISA had a 98% recovery upon addition. Coefficients of variation were 3.4% within assay, 2.2% between assays and 3.9% in total assay.

**Statistical analysis**

All values are means ± S.E.M. for the number of experiments given. Each experiment represents one animal, and experiments were performed on different days. Statistical comparisons were made with ANOVA (SigmaStat; SSPD) in conjunction with Bonferroni’s post-hoc test. \( P < 0.05 \) was deemed statistically significant.

**RESULTS**

**Effects of captopril, irbesartan and pravastatin on blood flow in female rats**

Intravenous injection of pravastatin (0.5 mg/kg of body weight), captopril (3 mg/kg of body weight) and irbesartan (3 mg/kg of body weight) significantly enhanced PBF (Figure 1A), the action of the statin being the most pronounced. IBF was also significantly augmented by all three substances (Figure 1B). Only irbesartan and captopril preferentially increased IBF, i.e. relatively more than PBF (IBF as a percentage of PBF; Figure 1C). KBF (kidney blood flow) was also substantially increased after administration of all three agents (Figure 2A). Irbesartan and pravastatin augmented ABF (adrenal blood flow) significantly, whereas captopril did not (Figure 2B). Interestingly and quite unexpectedly, the vascular response to pravastatin was more pronounced than that occurring after treatment with agents interfering with RAS in all of the organs tested. Additionally, this effect occurred exclusively in female rats and not in our male rats studied previously [6]. This indicates significant vasoactive actions of pravastatin that have hitherto not received much attention, but that may be involved in the anti-atherogenic effects of the statin. This finding may also have implications for gender-specific differences in the vasoactive responses to these drugs described previously [9,10].

**Blood glucose concentrations, serum insulin levels and MABP**

None of the treatments significantly influenced non-stimulated serum insulin levels (Figure 3A). Blood glucose concentrations also did not differ between any of the treatment groups in the non-stimulated state (Figure 3B). In contrast, during an IPGTT, late-phase insulin secretion was substantially augmented (Figure 3A) and glucose tolerance was significantly improved (Figure 3B) in animals treated with captopril or pravastatin. Separate calculations of the total amount of

© 2007 The Biochemical Society
Following intravenous injection of captopril (3 mg/kg of body weight), irbesartan (3 mg/kg of body weight) or pravastatin (0.5 mg/kg of body weight) into healthy female rats, rates of blood perfusion in the kidneys (A) and adrenals (B) were measured using a microsphere technique. Values are means ± S.E.M. for eight independent experiments. *P < 0.05 and **P < 0.01 compared with controls, as determined by ANOVA.

Figure 2 Enhance of KBF and ABF by captopril, irbesartan and pravastatin
Following intravenous injection of captopril (3 mg/kg of body weight), irbesartan (3 mg/kg of body weight) or pravastatin (0.5 mg/kg of body weight) into healthy female rats, rates of blood perfusion in the kidneys (A) and adrenals (B) were measured using a microsphere technique. Values are means ± S.E.M. for eight independent experiments. *P < 0.05 and **P < 0.01 compared with controls, as determined by ANOVA.

insulin secreted during the 120 min IPGTT (area under the curve) revealed that insulin secretion was significantly higher in rats treated with pravastatin (P < 0.01) or captopril (P < 0.001) than in control rats receiving solvent only (Figure 3C). As shown in Figure 3(D), there was also a trend towards a decrease in post-load glycaemia, expressed as the area under the curve, although this difference did not reach statistical significance. No effects on MABP (mean, 110 mmHg) were detected following any of the treatments (results not shown).

DISCUSSION

Type 2 diabetes is increasing in the Western world and is seen in ever-younger age groups [11]. We can expect this to lead to momentous public health problems, especially in the form of premature cardiovascular morbidity. In terms of quantity, the most important complications of Type 2 diabetes are macro-angiopathies, i.e. myocardial infarction and stroke, which cause some 70% of the deaths related to Type 2 diabetes [11,12]. In contrast with micro-angiopathies (e.g. nephropathy and retinopathy), where the causal relation to hyperglycaemia is well supported, the link between hyperglycaemia and macro-angiopathy is uncertain, at least in terms of the possibility of reducing macrovascular morbidity solely by reducing hyperglycaemia [12]. To improve prognosis of diabetic patients, several other risk factors [e.g. hypertension, albuminuria (a biomarker of generalized endothelial dysfunction), dyslipidaemia etc.] need to be treated as well [12]. The disease is characterized not only by hyperglycaemia, but also by insulin resistance with attendant dyslipidaemia and increased levels of circulating NEFAs (non-esterified fatty acids). Diabetic patients whose risk factor profile is well controlled are thus being treated with one, or more, antidiabetic drug, a lipid-lowering statin and an ACE inhibitor or Ang II receptor antagonist against hypertension and albuminuria. These drugs also exert beneficial metabolic effects, causing an improved glucose tolerance in patients, but the precise nature of the mechanisms by which this is achieved remains elusive [5]. We have now studied whether these drugs influence islet blood perfusion, glycaemia and insulin levels *in vivo* in female rats.

