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 (AT_1R) and vasodilatation through the type 2 receptor (AT_2R). 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 AT_1R 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 AT_2R antagonist PD123319 significantly decreased muscle MBV by $\geq$80% ($P<0.001$). This was associated with a significant decrease in hindleg glucose extraction and muscle oxygen saturation. AT_2R 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 AT_1R and AT_2R to regulate basal muscle microvascular perfusion. Basal AT_1R tone restricts muscle MBV and glucose extraction, whereas basal AT_2R activity increases muscle MBV and glucose uptake. Pharmacological manipulation of the balance of AT_1R and AT_2R 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 (AT_1R) and type 2 receptor (AT_2R). In small resistance vessels, activation of AT_1Rs promotes vasoconstriction and smooth muscle proliferation, whereas AT_2R stimulation activates an autacoid vasodilator cascade composed of bradykinin, NO, and cGMP, leading to vasodilation and opposing the vasoconstrictor actions of Ang II via the AT_1R.[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 AT_1Rs and AT_2Rs 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 AT_1Rs and/or AT_2Rs. 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 $[(V)-(A)]=[(I)-(A)]\times[1-e^{-PS/Q}]$, 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 skeletal[13–17] and cardiac[18] muscles, and this action accounts for $\approx$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

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shown that Ang II impairs insulin action in cultured cell systems.[21–23] Numerous clinical trials using either Ang-converting enzyme inhibitors or AT_1R blockers have demonstrated an improved insulin sensitivity and/or decreased incidence of new-onset type 2 diabetes mellitus.[4,24] Surprisingly, acutely raising Ang II systemically improves muscle glucose use.[25,26]

We considered that these seemingly discordant findings may reflect the differential effects of Ang II via AT_1Rs and AT_2Rs. In the present study, we assessed the roles of Ang II receptors (AT_1Rs and AT_2Rs) in the acute regulation of muscle microvascular perfusion and glucose uptake. Our results indicate that AT_1Rs and AT_2Rs each regulate basal muscle microvascular perfusion. Basal AT_1R tone restricts muscle microvascular blood volume (MBV) and glucose extraction, whereas basal AT_2R 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 $22 \pm 2°C$, 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 through a midline neck incision and after a 30-minute baseline period to assure hemodynamic stability and a stable level of anesthesia, rats were studied under 1 of the following 6 groups: (1) group 1 received an IV infusion of Ang II at 100 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_1R blocker, 0.3 mg/kg); (4) group 4 received systemic infusion of PD123319 (AT_2R blocker, 50 μg/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 N^G-nitro-L-arginine methyl ester (L-NAME; 50 μg/kg per minute).

Muscle MBV (an indicator of microvascular perfusion and surface exchange area), femoral artery blood flow (FBF), hindleg glucose extraction, and muscle interstitial partial pressure of oxygen were measured as described below. Throughout the study, mean arterial blood pressure (MAP) was monitored via a sensor connected to the carotid arterial catheter (Harvard Apparatus). Pentobarbital sodium was infused at a variable rate to maintain steady levels of anesthesia and blood pressure throughout the study. Euthermia was maintained with a heating pad. Ang II, losartan, PD123319, and L-NAME were obtained from Sigma Chemicals. Losartan at the dose selected does not significantly alter systemic blood pressure.[27] L-NAME at the dose selected raises MAP by 20 to 30 mm Hg above baseline without affecting the heart rate and completely inhibits insulin-mediated increases in both FBF and muscle MBV.[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.[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 μL/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.[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.[17,18,30,31]

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 fiber-optic 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 fibro-optic 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)$\times$21%.

