Influences of AT1 receptor blockade on tissue metabolism in obese men

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Boschmann, Michael, Stefan Engeli, Frauke Adams, Gabriele Franke, Friedrich C. Luft, Arya M. Sharma, and Jens Jordan. Influences of AT1 receptor blockade on tissue metabolism in obese men. Am J Physiol Regul Integr Comp Physiol 290: R219–R223, 2006. First published August 18, 2005; doi:10.1152/ajpregu.00341.2005.—ANG II applied to the interstitial space influences carbohydrate and lipid metabolism in a tissue-specific fashion. Thus endogenous ANG II may have a tonic effect on tissue metabolism that could be reversed with ANG II type 1 (AT1) receptor blockade, particularly during adrenergic stimulation. We studied 14 obese men. They were treated for 10 days with the AT1 receptor blocker irbesartan or with placebo in a double-blind and cross-over fashion. At the end of each treatment period, we assessed skeletal muscle and adipose tissue metabolism using the microdialysis technique. The ethanol dilution technique was applied to follow changes in tissue blood flow. Measurements were obtained at baseline and during application of incremental isoproterenol concentrations through the microdialysis catheter. Blood pressure decreased from 133 ± 3/84 ± 3 to 128 ± 3/79 ± 2 mmHg for systolic and diastolic blood, respectively (P = 0.02 and 0.006, respectively) with AT1 receptor blockade. Isoproterenol perfusion caused a dose-dependent increase in dialysate glycerol in adipose tissue and in skeletal muscle. Irbesartan slightly reduced the isoproterenol-induced glycerol response in adipose tissue (P < 0.05 by ANOVA). Ethanol ratio, interstitial glucose supply, and lactate production in adipose tissue and skeletal muscle were similar with placebo and irbesartan. We conclude that AT1 receptor blockade in obese men does not reveal a major tonic ANG II effect on interstitial glucose supply, lipolysis, or glycolysis in skeletal muscle, either at rest or during β-adrenergic stimulation. Endogeneous ANG II may slightly increase adipose tissue lipolysis. The mechanism may promote the redistribution of triglycerides from adipose tissue toward other organs.

adipose tissue; angiotensin II; insulin

HEMODYNAMIC AND CARDIOVASCULAR EFFECTS of angiotensin (ANG) II are universally recognized; however, metabolic effects of ANG II are less well appreciated. These effects may be particularly germane to obesity. Obesity is associated with activation of the systemic renin-ANG system (9, 17). The renin-ANG system activity may be further increased in adipose tissue (3, 9, 12). Renin-ANG system activation may contribute to obesity-associated hypertension (4, 17). The activation may also promote metabolic disease through an unknown mechanism. Indeed, ANG subtype 1 (AT1) receptor blockers and ANG-converting enzyme inhibitors decreased the risk for new-onset diabetes mellitus by >20% in several large studies (16). The phenomenon could be related in part to a direct effect of ANG II on carbohydrate and lipid metabolism at the tissue level (1). ANG II in pharmacological doses through a microdialysis catheter increased adipose tissue lipolysis in a dose-dependent fashion. The increase was probably related to ANG II-mediated norepinephrine release from adrenergic neurons. In contrast, skeletal muscle lipolysis decreased with ANG II. In another study, ANG II inhibited lipolysis in both tissues (11). Local ANG II also modulated carbohydrate metabolism. In muscle, ANG II increased dialysate pyruvate, whereas the lactate-to-pyruvate ratio decreased. We hypothesized that ANG II may have a tonic effect on tissue metabolism that could be reversed with AT1 receptor blockade. To address this issue, we tested the effect of AT1 receptor blockade in obese men on adipose tissue and skeletal muscle metabolism, both at rest and during local β-adrenergic stimulation with isoproterenol.

MATERIALS AND METHODS

Subjects. We recruited 14 obese men on no medications (age: 35 ± 2 yr; body mass index: 32 ± 1 kg/m2; waist circumference: 106 ± 2 cm). Written, informed consent was obtained before study entry. Our institutional review board approved the study.