Our present study shows a substantial increase in PBF, pancreatic IBF, KBF and ABF following pravastatin treatment. Along with these beneficial effects, by preferentially increasing IGF, insulin secretion was augmented (preferentially late-phase secretion) and glycaemia was improved following an IPGTT. Future work, involving diabetic animals, will reveal whether stimulation of IGF by similar means is sufficient to reverse experimental hyperglycaemia. The antithrombotic and anti-inflammatory effects of pravastatin may play a role in enhancing endothelium-dependent vasodilation [13], lending support to our present findings of increased blood flow after pravastatin administration. Conversely, dyslipidaemia, a salient feature in Type 2 diabetes, is known to impair endothelium-mediated vasodilation [14], and impaired endothelial function has been shown to result in diminished capillary recruitment [15]. Pravastatin has known beneficial effects on endothelial function [16–18]; therefore pravastatin may significantly enhance selective tissue perfusion by restoring endothelial function. Beyond simply lowering cholesterol, it also exerts beneficial antithrombotic actions by inhibiting platelet aggregation and promoting local NO synthesis [19–21]. Thus it is possible, albeit not proven, that increased local NO formation might be involved in the salutary effects of pravastatin observed in the present study. There appear to be important differences between different statins in this regard, as pravastatin is in a class of its own in terms of preventing diabetes [5,22,23]. Pravastatin is not metabolized by hepatic CYP450 (cytochrome p450) enzymes, shows very little
Islet blood flow regulation

Figure 3  Captopril and pravastatin augment insulin secretion and improve glycaemia during an IPGTT
Following an intravenous injection of captopril (3 mg/kg of body weight) or pravastatin (0.5 mg/kg of body weight) into healthy female rats, serum insulin concentrations (A) and blood glucose levels (B) were measured by ELISA and test reagent strips respectively, during a simultaneous IPGTT over 120 min. Total amount of insulin secreted (C) and blood glucose (D) during the 120 min IPGTT was calculated as the area under the curve (AUC). Values are means ± S.E.M. for eight independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with controls, as determined by ANOVA.

binding to proteins and is markedly hydrophilic. Whether these characteristics or other attributes, such as anti-inflammatory actions, underlie the anti-diabetogenic effect of pravastatin remains to be shown. Other mechanisms are conceivable, for instance direct effects on the endocrine pancreas. In vitro studies have shown that lipophilic statins (simvastatin) inhibit glucose-stimulated insulin secretion by blocking voltage-gated L-type Ca\(^{2+}\) channels in insulin-secreting β-cells, whereas pravastatin has no such adverse effect [24]. Furthermore, pravastatin can prevent inflammation and rejection of transplanted islets of Langerhans [25], all actions that would contribute to improved glucose tolerance.

We recently reported that administration of pravastatin in male Wistar rats increased only pancreatic IBF [6]. Our present results indicate qualitative and quantitative gender-specific differences between male and female rats in terms of vascular responses to pravastatin and RAS-interfering agents, which may be due to the vasoactive features of oestrogen or other influences conferred by female gender. For instance, while in male rats captopril was the most potent stimulus of IBF [6], pravastatin
evoked the strongest stimulation of IBF in the female rats in the present study (Figure 1B). Likewise, captopril was a stronger stimulator of IBF in male than in female rats compared with irbesartan. Additionally, non-stimulated serum insulin levels were heightened by irbesartan and captopril in male rats [6], but not influenced by these drugs in the present study in female rats. It is becoming increasingly appreciated that gender differences exist in terms of susceptibility to and mortality from a variety of vascular diseases [10,26,27]. Oestrogen is known to increase NO production primarily through up-regulation of eNOS (endothelial NO synthase) gene expression [28]; also a gender difference exists in basal NO release by rabbit aorta [29,30]. Alternatively, it is possible that oestrogen may function by up-regulating eNOS and/or enhancing production of eNOS-derived NO [31–33], thereby producing a vasodilatory effect [27].

