Angiotensin II Type 1 and Type 2 Receptors Regulate Basal Skeletal Muscle Microvascular Volume and Glucose Use

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Abstract—Angiotensin II causes vasoconstriction via the type 1 receptor (AT1R) and vasodilatation through the type 2 receptor (AT2R). Both are expressed in muscle microvasculature, where substrate exchanges occur. Whether they modulate basal muscle microvascular perfusion and substrate metabolism is not known. We measured microvascular blood volume (MBV), a measure of microvascular surface area and perfusion, in rats during systemic infusion of angiotensin II at either 1 or 100 ng/kg per minute. Each caused a significant increase in muscle MBV. Likewise, administration of the AT1R blocker losartan increased muscle MBV by >3-fold (P<0.001). Hindleg glucose extraction and muscle interstitial oxygen saturation simultaneously increased by 2- to 3-fold. By contrast, infusing AT2R antagonist PD123319 significantly decreased muscle MBV by ≥80% (P<0.001). This was associated with a significant decrease in hindleg glucose extraction and muscle oxygen saturation. AT1R antagonism and inhibition of NO synthase each blocked the losartan-induced increase in muscle MBV and glucose uptake. In conclusion, angiotensin II acts on both AT1R and AT2R to regulate basal muscle microvascular perfusion. Basal AT1R tone restricts muscle MBV and glucose extraction, whereas basal AT2R activity increases muscle MBV and glucose uptake. Pharmacological manipulation of the balance of AT1R and AT2R activity affords the potential to improve glucose metabolism. (Hypertension. 2010; 55[part 2]:523-530.)

Key Words: angiotensin II receptors • microvascular blood volume • muscle • NO • glucose metabolism

The renin-angiotensin (Ang) system (RAS) plays pivotal roles in maintaining vascular health and hemodynamic stability.1-2 Ang II exerts its vascular actions via 2 G protein–coupled receptors, the type 1 receptor (AT1R) and type 2 receptor (AT2R). In small resistance vessels, activation of AT1Rs promotes vasoconstriction and smooth muscle proliferation, whereas AT2R stimulation activates an autacoid vasodilator cascade composed of bradykinin, NO, and cGMP, leading to vasodilation and opposing the vasconstrictor actions of Ang II via the AT1R.1-3-5 To date, studies on the regulation of arterial blood flow by the RAS have been focused on conduit arteries and/or resistance arterioles. Whether RAS also regulates the perfusion of muscle microvascular beds has not been studied.

Skeletal muscle microvascular perfusion is determined by precapillary terminal arterioles.6-7 Both AT1Rs and AT2Rs are present throughout the skeletal muscle microcirculation, including endothelial cells, vascular smooth muscle cells, and other vessel-associated cells.8 Thus, Ang II may regulate muscle microvascular perfusion via actions on AT1Rs and/or AT2Rs. This is of particular physiological importance, because the delivery of oxygen, hormones such as insulin, and nutrients such as glucose, amino acids, and fatty acids to skeletal muscle depends on total muscle blood flow, microvascular/capillary surface area, and vascular permeability, especially in patients with fixed blood flow, such as artery narrowing. Because substrate extraction takes place in the microcirculation where substrate extraction takes place and the rate of substrate extraction [V(A)]=(I(A))×[1−e−P/Q], where “V” is the venous plasma concentration, “I” is the interstitial concentration, “A” is the arterial plasma concentration, “P” is surface permeability, “S” is surface area, and “Q” is the plasma flow rate, a small change in the microvascular volume (ie, substrate exchange surface area) could markedly increase or decrease the substrate extraction into the skeletal muscle. Indeed, the regulation of microvascular insulin delivery appears to be the rate-limiting step in skeletal muscle insulin action.7-9-12 We and others have shown repeatedly that insulin at physiological concentrations potently enhances microvascular blood perfusion in both skeletal13-17 and cardiac18 muscles, and this action accounts for ~40% of insulin-stimulated muscle glucose uptake.19

