Angiotensin II potentiates α-adrenergic vasoconstriction in the elderly

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Abstract

Aging is characterized by increased sympatho-excitation, expressed through both the α-adrenergic and RAAS (renin–angiotensin–aldosterone) pathways. Although the independent contribution of these two pathways to elevated vasoconstriction with age may be substantial, significant cross-talk exists that could produce potentiating effects. To examine this interaction, 14 subjects ($n = 8$ young, $n = 6$ old) underwent brachial artery catheterization for administration of AngII (angiotensin II; 0.8–25.6 ng/dl per min), NE [noradrenaline (norepinephrine); 2.5–80 ng/dl per min] and AngII with concomitant α-adrenergic antagonism [PHEN (phentolamine); 10 μg/dl per min]. Ultrasound Doppler was utilized to determine blood flow, and therefore vasoconstriction, in both infused and contralateral (control) limbs. Arterial blood pressure was measured directly, and sympathetic nervous system activity was assessed via microneurography and plasma NE analysis. AngII sensitivity was significantly greater in the old, indicated by both greater maximal vasoconstriction (-59 ± 4% in old against -48 ± 3% in young) and a decreased EC_{50} (half-maximal effective concentration) (1.4 ± 0.2 ng/dl per min in old against 2.6 ± 0.7 μg/dl per min in young), whereas the maximal NE-mediated vasoconstriction was similar between these groups (-58 ± 9% in old and -62 ± 5% in young). AngII also increased venous NE in the old group, but was unchanged in the young group. In the presence of α-adrenergic blockade (PHEN), maximal AngII-mediated vasoconstriction in the old was restored to that of the young (-43 ± 8% in old and -39 ± 6% in young). These findings indicate that, with healthy aging, the increased AngII-mediated vasoconstriction may be attributed, in part, to potentiation of the α-adrenergic pathway, and suggest that cross-talk between the RAAS and adrenergic systems may be an important consideration in therapeutic strategies targeting these two pathways.

Key words: angiotensin II, blood flow regulation, cardiovascular system, elderly, sympathetic nervous system, vasoconstriction

INTRODUCTION

One principal alteration associated with age-related changes of the cardiovascular system is an elevation in vascular tone [1,2], which probably contributes to the attenuated skeletal muscle blood flow commonly observed in the elderly [3–8]. Although there are many regulatory pathways that collectively contribute to the age-related vasoconstriction, the effects of the α-adrenergic and RAAS (renin–angiotensin–aldosterone system) pathways are of particular interest. Specifically, there is evidence that α-adrenergic blockade in old individuals partially restores resting limb blood flow towards that of the young [1], suggesting an age-related alteration in this receptor group. Although less well studied, there is also evidence to suggest an important role of the RAAS pathway; indeed, we recently identified a significantly greater vasoconstriction in response to AngII (angiotensin

II) in the old compared with their young counterparts [7], implicating this pathway as an additional significant contributor to the regulation of resting skeletal muscle blood flow with advancing age.

Although the independent contribution of these two pathways to excessive vascular tone in the elderly may be substantial, significant cross-talk exists that could produce a potentiating effect. Specifically, circulating AngII acts as a potent vasoconstrictor through binding to AT_1 receptors (AngII subtype 1 receptors) located on the vascular smooth muscle [9]. Additionally, AngII induces a marked vasoconstriction by binding to AT_1 receptors located presynaptically on postganglionic sympathetic nerves, which enhances the release of NE [noradrenaline (norepinephrine)], attenuates the re-uptake of NE, and ultimately potentiates α-adrenergic vasoconstriction [10,11]. Considering the evidence for an age-related increase in circulating AngII and AT_1 receptor density [12,13], the direct and indirect (α-adrenergic-mediated) vasoconstricting effects of AngII in the elderly may be profound. This age-associated increase in RAAS activity is especially important in the context of underlying changes in autonomic function with age. Indeed, one of the most pronounced and reproducible observations associated with aging is a progressive increase in MSNA (muscle sympathetic nerve activity) [2,14,15], which is modulated peripherally by both the α-adrenergic and RAAS pathways. Thus it follows that, in the presence of elevated basal MSNA, the potentiating effect of circulating AngII may be even more pronounced. Considering this, presynaptic AT_1 receptors may be seen as uniquely positioned to act as a regulatory point for sympathetic nervous system control of skeletal muscle blood flow with advancing age.

The extent to which the potentiating effects of AngII on α-adrenergic vasoconstriction contribute to the enhanced AngII-mediated vasoconstriction in the elderly is currently unknown. Therefore the aim of the present study was to evaluate the peripheral haemodynamic consequences of age-related changes in AngII-mediated vasoconstriction in the presence and absence of α-adrenergic vasoconstriction. We hypothesized that AngII-mediated vasoconstriction would be greater in the old compared with the young, but this age effect would be attenuated in the presence of α-adrenergic antagonism.

MATERIALS AND METHODS

Subjects

Eight young (26 ± 1 years) and six old (68 ± 3 years) healthy subjects were enrolled in this study. All subjects were non-smokers and were normally active. Subjects were not taking any prescription medication and were free of overt cardiovascular disease, as indicated by a health history. Protocol approval and written informed consent were obtained according to the University of Utah and the Salt Lake City Veterans Affairs (VA) Medical Center Institutional Review Board requirements. All data collection took place at the VA Salt Lake City GRECC (Geriatric, Research, Education and Clinical Center) in the UVRL (Utah Vascular Research Laboratory). Subjects remained supine in a thermoneutral environment for the duration of the study.

