Possible interactions between angiotensin II and insulin: effects on glucose and lipid metabolism in vivo and in vitro

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Abstract

Angiotensin II (ANGII) increases insulin sensitivity in diabetic and non-diabetic subjects, even at subpressor doses, and because there is ‘crosstalk’ between ANGII and insulin-signaling pathways the underlying mechanism may not be due solely to changes in regional blood flow. A series of experimental studies was undertaken to evaluate the effects of ANGII on glucose and lipid metabolism in vivo and in vitro. Groups of fructose-fed, insulin-resistant Sprague–Dawley (SD) rats were pre-treated with 0.3 mg/kg per day of the AT\(_1\)-receptor antagonist L-158 809 (\(n=16\)), or vehicle (\(n=16\)), by oral gavage. This was prior to an oral glucose tolerance test (day 5) and measurement of the effects of ANGII infusion (20 ng/kg per min i.v. for 3 h) on whole-body insulin sensitivity using the insulin suppression test (day 7). The effect of ANGII infusion on total triglyceride secretion rate (TGSR) was evaluated in normal SD rats pretreated for 7 days with L-158 809 (\(n=12\)) or vehicle (\(n=12\)). AT\(_1\)- and AT\(_2\)-receptor mRNA expression and \([\text{3H}]\)-deoxyglucose uptake were assessed in cultured L6 myoblasts. Short-term treatment with L-158 809 had no effect on glucose tolerance or fasting triglyceride levels in fructose-fed rats pretreated with vehicle (steady-state plasma glucose (SSPG) values 8.1 ± 1.6 vs 8.4 ± 0.4 mmol/l), but pretreatment with L-158 809 resulted in ANGII having a modest insulin antagonist effect on TGSR (e.g. 24.6 ± 1.4 vs 28.4 ± 0.9 mg/100 g per h in vehicle-treated animals). RT-PCR analysis showed that L6 cells express both AT\(_1\)- and AT\(_2\)-receptor mRNA. Incubation with ANGII (10\(^{-9}\) and 10\(^{-8}\) M) had no significant effect on the dose–response curve for insulin-stimulated \([\text{3H}]\)-deoxyglucose uptake. For example, C\(_{1200}\) values (dose of insulin required to increase glucose uptake by 200%) were 4.5 × 10\(^{-9}\) M (control) vs 3.9 × 10\(^{-9}\) M and 6.2 × 10\(^{-9}\) M, whereas the positive control (glucagon-like peptide-1) increased insulin sensitivity. Thus, ANGII infusion may have a modest insulin antagonist effect on glucose disposal in insulin-resistant fructose-fed rats pretreated with an AT\(_1\)-blocker, but ANGII has no effect on TGSR or in vitro glucose uptake in L6 myoblasts. These findings are relevant to recent clinical discussions about the metabolic effects of ANGII and renin–angiotensin system blockade.


Introduction

Activation of the circulating endocrine renin–angiotensin system (RAS), as well as the tissue-based autocrine/paracrine RAS, plays an important role in the haemodynamic abnormalities associated with insulin-resistant states such as diabetes, heart failure and hypertension. However, potentially important interactions between the RAS and pathways of glucose and lipid metabolism have been less clearly defined. Previous studies from our own group and others have shown that i.v. infusion of angiotensin II (ANGII) increases insulin sensitivity in normal volunteers (Townsend & DiPette 1993, Buchanan et al. 1993, Fliser et al. 1993) and patients with type II diabetes (Morris et al. 1994). Increases of 20–40% in whole-body insulin-mediated glucose disposal, assessed using the euglycaemic hyperinsulinaemic clamp, were primarily due to an increase in peripheral (i.e. muscle/fat) insulin sensitivity, since ANGII had no effect on hepatic glucose production (Buchanan et al. 1993). However, the underlying mechanism for this remains unclear (Morris & Donnelly 1996).
It has been suggested that the insulin-sensitizing effect of ANGII may be secondary to differential vasoconstriction in different vascular beds, which results in a redistribution of blood flow away from organs such as the kidney and a net increase in perfusion of insulin-sensitive tissues such as skeletal muscle (Buchanan et al. 1993). In support of this blood flow hypothesis, Townsend & DiPette (1993) showed that ANGII produces parallel dose-dependent increases in blood pressure (BP) and insulin-stimulated glucose transport and oxidation in skeletal muscle of healthy volunteers. However, several studies have shown that ANGII exerts metabolic effects even at subpressor doses. For example, Morris et al. (1994) found that a low-dose ANGII infusion (2.5 ng/kg per min) had no effect on arterial BP, but increased insulin sensitivity by 25% in patients with type II diabetes. Buchanan et al. (1993) found that a subpressor dose of ANGII increased insulin-stimulated glucose disposal in normal volunteers.

