Angiotensin II in the Elderly
Impact of Angiotensin II Type 1 Receptor Sensitivity on Peripheral Hemodynamics

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Abstract—Exercise hyperemia is attenuated in the elderly, which may be attributed to local vasoregulatory pathways within the skeletal muscle vasculature. Therefore, we sought to determine whether healthy aging is associated with changes in angiotensin II (Ang II) receptor sensitivity through measurements of leg blood flow in resting and exercising skeletal muscle. In 12 (n=6 young, 24±1 years; n=6 older, 68±3 years) healthy volunteers, we determined changes in leg blood flow (ultrasound Doppler) before and during intra-arterial infusion of Ang II (0.8 ng/mL of leg blood flow per minute). Heart rate, arterial blood pressure, common femoral artery diameter, and mean blood velocity were measured at rest and during knee-extensor exercise at 20% and 40% of the maximal work rate (WRmax). At rest, Ang II infusion decreased leg blood flow to a greater extent in older (−61±8%) subjects compared with younger subjects (−31±5%). Compared with rest, Ang II–mediated vasoconstriction (leg blood flow) during exercise was diminished in both older and younger subjects at 20% (older: −7±5%; younger: −21±2%) and 40% WRmax (older: −5±4%; younger: −9±3%). These data identify a clear age-related hypersensitivity to Ang II in the resting leg, which may contribute to the recognized decrement in leg blood flow in this cohort. However, the diminished vasoconstriction to Ang II during exercise suggests that the elevation in Ang II type 1 receptor sensitivity documented at rest does not contribute significantly to the blunted exercise hyperemia experienced with advancing age. (Hypertension. 2008;51:1-6.)

Key Words: basic science ■ elderly ■ blood flow regulation ■ exercise ■ angiotensin receptors

Angiotensin II (Ang II) acts as a potent endogenous vasoconstrictor through binding to the Ang II type 1 (AT1) receptor on arteriolar vascular smooth muscle. With advancing age, there is a notable decline in plasma renin activity1 accompanied by small decrements in circulating Ang II2 and an increase in AT1 receptor density.3,4 However, the functional consequence of this age-related adaptation of the renin-angiotensin system on the peripheral circulation is not well understood. Although the pressor response to systemic Ang II infusion is elevated with age, no age-specific adaptation in AT1 receptor sensitivity has been observed with local, intra-arterial Ang II infusion in the arm.6 Together, these studies indicate a general decline in renin-angiotensin system function with age but with uncertainty as to the impact of these changes on end-organ function and skeletal muscle hemodynamics.

AT1 receptor sensitivity to Ang II in the elderly may be especially important in the context of underlying changes in autonomic function. It is well established that elevated vasoconstrictor tone as a consequence of high sympathetic nerve activity is present in healthy, older adults,7-9 despite a reduction in the sensitivity of postjunctional adrenergic vascular receptors.10 This capacity for sustained sympathetic vasoconstriction in the face of reduced adrenergic responsiveness may be suggestive of an age-related change in nonadrenergic vasoconstrictor pathways, such as Ang II. Clinically, further characterization of age-related changes in AT1 receptor function are particularly important, given that the combination of sympathetic and renin-angiotensin system activation adversely affects prognosis in renal and cardiovascular diseases.11

The age-related elevation in vascular tone and subsequent decline in resting limb blood flow subsists during exercise, with evidence from our group12,13 and others,14,15 for a reduction in exercise hyperemia in the leg of elderly subjects. The mechanisms responsible for this apparent reduction in vasodilatory capacity remain unknown, although studies in the forearm suggest that the augmented sympathetic vasoconstriction seen at rest may carry over during handgrip exercise in older individuals, resulting in a lesser “magnitude of sympatholysis” in older individuals.16,17 Again, the relatively higher vasoconstriction present during exercise in older individuals may not be adrenergic in nature, but rather may be partially attributed to altered sensitivity of nonadrenergic
vasoconstrictor pathways. However, the degree to which Ang II–mediated vasoconstriction may be blunted during exercise has not been evaluated in the elderly.