The effects of the RAS on muscle glucose metabolism remain to be defined. It is well known that the RAS is chronically upregulated in insulin-resistant states, including type 2 diabetes mellitus.4,20 In vitro studies have repeatedly
shown that Ang II impairs insulin action in cultured cell systems.\textsuperscript{21-23} Numerous clinical trials using either Ang-converting enzyme inhibitors or AT\textsubscript{2}R blockers have demonstrated an improved insulin sensitivity and/or decreased incidence of new-onset type 2 diabetes mellitus.\textsuperscript{4,24} Surprisingly, acutely raising Ang II systemically improves muscle glucose use.\textsuperscript{25-26}

We considered that these seemingly discordant findings may reflect the differential effects of Ang II via AT\textsubscript{1}Rs and AT\textsubscript{2}Rs. In the present study, we assessed the roles of Ang II receptors (AT\textsubscript{1}Rs and AT\textsubscript{2}Rs) in the acute regulation of muscle microvascular perfusion and glucose uptake. Our results indicate that AT\textsubscript{1}Rs and AT\textsubscript{2}Rs each regulate basal muscle microvascular perfusion. Basal AT\textsubscript{1}R tone restricts muscle microvascular blood volume (MBV) and glucose extraction, whereas basal AT\textsubscript{2}R activity increases muscle MBV and glucose uptake. These effects are independent of changes in either systemic blood pressure or conduit artery blood flow.

Materials and Methods

Animal Preparations and Experimental Protocols

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 220 to 320 g were studied after an overnight fast. Rats were housed at 22 ± 2°C, on a 12-hour light-dark cycle, and fed standard laboratory chow and water ad libitum before study entry. Rats were anesthetized with pentobarbital sodium (50 mg/kg IP; Abbott Laboratories), placed in a supine position on a heating pad, and intubated with a polyethylene 90 tubing to ensure a patent airway. After cannulating the carotid artery and external jugular vein for arterial and jugular venous blood sampling, respectively, rats were studied under 1 of the following 6 groups: (1) group 1 received an IV infusion of Ang II at 1 ng/kg per minute for 120 minutes; (2) group 2 received an IV infusion of Ang II at 1 ng/kg per minute for 120 minutes; (3) group 3 received IV injection of losartan (AT\textsubscript{2}R blocker, 0.3 mg/kg); (4) group 4 received systemic infusion of PD123319 (AT\textsubscript{1}R blocker, 50 \mu g/kg per minute); (5) group 5 received losartan injection 10 minutes after the initiation of PD123319 infusion; and (6) group 6 received losartan injection 30 minutes after beginning systemic infusion of NO synthase inhibitor L-NAME at the dose selected raises MAP by 20 to 30 mm Hg above baseline without affecting heart rate and completely inhibits insulin-mediated increases in both FBF and muscle MBV.\textsuperscript{19} The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. 85-23, revised 1996). The study protocol was approved by the animal care and use committee of the University of Virginia.

Contrast-Enhanced Ultrasound

The skin overlying the proximal hindlimb adductor muscles was shaved, and muscle MBV, a measure of microvascular surface area and perfusion, was measured using contrast-enhanced ultrasound (CEU) imaging, as described previously.\textsuperscript{28} In brief, CEU was performed using a HDI-5000 ultrasound system and a L7-4 linear array transducer (Philips Medical Systems) on the proximal adductor muscles (adductor magnus and semimembranosus) of the right hindlimb at a transmission frequency of 3.3 MHz. The contrast agent (Definity, Lantheus Medical Imaging), microbubbles composed of a lipid shell filled with a perfluorocarbon gas, was infused IV at a rate of 16 \mu L/min. After 10 minutes of continuous infusion of the microbubbles to allow the microbubbles to reach steady state within the blood pool, images were digitally acquired, as described previously.\textsuperscript{29} A mechanical index of 0.8 was used that is capable of destroying all of the microbubbles within the ultrasound beam. Depth, focus, and gains (overall gain, time-gain compensation, and lateral-gain compensation) were optimized at the beginning of each study and held constant throughout each study. All of the muscle CEU images were analyzed using the QLAB software (Philips Medical Systems). Background-subtracted video intensity in the region of interest was determined as described previously.\textsuperscript{27,18,30,31}

Measurement of FBF

After removing overlaying skin, the femoral artery was carefully separated from the femoral vein and nerve. Ultrasound transmission gel was then applied, and a flow probe (VB series 0.5 mm; Transonic Systems) was positioned around the femoral artery to measure the FBF, and the results were expressed as milliliters per minute.

