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Acute angiotensin II receptor blockade improves insulin-induced microvascular function in hypertensive individuals

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ABSTRACT

Objective: An effect of insulin that is crucial for stimulating glucose uptake is its ability to increase the number of perfused capillaries, and thereby enhance its own delivery, and that of glucose, to muscle cells. To unravel possible mechanisms involved in the insulin-sensitizing effects of angiotensin II receptor blockers (ARBs) in hypertensive individuals we investigated the effect of single-dose ARB administration on insulin-mediated microvascular perfusion in hypertensive individuals.

Methods: We examined the effects of ARB administration on hyperinsulinemia-associated capillary density by measuring baseline skin capillary density, capillary density during reactive hyperemia (hyperemic capillary recruitment), and capillary density during venous congestion in 17 hypertensive individuals in the basal state, during a hyperinsulinemic euglycemic clamp, and during a hyperinsulinemic clamp with acute ARB administration (600 mg irbesartan), acute calcium channel blockade (CCB; 10 mg felodipine ER), as a control for the reduction in blood pressure, or placebo. In addition, insulin sensitivity and blood pressure were measured.

Results: Compared to the basal state, hyperinsulinemia increased baseline capillary density (57.3 ± 6.8 vs. 60.3 ± 7.9 n/mm^2, $P<0.01$), but not hyperemic capillary recruitment. ARB and CCB treatment induced similar blood pressure reductions. Compared to placebo, ARB, but not CCB, increased hyperinsulinemia-associated baseline capillary density ($+2.3 \pm 3.4$ ($P=0.02$) and -0.4 ± 4.4 n/mm^2, respectively). Hyperinsulinemia-associated hyperemic capillary recruitment was not altered by either treatment. Compared to placebo, neither ARB nor CCB treatment enhanced insulin sensitivity.

Conclusions: Acute ARB administration increases insulin-induced microvascular perfusion in mildly hypertensive individuals; this beneficial effect on microvascular perfusion was however not associated with increased insulin-mediated glucose uptake.

Introduction

A growing number of clinical trials have demonstrated that antihypertensive treatment with drugs that inhibit the renin–angiotensin system (RAS) reduces the risk for developing type 2 diabetes in hypertensive individuals (Andraws and Brown, 2007; Elliott and Meyer, 2007). The antidiabetogenic action of angiotensin II receptor blockers (ARBs) has been largely attributed to improvements in peripheral insulin sensitivity (Aksnes et al., 2006; Fogari et al., 2008, 2009; Furuhashi et al., 2003; Paolisso et al., 1997). However, although several potential mechanisms have been proposed for these insulin-sensitizing effects of ARBs (e.g. adipose tissue modification (Lee et al., 2008), reduced sympathetic activity (Aksnes et al., 2008), activation of peroxisome proliferator-activated receptor gamma (Benndorf et al., 2006), and reduced oxidative stress) (Zhou et al., 2009) so far, the exact mechanisms, at molecular and organ systems levels, have not been fully explained.

It has become clear that, in addition to muscle cells, the vasculature is an important physiological target for insulin (Barrett et al., 2009; Clark, 2008). Insulin acts at key sites in the vasculature of muscle to increase the number of perfused capillaries. This insulin-induced increase in microvascular perfusion is mediated via activation of the phosphatidylinositol 3-kinase (PI3-kinase)/Akt pathway and the production of nitric oxide (NO) in the vascular endothelium (Eringa et al., 2002), and has

Abbreviations: ARB, angiotensin II receptor blocker; CCB, calcium channel blocker; RAS, renin–angiotensin system; PI3-kinase, phosphatidylinositol 3-kinase; NO, nitric oxide; ABPM, ambulatory blood pressure measurement; M value, whole body glucose uptake; M/I value, insulin sensitivity; PRH, postocclusive reactive hyperemia; ROS, reactive oxygen species.

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been shown to be a significant regulator of overall insulin-mediated glucose uptake (Newman et al., 2009; Vincent et al., 2003). Since there is substantial evidence for a physiologically relevant crosstalk between the insulin and RAS signaling pathways (Velloso et al., 1996; Wei et al., 2007), we hypothesized that an improvement in the ability of insulin to increase the number of perfused capillaries may be a possible mechanism contributing to the metabolic benefits of ARBs.

Skeletal muscle precapillary arterioles are a major site of RAS action in vivo (Chai et al., 2010) and *in vitro* studies have repeatedly shown that angiotensin II (AngII) impairs vascular insulin signaling and reduces insulin-stimulated NO production (Andreozzi et al., 2004; Wei et al., 2007). In addition, *ex vivo* studies demonstrated impaired vascular insulin PI3-kinase/Akt signaling and impaired insulin-stimulated NO-dependent vasodilation (Li et al., 2010; Potenza et al., 2005; Zhou et al., 2009) in different animal models of hypertension. Therefore, we specifically hypothesized that chronic overactivity of the RAS in hypertension leads to an impaired action of insulin to increase the number of capillaries perfused (i.e. microvascular insulin resistance) and consequently to the development of metabolic insulin resistance. Conversely, we hypothesized that ARB treatment will improve the capillary responses to insulin and, subsequently, insulin-mediated glucose uptake in hypertensive individuals. To date, the effects of ARBs on microvascular perfusion during hyperinsulinemia have not been studied.

Therefore, in the present study we examined the effects of single-dose ARB administration on insulin-induced microvascular perfusion and insulin-stimulated glucose uptake in hypertensive individuals.