Thus, the current study sought to evaluate the functional consequence of age-related changes in AT1 receptor sensitivity through determination of vasoconstriction in response to intra-arterial administration of exogenous Ang II at rest and during knee-extensor exercise. Specifically, we hypothesized the following: (1) AT1 receptor sensitivity would be elevated in older individuals compared with their younger counterparts; (2) knee-extensor exercise would blunt Ang II–mediated vasoconstriction in an intensity-dependent manner in both groups; and (3) older subjects would remain relatively more sensitive to Ang II than the young group during exercise.

Methods

Subjects and General Procedures

Twelve healthy subjects (n=6 young; 24±1 years; n=6 old, 68±3 years) participated in the current study. All of the subjects were nonsmokers, normotensive (<140/90 mm Hg), normally active, and free of overt cardiovascular disease, as identified by responses to a health history questionnaire and a physical examination. Protocol approval and informed consent were obtained according to the University of California San Diego Human Subjects Protection Program requirements. Subjects reported to the laboratory on a preliminary day to complete health histories, physical examinations, and to perform a graded single-leg knee-extensor test to determine maximal work rate (WRmax).

Experimental Protocol

Subjects reported to the laboratory in a fasted state at 8 AM on the experimental day. A catheter was placed in the common femoral artery using manual auscultation (Table 1). After catheter placement, real-time blood flow was determined using ultrasound Doppler, and infusion rate was blood flow adjusted according to these "on-the-fly" blood flow values to ensure similar effective concentrations of infused drugs across subjects, both at rest and during exercise. Thus, infusion rates varied between 0.5 and 15.0 mL/min, achieved using a constant speed infusion pump (Harvard Apparatus). For the present study, we selected a dose that would elicit significant vasoconstriction but limit the risk of systemic spillover. In addition, in several subjects, we reproduced the dose–response curves for Ang II that we have documented previously in the young.

In both age groups, initial studies identified a tendency for increased mean arterial blood pressure after 60 to 90 seconds of continuous infusion during exercise, suggesting that Ang II effects were no longer limited to the leg. Thus, to avoid a potential pressor effect, Ang II infusion was stopped after 90 seconds at 20% WRmax and after 60 seconds at 40% WRmax.

Exercise Model

A knee-extensor exercise paradigm was implemented in this study,20 because this approach allows examination of exercising skeletal muscle hemodynamics without central cardiovascular limitations. Subjects were seated on an adjustable chair, with a cycle ergometer (model 828e, Monark Exercise AB) placed behind them. Resistance was provided by friction on the flywheel, which was turned by the subject via a metal bar connected to the crank of the ergometer and a boot attached to the ankle of the subject. Sixty contractions per minute were maintained at each work rate. The 20% and 40% WRmax exercise intensities were selected to minimize the amount of infused drug and, thus, to limit systemic spillover and also to avoid exercise-induced increases in sympathetic nerve activity, which could confound the observed vasoconstriction in response to Ang II infusion.

Measurements

Leg blood flow was evaluated using an ultrasound Doppler device (Logiq 7, GE Medical Systems) equipped with a linear array transducer operating at an imaging frequency of 10 MHz. The common femoral artery was insonated 2 to 3 cm proximal to the bifurcation of the common femoral artery into the superficial and deep branches. The blood velocity profile was obtained using the same transducer with a Doppler frequency of 4.0 to 5.0 MHz, operated in the high-pulsed repetition frequency mode (2 to 25 kHz), and the sample volume was placed at a depth of 1.5 to 3.5 cm and maximized according to vessel size. All of the blood velocity measurements were obtained with the probe appropriately positioned to maintain an insolation angle of ≤60°. At all of the sample points, arterial diameter and angle-corrected, intensity-weighted mean blood velocity (Vmean) values were calculated using commercially available software (Logiq 7, GE Medical Systems). Using measured artery diameter and Vmean, blood flow was calculated as follows: blood flow (mL/min)=Vmean×π×(vessel diameter/2)²×60.

