Sympathetic nervous system activity and α-adrenergic responsiveness in older hypertensive humans

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Division of Geriatric Medicine, Department of Internal Medicine, and Institute of Gerontology, University of Michigan, and Geriatric Research, Education, and Clinical Center, Department of Veterans Affairs Medical Center, Ann Arbor, Michigan 48105

Supiano, Mark A., Robert V. Hogikyan, Mohamad A. Sidani, Andrzej T. Galecki, and Jodi L. Krueger. Sympathetic nervous system activity and α-adrenergic responsiveness in older hypertensive humans. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E519–E528, 1999.—We have previously demonstrated in normotensive humans an age-associated increase in sympathetic nervous system (SNS) activity combined with appropriate downregulation of α-adrenergic responsiveness. Impaired downregulation of α-adrenergic responsiveness, despite a comparable level of SNS activity, could contribute to higher blood pressure in older hypertensive humans. We measured arterial plasma norepinephrine (NE) levels and the extravascular NE release rate (NEq) derived from [3H]NE kinetics (to assess systemic SNS activity), and platelet and forearm arterial adrenergic responsiveness in 20 normotensive (N) and in 24 hypertensive (H), otherwise healthy, older subjects (60–75 yr). Although plasma NE levels were similar (N 357 ± 27 vs. H 322 ± 22 pg/ml; P = 0.37), NEq tended to be greater in the hypertensive group (H 2.23 ± 0.21 vs. N 1.64 ± 0.20 µg·min⁻¹·m⁻²; P = 0.11), and the NE metabolic clearance rate was greater (H 1,100 ± 30 vs. N 900 ± 50 ml·m⁻²; P = 0.004). In the hypertensive group, there was a greater α-agonist-mediated inhibition of platelet membrane adenylyl cyclase activity and a NE- but not ANG II-mediated decrease in forearm blood flow. Compared with normotensive subjects, in older hypertensive subjects 1) NE metabolic clearance rate is increased, 2) systemic SNS activity tends to be increased, and 3) arterial and platelet α-adrenergic responsiveness is enhanced. These results suggest that heightened SNS activity coupled with enhanced α-adrenergic responsiveness may contribute to elevated blood pressure in older hypertensive humans.

norepinephrine; hypertension; aging

There is an age-related increase in the prevalence of hypertension that is associated with significant morbidity and mortality from related cardiovascular and cerebrovascular disease (36). Although it is clear that hypertension is an important disease in elderly humans, there is a limited understanding of the factors that contribute either to the development or to the maintenance of elevated blood pressure in this population. The potential relationship between the level of sympathetic nervous system (SNS) activity and blood pressure elevation in the elderly has not been well characterized. There are many complexities involved in the definition of SNS activity. Additionally, the interaction between SNS activity and an elevated blood pressure is multifaceted, mediated in part through vascular adrenergic responsiveness to the SNS input.

A number of studies using various methodologies to measure systemic SNS activity (plasma norepinephrine (NE) levels (40); the rate of NE release from studies of [3H]NE kinetics (33, 48, 51); and microneurography (18)) have concluded that there is an age-related increase in SNS activity. There remains controversy about the level of SNS activity in patients with essential hypertension (11, 14, 30). It appears that significant increases in SNS activity are found in some young hypertensive patients (5, 28) but that a hypertensive-normotensive difference in SNS activity has not generally been identified in older hypertensive patients (11, 30, 43).

We have interpreted results from our previous studies of adrenergic receptor responsiveness in aging, namely an age-associated decrease in platelet (46, 49), vasoconstriction (47), and arterial vasoconstritor (16) responses to α-adrenergic stimulation, to represent downregulation of α-adrenergic responsiveness, which is appropriate given heightened SNS activity in aging. In support of this interpretation, we found that when SNS activity is suppressed in older normotensive humans there is appropriate upregulation of their vascular α-adrenergic responsiveness (16). Studies of adrenergic responsiveness in hypertension have led to the suggestion that there may be an increase in α-adrenergic responsiveness to catecholamine stimulation, although to date these studies have not been conducted in an older hypertensive subject population (1, 3, 4, 23).

This study was performed to address the hypothesis that there is impaired desensitization of α-adrenergic receptor responsiveness in older hypertensive humans. Two primary research questions were addressed: compared with older normotensive subjects, do older hypertensive subjects have 1) similar levels of systemic SNS activity (determined by the rate of NE release into an extravascular compartment derived from compartmental analysis of [3H]NE kinetics studies) and 2) increased platelet and vascular α-adrenergic receptor responsiveness? Epinephrine-mediated inhibition of in vitro platelet membrane adenyl cyclase activity and the decrease in forearm blood flow resulting from in vivo intra-arterial NE infusion were determined to characterize platelet and vascular α-adrenergic receptor responsiveness, respectively. We report that compared with older normotensive subjects, older hypertensive subjects tended to have a further increase in...
systemic SNS activity, and despite equal or greater SNS activity, an increase in both platelet and vascular α-adrenergic receptor responsiveness.