Thus, although changes in regional blood flow undoubtedly occur at higher doses of ANGII, it remains unclear whether ANGII has additional, direct effects on insulin-mediated pathways of glucose and lipid metabolism, perhaps via AT1 or AT2 receptors on skeletal muscle. It is also unclear whether the metabolic responses to ANGII are different in insulin-sensitive versus insulin-resistant states (Morris & Donnelly 1996). The local tissue-based RAS plays a vital role in adipocyte differentiation (Karlsson et al. 1998) and recent studies have shown significant ‘crosstalk’ between intracellular pathways of ANGII and insulin signaling in muscle, especially at the level of the insulin receptor substrate (IRS) proteins (Folli et al. 1999).

This series of experiments was designed to extend earlier observations and to investigate possible interactions between ANGII (via AT1 and AT2 receptors) and insulin-mediated pathways of glucose and lipid metabolism both in vivo and in vitro.

Materials & Methods

Animals

Male Sprague–Dawley (SD) rats were obtained from The Combined University Laboratory Animal supply (NSW, Australia) at 8 weeks of age and housed under controlled conditions of temperature (21°C) and lighting (12 h light–darkness cycle, lights on 0600 h) with free access to water and food. Normal rats were fed standard laboratory chow throughout the study (Young Stock Feed, NSW, Australia) while fructose–fed animals were switched to a pelleted, high-fructose diet containing 66% fructose, 22% protein and 12% fat (TD78463, Teklad Diets, Madison, WI, USA). The fructose-fed SD rat is a well established non-obese model of dietary-induced insulin resistance characterised by decreased insulin-mediated peripheral glucose uptake, hypertriglyceridaemia and hypertension (Tobey et al. 1982). All experiments involving animals were approved by the University of Sydney Animal Care and Ethics Committee.

Effects of oral AT1-receptor antagonist and i.v. ANGII in fructose-fed SD rats

Normal SD rats (n=32) initially weighing 180–200 g were switched to a pelleted high-fructose diet for 2 weeks. Thereafter, they were divided into 2 groups and given the AT1-receptor antagonist L-158 809 at 0.3 mg/kg per day (Sieg et al. 1992) (Merck Laboratories, NJ, USA) (n=16), or vehicle (2% carboxymethylcellulose) (n=16), by once-daily oral gavage at 0830 h. The fructose diet and treatment with L-158 809 (or vehicle) were continued for the remainder of the study (Fig. 1). Measurements of oral glucose tolerance (OGTT) were performed prior to starting treatment with L-158 809 and after 5 days dosing, and on day 7 in vivo insulin sensitivity was measured in groups of L-158 809- and vehicle-treated rats with and without co-infusion of i.v. ANGII at 20 ng/kg per min (Sigma, Poole, UK) (Fig. 1).

Oral glucose tolerance

OGTTs were performed in fructose-fed rats prior to starting treatment with L-158 809 or vehicle, and after 5 days. On each occasion, following an overnight fast, conscious animals were administered oral glucose at 2 g/kg by gavage. Tail-vein blood samples were collected prior to giving glucose, and at 30, 60, 90 and 120 mins following the glucose challenge for measurement of serum glucose and insulin concentrations. The scheduled daily dose of L-158 809 or vehicle was administered 2 h prior to the second OGTT.