Before catheterization, arterial blood pressure was determined using manual auscultation (Table 1). After catheter placement, arterial blood pressure measurements were collected continually from within the femoral artery (Table 2), with the pressure transducer placed at the level of the catheter (Transpac IV, Abbot Laboratories). Mean arterial pressure (mm Hg) was calculated as follows: diastolic arterial pressure + (arterial pulse pressure×0.33). Leg vascular conductance (mL·min⁻¹·mm Hg⁻¹) was calculated as follows: leg blood flow/mean arterial pressure. Heart rate was monitored from a standard 3-lead ECG, recorded in duplicate on the data acquisition device (BIOPAC Systems Inc) and as an integral part of the Doppler system (Logiq 7, GE Medical Systems).

Data Analysis and Statistics

Ultrasound images and Doppler velocity waveforms were measured continuously, with repeated 45-second segments recorded digitally before and during drug infusions. For each 45-second ultrasound Doppler segment, Vmean was averaged across 15-second intervals of each recorded clip, with intima-to-intima diameter measurements.
evaluated during diastole, as described previously. Because of the anticipated decline in maximal knee-extensor exercise capacity in older subjects, the a priori design was to assess responses at similar relative exercise intensities. However, posthoc analysis of responses using a single absolute work rate of 10 W was also performed to evaluate potential age-related differences at comparable levels of leg O2 consumption.

Statistics were performed with the use of commercially available software (SigmaStat 3.10, Systat Software Inc). For both groups, sample size determination was performed based on Ang II–induced changes in measured variables at 10% level of significance. Repeated-measure ANOVA, ANOVA, and Student t tests were used to identify significant changes in measured variables within and between groups and across exercise intensities, with the Holm-Sidak test used for posthoc analysis when a significant main effect was found. All of the group data are expressed as means±SE.

## Results

### Subjects

Subject characteristics are presented in Table 1.

### Ang II at Rest and During Exercise

At rest, continuous infusion of Ang II (0.8 ng/mL of leg blood flow per minute) did not significantly change the heart rate, femoral artery diameter, or mean arterial blood pressure in either group after 2.5 minutes of continuous infusion (Table 2). However, this infusion did provoke a significant and marked reduction in leg blood flow (−117±25 mL·min⁻¹·m⁻²·mmHg; young; −183±22 mL·min⁻¹·m⁻²·mmHg, old) and leg vascular conductance (−1.2±0.1 mL·min⁻¹·m⁻²·mmHg, young; −2.1±0.2 mL·min⁻¹·m⁻²·mmHg, old), which were significantly greater in older subjects compared with their younger counterparts (Table 2 and Figure 1). Dose-response curves assessed in several subjects revealed a near-maximal response to Ang II–mediated vasoconstriction for both groups at the flow-adjusted dose used in the present study.

Similar to rest, Ang II infusion for 90 seconds (20% WRmax) or 60 seconds (40% WRmax) during exercise did not elicit changes in heart rate, mean arterial blood pressure, or common femoral artery diameter in either group (Table 2). However, Ang II–mediated vasoconstriction was blunted during knee extensor exercise in both young and old subjects. At 20% WRmax, Ang II significantly reduced leg blood flow (−474±80 mL·min⁻¹·m⁻²·mmHg; −21% decrease) and leg vascular conductance (−5±1 mL·min⁻¹·m⁻²·mmHg; −22% decrease) in the young group, whereas no significant vasoconstriction was observed in older volunteers. At 40% WRmax, vasoconstriction to Ang II was abolished in both the young and older groups (Figure 1). Posthoc examination of responses to Ang II at a similar absolute work rate (10±1 W, young; 11±1 W, older) again identified a significantly greater reduction in both leg blood flow and leg vascular conductance between young and old (Figure 2).