**METHODS**

Subjects. Twenty normotensive and twenty-four hypertensive older (>60 yr) subjects in otherwise good general health were recruited through the Human Subjects Core of the University of Michigan Geriatrics Center, as well as through newspaper advertisement. Descriptive characteristics of each subject group are provided in Table 1. Subjects were screened before study entry with a medical history, physical examination, and laboratory tests, including a complete blood count, routine chemistries, and an electrocardiogram (ECG). Subjects were excluded from participation in either group if they exceeded 150% of ideal body weight (Metropolitan Life Insurance tables, 1983), were taking any medication known to interact with SNS function, or had evidence from either history, physical exam, or laboratory results of significant underlying illness. Normotensive subjects gave no prior history of hypertension and had a resting seated blood pressure <160 mmHg systolic and <90 mmHg diastolic at the screening visit. Subjects with mild to moderate hypertension whose blood pressure was well controlled on monotherapy were recruited for the hypertensive group such that their blood pressure would likely remain below a limit of 200 mmHg systolic and 110 mmHg diastolic throughout a 4-wk antihypertensive medication washout period. Individuals whose blood pressure exceeded these limits during the washout discontinued their participation in the study and resumed their antihypertensive medication. At the completion of the 4-wk antihypertensive washout period, hypertensive subjects were required to demonstrate a resting seated diastolic blood pressure of >90 mmHg. Each subject gave written informed consent that was approved by the University of Michigan Human Use Committee.

Study Protocol. All subjects reported to the General Clinical Research Center of the University of Michigan Medical Center at 0730 to control for any diurnal variation in NE metabolism (41) or arterial α-adrenergic tone (39). Subjects were instructed to fast from 2200 the night before and to abstain from cigarettes, caffeine, and other known modulators of catecholamines for 12 h before each study began. Subjects were studied in the supine position in a quiet room maintained at a constant temperature of 23–25°C, to facilitate achieving an adequate baseline forearm blood flow (FABF). The proportion of body fat was determined by bioelectrical impedance (RJL Systems, Mt. Clemens, MI) (26), and the waist-to-hip ratio was determined from an individual’s waist and hip circumferences taken at the level of the umbilicus and the largest gluteal circumference, respectively.

Forearm volume (FAV) was measured using water displacement. A 20-gauge, 1.25-in. Insyte catheter was placed into the brachial artery of the nondominant arm. The catheter was connected to a pressure transducer (Hewlett-Packard 1290A quartz transducer; Hewlett-Packard, Andover, MA). An intra-arterial catheter was placed in the contralateral arm for infusion of [3H]NE. Before this infusion was begun, venous blood from the site was collected into a plastic syringe containing 1 ml of 0.25 M EDTA to be used to prepare platelet membranes. Beginning 30 min after insertion of the catheters, the [3H]NE kinetics protocol was carried out as previously described (15, 16) with sampling from the brachial arterial catheter. Arterial samples were obtained for catecholamine levels every 10 min beginning 40 min into the infusion. One of the three basic ECG limb leads was monitored.

Seven of the normotensive subjects did not participate in the arterial NE kinetics and FABF studies described below. In these subjects, after the same study preparation, a scalp vein needle was inserted retrogradely into a dorsal hand vein, and the hand was placed into a warming box heated to 60°C to obtain arterialized venous blood (9). A 5-ml arterialized venous blood sample for catecholamines was obtained from this site 20 min later. Blood for platelet membrane preparation was then obtained by venipuncture of an antecubital vein of the contralateral arm as described above.

FABF protocol. After the tracer [3H]NE infusion protocol, FABF was measured using venous occlusion plethysmography during an intra-arterial infusion protocol we have previously described (15, 16). To establish a stable baseline, FABF readings were taken until three consecutive readings representing similar FABF were obtained. To determine the effect of intra-arterial infusions of NE on FABF, NE (Levophed bitartrate, Sterling Drug) was diluted in 5% dextrose to achieve stepwise increasing infusion doses of 1.25, 5, 20, 80, and 240 ng·100 ml FAV⁻¹·min⁻¹. Each NE dose was administered by an infusion pump (Harvard model 970T; Harvard Apparatus, South Natick, MA) for 4 min before FABF was recorded during the 5th minute of each infusion. After the baseline measurement at the 240-ng dose, the NE infusion was stopped.

After a 10-min washout period, repeat measurement of baseline FABF was carried out as described above. To determine the effect of a nonadrenergic vasoconstrictor on FABF, ANG II (Hypertensin, Ciba-Geigy, Summit, NJ) was diluted in 0.9% normal saline to achieve stepwise increasing infusion doses of 0.125, 0.5, 2, and 8 ng·100 ml FAV⁻¹·min⁻¹. FABF was measured as described for the NE infusions.

Platelet membrane preparation. Platelet membrane lysates were prepared from 50–200 ml whole venous blood as previously described (46, 49). An aliquot of the freshly prepared membrane lysate was used for adenyl cyclase assays. The remainder of the sample was quick-frozen in liquid nitrogen and stored at −70°C; radioligand binding studies were performed within 2 wk of membrane preparation.