Subjects and methods

Subjects

Seventeen non-obese (body mass index <30 kg/m^2) individuals with essential hypertension participated in this study. All subjects were Caucasian, non-smokers, non-diabetic, as defined by fasting plasma glucose levels <7.0 mmol/l (Anon, 2003), and had no signs or symptoms of cardiovascular or other concomitant disease. Fifteen subjects were taking antihypertensive medication at the time of inclusion. Six of these were treated with an ARB alone ($n=1$), or in combination with a β-blocker ($n=1$), a calcium channel blocker (CCB; $n=2$), a thiazide diuretic ($n=1$), or a β-blocker with a CCB ($n=1$); seven subjects were treated with angiotensin-converting enzyme inhibitors alone ($n=2$), or in combination with a thiazide diuretic ($n=3$), or a CCB with ($n=1$), or without a diuretic ($n=1$); two subjects were treated with CCBs in combination with a β-blocker ($n=1$) or in combination with a diuretic ($n=1$). All antihypertensive medication was discontinued three weeks before the studies. Thereafter all subjects underwent a 24-h ambulatory blood pressure measurement (ABPM). The average daytime blood pressure levels of all subjects were >135/85 mm Hg. None of the participants used any medication during the studies. All participants gave informed consent for the study. The study was approved by the institutional review committee and performed in accordance with the Declaration of Helsinki.

Study design

All individuals were allocated to three interventions in random order according to a double-blind design, i.e. a euglycemic hyperinsulinemic clamp with administration of a single oral dose of 600 mg irbesartan (ARB); a euglycemic hyperinsulinemic clamp with administration of a single oral dose of 10 mg felodipine ER (CCB), as a control for the reduction in blood pressure; or a euglycemic hyperinsulinemic clamp with placebo administration, as a hyperinsulinemic time-control (Fig. 1). Irbesartan was chosen as it is a highly specific competitive antagonist of AngII type 1 receptors with a high receptor affinity and a rapid onset of action (Ribstein et al., 2001). A high dose was chosen in order to obtain maximal AngII type 1 receptor blockade. Indeed, before conducting these experiments, we confirmed, in six normotensive healthy individuals, that this dose was sufficient to fully block the response to exogenous AngII. Felodipine was chosen as a comparator for the vasodilator effects of irbesartan, as CCBs are considered to have a neutral effect on insulin sensitivity (Elliott and Meyer, 2007). In the same six individuals we confirmed that treatment with 600 mg irbesartan and 10 mg felodipine ER resulted in identical blood pressure reductions. The interval between each of the three study days was one week. In order to standardize the activity of the endogenous RAS, subjects adhered to a moderately sodium restricted diet (100 mmol/day) for one week prior to the first visit, and also prior to the following visits. Compliance with the diet was assessed by two measurements of 24-h urinary sodium excretion.

All measurements were conducted in a quiet, temperature-controlled room ($T=23.4\pm0.5$ °C) at 8.00 a.m., after a 10-h fast, with the subjects in the supine position. Subjects were asked to refrain from drinking alcohol for a period of 24-h before each study day and to perform no strenuous exercise for a period of 48-h before each study day. Baseline measurements were obtained after allowing 30 min of rest and acclimatization after insertion of two i.v. catheters (Venflon, Viggo, Gotenborg, Sweden). Skin temperature was monitored during the tests.

Hyperinsulinemic euglycemic clamp

Insulin sensitivity was assessed by the hyperinsulinemic, euglycemic clamp method, using a modification of the method described by DeFronzo (DeFronzo et al., 1979). Briefly, insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) was infused in a primed continuous manner at a rate of 50 mU/kg/h. Mean fasting glucose concentrations were determined from three glucose concentrations measured before $t=0$. Normoglycemia was maintained by adjusting the rate of a 20% D-glucose infusion based on plasma glucose measurements performed at 5–10 min intervals. Whole body glucose uptake (M) was calculated from the glucose infusion rates during the last 60 min of hyperinsulinemia

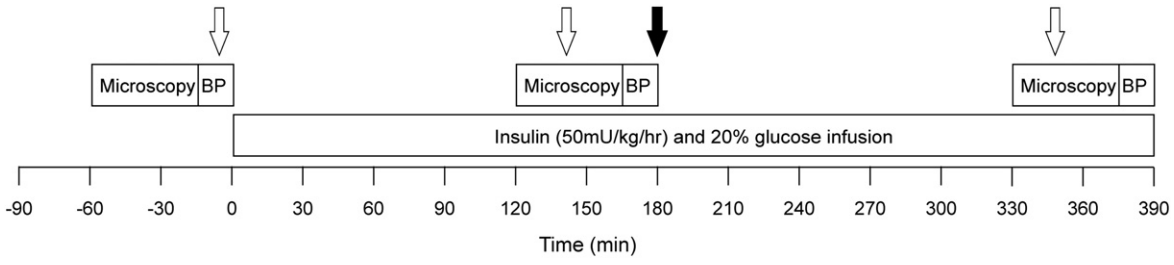

Fig. 1. Design of the study. There were three interventions: hyperinsulinemic clamp with single-dose ARB administration, hyperinsulinemic clamp with single-dose CCB administration, and hyperinsulinemic clamp with placebo administration. Microscopy indicates capillary microscopy of the skin of the finger; BP, blood pressure measurements; ⇩, blood samples for measurements of insulin concentrations; ⬇, intake of ARB, CCB or placebo. Baseline microvascular measurements ($t=-60$–0 min) were performed on only one of the experimental days (randomly assigned). Microvascular measurements during the hyperinsulinemic clamp and during clamp plus drug administration were performed during all three study days.