Statistic Analysis

All of the data are presented as mean$\pm$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
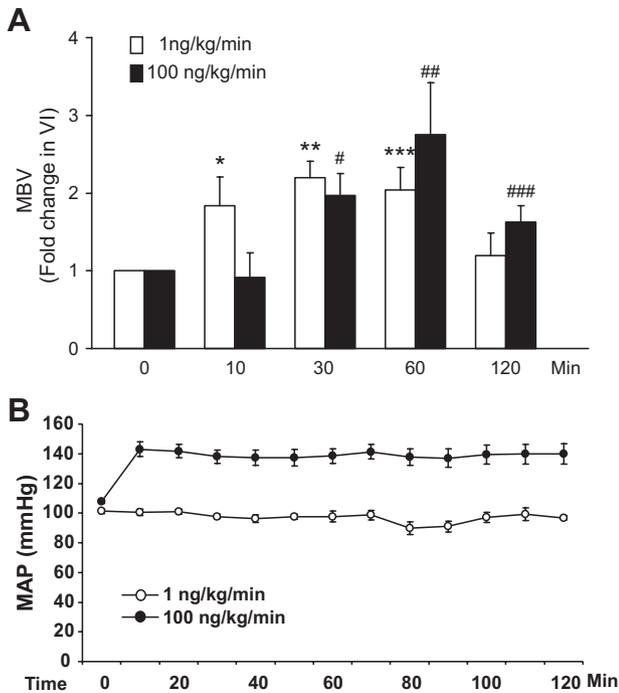
Figure 1. Ang II infusion increases muscle MBV independent of changes in blood pressure. A, Changes in muscle MBV. B, Changes in MAP. Compared with basal level, *$P<0.02$, **$P<0.001$, ***$P<0.005$, #$P<0.02$, ##$P<0.05$, and ###$P<0.03$. n=4 for 100 ng/kg per minute and 7 for 1 ng/kg per minute.

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 AT_2R or initial preferential activation of AT_2R and later dual stimulation on both AT_1R and AT_2R 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 AT_1R 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 AT_1R and AT_2R affected muscle microvascular perfusion and whether this impacted muscle glucose use. Losartan was given intravenously to address whether the AT_1R regulated basal muscle vascular tone. As shown in Figure 2, AT_1R 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.
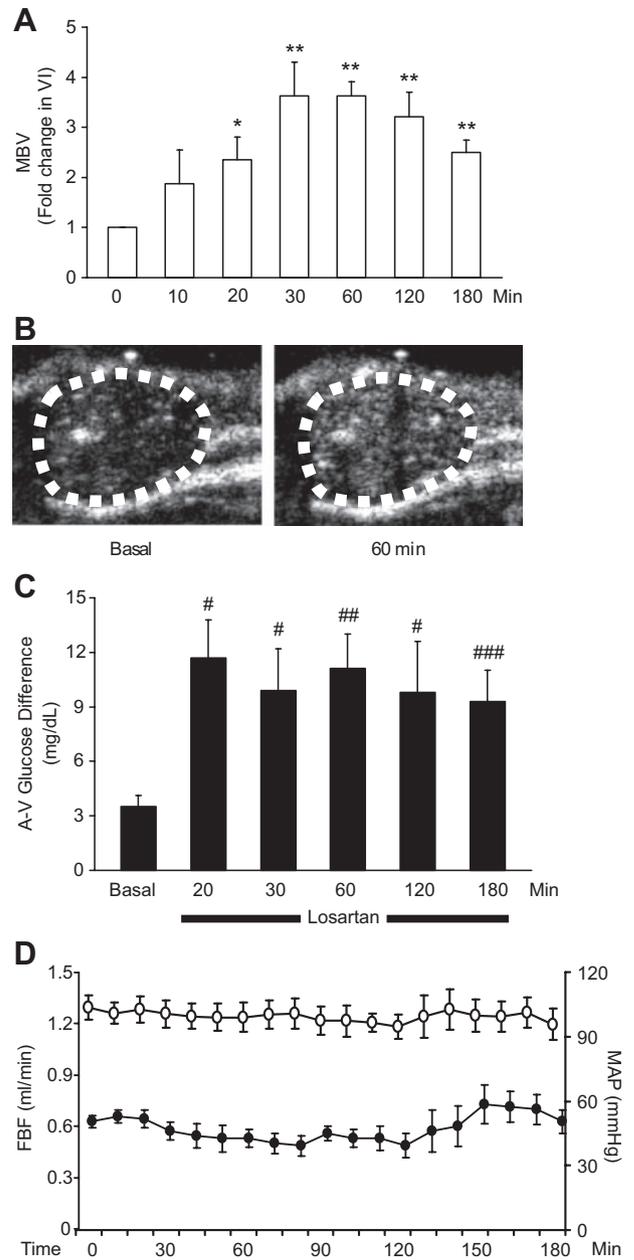
Figure 2. AT_2R activity increases muscle MBV and glucose extraction. Each rat received an IV injection of losartan (0.3 mg/kg) at time 0 to block AT_1R activity and to reveal AT_2R activity. A, Changes in muscle MBV. B, Example of CEU images before and after losartan injection. The dotted line denotes the region of interest. C, Changes in hindleg A-V glucose differences. D, FBF (●) and MAP (○). n=6. Compared with baseline (time 0), *$P<0.01$, **$P<0.001$, #$P<0.05$, ##$P<0.02$, and ###$P<0.01$.