Protocol. The study protocol is illustrated in Fig. 1. Patients were treated with the AT1 receptor blocker irbesartan (Aprovel; Sanofi-Aventis) and with placebo in a randomized, double-blind, and cross-over fashion. Patients were treated over a 10-day period with placebo or irbesartan. Between treatments, patients were submitted to a 2- to 4-wk washout period. Irbesartan was begun at a dose of 75 mg/day and increased to 150 mg/day after 3 days. The metabolic evaluation was scheduled on the last day of the treatment phase. On this day, patients presented to the Franz-Volhard Clinical Research Center after an overnight fast. They ingested the last dose of the study medication in the morning with 100 ml of water.

Assessment of tissue metabolism. Studies were conducted with the patients in the supine position. One microdialysis probe was inserted into abdominal subcutaneous adipose tissue. Another microdialysis probe was inserted into skeletal muscle (quadriiceps femoris, vastus lateralis) as described elsewhere (CMA/60 microdialysis catheters and CMA/102 microdialysis pumps; CMA Microdialysis, Solna, Sweden) (14). After probe insertion, tissue perfusion with lactate-free Ringer solution supplemented with 50 mM ethanol was started at a flow rate of 2 μl/min. After instrumentation, the patients recovered for 60 min (“baseline”). Both microdialysis probes were then perfused with incremental isoproterenol concentrations (0.01, 0.1, and 1.0 μM) (Isolep; Abbot, Ottignies, France).

Analytical methods. Ethanol concentrations in microdialysis perfusate (inflow) and dialysate (outflow) were measured with a standard enzymatic assay. Microdialysate glucose, lactate, pyruvate, and glycerol concentrations were measured with the CMA/600 analyser (CMA Microdialysis). Changes in blood flow were determined using the ethanol dilution technique, which is based on Fick’s principle (13). A decrease in the outflow-to-inflow ratio (“ethanol ratio”) is equivalent to an increase in blood flow and vice versa. Changes in glycerol were used to assess changes in lipolysis and/or lipid mobilization; changes

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in glucose and lactate were used to assess changes in carbohydrate metabolism. In situ recovery for glycerol, glucose, and lactate in the dialysate was assessed by near-equilibrium dialysis. For glucose, lactate, and pyruvate, we found recoveries of $\sim30\%$ in adipose tissue and 50% in muscle. Recovery of glycerol was $\sim30\%$ in adipose tissue and 80% in skeletal muscle. Recovery for all metabolites did not differ between placebo and irbesartan.

Statistics. All data are expressed as means $\pm$ SE. Repeated-measures ANOVA testing was performed for multiple comparisons using the statistical program InStat, version 3.0 (GraphPad Software, San Diego, CA). A value of $P < 0.05$ was considered significant.

RESULTS

Patients tolerated the treatment well. They experienced no adverse events during the study. On placebo, five patients were classified as hypertensive with a systolic blood pressure of $>140$ mmHg and/or diastolic blood pressure of $>90$ mmHg. Blood pressure was $133 \pm 8/43 \pm 3$ mmHg with placebo and $128 \pm 3/79 \pm 2$ mmHg with irbesartan ($P = 0.02$ and 0.006 for systolic and diastolic blood pressure, respectively). Heart rate was $80 \pm 3.4$ beats/min with placebo and $83 \pm 3.4$ beats/min with irbesartan. Venous glucose, insulin, and triglyceride concentrations were similar with placebo and irbesartan.

Ethanol ratio and dialysate glucose concentrations at baseline and during isoproterenol perfusion in adipose tissue and in skeletal muscle are illustrated in Fig. 2. In adipose tissue, the ethanol ratio decreased substantially during isoproterenol perfusion ($P < 0.001$ by ANOVA). With isoproterenol, the ethanol ratio did not change significantly in skeletal muscle. In both tissues, irbesartan had no effect on ethanol ratio, both at baseline and during incremental isoproterenol perfusion. Dialysate glucose concentration did not change with isoproterenol perfusion. Dialysate glucose responses were similar with placebo and with irbesartan.