Protocols

Subjects reported to the UVRL fasted at 08.00 h on the experimental day. Catheters (18 g, 20 cm; Arrow) were placed in the BA (brachial artery) and basilic or cephalic vein using sterile technique, with the site of BA catheter insertion approximately 10 cm distal to the axilla and both catheters advanced 6–8 cm in the proximal direction. The arterial catheter was placed in this region of the upper arm to ensure that study drugs entered the artery 'upstream' (~ 12 cm) of the ultrasound Doppler sample volume, allowing assessment of drug effects on BA diameter and blood velocity [16]. A microelectrode was placed in the peroneal nerve for direct assessment of basal MSNA, as described previously [17]. Following catheter and microelectrode placement, the drug infusion protocol was performed, as illustrated in Figure 1.

Study drugs

Lower and upper arm volumes were determined anthropometrically, and then used for the calculation of drug dosing. Total limb volume receiving infusate was calculated as:

Total volume (dl) = forearm volume + (upper arm × 0.5)

A portion of the upper arm was included in this calculation due to the proximal location of the arterial catheter [16]. Prior to each drug infusion trial, baseline measurements were taken in order to account for any changes in baseline values between the trials.

AngII (Bachem) was diluted from 50 μg of freeze-dried powder in normal saline (0.9 % NaCl) to a concentration of 0.125 μg/ml. The drug was infused intra-arterially at 0.8–1.6–3.2–6.4–12.8 μg/dl per min (3 min for each dose). NE (norepinephrine bitartrate; Levophed; Hospira) was diluted from 4 mg of suspension in 5 % dextrose to a concentration of 1 μg/ml. The drug was infused intra-arterially at 2.5–5–10–20–40 μg/dl per min (3 min for each dose). If a subject did not exhibit a plateau in BA blood flow and BA diameter after the fifth dose of AngII or NE, a sixth doubling dose (AngII, 25.6 μg/dl per min; NE, 80 μg/dl per min) was administered of the respective drug. PHEN (phentolamine mesylate; Regitine, Bedford Labs) was diluted from 5 mg of freeze-dried powder in normal saline (0.9 % NaCl) to a concentration of 83.3 μg/ml. The drug was infused at a rate of 10 μg/dl per min for 20 min followed by a maintenance dose 5 μg/dl per min during the remainder of the protocol. In a subset of subjects ($n = 4$ young, $n = 4$ old), NE was also infused at 5 and 20 μg/dl per min (3 min for each dose) at the end of the α-adrenergic blockade portion of the protocol to challenge the α-receptor blockade (Figure 1).

Measurements

Simultaneous measurements of BA blood velocity and vessel diameter were performed in both the infused and control arms using Logiq 7 and Logic e ultrasound Doppler systems (GE Medical Systems) operating in duplex mode. The Logic 7 and Logic e were equipped with linear array transducers operating at imaging frequencies of 14 and 12 MHz, respectively. Blood velocity was obtained using the same transducers with a Doppler frequency of 5 MHz. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of $60°$ or less. The sample volume was
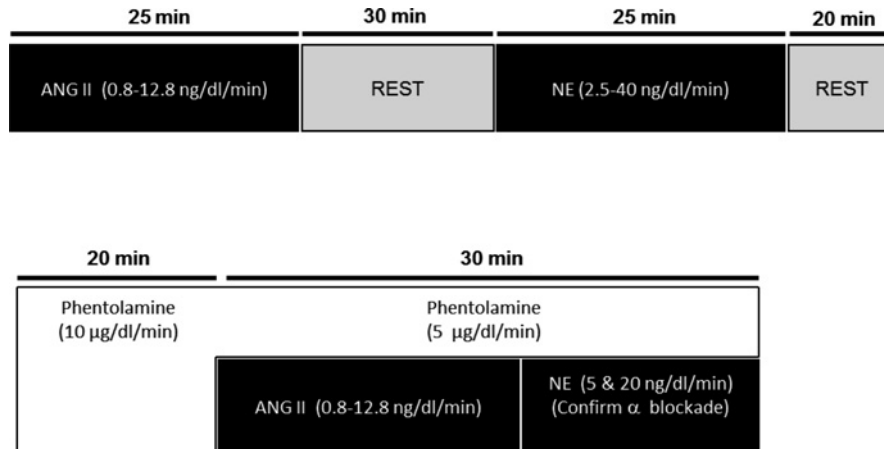
Figure 1   Protocol timeline
The order of AngII and NE administration was balanced, but the remainder of the protocol was ordered due to lasting effects of PHEN on BA blood flow.

maximized according to vessel size and was centered within the vessel on the basis of real-time ultrasound visualization. Mean velocity values (V_{mean}, angle-corrected and intensity-weighted area under the curve) were calculated using commercially available software (Logic 7 and Logic e). End-diastolic, ECG R-wave-gated images were collected via video output from the Logic 7 and Logic e for off-line analysis of BA vasodilation using automated edge-detection software (Medical Imaging Applications). Using BA diameter and V_{mean}, BA blood flow was calculated according to the equation:

$$BA\ blood\ flow = [V_{mean} \cdot \pi (vessel\ diameter/2)^2 \cdot 60]$$

Basal MSNA was recorded directly from the peroneal nerve, as described previously [18–20]. Briefly, a tungsten microelectrode was inserted into fascicles of the peroneal nerve near the fibular head of the left leg. Neural signals were processed by a preamplifier and amplifier (Nerve traffic analyzer model 662C-3) with a total gain of 90 000. Amplified signals were filtered (bandwidth, 700-2000 Hz), rectified, and integrated (time constant, 0.l s) to obtain mean voltage neurograms.