Radioligand equilibrium binding assays. [methyl-3H]yohimbine (72.5–90.0 Ci/mmol; Amersham, Arlington Heights, IL), a specific α₂-adrenergic receptor antagonist, was used to determine platelet membrane α₂-adrenergic receptor antagonist binding properties, and the imidazoline full α₂-adrenergic receptor agonist, [3H]labeled 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine[imidazolyl-4,5-\(\text{H}\)]UK-14,304 or [3H]bromoxidine; 60.0–93.9 Ci/mmol, New England Nuclear, Boston, MA) was used to determine platelet membrane α₂-adrenergic receptor agonist binding properties.

**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>65 ± 1</th>
<th>65 ± 1</th>
<th>0.92</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, M:F</td>
<td>8:12</td>
<td>15:9</td>
<td>0.20</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.2 ± 0.8</td>
<td>27.6 ± 0.7</td>
<td>0.008</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>24.1 ± 1.9</td>
<td>28.6 ± 1.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.80 ± 0.03</td>
<td>0.87 ± 0.03</td>
<td>0.78</td>
</tr>
<tr>
<td>Mean arterial BP, mmHg</td>
<td>98 ± 2</td>
<td>120 ± 2</td>
<td>0.92</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>62 ± 1</td>
<td>66 ± 2</td>
<td>0.33</td>
</tr>
<tr>
<td>Baseline FABF, ml/min</td>
<td>3.8 ± 0.5</td>
<td>3.9 ± 0.3</td>
<td>0.75</td>
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</table>

Values are means ± SE; n = no. of subjects. Body fat was measured by bioelectrical impedance method. Mean arterial blood pressure (BP) values were derived from supine intra-arterial BP recording. For baseline forearm blood flow (FABF) value, normotensive subject number is 13. M:F, male:female. P values are adjusted for gender effect.
using methods we have previously described (46). In human platelet membranes [3H]bromoxidine has been demonstrated to bind to one high-affinity site (37), which is functionally coupled to adenyl cyclase (50). We have previously shown that analysis of [3H]bromoxidine specific binding over the concentration range and assay conditions utilized in these experiments identifies the high-affinity binding state (46).

Adenyl cyclase assays. Platelet membrane basal adenyl cyclase activity was determined using freshly prepared membranes at the beginning (time 0) and at the conclusion of a 15-min incubation at 30°C as previously described (46, 49). Stimulation of adenyl cyclase activity was achieved with the addition of 25 mM NaF and its α2-adrenergic receptor-mediated inhibition by 10−8–10−4 M epinephrine (Epi). The concentration of cAMP in the assay tubes was measured by radioimmunoassay (49). The concentration of cAMP in the time 0 basal activity condition was subtracted from the 15-min basal, NaF-stimulated, and Epi-inhibition conditions so that the values would reflect only the accumulation of cAMP over the 15-min incubation period. The extent of Epi-mediated inhibition at each Epi concentration was determined as the percent decrease in cAMP accumulation in the presence of Epi and NaF from the NaF-stimulated activity without Epi.

SDS-PAGE and Western immunoblotting for G-binding protein content. On the day of platelet membrane preparation, 0.1 ml of resuspended platelet membranes was added to an equal volume of a 4% cholate buffer [50 mM Tris·HCl, 1 mM Na2EDTA, 2 mM dithiothreitol (DTT), pH 7.6]. This solution was shaken on ice for 1 h and then subjected to ultracentrifugation at 38,000 rpm in a 60 Ti rotor for 1 h (Beckman L8–70M ultracentrifuge, Beckman Instruments, Fullerton, CA). A 0.1-ml aliquot of the supernatant was added to 0.9 ml of buffer containing 0.05% Lubrol (20 mM Tris·HCl, 0.1% Tween 20) and 3% Carnation nonfat dry milk three times for 20 min each. Immunoblotting was performed with a solution containing Tris-buffered saline with Tween 20 (20 mM Tris base, 500 mM NaCl, pH 7.4, 0.1% Tween 20) and 3% Carnation nonfat dry milk three times for 20 min each. Immunoblotting was performed with an antibody against Gα2 (AS/7; Du Pont/NEN, Wilmington, DE). Each blot was incubated with antibody at a dilution of 1:1,000 for 18 h at room temperature. Anti-G protein antibody binding to the transfer membrane was visualized by incubating each blot with 1 µCi of goat anti-rabbit 125I-labeled immunoglobulin (Amersham, Arlington Heights, IL) for 2.5 h at room temperature and then exposing the membrane to Kodak X-OMAT film with an intensifying screen at ~80°C for 24–96 h depending on the activity. The bands of appropriate molecular mass (41 kDa) were then cut from the 125I-labeled membranes and counted on a gamma counter (Tm Analytic, Elk Village, IL). Total counts per minute were recorded for background, and specific counts were normalized for that gel’s control value such that results are expressed as percentage of control.

