Candesartan Acutely Recruits Skeletal and Cardiac Muscle Microvasculature in Healthy Humans

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Context: Angiotensin II type 1 receptor (AT1R) tone restricts muscle microvascular blood volume (MBV) and decreases muscle insulin delivery and glucose use.

Objective: The objective of the study was to examine whether acute AT1R blockade alters microvascular perfusion in skeletal and cardiac muscle in humans.

Setting: The study was conducted at the General Clinical Research Center at the University of Virginia.

Methods: Eight overnight-fasted healthy young adults were studied thrice in random order. In study 1, each subject received candesartan (32 mg) orally at time 0. In study 2, each subject received placebo at time 0 and a 1 mU/min/1.85 kg euglycemic insulin clamp from time 240 to 360 min. In study 3, each subject received candesartan (32 mg) orally at time 0 and insulin infusion from 240 to 360 min. Forearm skeletal and cardiac muscle MBV, microvascular flow velocity, and microvascular blood flow (MBF) were determined at baseline and at 240 and 360 min.

Results: Candesartan treatment acutely recruited microvasculature in both skeletal and cardiac muscle by significantly increasing MBV ($P<0.03$ and $P=0.02$, respectively) and MBF ($P<0.03$ for both) without altering microvascular flow velocity. Insulin infusion significantly increased cardiac MBV ($P<0.02$) and MBF ($P<0.02$). Superimposing insulin infusion 4 h after candesartan ingestion did not further recruit microvasculature. Insulin-mediated whole-body glucose disposal did not differ with or without candesartan pretreatment.

Conclusions: Acute AT1R blockade with candesartan recruits skeletal as well as cardiac muscle microvasculature in healthy humans without altering insulin-mediated whole-body glucose disposal. This may contribute to the observed improvement in the cardiovascular outcomes in patients receiving prolonged treatment with AT1R blockers. (J Clin Endocrinol Metab 97: E1208–E1212, 2012)

The role of the renin-angiotensin system in the microcirculation has not been well defined. Superimposing angiotensin II infusion on insulin infusion clearly increases insulin-mediated glucose disposal in humans (5–7). Angiotensin II acts via two receptors, the type 1 (AT1R) and type 2 (AT2R) receptors. Both are expressed abundantly in the vascular endothelium (8–10). We have recently reported in laboratory animals that these receptors play important roles in the regulation of muscle microvascular perfusion and insulin delivery/action.

Abbreviations: AT1R, Angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor; FFA, free fatty acid; MBF, microvascular blood flow; MBV, microvascular blood volume; MFV, microvascular flow velocity; NO, nitric oxide.
Although blockade of the AT₁R potently recruits muscle microvasculature, AT₁R antagonism leads to exactly the opposite effect, causing a significant (up to 80%) microvascular decruitment and suppressed muscle insulin delivery and action (3, 11).

In the current study, we hypothesized that acute blockade of the AT₁R in healthy adult humans would affect skeletal and/or cardiac muscle microvascular perfusion, which may impact the body’s metabolic response to insulin. Our results indicate that candesartan treatment acutely recruits microvasculature in both skeletal and cardiac muscle but does not alter insulin-mediated whole-body glucose disposal.

Materials and Methods

Human subjects and study protocols

Healthy, young (18–35 yr old) volunteers were screened at the General Clinical Research Center. Exclusion criteria included a personal or family history of diabetes, smoking, use of vasoactive medications, body mass index greater than 25 kg/m², hyperlipidemia, hypertension, or chronic illness. The study protocol was approved by the Institutional Review Board at the University of Virginia. All subjects provided written informed consent at the screening visit.

A total of eight (five males, three females) subjects (ages 22.8 ± 2.1 yr, body mass index 22.6 ± 0.6 kg/m²) were studied randomly under three separate protocols, spaced several weeks apart. For each study, each subject was admitted to General Clinical Research Center the night before and fasted for 12 h. A catheter was placed in the antecubital vein of the right arm next morning for infusions of normal saline, dextrose, microbubbles, and insulin. A second catheter was placed distal to the antecubital vein for blood sampling. During each study, vital signs were measured every 30 min. Blood samples were obtained for insulin, free fatty acid (FFA), and nitric oxide (NO) measurements at baseline, 240 min, and 360 min.