Determination of Hindleg Glucose Uptake

Carotid arterial and femoral venous blood glucose concentrations were determined using an Accu-Chek Advantage blood glucometer (Roche). Glucose levels were determined 2 to 4 times per time point, and the numbers were averaged. Hindleg glucose uptake (milligrams per deciliter) was calculated as the arterial-venous (A-V) glucose differences.

Quantitation of Muscle Interstitial Oxygen Saturation

Muscle interstitial oxygen saturation was measured using a fiberoptic oxygen meter (OXY-MICRO-AOT; World Precision Instruments). The measurement was on the basis of the effect of dynamic luminescence quenching by molecular oxygen. Briefly, a needle housing the fiberoptic oxygen microsensor was inserted into the right hindlimb skeletal muscle. Then the glass fiber with its oxygen-sensitive tip inside the needle was extended into muscle interstitium by carefully pressing the syringe plunger. Measurements were taken every 10 seconds, and 30-minute average values were calculated. The readings (percentage of air saturation) were converted to oxygen saturation using the following formula: oxygen saturation (percentage) = air saturation (percentage) × 21%. Statistical Analysis

All of the data are presented as mean ± SEM. Statistical analyses were performed with SigmaStat 3.1.1 software (Systat Software, Inc), using 1-way repeated-measures ANOVA or the 2-tailed t test where appropriate. A P value of <0.05 was considered statistically significant.

Results

Effect of Ang II on Muscle MBV and MAP

We first tested whether systemic infusion of Ang II altered muscle MBV and, if so, whether this effect was associated with changes in MAP. Rats received systemic infusions of Ang II at 2 concentrations, 1 or 100 ng/kg per minute (Figure 1). We initially carried out a dose-response study of Ang II (1, 10, 50, and 100 ng/kg per minute) with MAP as the end point. We then selected these 2 doses, because we wanted to address the role of Ang II in regulating muscle microvasculature in the presence and absence of changes in systemic blood pressure. Ang II at 1 ng/kg per minute did not alter MAP but
increased muscle MBV by ≈2-fold. This effect appeared 10 minutes after the initiation of Ang II infusion and lasted for ≈60 minutes. Muscle MBV returned back to baseline by 120 minutes despite continued Ang II infusion at 1 ng/kg per minute. This could be secondary to either tachyphylaxis of the Ang II effect on AT2R or initial preferential activation of AT1R and later dual stimulation on both AT1R and AT2R in the microcirculation. Infusing Ang II at 100 ng/kg per minute increased MAP by 35 mm Hg at 10 minutes ($P_{<0.001}$), and it remained elevated throughout the 120-minute infusion ($P_{<0.001}$, ANOVA). Muscle MBV increased by ≈2-fold at 30 minutes and remained elevated throughout this high-dose Ang II infusion.

**Effect of Basal AT1R Activity on Muscle MBV and Glucose Use**

As the low-dose Ang II infusion rate (1 ng/kg per minute) significantly raised MBV in the absence of changes in blood pressure, we next explored whether the basal tone of AT1R and AT2R affected muscle microvascular perfusion and whether this impacted muscle glucose use. Losartan was given intravenously to address whether the AT1R regulated basal muscle vascular tone. As shown in Figure 2, AT1R blockade increased muscle MBV >3-fold, and this effect lasted for ≈3 hours. There was no change in either FBF or MAP after losartan injection. The increase in MBV was associated with a >3-fold increase in muscle glucose extraction, as evidenced by a significant increase in hindleg A-V glucose differences. The increase in muscle glucose extraction also lasted for ≈3 hours after losartan injection, paralleling the increase in muscle MBV.
starting the PD123319 infusion, muscle MBV was only 20% of the baseline value. Despite this change in MBV, FBF remained constant during PD123319 infusion. The decrease in muscle MBV during PD123319 infusion was accompanied by a significant decrease in muscle glucose uptake. As shown in Figure 3, the hindleg A-V glucose differences decreased by 70% at 30 minutes (P<0.004) and ≈60% at 60 minutes (P<0.01). At 120 and 180 minutes, the A-V glucose differences remained ≈30% lower than the baseline value, although these differences were not statistically significant (P=0.22 and 0.13 for 120 minutes and 180 minutes, respectively).