Effect of Basal AT_2R Activity on Muscle MBV and Glucose Use

Figure 3 illustrates the effect of basal AT_2R activity on muscle MBV and glucose extraction. We infused PD123319 continuously to block AT_2Rs and to unmask the basal tonic effect of AT_2Rs. Opposite to results observed with losartan treatment, PD123319 caused a time-dependent decrease of muscle MBV. This effect started by ≈30 minutes and lasted through 180 minutes. Remarkably, by 180 minutes after
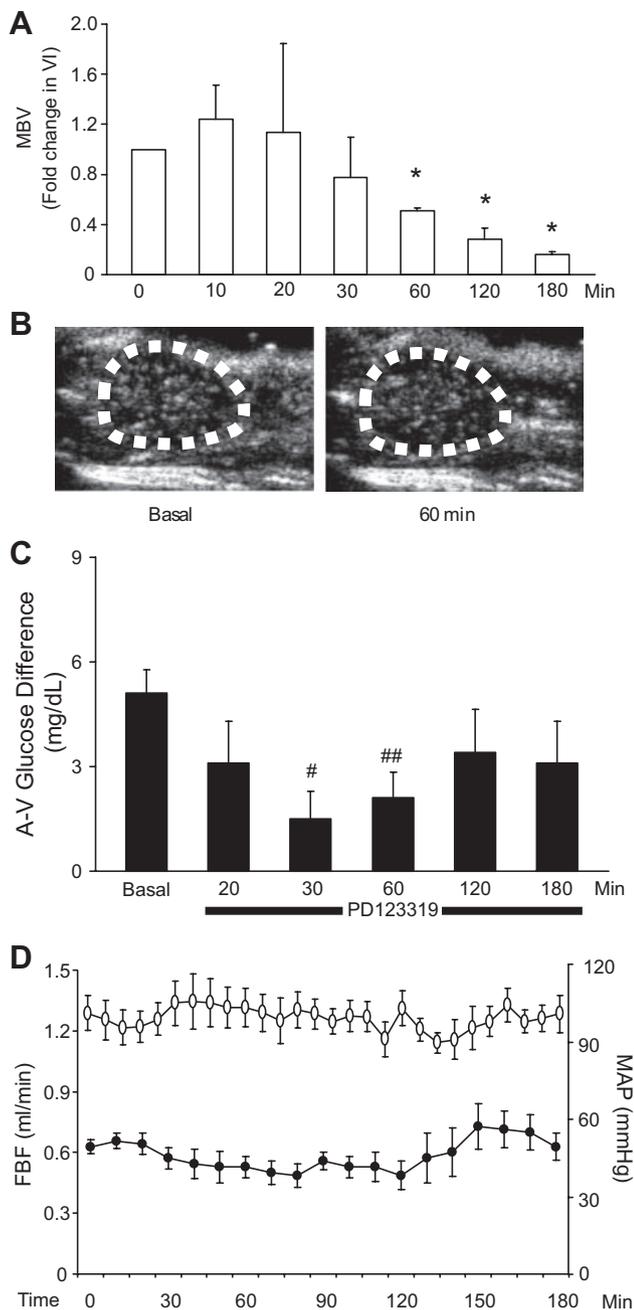
Figure 3. AT$_1$R activity decreases muscle MBV and glucose extraction. Each rat received continuous IV infusion of PD123319 (50 μg/kg per minute), begun at time 0, to block AT$_2$R activity and to reveal AT$_1$R activity. A, Changes in muscle MBV. B, Example of CEU images before and 60 minutes after 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.