The systemic RAS plays a crucial role in the regulation of arterial BP. Over the past few years, it has become increasingly clear that a local RAS also exists in various tissues, implying that high local levels of Ang II might exert paracrine influences on neighbouring cells [34–38]. In the pancreas of several species, mRNA encoding angiotensinogen and renin, as well as substantial levels of Ang II, have been detected [34–38]. Ang II has been shown to adversely influence PBF and IBF through vasoconstrictive effects [39,40]. Also, high-affinity binding sites for Ang II were recently localized specifically to islet β-cells by double immunostaining [35], and Ang II was found to block glucose-stimulated insulin secretion, an event fully reversible by losartan [39,40]. 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 [35,39,40]. This may be of particular importance in diabetic patients, since hypertension is markedly over-represented in these individuals [5,23], and angiotensinogen expression appears to be up-regulated by hypertension [5,23]. Hence many diabetic patients are treated with ACE inhibitors or Ang II receptor antagonists for hypertension or as part of a renal protection strategy. It has become clear that oestrogen can influence RAS [41–47] and that this effect is mediated by down-regulating renin, ACE and Ang II receptor expression [48]. An interesting review has reported that gender and sex hormones affect the components of RAS by multifarious mechanisms [9]. In general, oestrogen inhibits Ang II action and has been shown to attenuate vascular contractility [49]. Irbesartan is an Ang II receptor antagonist, characterized by high selectivity and insurmountable blockade of the AT1 (type 1 Ang II) receptor. In our present study, intravenous injection of irbesartan or the ACE inhibitor captopril induced robust increases in blood flow not only in the pancreas and pancreatic islets, but also (as expected) in the kidney. PBF was preferentially diverted to the endocrine part, i.e. the islets. This can be explained by a local RAS operative in the pancreas [4]. Such a system enables local production of Ang II, yielding Ang II concentrations much higher than those encountered in peripheral blood. Some observations have shown that IBF can be suppressed by locally produced Ang II [12,35], lending support to our present findings. Evidence also suggests that certain Ang II receptor antagonists have the capacity to enhance vascular vasodilation [12,35], which might be another mechanism contributing to our current findings. Additionally, it has been demonstrated recently [50] that the Ang II receptor antagonist losartan selectively improved glucose-induced insulin release and insulin biosynthesis in islets from spontaneously diabetic mice. Oral losartan treatment delayed the onset of diabetes and improved glucose tolerance in the diabetic mice, but did not affect the insulin sensitivity of peripheral tissues, suggesting that Ang II receptor antagonism improves β-cell function and glycaemia in young Type 2 diabetic mice. It was also shown recently [51] that another potent Ang II receptor antagonist, candesartan, when given orally over several weeks increased β-cell mass, increased staining intensity of insulin and decreased oxidative stress markers in β-cells. Candesartan treatment also reduced fibrosis in and around the islets and prevented the loss of endothelial cells in islets, indicating that candesartan partially prevents deterioration of glucose tolerance by providing protection against progressive β-cell damage in diabetic mice.

In conclusion, we have shown that vasoactive drugs that are frequently given to diabetic patients and improve their glucose tolerance have beneficial effects by enhancing pancreatic IBF, augmenting insulin secretion and improving glycaemia. Moreover, our findings indicate qualitative gender differences in the vascular response to these drugs, which might be related to the ability of oestrogen to produce vasodilation. The results may prove useful in tailoring gender-specific treatment strategies that improve islet function, contributing to an improved glycaemic control in diabetic patients or in subjects at risk of developing glucose intolerance.

ACKNOWLEDGMENTS

The skilled technical assistance of Birgitta Bodin and Astrid Nordin is gratefully acknowledged. Financial support was provided through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and the Karolinska Institute. Financial support was also given by the Swedish Research Council (grants 72X-109, 72X-12550, 72X-14507, 72X-11564, 72P-12995, 72X-00034, 72X-09890, 72XS-12708, 72P-14787 and 12P-10151), EFSD
(European Foundation for the Study of Diabetes)/Novo Nordisk Programme in Type 2 Diabetes, the Swedish Diabetes Association, the Novo Nordic Research Fund, the Family Ermfors Fund, Petrus and Augusta Hedlund’s Foundation, the Swedish Society of Medicine, Trygg-Hansa’s Research Foundation, the Janne Elgqvist Family Foundation, the Sigurd and Elsa Golje Memorial Foundation, Svenska Försäkringsförening, Svenska Diabetesföreningen, Åke Wiberg’s Foundation, Torsten and Ragnar Söderberg’s Foundations, Berth von Kantzow’s Foundation, Harald Jeansson’s and Harald and Greta Jeansson’s Foundations, Tore Nilsson’s Foundation for Medical Research, Fredrik and Inger Thuring’s Foundation, and Syskonen Svensson’s Fund.

REFERENCES