**Effect of AT2R Blockade on Losartan-Mediated Changes in Muscle MBV and Glucose Use**

Because specific blockade of AT1R by losartan allows unopposed AT2R stimulation by endogenous Ang II, we next examined whether the losartan-induced MBV increase depended on AT2R activity. We started to infuse PD123319 10 minutes before losartan injection and then followed muscle MBV and glucose extraction over time. As shown in Figure 4, blockade of AT2R with PD123319 infusion completely abolished losartan-induced increases in both muscle MBV and glucose uptake. There were no changes in either FBF (1.00±0.12 versus 0.98±0.12 mL/min, baseline versus 60 minutes; P=0.43) or MAP (90±7 versus 83±5 mm Hg, baseline versus 60 minutes; P=0.41) in the presence of the combined blockade of AT1R and AT2R.

**Role of NO in Losartan-Induced Increases in Muscle MBV and Glucose Use**

Earlier work has confirmed that AT2R activation leads to vasodilation via the bradykinin-NO signaling pathway in isolated small blood vessels.5,32 To examine the role of NO production in AT2R activity–induced increases in muscle MBV and glucose uptake, we gave L-NAME via continuous infusion starting 30 minutes before losartan administration. As expected, systemic infusion of L-NAME raised the MAP from 103±6 to 119±6 mm Hg (P<0.04) without changing

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**Figure 3.** AT1R activity decreases muscle MBV and glucose extraction. Each rat received continuous IV infusion of PD123319 (50 μg/kg per minute), begun at time 0, to block AT1R activity and to reveal AT1R activity. A, Changes in muscle MBV. B, Example of CEU images before and 60 minutes after initiation of PD123319 infusion. The dotted line denotes the region of interest. C, Changes in hindleg A-V glucose differences. D, FBF (•) and MAP (○). n=4 to 7. Compared with baseline (time 0), *P<0.001, #P<0.004, and ##P<0.01.

**Figure 4.** AT2R blockade abolishes losartan-induced increase in muscle MBV and glucose extraction. Each rat received an IV injection of losartan (0.3 mg/kg) 10 minutes after the initiation of a continuous IV infusion of PD123319 (50 μg/kg per minute). A, Changes in muscle MBV. B, Changes in hindleg A-V glucose differences. n=5.
increase in muscle interstitial oxygen saturation. As shown in Figure 6, AT1R blockade with losartan was associated with a steady increase in muscle MBV and glucose extraction. Each rat received an IV injection of losartan (0.3 mg/kg) 30 minutes after the initiation of a continuous intravenous infusion of L-NAME (50 μg/kg per minute). A, Changes in muscle MBV. B, Changes in hindleg A-V glucose differences. n=4.

**Figure 5.** Inhibition of NO synthase abrogates losartan-induced increase in muscle MBV and glucose extraction. Each rat received an IV injection of losartan (0.3 mg/kg) 30 minutes after the initiation of a continuous intravenous infusion of L-NAME (50 μg/kg per minute). A, Changes in muscle MBV. B, Changes in hindleg A-V glucose differences. n=4.

FBF (0.90±0.09 versus 0.88±0.13 mL/min, baseline versus 30 minutes; P=0.87). However, as shown in Figure 5, L-NAME completely abolished the losartan-mediated increase in muscle MBV and glucose uptake.

**Effect of AT1R or AT2R Blockade on Muscle Interstitial Oxygen Saturation**

Finally, we assessed whether the changes in the muscle microvascular perfusion induced by AT1R or AT2R blockade led to changes in muscle oxygenation. As shown in Figure 6, AT1R blockade with losartan was associated with a steady increase in muscle interstitial oxygen saturation (P<0.001, ANOVA). Conversely, AT2R antagonism led to a significant decrease in the oxygen saturation in the muscle interstitium (P<0.001, ANOVA). Thus, the changes in muscle interstitial oxygen saturation paralleled the changes in muscle MBV.