**Candesartan protocol**

After obtaining baseline blood samples, baseline skeletal and cardiac muscle microvascular parameter measurements including microvascular blood volume (MBV), microvascular flow velocity (MFV), and microvascular blood flow (MBF) were done using contrast-enhanced ultrasound and myocardial contrast echocardiography, as described previously (12–15). Brachial artery diameter and blood flow velocity were quantified, as described previously (12). Each subject then ingested 32 mg candesartan. Blood samples were collected and microvascular and brachial arterial parameters were determined again at 240 and 360 min.

**Euglycemic hyperinsulinemic clamp protocol**

After obtaining blood samples and microvascular and brachial artery parameter measurements at baseline and again at 240 min, each subject received a 2-h infusion of insulin at 1 mU/min · kg. Plasma glucose concentrations were monitored every 5 min and maintained at approximately 10 mg/dl below the baseline level to avoid arterial hyperglycemia using 20% dextrose infused at variable rates. Blood samples were collected and microvascular and brachial artery parameters determined again at 360 min.

Candesartan + euglycemic hyperinsulinemic clamp protocol

This protocol was essentially the combination of the two protocols mentioned above. Blood samples were collected and microvascular and brachial arterial parameters were determined before and 240 min after ingestion of 32 mg candesartan. A hyperinsulinemic-euglycemic clamp (1 mU/kg · min) was then performed for 2 h and all parameters were determined again at the end of clamp.

Analytical methods

Biochemical and statistical analysis was performed as previously described (3, 13).

Results

Subject characteristics and biochemical parameters

All subjects were normotensive with normal lipid profiles. Table 1 shows the changes in the various parameters during the course of each of the three studies. Candesartan, insulin, or candesartan + insulin did not have any significant effects on blood pressure or heart rate. Candesartan treatment did not significantly alter plasma insulin, glucose, or FFA concentrations, nor did it change plasma insulin concentration or plasma FFA response to insulin during insulin clamp. Plasma NO levels, likely contributed by all isoforms of NO synthase, decreased progressively throughout each study with no significant differences among the three protocols.

Candesartan treatment had no significant effect on insulin-stimulated whole-body glucose disposal in either steady-state glucose infusion rate (5.9 ± 0.7 vs. 5.3 ± 0.7 mg/kg · min, insulin vs. candesartan + insulin, P = 0.14) or the glucose infusion rate area under the curve during the insulin infusion (112.9 ± 12.2 vs. 105.7 ± 11.8, insulin vs. candesartan + insulin, P = 0.33).

Effect of candesartan ± insulin on skeletal muscle microvascular parameters (Fig. 1)

Candesartan administration increased skeletal muscle MBV by approximately 50% (P = 0.07) at 240 min and 68% (P < 0.03) at 360 min during the candesartan alone study. The increase from baseline to 240 min was similar (58%, P = 0.053) during the candesartan + insulin protocol. Pooled together, the increase from baseline to 240 min after candesartan ingestion was highly significant (P = 0.006). There was no significant alteration in MFV. As a result, muscle MBF increased by 45% (P = 0.07) at 240 min and 51% (P < 0.03) at 360 min during the candesartan + insulin protocol.
TABLE 1. Study parameters

<table>
<thead>
<tr>
<th></th>
<th>Candesartan protocol</th>
<th>Candesartan + insulin protocol</th>
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<tr>
<td><strong>Baseline</strong></td>
<td></td>
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</tr>
<tr>
<td>SBP [mm Hg]</td>
<td>120 ± 6.9</td>
<td>114 ± 4.3</td>
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<tr>
<td>DBP [mm Hg]</td>
<td>66 ± 2.2</td>
<td>65 ± 3.3</td>
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<tr>
<td>Heart rate [bpm]</td>
<td>63 ± 2.4</td>
<td>64 ± 3.3</td>
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<tr>
<td>Plasma [FFA] [mM]</td>
<td>0.84 ± 0.3</td>
<td>0.72 ± 0.2</td>
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<tr>
<td>Plasma [glucose] [mM]</td>
<td>92 ± 3.1</td>
<td>94 ± 5.2</td>
</tr>
<tr>
<td>Plasma [insulin] [muU]</td>
<td>19 ± 7.6</td>
<td>25 ± 11</td>
</tr>
<tr>
<td>Plasma [NO] [mM]</td>
<td>42.3 ± 2.5</td>
<td>40.0 ± 3.1</td>
</tr>
<tr>
<td>BA diameter [cm]</td>
<td>90 ± 2.2</td>
<td>94 ± 4.5</td>
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<tr>
<td>BA flow velocity [cm/sec]</td>
<td>12.9 ± 1.0</td>
<td>13 ± 1.2</td>
</tr>
<tr>
<td>BA blood flow [mL/min]</td>
<td>29.1 ± 1.6</td>
<td>23.6 ± 4.5</td>
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SBP, Systolic blood pressure; DBP, diastolic blood pressure; BA, brachial artery.