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
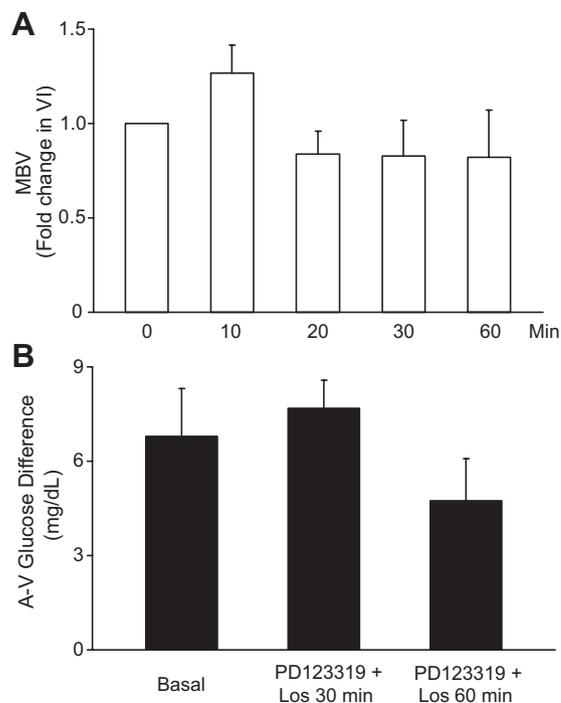
Figure 4. AT$_2$R blockade abolishes losartan-induced increase in muscle MBV and glucose extraction. Each rat received an IV injection of losartan (0.3 mg/kg) 10 minutes after the initiation of a continuous IV infusion of PD123319 (50 μg/kg per minute). A, Changes in muscle MBV. B, Changes in hindleg A-V glucose differences. n=5.

≈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 AT$_2$R Blockade on Losartan-Mediated Changes in Muscle MBV and Glucose Use

Because specific blockade of AT$_1$R by losartan allows unopposed AT$_2$R stimulation by endogenous Ang II, we next examined whether the losartan-induced MBV increase depended on AT$_2$R 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 AT$_2$R 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 AT$_1$R and AT$_2$R.