Plasma catecholamine analytic methods. Arterial or arterialized-venous blood samples were collected into chilled plastic tubes containing EGTA and reduced glutathione. The tubes were kept on ice until centrifugation at 4°C. Plasma samples were stored at −70°C until assayed. Plasma NE and Epi were quantified by a single-isotope radioenzymatic assay, with all samples from a given subject analyzed in the same assay (6). The intra-assay coefficient of variation for NE in this assay is 5%. Alumina extraction of plasma samples and measurement of [3H]NE levels were carried out as previously described (25, 33).

Data and statistical analysis. Steady-state, one-compartment kinetic parameters [the rate of NE appearance into the circulation (NEAP) and clearance from the circulation (NECL)] were calculated from steady-state plasma levels of [3H]NE and NE as previously described (25). Compartmental analysis of NE kinetics was performed using the previously described minimal two-compartment model (25). The quantity of NE in each compartment [NE mass in the intravascular compartment (Q1) and in the extravascular compartment (Q2)], the rate of NE appearance into each compartment (R12 into compartment 1 and NE2 into compartment 2), the NE metabolic clearance rate from compartment 1 (MCR1), the NE spillover fraction (NESF), and the volume of distribution of NE in compartment 1 (V1) were calculated from the two-compartment model as functions of the estimated transfer rate coefficients as previously described (25).

Dose-response data for NE and ANG II were analyzed as the percent change in FABF from the baseline value obtained before the infusion of each drug, to control for potential differences between groups in baseline FABF utilizing linear mixed-effects analysis (using SAS/PROC MIXED; SAS Institute, Cary, NC). This analysis was chosen to adjust group differences for gender, to accommodate unbalanced data, and to permit dose to be tested either as a linear effect or as a factor with several levels (19). Mean arterial pressure (MAP) was determined from the electronically integrated area under the intra-arterial blood pressure curve from the Marquette telemetry system (series 7700, Marquette Electronics, Milwaukee, Wisconsin) just before each FABF measurement. Forearm vascular resistance (FVR) was calculated as the MAP divided by the FABF and is presented in arbitrary units.

An unweighted nonlinear least-squares fit of the specific binding data for [3H]yohimbine and [3H]bromoxidine was made to a hyperbolic binding curve, $Y = (A \times X)/(B + X)$, where $Y$ is specific binding (in fmol/mg protein), $X$ is free [3H]yohimbine or bromoxidine concentration (in nM), $A$ is the maximum receptor density $B_{max}$, and $B$ is the apparent dissociation constant $K_d$ (InPlot 3.1, GraphPAD Software, San Diego, CA).

Values are presented as means ± SE. Statistical analysis was performed using SAS (SAS Institute). A value of P < 0.05 was selected to indicate statistical significance. One-tailed tests were employed to test for the hypothesized increase in α-adrenergic responsiveness in the hypertensive group. Normotensive-hypertensive group differences were analyzed using a two-factor ANOVA to adjust for the effect of gender and by analysis of covariance (bromoxidine density). Linear mixed-effects analysis was used to compare FABF and adenyl cyclase dose-response results. Simple linear regressions were performed to compare relationships between MAP and body mass index (BMI) with NE kinetic parameters.
RESULTS

Subject characteristics. Characteristics of the normotensive and hypertensive subject groups are compared in Table 1. The groups were similar with respect to age and gender distribution. Although subjects who exceeded 150% of their ideal body weight were excluded, the BMI and percent body fat of the hypertensive group, after we adjusted for gender, were significantly greater than for the normotensive group. There was no statistically significant group difference in waist-to-hip ratio.

Plasma catecholamine levels and NE kinetics. As summarized in Table 2, there were no normotensive-hypertensive group differences for either arterial plasma NE or Epi levels. NE kinetics results were not obtained from one hypertensive subject because of technical difficulties. One-compartment model analysis indicated a significantly greater $\text{NE}_{\text{CL}}$ in the hypertensive group and no group difference in the $\text{NE}_{\text{sp}}$. The analogous results from the two-compartment model analysis were similar; $\text{MCR}_{1}$ was significantly greater in the hypertensive group, although this difference was not statistically significant ($P = 0.11$). The $\text{NE}_{\text{SF}}$ from compartment 2 into 1 was significantly less in the hypertensive group. There were no normotensive-hypertensive group differences for NE mass in either compartment 1 or 2 (although $Q_{2}$ tended to be greater in the hypertensive group) or for the NE volume of distribution in compartment 1, $V_{1}$. There were no relationships identified between BMI and NE in both the normotensive and hypertensive subject groups.