### Discussion

This study sought to determine whether healthy aging is associated with changes in Ang II receptor sensitivity and to characterize the functional consequence of such changes through the evaluation of leg blood flow in resting and exercising skeletal muscle. We identified a profound age-related increase in AT1 receptor sensitivity in the vasculature of the resting leg, as determined by a greater vasoconstriction and subsequent reduction in leg blood flow in response to
exogenous Ang II. Interestingly, Ang II–mediated vasoconstriction was greatly reduced in both groups during isolated, small muscle mass exercise, with AT$_1$ receptor sensitivity under these conditions attenuated to a greater degree in the older subjects. Together, these data identify a differential role of the Ang II pathway in the resting and exercising muscle vasculature, indicating that age-related elevations in resting AT$_1$ receptor sensitivity may play a role in the attenuated resting leg blood flow with age but likely do not contribute to the blunted exercise hyperemia widely observed in the elderly.

**AT$_1$ Receptor Sensitivity at Rest**

Using equivalent doses of Ang II between groups, the present study has identified a greater Ang II–mediated vasoconstriction in healthy, older adults compared with their younger counterparts at rest (Table 2 and Figure 1). This finding is in contrast to those of Hogikyan and Supiano, who reported similar percentage changes in forearm blood flow between young and older volunteers to intra-arterial Ang II infusion in the brachial artery, a disparity that may be attributed to significant differences in methodology between studies. This previous study was performed in the forearm, used venous occlusion plethysmography and administered Ang II in incrementally increasing doses that evoked a systemic pressor response. In contrast, we performed ultrasound Doppler measurements of leg blood flow to a single, blood flow–adjusted dose on Ang II with no indication of systemic spillover. In addition, several recent studies have identified clear limb-specific responses to pharmacological and physiological stimuli, as well as significant differences in tonic vasoconstriction between limbs in the elderly. Thus, the current data extend this earlier work in the forearm, demonstrating for the first time a significant increase in AT$_1$ receptor sensitivity in the leg vasculature of healthy elderly individuals at rest.

Although the mechanism for increased AT$_1$ responsiveness may be as simple as receptor upregulation in response to reduced substrate, this pathway may become an increasingly important regulator of peripheral hemodynamics with advancing age. Recent studies have identified a reduction in postjunctional $\alpha$-adrenergic receptor sensitivity in both the arm and leg of older individuals, yet $\alpha$-adrenergic blockade in this population does not completely restore hemodynamics to that of the young. We speculate that this observation is suggestive of a significant contribution from other, nonadrenergic receptor groups, such as Ang II; however, further studies involving pharmacological blockade of AT$_1$ receptors are required to more completely address the contribution of this pathway to the reduced skeletal muscle blood flow in the elderly.

Additional considerations for the observed increase in Ang II–mediated vasoconstriction with age include the potential role of alternate AT$_1$ and ANG II type 2 receptor populations and the contribution of age-specific variations in endothelial–dependent vasodilation on the observed responses. Regarding the previous, it is important to consider the potential impact of age on AT$_1$ receptors located on postganglionic sympathetic nerves, which, when bound, can potentiate release and inhibit reuptake of the sympathetic neurotransmitter norepinephrine. If this receptor group were to demonstrate an increased sensitivity with age, Ang II–mediated sympathetic vasoconstriction may have been augmented to a greater extent in the elderly. However, any potential amplifying effect on Ang II–mediated vasoconstriction would be miti-
Ang II–Mediated Vasoconstriction During Exercise

During exercise, perfusion to active skeletal muscle must increase according to the metabolic demand of the tissue. Although the mechanisms governing this event are far from certain, there exists evidence in humans for a reduction in the effectiveness of both adrenergic31–33 and nonadrenergic18,34 vasoconstrictor pathways to facilitate the requisite hyperemic response. The potential involvement of endogenous Ang II in the regulation of exercise hyperemia is supported by the acute increase in circulating Ang II during exercise,35 as well as the profound hemodynamic effect seen when AT1 receptor blockade is applied during exercise. Indeed, pretreatment with the AT1 antagonist losartan has been shown to significantly decrease systemic vascular resistance and mean arterial pressure during exercise compared with the unblocked state,36,37 indicating that Ang II is an important component of the systemic cardiovascular response to dynamic exercise.