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

Earlier work has confirmed that AT$_2$R 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 AT$_2$R 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
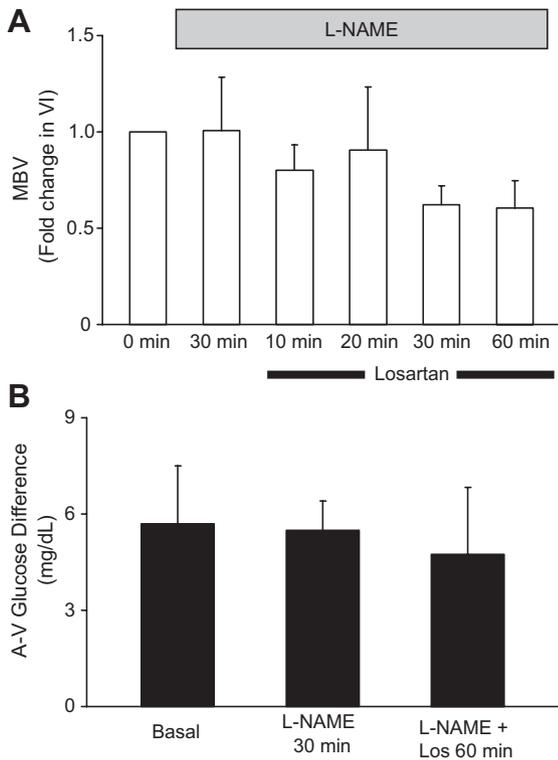
Figure 5. Inhibition of NO synthase abrogates losartan-induced increase in muscle MBV and glucose extraction. Each rat received an IV injection of losartan (0.3 mg/kg) 30 minutes after the initiation of a continuous intravenous infusion of L-NAME (50 μg/kg per minute). A, Changes in muscle MBV. B, Changes 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 AT_1R or AT_2R Blockade on Muscle Interstitial Oxygen Saturation

Finally, we assessed whether the changes in the muscle microvascular perfusion induced by AT_1R or AT_2R blockade led to changes in muscle oxygenation. As shown in Figure 6, AT_1R blockade with losartan was associated with a steady increase in muscle interstitial oxygen saturation ($P<0.001$, ANOVA). Conversely, AT_2R antagonism led to a significant
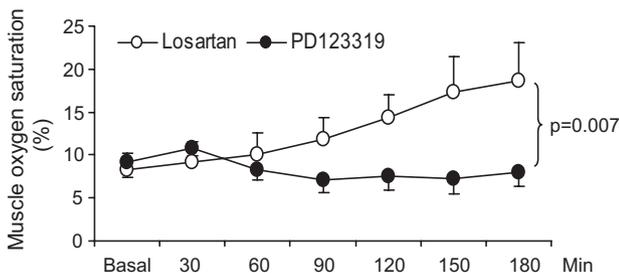
Figure 6. AT_1R blockade increases, whereas AT_2R blockade decreases muscle interstitial oxygenation. Each rat received either an IV injection of losartan (0.3 mg/kg) or a continuous IV infusion of PD123319 (50 μg/kg per minute). n=5 to 6.

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 AT_1R and AT_2R 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 AT_2Rs. Such infusions are known to stimulate glucose uptake by human skeletal muscle,[25,26,33] although their effects on microvascular perfusion have not been defined in humans. The current findings suggest that basal AT_1R and AT_2R tone exert opposite effects on muscle microvascular perfusion and glucose use. These effects are independent of changes of blood pressure or FBF.

The AT_2R 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.[3,32,34–36] In rat hearts, chronic candesartan treatment induced coronary vasodilation, which was abolished with either L-NAME or PD123319 treatment.[37] Similarly, in isolated human coronary microarteries, Ang II–induced contraction was prevented by AT_1R blockade and potentiated by AT_2R antagonism.[5] Our results provide a first demonstration that skeletal muscle precapillary arterioles are a major site of AT_2R action in vivo. Indeed, in the presence of losartan, we observed a 3-fold increase in muscle MBV, whereas blockade of the AT_2R rendered an $\approx80\%$ reduction in muscle MBV. That the losartan-induced increase in muscle MBV was abolished by AT_2R blockade further suggests that this microvascular effect of losartan was attributable to unopposed AT_2R stimulation by basal levels of Ang II in the presence of AT_1R blockade.