Table 2. Plasma catecholamines and NE kinetic parameters

<table>
<thead>
<tr>
<th></th>
<th>Normotensive (n = 13)</th>
<th>Hypertensive (n = 23)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma NE, pg/ml</td>
<td>357 ± 27</td>
<td>322 ± 22</td>
<td>0.37</td>
</tr>
<tr>
<td>Plasma Epi, pg/ml</td>
<td>77 ± 8</td>
<td>69 ± 6</td>
<td>0.55</td>
</tr>
<tr>
<td>One-compartment model</td>
<td></td>
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<tr>
<td>$\text{NE}_{\text{sp}}$, µg·min$^{-1}$·m$^{-2}$</td>
<td>0.35 ± 0.03</td>
<td>0.32 ± 0.02</td>
<td>0.51</td>
</tr>
<tr>
<td>$\text{NE}_{\text{CL}}$, ml·min$^{-1}$·m$^{-2}$</td>
<td>1,710 ± 110</td>
<td>2,050 ± 70</td>
<td>0.004</td>
</tr>
<tr>
<td>Two-compartment model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{NE}_{2}$, µg·min$^{-1}$·m$^{-2}$</td>
<td>1.64 ± 0.2</td>
<td>2.23 ± 0.21</td>
<td>0.11</td>
</tr>
<tr>
<td>$\text{MCR}_{1}$, ml·min$^{-1}$·m$^{-2}$</td>
<td>0.110 ± 30</td>
<td>1.100 ± 30</td>
<td>0.004</td>
</tr>
<tr>
<td>$Q_{2}$, µg·m$^{-2}$</td>
<td>0.40 ± 0.07</td>
<td>0.46 ± 0.04</td>
<td>0.58</td>
</tr>
<tr>
<td>$Q_{2}$, µg·m$^{-2}$</td>
<td>39 ± 5</td>
<td>55 ± 7</td>
<td>0.12</td>
</tr>
<tr>
<td>$\text{NE}_{\text{SF}}$, %</td>
<td>21 ± 1</td>
<td>17 ± 1</td>
<td>0.03</td>
</tr>
<tr>
<td>$V_{1}$, ml/m$^{2}$</td>
<td>2.4 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of subjects. Compartment 1, vascular-containing compartment; compartment 2, extravascular compartment. NE, norepinephrine; Epi, epinephrine; $\text{NE}_{\text{sp}}$, rate of NE appearance into circulation; $\text{NE}_{\text{CL}}$, rate of NE clearance from circulation; $\text{NE}_{2}$, extravascular NE release rate; $\text{MCR}_{1}$, rate of NE appearance into compartment 1; $\text{MCR}_{2}$, NE metabolic clearance rate from compartment 1; $Q_{2}$ and $Q_{2}$, NE mass in compartments 1 and 2, respectively; $\text{NE}_{\text{SF}}$, NE spillover fraction; $V_{1}$, NE volume of distribution in compartment 1. P values are adjusted for gender effect.

FABF during vasoactive infusions. There were no normotensive-hypertensive group differences in baseline FABF measured before the intra-arterial infusion protocol (Table 1). There was also no significant difference between groups in FAVR before the infusion protocol (normotensive $31 ± 4$ vs. hypertensive $36 ± 3$; $P = 0.35$). There were modest, although statistically significant, increases in MAP during the NE-infusion protocol in each group (MAP measured during the highest NE-infused dose was $103 ± 1$ mmHg in the normotensive group and $130 ± 3$ mmHg in the hypertensive group, each change $P < 0.001$). The change in FABF from baseline in response to each NE intra-arterial infusion dose is shown for each of the subject groups in Fig. 1 as the percent decrease from baseline FABF. Complete NE dose-response results were not available for one hypertensive subject. In addition to a significant effect of NE dose ($P < 0.0001$), the linear mixed-effects analysis indicated a significant group difference in the NE-mediated decrease in FABF, with the dose response for the hypertensive subject group being significantly shifted to the left of the normotensive group ($P < 0.05$). The addition of gender as a covariate in the linear mixed-effects model revealed a significant gender effect, with males having enhanced response compared with females ($P = 0.0001$). The gender-adjusted normotensive-hypertensive group difference was similar but no longer achieved statistical significance ($P = 0.12$).

There was no difference between groups in baseline FABF measured before the ANG II intra-arterial infusion protocol (normotensive $4.3 ± 0.4$ vs. hypertensive $4.8 ± 0.3$ ml/min; $P = 0.40$). There were modest, although statistically significant, increases in MAP during the NE-infusion protocol in each group (MAP measured during the highest ANG II-infused dose was $113 ± 3$ mmHg in the normotensive group and $136 ± 3$ mmHg in the hypertensive group, each change $P < 0.001$). Figure 2 presents results for the FABF response to ANG II for each subject group. Six normotensive subjects did not receive ANG II infusions. Although
there was a significant effect of ANG II dose (P < 0.0001), the linear mixed-effects analysis did not demonstrate a group difference in the ANG II-mediated decrease in FABF (P = 0.76).