**Discussion**

Using CEU, we found important basal tonic effects of AT1R and AT2R on microvascular volume in skeletal muscle. This implies an important role for circulating or tissue-derived Ang II in the regulation of basal muscle microvascular perfusion. Importantly, this basal tone had a significant impact on skeletal muscle glucose use and oxygenation in vivo. Our results also confirm that infusing Ang II acutely increases microvascular perfusion, presumably by selectively or predominately activating the AT1Rs. Such infusions are known to stimulate glucose uptake by human skeletal muscle, although their effects on microvascular perfusion have not been defined in humans. The current findings suggest that basal AT1R and AT2R tone exert opposite effects on muscle microvascular perfusion and glucose use. These effects are independent of changes of blood pressure or FBF.

The AT1R is a G protein–coupled receptor, and it has clearly been established as a vasodilator receptor in both resistance arterioles and in large capacitance vessels, such as uterine arteries, mesenteric arteries, coronary arterioles, and the thoracic aorta. In rat hearts, chronic candesartan treatment induced coronary vasodilation, which was abolished with either L-NAME or PD123319 treatment. Similarly, in isolated human coronary microarteries, Ang II–induced contraction was prevented by AT1R blockade and potentiated by AT2R antagonism. Our results provide a first demonstration that skeletal muscle precapillary arterioles are a major site of AT1R action in vivo. In the presence of losartan, we observed a 3-fold increase in muscle MBV, whereas blockade of the AT1R rendered an 80% reduction in muscle MBV. That the losartan-induced increase in muscle MBV was abolished by AT1R blockade further suggests that this microvascular effect of losartan was attributable to unopposed AT1R stimulation by basal levels of Ang II in the presence of AT1R blockade.

Many studies have confirmed the involvement of the bradykinin-NO-cGMP signaling cascade in the vasorelaxant action of AT2R, because AT2R antagonist PD123319, bradykinin-B2 receptor antagonist icatibant, and the NO synthase inhibitor L-NAME each independently blocks this action, and arterial cGMP concentrations is increased by Ang II 25,32,36. Our finding that L-NAME treatment abolished losartan-induced increases in MBV and glucose extraction strongly suggest that, in the muscle microcirculation, the AT1R regulates precapillary arteriole vasodilation via this pathway.

Our observation that changes in muscle glucose extraction and oxygenation closely parallel changes in muscle MBV after losartan administration further demonstrates the importance of microvascular perfusion in the regulation of muscle glucose metabolism. That neither FBF nor MAP changed after losartan administration and NO synthase inhibition with L-NAME completely abolished losartan-stimulated muscle glucose extraction strongly suggest that the changes in
Late-stage trending up of muscle glucose uptake might be cose uptake remain to be elucidated. It is conceivable that the mechanisms underlying this partial recovery in muscle glucose uptake after the first hour despite continued infusion. Although the overall glucose extraction rates remained 30% lower at 180 minutes (Figure 3), this was in the presence of an 80% decrease in muscle MBV and was not significantly below the baseline value. The lack of changes in FBF in the presence of markedly increased MBV would mean even bigger exchange surface area exposure to the same amount of blood. It has been reported that selective expansion of muscle MBV without increasing femoral arterial blood flow significantly increases muscle delivery of insulin. Although the A-V concentration differences of both glucose and insulin are small in the fasting state, the arterial-to-interstitial concentration differences in muscle are as much as 2-fold for each. Increasing MBV expands the surface area available for insulin and glucose exchange with muscle and by Fick equation can increase delivery of both insulin and glucose to muscle interstitium. Thus, our results indicate that AT1R activity plays an active and important role in the regulation of muscle glucose use through its effect on muscle microvascularity.