Many studies have confirmed the involvement of the bradykinin-NO-cGMP signaling cascade in the vasorelaxant action of AT_2R, because AT_2R antagonist PD123319, bradykinin-B_2 receptor antagonist icatibant, and the NO synthase inhibitor L-NAME each independently blocks this action, and arterial cGMP concentrations is increased by Ang II.[3,5,32,38] Our finding that L-NAME treatment abolished losartan-induced increases in MBV and glucose extraction strongly suggest that, in the muscle microcirculation, the AT_2R 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
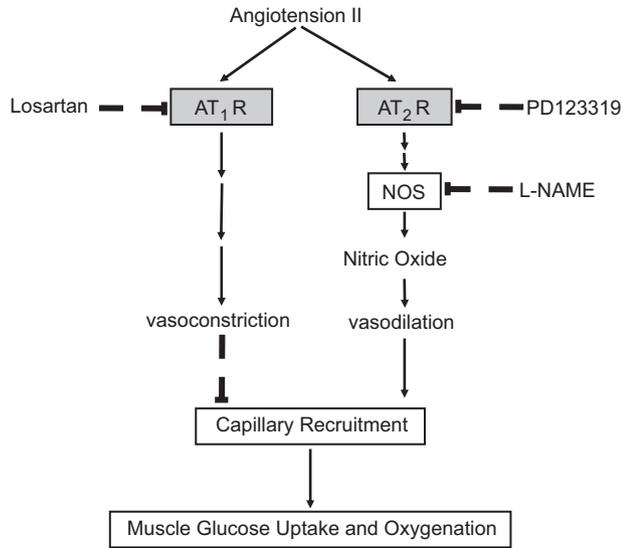
Figure 7. Proposed mechanisms of Ang II action in muscle microvasculature. Solid line indicates stimulation; dashed line, inhibition.

Disclosures

None.