Platelet membrane adenylyl cyclase activity. The basal rate of cAMP production in the platelet membrane lysates over the 15-min incubation period (after the cAMP concentration present in the membrane samples at time 0 of the assay was subtracted) was significantly less in the hypertensive subject group (normotensive 690 ± 40 vs. hypertensive 553 ± 45 pmol·mg⁻¹·min⁻¹; P = 0.04), although there was no difference in the NaF-stimulated rate of cAMP production (normotensive 2,230 ± 110 vs. hypertensive 2,190 ± 100 pmol·mg⁻¹·min⁻¹; P = 0.81). The dose-response effect for Epi-mediated inhibition of NaF-stimulated adenylyl cyclase activity is presented in Fig. 3. In addition to a significant overall dose effect for Epi-mediated inhibition (P < 0.0001), the linear mixed-effects analysis indicated a significant group effect observed for the percent inhibition from the NaF-stimulated level, with greater inhibition in the hypertensive group (P = 0.006). The hypertensive group was also found to have significantly greater maximal percent inhibition (normotensive 41 ± 2 vs. hypertensive 53 ± 2%; P < 0.001).

Equilibrium binding studies. The receptor binding densities from equilibrium binding studies for the antagonist [³H]yohimbine and the agonist [³H]bromoxidine and the ratio of the bromoxidine to yohimbine binding density for the subject groups are presented in Fig. 4. There was no group difference in either receptor density (normotensive 128 ± 9 fmol/mg; P = 0.99) or antagonist binding affinity (normotensive 2.6 ± 0.2 vs. hypertensive 2.1 ± 0.2 nM; P = 0.18) detected for [³H]yohimbine. An insufficient platelet protein yield prevented [³H]bromoxidine studies from being done in 4 normotensive and 16 hypertensive subjects. The [³H]bromoxidine receptor density tended to be greater in the hypertensive group (normotensive 29 ± 4 vs. hypertensive 42 ± 6 fmol/mg; P = 0.08), although there was no group difference noted for its binding affinity (normotensive 2.4 ± 0.3 vs. hypertensive 3.1 ± 0.8 nM; P = 0.34). Analysis of covariance indicated that subject group (P = 0.07) and yohimbine Bmax (P = 0.002) were each significantly associated with bromoxidine Bmax.

G protein content. Western immunoblot studies utilizing an anti-Goα12 antibody demonstrated a single band at an approximate molecular mass of 41 kDa for the known Gi standard and the cholate extracts from the subjects’ platelet membrane lysates. There was no cross-reactivity against a known Gs standard. Protein content could be determined in 11 of the normotensive and 22 of the hypertensive subjects. There was significantly less Gi protein content detected in cholate extracts from hypertensive compared with normotensive subjects (normotensive 84 ± 10 vs. hypertensive 64 ± 4% of control; P = 0.03).

**DISCUSSION**

The results from this study in older hypertensive humans provide evidence for enhanced platelet and vascular α-adrenergic receptor responsiveness despite systemic SNS activity equal to or greater than that of normotensive age-matched subjects. These results support the hypothesis that there is impaired desensitization of α-adrenergic receptor responsiveness in older hypertensive humans and suggest that altered SNS function may contribute in part to the pathophysiology of hypertension in the elderly.

The role of enhanced SNS activity in the pathogenesis of essential human hypertension remains controversial (28). A significant component of this controversy is related in part to limitations in each of the available methods to assess SNS activity in humans. Studies that have measured normotensive-hypertensive differences in plasma catecholamines have been reviewed in detail by Goldstein (10). The overall conclusion derived from these studies is that although there are some hypertensive patients who exhibit significant increases in plasma NE, namely young patients with persistent...
hypertension (5), the hypertensive-normotensive difference in plasma NE is generally not apparent in older hypertensive patients (11, 30, 43). One report that studied 24-h plasma catecholamine levels found that the mean plasma NE level over a 24-h period was lower in older hypertensive compared with normotensive patients (45). Consistent with these previous reports, we did not detect an increase of arterial plasma NE levels in our older hypertensive subject population.

However, plasma NE levels provide at best only an indirect index of systemic SNS activity. The plasma NE concentration reflects the net balance between NE appearance and removal mechanisms and provides no information concerning the complex metabolic fate of NE after its release from presynaptic sympathetic nerve terminals. Tracer NE kinetics studies utilizing isotope dilution methods have been developed to estimate systemic rates for NE AP into and NE CL from plasma. Esler et al. (5) have examined the effect of age on [3H]NE kinetic parameters in 34 patients with essential hypertension from age 22 to 74 yr using the isotope dilution technique (5). In this study, although an increase in NE AP was noted overall in the hypertensive group compared with controls, the NE AP of the older (i.e., >40 yr) hypertensive subjects was similar to their age-matched controls, suggesting that SNS activity of older hypertensive subjects is similar to that of age-matched controls. The NE kinetics results from the present study are consistent with this interpretation because there was no normotensive-hypertensive group difference identified in NE AP or in the analogous NE kinetic parameter from the two-compartment model analysis, the rate of NE appearance into the vascular compartment, R12.