Insulin suppression test

The acute effects of i.v. ANGII infusion (20 ng/kg per min) on in vivo insulin sensitivity were assessed in groups of fructose-fed SD rats pre-treated with L-158 809 or vehicle (Fig. 1). Insulin sensitivity was evaluated using a constant-rate infusion of soluble insulin (4 mU/kg per min), glucose (11 mg/kg per min) and somatostatin (300 pmol/kg per min) in 1% bovine serum albumin, 140 mM NaCl and 5 mM KCl, as described previously (Mondon & Reaven 1988). ANGII 20 ng/kg/min or normal saline was co-infused with the insulin–glucose–somatostatin mixture in groups of animals pretreated with the AT1 blocker or vehicle.

Animals were fasted overnight and anaesthetised (xylazine 10 mg/kg and ketamine 50 mg/kg i.p., Sigma) prior to giving glucose.
to the insertion of a cannula in the right jugular vein. The combined infusion was administered at a rate of 1 ml/h for 180 min using a Harvard infusion pump, and blood samples were collected from the tip of the tail at 0, 160, 170 and 180 min to calculate steady-state plasma glucose and insulin concentrations (Mondon & Reaven 1988).

Effects of oral AT₁-receptor antagonist and i.v. ANGII on triglyceride secretion rate

Groups of normal chow-fed SD rats were treated for 7 days with 0·3 mg/kg per day of the AT₁-receptor antagonist L-158 809 (n=12), or vehicle (n=12), by oral gavage. This was prior to measurements of total triglyceride secretion rate (TGSR) during acute i.v. infusion of ANGII at 20 ng/kg per min for 120 min.

Total TGSR was measured in L-158 809- and vehicle-treated SD rats according to the method of Otway & Robinson (1967a, b), as previously described in detail by our group (Donnelly et al. 1994). In brief, TGSR is proportional to the rate of increase in plasma triglyceride (TG) concentrations 2 h after injection of Triton WR1339 (Sigma), which inhibits lipoprotein lipase-mediated TG clearance. Animals were anaesthetised and a cannula inserted into the right jugular vein. Triton WR1339 (300 mg/ml) was injected into the proximal tail vein in a dose of 800 mg/kg, and a constant i.v. infusion of 20 ng/kg per min ANGII (or normal saline) was administered through the jugular venous cannula for 120 min.

Effects of AT₁-receptor antagonist and ANGII on insulin-stimulated [³H]2-deoxyglucose uptake in L6 myoblasts

2-Deoxyglucose uptake was measured in cultured L6 myoblasts, a rat skeletal muscle-derived insulin-responsive cell line (Walker et al. 1989), as described previously (Yamasaki et al. 1996). Dose–response curves were constructed for insulin-stimulated glucose uptake and C₁₂₀₀ values (concentration of insulin required to produce a 200% increase in glucose uptake) derived for each experiment. Cells were exposed to insulin and ANGII for 24 h. Incubation of L6 cells with 100 nM glucagon-like peptide-1 (GLP-1) was used as a positive control (Yang et al. 1998).

RT-PCR analysis of AT₁- and AT₂-receptor mRNA in L6 cells

The expression of AT₁- and AT₂-receptor mRNA in L6 cells was investigated using polymerase chain reaction (PCR) amplification of reverse-transcribed (RT) cDNA, as described previously (Paul et al. 1993). In brief, total RNA was extracted from L6 myoblasts using the method of Chomczynski & Sacchi (1987) and reverse transcribed prior to PCR amplification. For amplification of AT₁-cDNA the antisense primer was GCCCTGTCCACAATATCTGC (extending from base 1108 to base 1127) and the sense primer was ACCTGAGCATATTTCTCGGC (base 682 to base 700).
703) and the sense primer was TGAGTCCGATTT AACTGC (base 227 to base 245). The PCR conditions were as described by Paul et al. (1993).

Biochemical assays

Serum glucose and TG levels were measured using enzymatic calorimetric methods (Trinder glucose kit and GPO-Trinder triglyceride kit, Sigma).

Statistical analysis

Biochemical measurements, including SSPG values and the area-under-curve (AUC) for glucose concentration–time profiles after the OGGT, were compared between treatments by two-way analysis of variance. Dose–response curves for insulin-stimulated [3H]2-deoxyglucose uptake in L6 cells were fitted to a quadratic function to derive CI200 values, which were compared between treatments by analysis of variance. Results throughout are presented as mean ± s.e.m.