References

1. Carey RM, Siragy HM. Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation. *Endocr Rev*. 2003;24:261–271.
2. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. *Physiol Rev*. 2006;86:747–803.
3. Carey RM. Cardiovascular and renal regulation by the angiotensin type 2 receptor: the AT2 receptor comes of age. *Hypertension*. 2005;45:840–844.
4. Liu Z. The renin-angiotensin system and insulin resistance. *Curr Diab Rep*. 2007;7:34–42.
5. Batenburg WW, Garrelds IM, Bernasconi CC, Juillerat-Jeanneret L, van Kats JP, Saxena PR, Danser AHJ. Angiotensin II type 2 receptor-mediated vasodilation in human coronary microarteries. *Circulation*. 2004;109:2296–2301.
6. Segal SS. Regulation of blood flow in the microcirculation. *Microcirculation*. 2005;12:33–45.
7. Barrett E, Eggleston E, Inyard A, Wang H, Li G, Chai W, Liu Z. The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. *Diabetologia*. 2009;52:752–764.
8. Nora EH, Munzenmaier DH, Hansen-Smith FM, Lombard JH, Greene AS. Localization of the ANG II type 2 receptor in the microcirculation of skeletal muscle. *Am J Physiol Heart Circ Physiol*. 1998;275:H1395–H1403.
9. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. *Endocr Rev*. 2007;28:463–491.
10. Chiu JD, Richey JM, Harrison LN, Zuniga E, Kolka CM, Kirkman E, Ellmerer M, Bergman RN. Direct administration of insulin into skeletal muscle reveals that the transport of insulin across the capillary endothelium limits the time course of insulin to activate glucose disposal. *Diabetes*. 2008;57:828–835.
11. Yang YJ, Hope ID, Ader M, Bergman RN. Insulin transport across capillaries is rate limiting for insulin action in dogs. *J Clin Invest*. 1989;84:1620–1628.
12. Miles PD, Levisetti M, Reichart D, Khoursheed M, Moossa AR, Olefsky JM. Kinetics of insulin action in vivo: identification of rate-limiting steps. *Diabetes*. 1995;44:947–953.
13. Rattigan S, Clark MG, Barrett EJ. Hemodynamic actions of insulin in rat skeletal muscle: evidence for capillary recruitment. *Diabetes*. 1997;46:1381–1388.
14. Clerk LH, Rattigan S, Clark MG. Lipid infusion impairs physiologic insulin-mediated capillary recruitment and muscle glucose uptake in vivo. *Diabetes*. 2002;51:1138–1145.
15. Coggins M, Lindner J, Rattigan S, Jahn L, Fasy E, Kaul S, Barrett E. Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. *Diabetes*. 2001;50:2682–2690.
16. Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ. Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. *Diabetes*. 2006;55:1436–1442.
17. Liu Z, Liu J, Jahn LA, Fowler DE, Barrett EJ. Infusing lipid raises plasma free fatty acids and induces insulin resistance in muscle microvasculature. *J Clin Endocrinol Metab*. 2009;94:3543–3549.
18. Liu Z. Insulin at physiological concentrations increases microvascular perfusion in human myocardium. *Am J Physiol Endocrinol Metab*. 2007;293:E1250–E1255.
19. Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S. Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am J Physiol Endocrinol Metab*. 2003;285:E123–E129.
20. Lim HS, MacFadyen RJ, Lip GYH. Diabetes mellitus, the renin-angiotensin-aldosterone system, and the heart. *Arch Intern Med*. 2004;164:1737–1748.
21. Andreozzi F, Laratta E, Sciacqua A, Perticone F, Sesti G. Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser312 and Ser616 in human umbilical vein endothelial cells. *Circ Res*. 2004;94:1211–1218.
22. Velloso LA, Folli F, Sun XJ, White MF, Saad MJA, Kahn CR. Cross-talk between the insulin and angiotensin signaling systems. *Proc Natl Acad Sci USA*. 1996;93:12490–12495.
23. Folli F, Kahn CR, Hansen H, Bouchie JL, Feener EP. Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels: a potential role for serine phosphorylation in insulin/angiotensin II crosstalk. *J Clin Invest*. 1997;100:2158–2169.
24. Scheen AJ. Prevention of type 2 diabetes mellitus through inhibition of the renin-angiotensin system. *Drugs*. 2004;64:2537–2565.
25. Buchanan TA, Thawani H, Kades W, Modrall JG, Weaver FA, Laurel C, Poppiti R, Xiang A, Hsueh W. Angiotensin II increases glucose utilization during acute hyperinsulinemia via a hemodynamic mechanism. *J Clin Invest*. 1993;92:720–726.
26. Jamerson KA, Nesbitt SD, Amerena JV, Grant E, Julius S. Angiotensin mediates forearm glucose uptake by hemodynamic rather than direct effects. *Hypertension*. 1996;27:854–858.
27. Ye S, Zhong H, Duong VN, Campese VM. Losartan reduces central and peripheral sympathetic nerve activity in a rat model of neurogenic hypertension. *Hypertension*. 2002;39:1101–1106.
28. Vincent MA, Clerk LH, Lindner JR, Klibanov AL, Clark MG, Rattigan S, Barrett EJ. Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake in vivo. *Diabetes*. 2004;53:1418–1423.
29. Inyard AC, Clerk LH, Vincent MA, Barrett EJ. Contraction stimulates nitric oxide independent microvascular recruitment and increases muscle insulin uptake. *Diabetes*. 2007;56:2194–2200.
30. Wei K, Jayaweera AR, Firoozan S, Linka A, Skyba DM, Kaul S. Quantification of myocardial blood flow with ultrasound-induced destruction of microbubbles administered as a constant venous infusion. *Circulation*. 1998;97:473–483.
31. Wei K, Ragosta M, Thorpe J, Coggins M, Moos S, Kaul S. Noninvasive quantification of coronary blood flow reserve in humans using myocardial contrast echocardiography. *Circulation*. 2001;103:2560–2565.
32. Hannan RE, Davis EA, Widdop RE. Functional role of angiotensin II AT2 receptor in modulation of AT1 receptor-mediated contraction in rat uterine artery: involvement of bradykinin and nitric oxide. *Br J Pharmacol*. 2003;140:987–995.
33. Fliser D, Arnold U, Kohl B, Hartung R, Ritz E. Angiotensin II enhances insulin sensitivity in healthy volunteers under euglycemic conditions. *J Hypertens*. 1993;11:983–988.
34. Yayama K, Hiyoshi H, Imazu D, Okamoto H. Angiotensin II stimulates endothelial NO synthase phosphorylation in thoracic aorta of mice with abdominal aortic banding via type 2 receptor. *Hypertension*. 2006;48:958–964.