a P < 0.001 compared with baseline.
b P < 0.03 compared with baseline.
c P = 0.008 compared with baseline.
d P = 0.003 compared with baseline.
e P < 0.04 compared with baseline.
f P < 0.02 compared with baseline.

candesartan-alone study and 51% (P = 0.09) at 240 min during the candesartan+insulin study. Similar to MBV, the pooled data of the two candesartan protocols showed a significant increase in MBF from baseline to 240 min (P = 0.013). During the insulin-alone study, muscle MBV trended upward, but the increase was not statistically significant (P = 0.14). Superimposing insulin on candesartan did not further increase muscle MBF from 240 to 360 min.

Effect of candesartan ± insulin on cardiac muscle microvascular parameters (Fig. 1)

Similarly to skeletal muscle, candesartan significantly increased cardiac muscle MBV at 240 min in both protocols (P = 0.017 for the combined protocols). Cardiac MBV trended up further from 240 to 360 min during the candesartan-alone study. Candesartan administration did not alter cardiac MFV. Thus, cardiac MBF increased significantly at both 240 min (P < 0.04) and 360 min (P < 0.03). Insulin infusion significantly increased cardiac muscle MBV (P = 0.02) and MBF (P < 0.02). Although both MBV and MBF trended up during the candesartan + insulin study, none of the changes was statistically significant.

Effect of candesartan ± insulin on brachial artery diameter and flow

Brachial artery diameter, flow velocity, and blood flow results are shown in Table 1. Candesartan treatment significantly increased brachial artery diameter during the candesartan-alone admission (P < 0.03), but the increase was not statistically significant during the candesartan + insulin admission (P = 0.08) at 240 min. When the measurements from the two admissions were pooled together, the brachial artery diameter increase remained statistically significant (P = 0.015). Blood flow velocity did not change significantly (P = 0.355). As a result, brachial artery blood flow increased significantly at 240 min (P < 0.03). Insulin infusion for 2 h had no significant effect on brachial artery parameters. The candesartan and insulin combination did not further increase brachial artery diameter or flow beyond what was observed with candesartan alone.

Discussion

The current study demonstrates that acute AT1R blockade with candesartan significantly recruits microvasculature in both skeletal and cardiac muscle in healthy humans. Because increased microvascular exchange surface area enhances the delivery of nutrients, oxygen, and hormones to tissue interstitium, our data suggest that angiotensin II receptors could play significant roles in energy metabolism and tissue/organ function in humans.

Angiotensin II interacts with AT1R and AT2R with equal affinity to balance tissue perfusion, and candesartan exerts an unsurmountable antagonism against AT1R, leading to an unopposed action of endogenous angiotensin II on AT2R. Although AT1R blockade inhibits the production of reactive oxygen species and inflammation (16), which could contribute to improved vasorelaxation, evidence suggests a pivotal role of AT2R activation in this process (17). We have recently demonstrated in rats that AT1R blockade with losartan leads to significant microvascular recruitment and muscle glucose use via an AT2R-
mediated, NO-dependent pathway (11). Our observation of a significant increase in MBV in both skeletal and cardiac muscle in humans is consistent with the data obtained from laboratory animals except the absolute increase in muscle MBV was much higher in rats compared with humans (3, 11). Several factors may have contributed to this discrepancy, including that candesartan was given orally in the current study, whereas the animals received losartan iv and general anesthesia, which may increase endogenous angiotensin II concentrations. Raising plasma concentrations of angiotensin II acutely increases muscle MBV in rats (11).

Although both candesartan and insulin increased skeletal muscle MBV, there was no additive effect in MBV when insulin was superimposed on candesartan ingestion. This suggests that a maximal effect may have been achieved by candesartan. However, we have surprisingly observed that in the cardiac muscle the combined effects of insulin and candesartan were actually less than each agent alone. Careful analysis of the data revealed that in three subjects cardiac MBV actually decreased during the study. This result should be interpreted with caution because a small sample size with a relatively large interindividual variation might have led to this observation.

It is not surprising that acute AT1R blockade alone with candesartan recruits microvasculature, thereby expanding the microvascular endothelial exchange surface area in both skeletal and cardiac muscle. Based on available data from animal and cell studies, this is likely secondary to an unopposed action of endogenous angiotensin II on AT2R and may play important roles in regulating energy metabolism and tissue function.
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References