Isoproterenol perfusion caused a dose-dependent increase in dialysate glycerol in adipose tissue ($P < 0.0001$ by ANOVA) and in skeletal muscle ($P = 0.005$ by ANOVA) (Fig. 3). In adipose tissue, the glycerol response to isoproterenol was slightly augmented during irbesartan treatment. In contrast, in skeletal muscle, glycerol responses were identical with placebo and with irbesartan. Dialysate lactate increased in skeletal muscle ($P < 0.0001$ by ANOVA) but not in adipose tissue. In skeletal muscle, dialysate pyruvate increased and the lactate-to-pyruvate ratio decreased with isoproterenol ($P < 0.0001$ by ANOVA for both) (Fig. 4). Except for a trend for decreased adipose tissue lactate, pyruvate and lactate responses were unchanged with irbesartan.

Fig. 2. Ethanol ratio (top) and microdialysis glucose concentrations (bottom) in adipose tissue (left) and skeletal muscle (right) on placebo (○) and irbesartan (●). Data are given at baseline and during incremental isoproterenol application through the microdialysis catheter. Values are means $\pm$ SE.
DISCUSSION

The microdialysis technique provides a unique tool to study tissue physiology in humans. We applied the technique to test the hypothesis that ANG II elicits a tonic metabolic response in obese men that could be reversed through AT1 receptor blockade. We assessed tissue metabolism at baseline and during local α1-adrenoreceptor stimulation with isoproterenol. We gave a dose of irbesartan commonly used to treat hypertension. The hypotensive response in our study documents that we achieved systemic AT1 receptor blockade.

Metabolite concentration in microdialysates is influenced by tissue perfusion, local metabolite production, and local metabolite utilization. Thus AT1 receptor blockade could influence tissue metabolism directly or indirectly through changes in tissue perfusion. We applied the ethanol dilution technique to account for possible indirect hemodynamic mechanisms. This method has been validated against direct blood flow measurements (13). Similar to previous studies, local isoproterenol application led to a decrease in the ethanol ratio that is consistent with increased tissue perfusion (14). In contrast, the ethanol ratio did not change in skeletal muscle. Tissue perfusion was similar with placebo and irbesartan. This observation is remarkable because it suggests that ANG II in the interstitial space may serve a different physiological purpose than intravascular ANG II. We determined the in situ recovery of the microdialysis probes. Recovery for all metabolites was similar with placebo and irbesartan. Thus any differences in metabolite concentrations between placebo and irbesartan are not explained by differences in tissue perfusion or in the recovery of the microdialysis probes.

We were particularly interested in the effect of AT1 receptor blockade on lipid mobilization at baseline and during isoproterenol application through the microdialysis catheter. Values are means ± SE. P values are given for the comparison of the 2 dose-response curves using a 2-way ANOVA.

Fig. 3. Microdialysate glycerol (A and B, top) and lactate concentrations (A and B, bottom) in adipose tissue (A) and skeletal muscle (B) on placebo (○) and irbesartan (●). Data are given at baseline and during incremental isoproterenol application through the microdialysis catheter. Values are means ± SE. P values are given for the comparison of the 2 dose-response curves using a 2-way ANOVA.

Fig. 4. Microdialysate pyruvate concentrations (A) and the lactate-to-pyruvate ratio (B) in skeletal muscle on placebo (○) and irbesartan (●). Data are given at baseline and during incremental isoproterenol application through the microdialysis catheter. Values are means ± SE.
terenol stimulation. Studies in animals and in humans suggested that ANG II modulates lipolysis. However, the results are controversial. We observed no change or an increase in lipolysis when ANG II was applied to adipose tissue (1, 2). Others observed decreased adipose tissue lipolysis using a similar approach (11). Decreased skeletal muscle lipolysis with interstitially applied ANG II is a consistent result throughout these studies (1, 11). Systemic ANG II application in rats through an osmotic minipump augments lipolysis, perhaps through interaction with the sympathetic nervous system (5). In our study, interstitial glycerol in skeletal muscle at baseline and during β-adrenergic stimulation was not influenced by AT1 receptor blockade. This observation suggests that endogenous ANG II did not restrain skeletal muscle lipolysis. AT1 receptor blockade slightly reduced adipose tissue lipolysis at least during β-adrenergic stimulation. We speculate that this phenomenon is related to blockade of presynaptic AT1 receptors on adrenergic neurons. Stimulation of these receptors increases norepinephrine release (1, 7, 8). The idea is supported by recent animal studies in which ANG II was applied chronically via an osmotic minipump (5). ANG II increased interstitial norepinephrine and glycerol concentrations in white adipose tissue (5). The response was reversed with AT1 receptor blockade.