35. Yayama K, Horii M, Hiyoshi H, Takano M, Okamoto H, Kagota S, Kunitomo M. Up-regulation of angiotensin II type 2 receptor in rat thoracic aorta by pressure-overload. *J Pharmacol Exp Ther*. 2004;308: 736–743.

36. Zhang C, Hein TW, Wang W, Kuo L. Divergent roles of angiotensin II AT1 and AT2 receptors in modulating coronary microvascular function. *Circ Res*. 2003;92:322–329.

37. Thai H, Wollmuth J, Goldman S, Gaballa M. Angiotensin subtype 1 receptor (AT1) blockade improves vasorelaxation in heart failure by up-regulation of endothelial nitric-oxide synthase via activation of the AT2 receptor. *J Pharmacol Exp Ther*. 2003;307:1171–1178.

38. Jun K, Masataka M. AT$_2$ receptor-dependent vasodilation is mediated by activation of vascular kinin generation under flow conditions. *Br J Pharmacol*. 2002;136:484–491.

39. Renkin EM. B. W. Zweifach award lecture: regulation of the microcirculation. *Microvasc Res*. 1985;30:251–263.

40. Clerk LH, Vincent MA, Lindner JR, Clark MG, Rattigan S, Barrett EJ. The vasodilatory actions of insulin on resistance and terminal arterioles and their impact on muscle glucose uptake. *Diabetes Metab Res Rev*. 2004;20:3–12.

41. Prasad A, Quyyumi AA. Renin-angiotensin system and angiotensin receptor blockers in the metabolic syndrome. *Circulation*. 2004;110: 1507–1512.

42. Zhang L, Cheng J, Ma Y, Thomas W, Zhang J, Du J. Dual pathways for nuclear factor κB activation by angiotensin II in vascular smooth muscle: phosphorylation of p65 by IκB kinase and ribosomal kinase. *Circ Res*. 2005;97:975–982.

43. Zhang L, Ma Y, Zhang J, Cheng J, Du J. A new cellular signaling mechanism for angiotensin II activation of NF-κB: An IκB-independent, RSK-mediated phosphorylation of p65. *Arterioscler Thromb Vasc Biol*. 2005;25:1148–1153.

44. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest*. 2005;115:1111–1119.

45. Vincent MA, Dawson D, Clark ADH, Lindner JR, Rattigan S, Clark MG, Barrett EJ. Skeletal muscle microvascular recruitment by physiological hyperinsulinemia precedes increases in total blood flow. *Diabetes*. 2002; 51:42–48.

46. Eggleston EM, Jahn LA, Barrett EJ. Hyperinsulinemia rapidly increases human muscle microvascular perfusion but fails to increase muscle insulin clearance: evidence that a saturable process mediates muscle insulin uptake. *Diabetes*. 2007;56:2958–2963.

47. Ye J, Gao Z, Yin J, He Q. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. *Am J Physiol Endocrinol Metab*. 2007;293: E1118–E1128.

48. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, Furukawa S, Tochino Y, Komuro R, Matsuda M, Shimomura I. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes*. 2007;56:901–911.

49. Ye J. Emerging role of adipose tissue hypoxia in obesity and insulin resistance. *Int J Obes*. 2008;33:54–66.

50. Yin J, Gao Z, He Q, Zhou D, Guo Z, Ye J. Role of hypoxia in obesity-induced disorders of glucose and lipid metabolism in adipose tissue. *Am J Physiol Endocrinol Metab*. 2009;296:E333–E342.

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