It is of interest to note that 2 human studies, each conducted more than a decade ago, demonstrated that Ang II increased muscle glucose uptake in the presence of insulin infusion. Both studies argued that the Ang II–mediated increase in muscle glucose use was secondary to the hemodynamic effects despite one reporting a significant increase and the other a significant decrease in arterial blood flow. Neither study examined the microvascular changes. As we have in our current study demonstrated that low-dose Ang II infusion acutely increases muscle MBV independent of blood pressure changes, it is very likely that, in both human studies, Ang II might have increased MBV, which led to increased muscle glucose uptake. Indeed, it has been suggested that insulin-mediated increase in the microvascular perfusion accounts for 40% of muscle glucose uptake during insulin infusion, and changes in total flow to muscle alone in the absence of exchange surface area expansion would not significantly augment a tissue glucose exchange within the skeletal muscle. Inasmuch as Ang II has been shown to increase insulin-stimulated muscle glucose uptake and NO can directly stimulate muscle glucose uptake, it remains possible that Ang II may have simultaneous direct vascular and metabolic effects that may not necessarily be coupled.

In rats treated with PD123319, both muscle MBV and glucose extraction decreased significantly. However, contrary to the synchronized increases in both muscle MBV and glucose uptake induced by AT1R blockade, PD123319 decreased muscle glucose uptake significantly only at 30 and 60 minutes. It appears that there was a partial recovery of muscle glucose uptake after the first hour despite continued PD123319 infusion. Although the overall glucose extraction rates remained 30% lower at 180 minutes (Figure 3), this was in the presence of an 80% decrease in muscle MBV and was not significantly below the baseline value. The mechanisms underlying this partial recovery in muscle glucose uptake remain to be elucidated. It is conceivable that the late-stage trending up of muscle glucose uptake might be secondary to some type of compensatory mechanism, such that the fractions of glucose being extracted from the capillary blood increase because of a marked decrease in the delivery of glucose to the leg muscle.

That blockade of the AT1R with losartan increased muscle MBV by 300% indicates that the basal AT1R activity exerts a potent tonic vasoconstriction effect on the muscle microcirculation. This finding is particularly important in patients with preexisting insulin resistance and/or diabetes mellitus, because patients with this condition have upregulated RAS in the cardiovascular system. Ang II via the AT1R has been shown to increase the production of both endothelin 1, a potent vasoconstrictor, and of superoxide, which promotes conversion of NO to peroxynitrite, thus reducing NO bioavailability. In addition, Ang II impairs insulin signaling and reduces insulin-stimulated NO production via the AT1R. Ang II also increases the expression of interleukin 6, tumor necrosis factor-α, and vascular adhesion molecules, as well as oxidative stress via the nuclear factor κB pathway, which may also impair insulin signaling. This is important, because we and others have repeatedly demonstrated a stimulatory effect of insulin on muscle microvascular perfusion in both laboratory animals and humans. Thus, insulin resistance and RAS activation could cooperatively facilitate vasoconstriction. This provides a plausible explanation for repeated clinical trial findings that AT1R blockade decreases blood pressure and improves insulin sensitivity in patients with insulin resistance and/or diabetes mellitus.

Our observations that AT1R blockade increased and AT2R antagonism decreased muscle interstitial oxygen saturation are consistent with the changes in muscle MBV induced by losartan or PD123319. This finding is potentially pathogenetically significant, because recent evidence has identified an important role for hypoxia in the genesis of insulin resistance and metabolic syndrome. Numerous clinical trials have confirmed the beneficial effects of AT1R blockers in decreasing cardiovascular morbidity and mortality in patients with diabetes mellitus. It is very likely that the increased tissue oxygenation may have played important role in this process.

Perspectives

Ang II, acting on both AT1R and AT2R, regulates basal skeletal muscle perfusion, glucose metabolism, and oxygenation. Basal AT1R tone restricts muscle MBV, glucose extraction, and oxygenation, whereas basal AT2R activity increases muscle MBV, glucose uptake, and oxygenation via the NO-dependent mechanism (Figure 7). Because the RAS is upregulated in the insulin-resistant states, including type 2 diabetes mellitus and hypertension, pharmacological manipulation of the balance between AT1R and AT2R activities affords the potential to improve muscle glucose metabolism and oxygenation and to reduce the cardiovascular complications of diabetes mellitus. Because anesthetized animals were used in the current study, caution should be introduced when extrapolating the current findings to humans.
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Disclosures
None.

References


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