Results

Effects of short-term oral treatment with L-158 809 and acute i.v. infusion of ANGII at 20 ng/kg per min (3 h) on oral glucose tolerance (AUCglu), insulin sensitivity (SSPG) and total triglyceride secretion rate (TGSR)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OGGT (AUCglu)</th>
<th>SSPG (mmol/l)</th>
<th>TGSR (mg/100 g body weight per h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-158 809</td>
<td>22.3 ± 0.4</td>
<td>9.6 ± 0.3*</td>
<td>24.0 ± 2.0</td>
</tr>
<tr>
<td>Days</td>
<td>20.1 ± 0.4</td>
<td>7.1 ± 0.6</td>
<td>20.7 ± 1.4</td>
</tr>
<tr>
<td>ANGII Control</td>
<td>21.9 ± 0.5</td>
<td>8.1 ± 1.6</td>
<td>24.6 ± 1.4</td>
</tr>
<tr>
<td>Control</td>
<td>19.2 ± 0.5</td>
<td>8.4 ± 0.4</td>
<td>28.4 ± 0.9</td>
</tr>
</tbody>
</table>

*P<0.03.

Effects of oral AT1-receptor antagonist and i.v. ANGII on total TGSR in normal rats

Treatment with the AT1 antagonist had no effect on fasting serum TG levels, and ANGII infusion had no significant effect on total TGSR, in normal SD rats pretreated with vehicle or L-158 809 (Table 1).

Effects of ANGII on [3H]2-deoxyglucose uptake in L6 myoblasts

 RT-PCR analysis was used to confirm that both AT1- and AT2-receptor mRNA are expressed in L6 cells (Fig. 2). ANGII at two different doses (10⁻⁹ and 10⁻⁸ M) had no direct effect on insulin-stimulated glucose uptake (Fig. 3), whereas a positive control, 100 nM GLP-1, increased insulin sensitivity (Fig. 4) as reported previously (Yang et al. 1998). CI200 values derived from the fitted insulin dose–response curves for individual experiments were similar for ANGII-treated and control cells: 4·5 × 10⁻⁹ M (control), 3·9 × 10⁻⁹ M (ANGII 10⁻⁹ M) and 6·2 × 10⁻⁹ M (ANGII 10⁻⁸ M).

Discussion

Clinical and experimental studies have shown that a functional RAS exists in both muscle and fat (Dzau 1987, Jonsson et al. 1994). In adipose tissue, activation of the local RAS plays an important role in adipocyte differentiation (Karlsson et al. 1998) and may contribute to the impaired lipolysis observed in insulin-resistant states (Hennes et al. 1996). However, the extent to which components of the RAS affect other metabolic pathways, especially in skeletal muscle, has been the subject of recent debate. That patients with a clustering of cardiovascular risk factors tend to have increased RAS activity suggests...
that metabolic as well as haemodynamic manifestations of Syndrome X may be influenced by local release of hormones such as ANGII (Egan 1994).

Evidence that ANGII increases whole-body insulin sensitivity independently of BP (Buchanan et al. 1993, Morris et al. 1994) first raised the possibility of a direct biochemical effect of ANGII on insulin-mediated glucose metabolism (Morris & Donnelly 1996). Recent studies have extended this discussion by showing that ‘crosstalk’ between ANGII and insulin-signalling pathways occurs at the level of IRS proteins (Folli et al. 1999). The present study has provided new information using in vitro and in vivo experimental techniques. In the fructose-fed SD rat – a well established rodent model of Syndrome X characterised by peripheral insulin resistance, dyslipidaemia and hypertension (Tobey et al. 1982) – short-term treatment with an AT₁-receptor antagonist had no effect on oral glucose tolerance (all of the classical effects of ANGII are mediated by the AT₁ receptor). Similarly, acute i.v. infusion of ANGII for 3 h had no effect on whole-body insulin sensitivity in untreated animals. However, ANGII had a modest insulin antagonist effect in fructose-fed rats pretreated with L-158 809.