Although this previous work using AT1 receptor blockade identified a significant role of endogenous Ang II during exercise, potential metabolic inhibition of this receptor group was not examined. Members of our group have recently addressed this issue though acute, intra-arterial administration of exogenous Ang II in younger subjects at rest and during light- and moderate-intensity exercise and reported a significant decrease in leg blood flow with Ang II infusion at rest, which was blunted in an intensity-dependent manner during knee-extensor exercise.38 Data from the present study extend these findings to an elderly cohort, who also experienced a diminished response to exogenous Ang II administration during exercise (Table 2 and Figure 1). It is noteworthy that the knee-extensor exercise capacity was reduced in older subjects (Table 1), and, thus, 20% and 40% WRmax represent lower absolute work in this group. However, between-group differences are only amplified when young and older groups are compared at a single absolute work rate (Figure 2), confirming the presence of an age-related diminution in Ang II–mediated vasoconstriction during exercise.

This age-dependent effect is even more pronounced when the change in AT1 sensitivity from rest to exercise is compared. In view of the greatly enhanced Ang II–mediated vasoconstriction in the elderly at rest, the “magnitude of attenuation” in leg blood flow from rest to 40% WRmax exercise is much greater in the older (50% to 60%) compared with the young (10% to 20%) group (Figures 1 and 2). These data are suggestive of a powerful mechanism within the active muscle tissue, likely multiple byproducts of aerobic and/or anaerobic metabolism, that is capable of overcoming the potent vasoconstrictor effects of Ang II and, again, indicates that age-related elevations in resting AT1 receptor sensitivity do not contribute significantly to the blunted exercise hyperemia widely observed in the elderly.12–15 Indeed, it seems that the profound “lysing” of both nonadrenergic (Figure 1) and adrenergic16 vasoconstriction may be nondiscriminate yet essential events in the overall series of reactions that collectively produce a sufficient increase in skeletal muscle blood flow, raising the degree of exercise hyperemia in the elderly cohort toward that of the young.

Perspectives

The present data demonstrating an elevated AT1 receptor sensitivity in healthy, older adults at rest may be particularly relevant to the progression of cardiovascular disease states characterized by an elevation in both sympathetic nerve activity and Ang II, such as hypertension and heart failure.38,39 Current recognition of the underlying AT1 sensitivity in the healthy elderly may present a pathway that is particularly susceptible to Ang II–mediated vasoconstriction and associated pressor effects. Indeed, a large number of clinical trials have been undertaken to examine AT1 receptor blockade and have shown this therapy to be efficacious in reducing resting arterial blood pressure,40 vascular compliance,41 and vascular tone42 in patients. Thus, we speculate that the present data identifying an apparent hypersensitivity of the AT1 receptor population in this older, normotensive cohort may provide evidence of a pathway by which hypertension may develop, although further studies are needed to further examine this issue.

Recent clinical trials examining the efficacy of AT1 blockade to improve exercise capacity in patients with hypertension or heart failure have produced mixed results, with evidence for43,44 and against45,46 the beneficial effects of this intervention. However, exercise capacity in these patients is often determined by a central cardiovascular limitation, and, thus, outcome of these studies may be due to improvements in cardiac rather than skeletal muscle function. This concept is supported by the observed improvement in myocardial blood flow during exercise after AT1 receptor blockade in animals.36 Nonetheless, the current finding of blunted AT1 receptor responsiveness during small muscle mass exercise in healthy, older adults suggests that elevated AT1 receptor sensitivity in the elderly at rest does not carry over to exercise, and, thus, this age-related adaptation does not appear to present a functional limitation to skeletal muscle vasodilation during exercise.

Experimental Considerations

It should be noted that, whereas the experimental paradigm of exogenous Ang II administration allows determination of AT1 receptor sensitivity, it is not designed to characterize the interaction of AT1 receptors with endogenous Ang II. For this reason, endogenous levels of Ang II were not assessed in the present study, and, thus, the effect of exercise on plasma [Ang II] in this paradigm remains uncertain.
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Disclosures
None.

References
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