A minimal two-compartment model developed to describe NE kinetics was used in the present study to determine the rate of NE release into the extravascular compartment (NE2) as a more proximate index of systemic SNS activity (25). We have previously demonstrated in normotensive subjects an age-associated increase in NE2 (48). Compared with older normotensives, the rate of NE release into the extravascular compartment tended to be greater in the older hypertensive group, although this gender-adjusted difference was not statistically significant (P = 0.11). There was also a trend toward greater NE mass in the extravascular compartment in the hypertensive group. These results suggest that systemic SNS activity in older hypertensive subjects is equal to or greater than that of older normotensive subjects. Our results are consistent with those of Yamada et al. (52), who reported an increase in muscle SNS activity (assessed using tibial nerve microneurography) in older (51–67 yr) hypertensive subjects compared with age-matched normotensive controls. Thus the age-associated increase in systemic SNS activity is present in older humans with established hypertension and may be exaggerated in this population.

Only a minority (<25%) of NE released into the synapse appears or spills over into the circulation; the majority of NE reenters the presynaptic terminal by neuronal reuptake mechanisms. In addition to providing an estimate of the rate of NE release into the extravascular compartment, the two-compartment model analysis also permits estimation of the spillover fraction. This analysis demonstrated that the spillover fraction was significantly lower in the hypertensive subject group, suggesting that NE reuptake mechanisms may be enhanced. The NE kinetics results also demonstrated significantly greater NE metabolic clearance rate in the hypertensive group. This finding coupled with the decrease in NE spillover fraction may explain why plasma NE levels were not elevated in the hypertensive group despite the trend for increased NE2. Therefore, at least in part because of the increase in NE clearance rate, the plasma NE level appears to underestimate the level of systemic SNS activity in older hypertensive subjects.

Vascular adrenergic tone represents the integration of SNS activity and vascular α- and β-adrenergic receptor responsiveness. A number of studies have suggested that there is an impairment in β-adrenergic-mediated vasodilation among hypertensive subjects (7, 35, 44). The present study focused on characterizing α-adrenergic receptor responsiveness because of our results in previous studies in older normotensive humans, which demonstrated that there is appropriate regulation of vascular α-adrenergic receptor responsiveness to perturbations in SNS activity (15, 16). We concluded from these observations that the age-associated decrease in platelet and vascular α-adrenergic receptor responsiveness is appropriate given the
age-associated increase in SNS activity. In the present study, vascular $\alpha$-adrenergic responsiveness was assessed concurrently with measures of systemic SNS activity in a population of older hypertensive subjects to permit interpretation of adrenergic responsiveness in the context of the prevailing level of SNS activity. In contrast to our observations in normotensive elderly subjects, the hypertensive elderly subject group demonstrated enhanced NE-mediated vasoconstriction despite evidence for an equal or a greater level of systemic SNS activity. Moreover, because there was no normotensive-hypertensive group difference noted in the decrease in FABF mediated by the nonadrenergic vasoconstrictor ANG II, the enhanced vasoconstrictor response appears to be specific for NE. This argues against the possibility of a structural or other nonadrenergic mechanism producing a nonspecific increase in vascular reactivity in the hypertensive group as an explanation for their enhanced response to NE.

The results demonstrating enhanced NE-mediated vasoconstriction in the older hypertensive group are consistent with other studies that have concluded that there is enhanced adrenergic receptor responsiveness in younger hypertensive subjects. Systemic NE infusions have been shown to decrease brachial artery diameter and blood flow to a greater extent in hypertensives compared with normotensive subjects (23). Additional studies have demonstrated augmentation of pressor responses to systemic infusions of adrenergic agonists (3), augmented vasoconstrictor response of postjunctional $\alpha_2$-receptors to epinephrine (1), and an increase in vascular $\alpha$-adrenergic tone (4) in hypertensives. Taken together, these studies support a conclusion that enhanced vascular adrenergic responsiveness may contribute to an increase in vascular resistance and blood pressure in hypertensive subjects. The increase in vascular adrenergic responsiveness may be of even greater physiological significance among older hypertensive subjects given the age-associated increased level of SNS activity. Although FAVR was higher among the hypertensive subject group, this difference was not statistically significant. The combination of equal or greater SNS activity and enhanced FABF responsiveness to NE infusion might be expected to result in greater FAVR. However, the regulation of FAVR is complex, involving a number of compensatory systems in addition to the adrenergic system, such that enhanced forearm vascular responses to NE may not directly translate to greater FAVR.