Heart rate was monitored from a standard three-lead ECG. Arterial blood pressure was acquired continually from within the BA, with the pressure transducer placed at the level of the catheter (Transpac IV; Abbott Laboratories).

Blood chemistry
A lipid panel was performed in all subjects by standard technique. In a subset of subjects ($n = 6$ young, $n = 5$ old), resting blood samples were obtained from the brachial artery and the basilic or cephalic vein. Arterial and venous plasma AngII concentration was assessed by radioimmunoassay (Bachem), and venous NE concentration by enzyme immunoassay (2-CAT Elisa; Labor Diagnostika Nord). An additional venous sample was collected at the highest AngII dose to test for changes in [NE] provoked by AngII.

Data analysis
Ultrasound images and Doppler velocity spectra were recorded continuously. During the last 60 s of each ultrasound Doppler segment (i.e. each 3 min drug infusion), V_{mean} was averaged across five 12 s intervals, which were matched with intima-to-intima BA diameter measurements evaluated during diastole in order to calculate BA blood flow. The $\log EC_{50}$ (half-maximal effective concentration) was calculated on an individual basis using a sigmoidal parameter $\{[a + (b - a)/(1 + 10(x - c)]\}]$ to estimate the vascular sensitivity to AngII (biodatafit v.1.02).

Maximal reductions in BA blood flow and BA diameter during AngII and NE infusions were identified on an individual basis from the three highest doses of AngII (6.4–12.8–25.6 μg/dl per min) and NE (20–40– 80 μg/dl per min). Mean arterial pressure was calculated using the time integral of the arterial waveform. Pulse-synchronous MSNA bursts were identified manually by an experienced microneurographer according to appearance and timing in relation to the preceding R-wave, and are expressed as burst frequency (bursts/min).

Statistical analyses were performed with the use of commercially available software (SigmaStat 3.10; Systat Software). Repeated-measure ANOVA was used to identify significant changes in measured variables within and between drug conditions, as well as between young and old. When a significant main effect was found, the Holm–Sidak method was used for α adjustment and post-hoc analysis. Student's t tests were used to identify significant differences in subject characteristics between young and old. Significance was established at $P < 0.05$. All group data are expressed as means $\pm$ S.E.M..

RESULTS

Subject characteristics are presented in Table 1. Resting MSNA measurements were attempted in all subjects, and an acceptable recording site was identified in nine of the 14 subjects ($n = 3$ young and $n = 6$ old). The old exhibited significantly higher

Table 1   Subject characteristics

Values are mean $\pm$ S.E.M. $*P < 0.05$ compared with young. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Characteristic	Young	Old
Age (years)	26 ± 1	$68 \pm 3*$
Height (cm)	176 ± 2	173 ± 4
Weight (kg)	74 ± 3	$84 \pm 4*$
Body mass index (kg/m^2)	24 ± 1	$28 \pm 1*$
Arm volume (dl)	16.7 ± 1.4	15.7 ± 0.8
Glucose (mg/dl)	79 ± 7	84 ± 2
Total cholesterol (mg/dl)	134 ± 22	146 ± 7
Triacylglycerol (mg/dl)	62 ± 10	$83 \pm 5*$
HDL (mg/dl)	47 ± 7	48 ± 7
LDL (mg/dl)	76 ± 12	83 ± 3
MSNA (bursts/min) ($n = 9$)	21 ± 3	$40 \pm 6*$
Plasma NE (venous) (pg/ml) ($n = 11$)	371 ± 54	$877 \pm 162*$
Plasma AngII (arterial) (pg/ml) ($n = 10$)	64.2 ± 8.7	58 ± 12.7

Figure 2 Dose–response for changes in BA blood flow (i.e. vasoconstriction) during intra-arterial AngII in young (circles) and old (squares) subjects

EC_{50} analysis revealed a significantly greater sensitivity to AngII in the elderly. $\dagger P < 0.05$ compared with young.

resting levels of MSNA and venous NE, whereas plasma AngII was similar between groups ($n = 6$ young, $n = 5$ old) (Table 1).
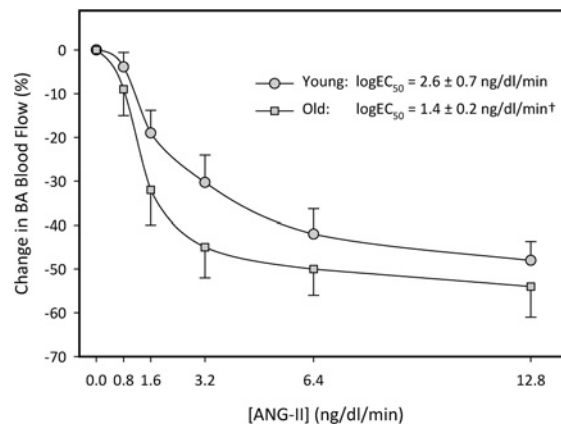
AngII

Changes in BA blood flow in response to AngII are presented in Figure 2. The calculated EC_{50} for AngII was significantly lower in old ($1.4 \pm 0.2 \, \mu$g/dl per min, $n = 6$) compared with young ($2.6 \pm 0.7 \, \mu$g/dl per min, $n = 7$). At the highest dose of AngII, a significant increase in venous NE was observed in the old, but remained unchanged in the young (Figure 3). The maximal reduction in BA blood flow (i.e. maximal vasoconstriction) induced by AngII was identified during doses 4–6 (6.4–12.8–25.6 μg/dl per min) and is shown in Figure 4. The maximal reduction in BA blood flow was significantly greater in the old ($-59 \pm 4\%$) compared with the young ($48 \pm 3\%$). Throughout the dose–response protocol, HR (heart rate), MAP (mean arterial pressure) and BA blood flow in the contra-lateral arm were unchanged (Table 2), confirming that the drug remained localized in the vasculature of the infused arm.