In the present study, we determined whether endogenous ANG II influences interstitial glucose metabolism, both in the absence and in the presence of isoproterenol. Isoproterenol increased tissue perfusion in a dose-dependent fashion, which increases interstitial glucose supply. The observation that dialysate glucose concentration did not change suggests an increase in cellular glucose uptake and metabolism. Indeed, dialysate lactate concentrations tended to increase. Interstitial glucose concentrations were similar in the presence and absence of AT1 receptor blockade. Thus the balance between glucose supply and glucose utilization was not altered. Similarly, application of the AT1 receptor blocker losartan through a microdialysis probe did not change the gradient between arterial and interstitial glucose concentrations (10). In contrast, local ANG-converting enzyme inhibition diminished the glucose gradient presumably through increased bradykinin concentrations (10). In a previous study (1), ANG II applied locally through a microdialysis probe elicited subtle changes in glucose metabolism. Dialysate lactate did not change with ANG II. However, dialysate pyruvate in skeletal muscle increased markedly, which led to a reduction in the lactate-to-pyruvate ratio (1). A decrease in lactate-to-pyruvate ratio is consistent with increased aerobic glycolysis. A decrease in the measurement is consistent with a hypoglycemic situation, namely the lower availability of glucose is compensated by a greater efficiency of glucose metabolism (18). Thus ANG II may have interfered with glucose uptake into skeletal muscle or with intracellular glucose utilization. Recent animal studies suggest that ANG II may interfere with insulin signaling downstream from phosphatidylinositol 3-kinase (15). In skeletal muscle, AT1 receptor blockade had no effect on lactate or pyruvate production in our study. In adipose tissue, AT1 receptor blockade tended to decrease lactate production during β-adrenergic stimulation. One possible explanation is that AT1 receptor blockade may have shifted the balance between lipolysis and lipogenesis toward increased lipogenesis. Glucose may have been utilized for α-glycerophosphate synthesis (key metabolite for triacylglycerol synthesis) rather than being degraded to lactate.

Our study has several limitations. The number of subjects was relatively small. However, in previous studies in which ANG II was applied locally through microdialysis probes, metabolic changes were observed in even smaller numbers of patients (1, 11). Only eight subjects were sufficient to demonstrate changes in tissue metabolism with local ANG-converting enzyme inhibition (10). The irbesartan dose applied in our study is commonly used for the treatment of arterial hypertension. Yet, even larger AT1 receptor blocker doses may be necessary for a more complete blockade of interstitial AT1 receptors. However, the significant blood pressure reduction with irbesartan suggests that we reached at least a partial AT1 receptor blockade. Indeed, even lower doses reduced blood pressure in previous studies (19). Finally, the full metabolic effect of ANG II may only be observed in conditions with a more pronounced renin-ANG system activation. Only five patients had blood pressure readings in the hypertensive range. We cannot exclude that the metabolic response to AT1 receptor blockade is more pronounced in patients with established hypertension. Despite these issues, we suggest that AT1 receptor blockade in obese men does not reveal a major tonic ANG II effect on interstitial glucose supply, lipolysis, or glycolysis in skeletal muscle, either at rest or during β-adrenergic stimulation. AT1 receptor blockade may slightly reduce adipose tissue lipolysis through inhibition of endogenous ANG II at least during adrenergic stimulation with isoproterenol. We speculate that, through this mechanism, AT1 receptor blockade may promote redistribution of triglycerides from skeletal muscle toward the physiological storage site. The physiology of the interstitial tissue ANG II in humans is still not fully understood and requires further studies.

GRANTS

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REFERENCES