The biological significance of these results is unclear and requires cautious interpretation. There is evidence of ‘crosstalk’ between the two receptor subtypes such that AT₁-receptor blockade can modify the response to AT₂-receptor stimulation (Searles & Harrison 1999). Recent
work has shown that ANGII has an inhibitory effect on insulin-stimulated phosphoinositide-3’-kinase (PI-3K) activity, which is blocked by saralasin (an AT\textsubscript{1}- and AT\textsubscript{2}-receptor antagonist) but not losartan (a selective AT\textsubscript{1}-antagonist) (Folli \textit{et al.} 1999). Thus, one tentative suggestion is that treatment with L-158 809 may have unmasked an inhibitory, AT\textsubscript{2}-receptor-mediated effect of ANGII on insulin-stimulated glucose uptake in this rodent model of dietary-induced insulin resistance, e.g. via ANGII-induced serine phosphorylation of insulin-receptor-substrate 1 (IRS1) and/or the insulin receptor itself (Folli \textit{et al.} 1999). Alternatively, since AT\textsubscript{1}-receptor blockade in the rat results in sympathetic activation (Takishita \textit{et al.} 1994), augmentation of catecholamine effects by ANGII could also account for the further reduction in insulin sensitivity in rats pretreated with L-158 809.

Dyslipidaemia, especially an increase in triglyceride levels with reduced HDL-cholesterol, is a characteristic feature of insulin-resistant states due to increased lipolysis and the resultant increase in circulating free fatty acid levels fuelling TG synthesis. The effects of ANGII on lipid metabolism have received much less attention, although it has been shown that RAS activation may contribute to the reduced anti-lipolytic effect of insulin in obese hypertensive subjects (Hennes \textit{et al.} 1996). The present study showed that neither ANGII infusion nor L-158 809 had any effect on TGSR.

To further explore possible interactions between ANGII and insulin, \textit{in vitro} studies were undertaken using L6 myoblasts, a skeletal muscle-derived cell line. It was established that L6 cells express both AT\textsubscript{1}- and AT\textsubscript{2}-receptor mRNA, but incubating the cells with ANGII had no significant effect on the dose–response curve for insulin-mediated [\textsuperscript{3}H]-2 deoxyglucose uptake. Neither insulin sensitivity nor insulin responsiveness was affected by ANGII, whereas GLP-1 increased insulin sensitivity in this cellular model, as reported previously (Yang \textit{et al.} 1998). This negative result is particularly relevant to previous discussions in which it has been suggested that ANGII may have direct biochemical effects on glucose uptake and metabolism in skeletal muscle (Morris & Donnelly 1996). ANGII stimulates glycogenolysis in isolated hepatocytes (DeWitt & Putney 1983) even though there is no effect on hepatic glucose production in clinical studies (Buchanan \textit{et al.} 1993), but in the present experiments there was no evidence of a direct insulin-sensitizing effect in skeletal muscle cells.

The results of these experiments are perhaps indirectly relevant to the interpretation of recent clinical studies showing that diabetic patients treated with angiotensin-converting enzyme (ACE) inhibitors are more prone to developing hypoglycaemia (Herings \textit{et al.} 1995), and the incidental finding in the HOPE and CAPP studies that ACE inhibitor therapy might even protect against the development of type II diabetes (HOPE investigators 2000, Hansson \textit{et al.} 1999). Since the present study shows a neutral effect of ANGII on insulin sensitivity, the ability of ACE inhibitors to increase kinin levels seems a more likely explanation for the clinical observations. Recent work has shown that bradykinin increases glucose uptake in skeletal muscle and L6 myoblasts (Miyata \textit{et al.} 1998).

In conclusion, the metabolic effects of ANGII and RAS blockade have been highlighted in a number of recent clinical studies. The major findings in this study were that ANGII has no direct insulin-sensitizing effect in cultured L6 cells, nor any effect on TGSR \textit{in vitro} but, in a rodent model of dietary-induced insulin resistance and dyslipidaemia (resembling Syndrome X in humans), ANGII had a modest insulin antagonist effect in animals pretreated with an AT\textsubscript{1}-receptor antagonist.

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