NE

The maximal reduction in BA blood flow induced by NE was identified during doses 4–6 (20–40–80 μg/dl per min). The maximal reduction in BA blood flow was similar between young and old (Figure 4). As with AngII, HR, MAP and BA blood flow in the contra-lateral arm were unchanged during NE infusion (Table 3), confirming that the drug remained localized in the vasculature of the infused arm.

AngII during non-specific α-adrenergic antagonism

α-Adrenergic receptor blockade by a continuous infusion of PHEN induced a significant age-independent increase in blood flow (young, $\Delta 149 \pm 39$ ml/min; old, $\Delta 182 \pm 47$ ml/min). NE was administered (5 and 20 μg/dl per min) at the end of the PHEN infusion ($n = 8$) to confirm α-adrenergic receptor blockade. Neither NE dose produced a significant change in BA blood
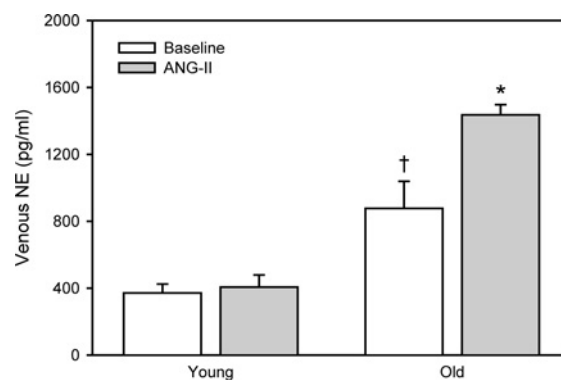
Figure 3 Venous NE concentrations before (white bars) and after (grey bars) administration of AngII ($n = 6$ young, $n = 5$ old)

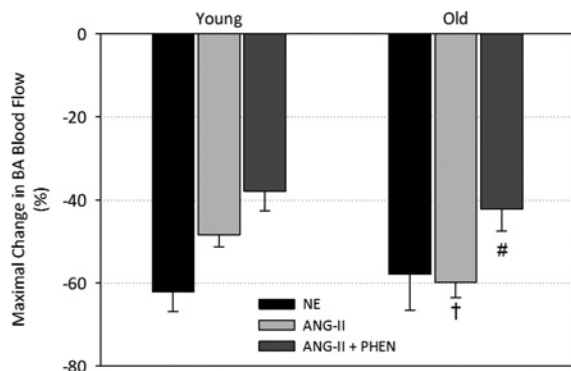$*P < 0.05$ compared with baseline; $\dagger P < 0.05$ compared with young.

flow (253 ± 92 ml/min at baseline; 241 ± 29 ml/min with NE at 5 μg/dl per min; 223 ± 3 ml/min with NE at 20 μg/dl per min).

Compared with AngII alone, the maximal reduction in BA blood flow during AngII + PHEN was similar in the young group, but was attenuated in the old (Figure 4 and Table 3), effectively abolishing the age-related differences in AngII-mediated vasoconstriction observed during AngII alone.

BA diameter

Similar reductions in BA diameter were induced by AngII and NE (Figure 5) in both the young (AngII, -0.57 ± 0.11 mm; NE, -0.51 ± 0.19 mm) and the old (AngII, -0.50 ± 0.13 mm; NE, -0.68 ± 0.06 mm). In the presence of α-adrenergic receptor antagonism, the AngII-mediated change in BA diameter was ablated in both the young (AngII + PHEN, -0.06 ± 0.03 mm) and the old (AngII + PHEN, 0.00 ± 0.02 mm) (Figure 5).

Figure 4 Maximal reductions in BA blood flow (i.e. vasoconstriction) induced by NE, AngII alone, and AngII after non-selective α-adrenergic blockade with PHEN
†$P < 0.05$ compared with young; #$P < 0.05$ compared with AngII alone.

DISCUSSION

To better understand the mechanisms regulating the peripheral circulation with advancing age, this study sought to examine the interaction between the α-adrenergic and RAAS systems. Significantly greater levels of SNS (sympathetic nervous system) activity were observed in the elderly, which was accompanied by an exaggerated vasoconstriction in response to intra-arterial AngII administration. A significant increase in venous NE was observed in the elderly during AngII infusion, suggesting a greater AngII potentiation of NE release with age. Following regional α-adrenergic blockade, the AngII-mediated vasoconstriction was similar to that of the young. Taken together, these data reveal that, with healthy aging, an increased AngII receptor sensitivity

may be attributed in part to a potentiation of the α-adrenergic-mediated vasoconstriction, and implicate the 'cross-talk' between the RAAS and adrenergic systems as an important consideration in therapeutic strategies targeting these two pathways. In the context of an age-related increase in SNS activity, these findings may also represent a potential mechanism contributing to enhanced α-adrenergic-mediated vascular tone associated with healthy aging.

AngII and blood flow regulation with age
This study has identified an enhanced sensitivity to AngII in healthy old individuals compared with their young counterparts, supported by both a reduced EC_{50} (Figure 2) and greater maximal reduction in BA blood flow (Figure 4) in the elderly cohort. These findings are in agreement with previous work from our group identifying a greater reduction in leg blood flow in old individuals compared with young following infusion of a single dose of AngII into the femoral artery [7]. Through the inclusion of an AngII dose–response protocol (Table 2) and subsequent EC_{50} determination, the assessment of circulating AngII, and control limb measurements, the present study expands on this previous work, confirming the apparent increased sensitivity to AngII in healthy old adults.

This exaggerated response to exogenous AngII in the old contrasts with the work of Hogikyan and Supiano [21], who observed a similar change in forearm blood flow in old and young individuals in response to BA infusion of AngII. This discrepancy may be due, in part, to the differences in methodology and drug dosing. The aforementioned study used venous occlusion plethysmography to measure forearm blood flow and administered a dose of AngII that elicited a systemic pressure response. In contrast, in the present study, ultrasound Doppler

Table 2   Haemodynamic responses to intra-arterial infusions of AngII
Values are means $\pm$ S.E.M. *$P < 0.05$ compared with baseline; †$P < 0.05$ compared with young.

Group	Baseline	AngII dose (ng/dl per min)				
		0.8	1.6	3.2	6.4	12.8
Young						
HR (beats/min)	63 ± 4	63 ± 4	67 ± 8	62 ± 3	62 ± 3	59 ± 2
MAP (mmHg)	99 ± 4	102 ± 4	100 ± 3	99 ± 5	100 ± 5	107 ± 4
BA diameter (mm)	3.69 ± 0.20	3.67 ± 0.19	3.62 ± 0.19	3.55 ± 0.19	$3.30 \pm 0.25^*$	$2.88 \pm 0.26^*$
BA velocity (cm/s)	6.5 ± 0.6	6.1 ± 0.6	$5.2 \pm 0.4^*$	$4.7 \pm 0.3^*$	$4.6 \pm 0.4^*$	$4.7 \pm 0.6^*$
BA blood flow (ml/min)	42 ± 6	39 ± 6	32 ± 8	$28 \pm 3^*$	$23 \pm 3^*$	$18 \pm 1^*$
BA blood flow (Δml/min)	0 ± 0	-3 ± 2	$-9 \pm 3^*$	$-14 \pm 4^*$	$-19 \pm 4^*$	$-16 \pm 3^*$
BA vascular conductance (ml/min per mmHg)	0.43 ± 0.06	0.39 ± 0.06	$0.33 \pm 0.05^*$	$0.30 \pm 0.04^*$	$0.26 \pm 0.04^*$	$0.20 \pm 0.03^*$
Non-infused BA blood flow (Δml/min)	0 ± 0	-2 ± 2	0 ± 1	3 ± 3	2 ± 2	-3 ± 5
Old						
HR (beats/min)	61 ± 5	63 ± 5	61 ± 5	64 ± 5	63 ± 5	61 ± 5
MAP (mmHg)	111 ± 3†	111 ± 3	111 ± 4	116 ± 7†	118 ± 3†	120 ± 4†
BA diameter (mm)	4.27 ± 0.38	4.24 ± 0.38	4.18 ± 0.40	4.00 ± 0.34	$3.83 \pm 0.36^*$†	$3.76 \pm 0.45^*$†
BA velocity (cm/s)	8.4 ± 1.6	8.0 ± 1.1	$5.7 \pm 0.9^*$	$5.1 \pm 0.9^*$	$5.0 \pm 0.9^*$	$5.1 \pm 0.9^*$
BA blood flow (ml/min)	65 ± 5†	63 ± 5	$42 \pm 4^*$†	$35 \pm 4^*$	$32 \pm 4^*$†	$31 \pm 3^*$†
BA blood flow (Δml/min)	0 ± 0	-3 ± 5	$-22 \pm 7^*$†	$-30 \pm 6^*$†	$-33 \pm 5^*$†	$-38 \pm 7^*$†
BA vascular conductance (ml/min per mmHg)	0.58 ± 0.04†	0.57 ± 0.05	$0.39 \pm 0.04^*$	$0.31 \pm 0.03^*$	$0.27 \pm 0.03^*$	$0.26 \pm 0.03^*$
Non-infused BA blood flow (Δml/min)	0 ± 0	0 ± 3	2 ± 2	2 ± 2	4 ± 4	-5 ± 8

Table 3   Haemodynamic responses to intra-arterial infusions of NE
Values are means $\pm$ S.E.M. *$P < 0.05$ compared with baseline.

| Group | Baseline | NE dose (ng/dl per min) | | | | |
		2.5	5	10	20	40
Young						
HR (beats/min)	$62 + 3$	$60 + 3$	$64 + 4$	$59 + 3$	$60 + 3$	$59 + 3$
MAP (mmHg)	$102 + 3$	$100 + 4$	$102 + 3$	$99 + 4$	$99 + 4$	$103 + 4$
BA diameter (mm)	$3.67 + 0.20$	$3.66 + 0.20$	$3.60 + 0.19$	$3.44 + 0.16^*$	$3.19 + 0.21^*$	$2.93 + 0.23^*$
BA velocity (cm/s)	$7.4 + 0.5$	$7.0 + 0.8$	$6.6 + 0.5$	$6.0 + 0.5^*$	$5.9 + 0.6^*$	$5.2 + 0.7^*$
BA blood flow (ml/min)	$49 + 7$	$46 + 7$	$41 + 5^*$	$34 + 4^*$	$28 + 3^*$	$21 + 4^*$
BA vascular conductance (ml/min per mmHg)	$0.48 + 0.07$	$0.47 + 0.80$	$0.41 + 0.06$	$0.35 + 0.04^*$	$0.28 + 0.04^*$	$0.21 + 0.04^*$
Non-infused BA blood flow (Δml/min)	$0 + 0$	$-2 + 3$	$2 + 2$	$8 + 8$	$2 + 4$	$6 + 6$
Old						
HR (beats/min)	$61 + 5$	$57 + 5$	$60 + 5$	$60 + 4$	$59 + 4$	$59 + 4$
MAP (mmHg)	$113 + 5$	$114 + 4$	$114 + 5$	$113 + 6$	$113 + 6$	$109 + 3$
BA diameter (mm)	$4.40 + 0.41$	$4.36 + 0.42$	$4.28 + 0.44$	$4.12 + 0.40^*$	$3.81 + 0.48^*$	$3.27 + 0.41^*$
BA velocity (cm/s)	$7.5 + 1.5$	$7.9 + 1.6$	$7.1 + 1.9$	$6.4 + 1.2^*$	$5.7 + 1.0^*$	$6.1 + 1.5^*$
BA blood flow (ml/min)	$63 + 8$	$65 + 10$	$54 + 10^*$	$49 + 9^*$	$36 + 5^*$	$33 + 11^*$
BA vascular conductance (ml/min per mmHg)	$0.56 + 0.07$	$0.57 + 0.08$	$0.47 + 0.10$	$0.43 + 0.07^*$	$0.25 + 0.07^*$	$0.21 + 0.10^*$
Non-infused BA blood flow (Δml/min)	$0 + 0$	$1 + 2$	$1 + 4$	$7 + 6$	$2 + 4$	$1 + 4$
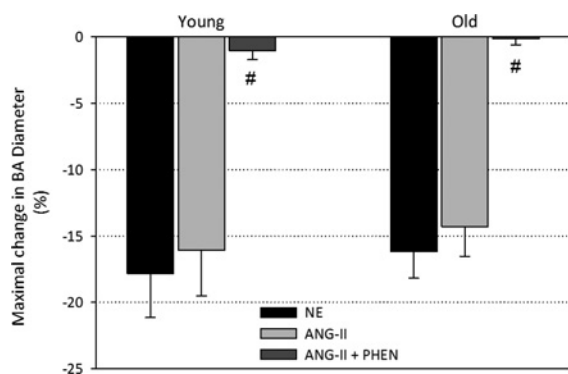
Figure 5 Maximal reductions in BA diameter induced by NE, AngII alone and AngII with concomitant α-adrenergic blockade via PHEN
#$P < 0.05$ compared with AngII alone.

was utilized to determine BA blood flow, there was no significant change in MAP during AngII administration, and BA blood flow in the contra-lateral (non-infused) arm during infusion of AngII was unchanged (Table 2). Therefore, through a controlled and comprehensive experimental design, the present findings build on these previous observations, now identifying a clear increase in AngII-mediated vasoconstriction in the elderly.

AngII interaction with the sympathetic nervous system

AngII acts as a potent vasoconstrictor not only by binding to AT_1 receptors located on the vascular smooth muscle [9], but also through the potentiation of sympathetic (i.e. α-adrenergic) vaso-constriction [10,11]. Indeed, previous studies have documented the presence of AT_1 receptors located presynaptically on post-

ganglionic sympathetic nerves, and identified the ability of this receptor group to potentiate the release and inhibit the reuptake of NE [11,22]. This apparent 'cross-talk' between the RAAS and adrenergic systems may be especially relevant in the elderly due to the well-described increase in resting SNS activity that accompanies healthy aging [2,14,15], predisposing this population to excess sympathetic vasoconstriction mediated by circulating AngII.

As anticipated, we observed greater MSNA and circulating catecholamines in the old compared with their younger counterparts (Table 1), confirming an age-associated rise in SNS activity. During maximal doses of AngII, we observed an increase in venous NE in the old, but not young (Figure 3), clearly indicating an exaggerated potentiation of NE release in response to AngII in the elderly. The functional role of these presynaptic AT_1 receptors was further established by repeating the AngII dose–response protocol with concomitant infusion of the non-selective α-adrenergic receptor antagonist PHEN. In support of our hypothesis, the age-associated increase in the AngII-mediated vaso-constriction was abolished after α-adrenergic blockade, such that the maximal reduction in blood flow in response to AngII was similar to that observed in the young group (Figure 4, Table 4). These findings thus indicate that α-adrenergic vasoconstriction is responsible for nearly 20% of the maximal reduction in blood flow induced by intra-arterial AngII infusion in the old (Figure 2), approximately double that observed in the young.

The interaction between the RAAS and α-adrenergic pathways in the peripheral circulation is well documented, though the exact site of action is less clear. Using an *in vivo* model, both Seidelin et al. [23] and Webb et al. [24] observed that BA infusions of low doses of AngII (at concentrations that do not affect forearm blood flow) enhanced the sympathetically mediated reduction in blood flow induced

Table 4   Haemodynamic responses to intra-arterial infusions of AngII with α-receptor antagonism
Values are means $\pm$ S.E.M. PHEN haemodynamics were used as baseline parameters for the AngII dose–response. *$P < 0.05$ compared with baseline; †$P < 0.05$ compared with young; ‡$P < 0.05$ compared with PHEN.

Group	Baseline	PHEN	AngII dose (ng/dl per min)				
			0.8	1.6	3.2	6.4	12.8
Young							
HR (beats/min)	61 ± 3	65 ± 3	65 ± 3	62 ± 3	60 ± 2‡	62 ± 2	62 ± 3
MAP (mmHg)	102 ± 2	102 ± 3	100 ± 3	100 ± 4	102 ± 5	110 ± 3‡	108 ± 3
BA diameter (mm)	3.71 ± 0.22	3.86 ± 0.22*	3.86 ± 0.22	3.86 ± 0.21	3.87 ± 0.21	3.83 ± 0.20‡	3.77 ± 0.23
BA velocity (cm/s)	10.7 ± 1.6	31.6 ± 5.2*	28.0 ± 4.4	29.2 ± 4.6	26.6 ± 4.3‡	23.6 ± 3.4‡	22.1 ± 2.0‡
BA blood flow (ml/min)	71 ± 13	220 ± 44*	192 ± 33	201 ± 35	180 ± 30‡	162 ± 29‡	150 ± 20‡
BA blood flow (Δml/min)	0 ± 0	149 ± 39*	-29 ± 11	-20 ± 10	-40 ± 19‡	-58 ± 20‡	-92 ± 26‡
BA vascular conductance (ml/min per mmHg)	0.70 ± 0.13	2.19 ± 0.44*	1.90 ± 0.32	2.00 ± 0.34	1.76 ± 0.28‡	1.51 ± 0.29‡	1.37 ± 0.19‡
Non-infused BA blood flow (Δml/min)	0 ± 0	4 ± 2	1 ± 3	-1 ± 2	1 ± 4	5 ± 4	4 ± 4
Old							
HR (beats/min)	64 ± 6	67 ± 2	65 ± 7	66 ± 6	65 ± 7	64 ± 6	65 ± 8
MAP (mmHg)	115 ± 5†	115 ± 5†	115 ± 4†	115 ± 4†	116 ± 4†	119 ± 5	123 ± 6
BA diameter (mm)	4.20 ± 0.46	4.32 ± 0.26*	4.34 ± 0.27	4.34 ± 0.27	4.36 ± 0.27‡	4.36 ± 0.27‡	4.38 ± 0.26‡
BA velocity (cm/s)	11.1 ± 1.8	26.7 ± 6.5*	21.1 ± 6.1	21.0 ± 6.3	19.3 ± 5.8‡	18.2 ± 5.4‡	14.1 ± 5.5‡
BA blood flow (ml/min)	93 ± 24	275 ± 43*	231 ± 30	228 ± 26‡	214 ± 31‡	198 ± 33‡	166 ± 40‡
BA blood flow (Δml/min)	0 ± 0	182 ± 47*	-44 ± 16	-47 ± 15‡	-61 ± 18‡	-77 ± 26‡	-110 ± 60‡
BA vascular conductance (ml/min per mmHg)	0.79 ± 0.18	2.26 ± 0.90	2.08 ± 0.93	1.91 ± 0.91	1.76 ± 0.86‡	1.59 ± 0.76‡	1.25 ± 0.76‡
Non-infused BA blood flow (Δml/min)	0 ± 0	-2 ± 3	2 ± 2	8 ± 8	2 ± 4	6 ± 6	7 ± 7

by lower body negative pressure, supporting a presynaptic effect of AngII in young, healthy humans. In contrast, both Qiu et al. [25] and Henrion et al. [26] demonstrated the ability of AngII treatment and inhibition to affect NE-induced vascular tone, supporting the existence of a postsynaptic 'cross-talk' between these two pathways in the absence of sympathetic innervation. However, when NE and AngII were infused concomitantly in humans, no potentiating effect of AngII was evident [23], increasing uncertainty as to the importance of postsynaptic potentiation in the intact, human model.

Using higher doses of AngII, Lyons et al. [10] demonstrated a marked reduction in forearm blood flow in response to BA infusion of AngII in the young that was significantly reduced ($\sim$36%) in the presence of PHEN. This response is in contrast with the present data in the young, who demonstrated a smaller, non-significant reduction in AngII-mediated vasoconstriction with concomitant PHEN administration (Figure 4 and Table 4). Although the reasons for this discrepancy are unclear, the use of significantly higher doses of AngII and the lack of blood pressure measurements to verify that AngII did not spill over in the former study limits a direct comparison with the present findings. Thus the present study expands upon previous work investigating the synergistic behavior between AngII and the α-adrenergic pathway in the young, demonstrating for the first time that the age-associated increase in AngII-mediated vasoconstriction is due, in part, to the heightened potentiating effect of AngII on the α-adrenergic pathway.

AT$_1$ and α-adrenergic receptor distribution

By design, all study drugs were infused into the BA proximal to where the vessel was imaged, allowing the unique opportunity to assess BA diameter changes in response to AngII, PHEN and NE. Independent of age, AngII produced a reduction of the BA diameter (Figure 5 and Table 2), which initially was thought to provide evidence for AT$_1$ receptors in the BA. However, during co-infusion with PHEN, AngII administration did not change BA diameter (Figure 5 and Table 4), indicating that the AngII-mediated reduction in BA diameter was, in fact, achieved through the α-adrenergic pathway. Thus it seems AT$_1$ receptors may be distributed heterogeneously throughout the arterial tree. Specifically, AT$_1$ receptors located on vascular smooth muscle appear to be located distal to the BA, whereas those located presynaptically on sympathetic adrenergic nerves may be located in both the proximal and distal portions of the arm vasculature.

The AngII-mediated reduction in BA diameter documented in this study is in contrast with earlier studies from our group performed in the leg. Specifically, we previously observed minimal changes in the diameter of the CFA (common femoral artery) in response to AngII infusion [7,27], whereas the α_1-adrenergic agonist phenylephrine provoked a significant reduction in CFA calibre [27–30]. The explanation for this disparity is most likely related to limb differences. Studies that have utilized reflex increases in MSNA have observed sympathetically mediated reductions in BA diameter [31,32], whereas the leg does not appear to have significant sympathetic innervation at the level of the CFA

[33–35], despite the presence of α_1-adrenergic receptors in this large conduit vessel [27–30]. Together, the previous and current findings indicate that AngII 'cross-talk' with the α-adrenergic pathway may be limited to those α-receptors that are sympathetically innervated, and thus support a presynaptic site of action for this potentiating effect of AngII on the α-adrenergic pathway, as reported previously [23,24].

Clinical implications

To our knowledge, this is the first study to demonstrate that a significant part of the vasoconstrictive action of AngII in the old is mediated through the α-adrenergic pathway, which may have important implications for the regulation of skeletal muscle blood flow in this population. Indeed, healthy aging is associated with a concomitant rise in MSNA (Table 1) and a reduction in skeletal muscle blood flow [3,6,8], and it has been suggested that upwards of 80% of the age-associated reduction in skeletal muscle blood flow can be explained by the α-adrenergic contribution to vascular tone [1]. In this light, the potentiating effects of AngII on NE release and α-adrenergic vasoconstriction with age may not only indicate an important site of AngII modulation of sympathetic-adrenergic vasoconstriction, but also a potential mechanism contributing to enhanced α-adrenergic-mediated vascular tone associated with healthy aging.

The interaction between the RAAS and sympathetic–adrenergic system may also be of particular relevance in cardiovascular disease states characterized by an elevation in activity of these two respective systems, such as hypertension and heart failure [36,37], a concept supported by the observed efficacy of AT_1 receptor blockade in reducing resting arterial blood pressure [38] and vascular tone [39] in these patients. These functional vascular changes are likely the consequence of a two-fold effect, whereby AT_1 receptors located on both the vascular smooth muscle and the presynaptic sympathetic nerves are antagonized, ablating both direct and indirect (i.e. α-adrenergic) effects of AngII respectively. In the same way, findings from the present study suggest that inhibition of the RAAS pathway in older healthy adults could potentially mitigate the age-associated increase in vascular tone mediated by the SNS, resulting in an overall improved vascular phenotype. Additionally, considering the known role of elevated SNS activity in the sequela of primary hypertension [40], we speculate that the present findings identifying an exaggerated cross-talk between AngII and the α-adrenergic pathway in prehypertensive, older adults may be an important contributor in the progression towards geriatric hypertension, although additional studies are required to further examine this concept.

Experimental considerations

Whether the exaggerated vasoconstriction in response to intra-arterial AngII is due to pre- or post-synaptic synergistic behaviour between AngII and NE cannot be definitely determined from the present study. However, previous evidence in younger subjects [23] and our observation that AngII cross-talk with the α-adrenergic pathway appears limited to those α-receptors that are sympathetically innervated support a presynaptic site of action. We also acknowledge that the contribution of the α-adrenergic

receptors to the AngII-mediated reduction in BA diameter is similar between groups (Figure 5) in the face of marked differences in venous NE following AngII administration (Figure 3), a discrepancy that is probably due to differing sensitivity between our functional (ultrasound) and quantitative (assay) measurements. Finally, it is noteworthy that, with administration of AngII, a non-selective agonist, the individual role of vascular AT_1 and AT_2 (AngII subtype 2 receptor) receptor subtypes cannot be determined. This is an important consideration, since AT_1 and AT_2 receptor groups appear to have antagonistic actions with regard to cell signalling, promoting vasoconstriction and vasodilation, respectively. Although AT_2 receptor inhibition does not appear to affect limb blood flow in young healthy adults [41], there is some evidence from the animal model demonstrating a transition of AT_2 receptor function towards vasoconstriction [42]. As such, we cannot exclude the possibility that age-related changes in AT_2 receptor function contributed to the observed increase in AngII-mediated vasoconstriction in the older group.

Conclusions

The results of the present study have identified an enhanced AngII-mediated vasoconstriction in healthy older individuals and have demonstrated that this apparent increase in sensitivity to AngII is due, at least in part, to an AngII-mediated potentiation of α-adrenergic vasoconstriction. These findings suggest that cross-talk between the RAAS and adrenergic systems may be an important regulator of resting vascular tone and muscle blood flow with advancing age.

CLINICAL PERSPECTIVES

- The present study was undertaken to examine the interaction between the α-adrenergic and RAAS pathways in the peripheral circulation with advancing age.
- The results indicate that the increase in AngII sensitivity in the elderly can be largely attributed to the potentiating effect of AngII on the α-adrenergic vasoconstrictor pathway.
- In the context of an age-related increase in SNS activity, these findings represent a potential mechanism contributing to enhanced α-adrenergic-mediated vascular tone associated with healthy aging. Furthermore, this interaction between the RAAS and sympathetic/adrenergic system may be of particular relevance in cardiovascular disease states characterized by an elevation in activity of these two respective systems, such as hypertension and heart failure.

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