In parallel with enhanced NE-mediated vasoconstriction, our results also indicated enhanced platelet $\alpha_2$-adrenergic responsiveness in the hypertensive subject group. The extent to which platelet $\alpha$-adrenergic receptor response provides a valid marker of vascular $\alpha$-adrenergic receptor responsiveness has been questioned. Given the inaccessibility of human vascular $\alpha$-adrenergic receptors, platelet membrane $\alpha_2$-adrenergic receptors have been utilized as a surrogate model system (31). The majority of investigations have reported only yohimbine (antagonist) binding properties in hypertensive and control subject populations. The results from these studies have not been consistent, demonstrating either increased (2, 32), decreased (20), or similar (17, 34, 38) total $\alpha_2$-receptor binding density. Several limitations need to be considered in interpreting results from platelet $\alpha_2$-adrenergic receptor antagonist binding studies (31). In particular, alterations in total receptor binding density may not convey any functional significance because only those receptors in the high-affinity (coupled) agonist binding state mediate inhibition of adenylyl cyclase. For this reason, we included measures of agonist binding properties and receptor-mediated adenylyl cyclase inhibition in the present study. Our results uniquely demonstrate a parallel increase in vascular and platelet $\alpha$-adrenergic responsiveness. In the present study, platelet membranes from the older hypertensive subjects demonstrated greater Epi-mediated inhibition of adenylyl cyclase activity relative to the older normotensive group. This finding is consistent with results from a study that reported a defect in the ability of adrenergic agonists to desensitize platelet $\alpha_2$-adrenergic receptors from younger hypertensive subjects (17). Given that there was not a normotensive-hypertensive group difference in plasma NE levels, the difference in platelet $\alpha_2$-receptor response cannot be accounted for by differences in plasma NE. This difference in response may be due to another sympathetic mechanism, such as platelet rather than plasma NE concentration (21), or a nonsympathetic mechanism. The enhanced response in platelet membranes from older hypertensive subjects appears not to be due to a greater density of platelet $\alpha_2$-adrenergic receptors but rather to an increase in the proportion of receptors in the high-affinity, or coupled, binding state. In the present study, the binding density for the direct $\alpha$-agonist bromoxidine tended to be higher among the hypertensive subjects. Receptor agonist affinity state has been examined in only one previous study; using analysis of agonist competition binding for $^3$H)lyphimbine, no significant normotensive-hypertensive group difference was found for the proportion of receptors in the high-affinity binding state (17).

Enhanced Epi-mediated inhibition of adenylyl cyclase activity in the hypertensive group could not be explained by an increase in $G_i$ binding protein content inasmuch as there was less $G_{i2}$ protein content detected in cholate extracts of the platelet membranes from the hypertensive subjects. This finding is consistent with another study that reported lower $G_{i2}$ protein levels in hypertensive subjects (27), although in another report no differences in $G_{i2}$ protein levels were noted (29). Our study was not designed to determine whether there were differences in content of other $G$ binding protein subtypes, $G_{i\alpha}$ or its subtypes, or the functional activities of these proteins. Stimulatory $G$ protein labeling by cholera toxin (but not by immunoblotting) has been shown to be reduced in lymphocyte preparations from younger hypertensive subjects in conjunction with a reduction in $\beta$-agonist-mediated stimulation of adenylyl cyclase activity (8). This reduction in lymphocyte $\beta$-adrenergic receptor responsiveness in hypertensive subjects has recently been associ-
ated with an increase in G protein-coupled receptor kinase activity (12). Therefore, additional studies need to be conducted to more completely examine the \( \alpha \)-adrenergic receptor-effector coupling pathway and its regulation in older hypertensive humans to attempt to elucidate the mechanism responsible for the apparent lack of appropriate desensitization.

We acknowledge several limitations inherent in our study primarily related to the heterogeneity of SNS activity and to the heterogeneous nature of hypertension in an older subject population. The NE kinetics methodology we employed assesses systemic SNS activity. Consequently, it is not possible to infer from our results whether there is greater regional, organ-specific (e.g., cardiac or renal) SNS activity in older hypertensive humans in addition to the systemic, whole body assessment. Future studies need to focus attention on this important question. Also, despite the similarity in vasoconstriction response to ANG II, our results cannot exclude the possibility of impaired withdrawal of SNS activity in the hypertensive group in the context of increased vasoconstriction due to a nonadrenergic (e.g., insulin resistance or impaired endothelial function) mechanism. We also recognize that despite our efforts to characterize a uniform older study population with mild to moderate essential hypertension who were studied after a 4-wk antihypertensive medication withdrawal period, the heterogeneous nature of this disorder (with respect to, for example, obesity, duration of illness, physical activity, racial background, ethnicity, insulin resistance, and sodium sensitivity of blood pressure) in this population may have influenced our results. An effect of gender was noted in the vasoconstriction response to NE (men having enhanced response), but no gender effects were identified for any of the other parameters we investigated. Several recent studies have reported an effect of estrogen supplementation to improve endothelium-dependent vasodilation (22) and to decrease NE-mediated vasoconstriction (24) in postmenopausal women, raising the possibility that these effects of estrogen may contribute to the gender difference we observed in the vasoconstriction response to NE. Among the other potential factors, we are able to comment only indirectly with respect to the influence of obesity. Studies that have observed associations between body mass index and percent body fat with muscle SNS activity have suggested that body fat may be an important regulator of SNS activity (13, 42). In our subject population, the hypertensive group had significantly greater body mass index and higher percent body fat but not waist-to-hip ratio. However, because there were no associations between body mass index and NE in our subject population, it seems unlikely that the trend toward increased NE in the hypertensive group may be ascribed to their higher body mass index. Future studies are needed to address potential interactions between other factors, including gender, obesity, and others cited above, and SNS function in older hypertensive populations.

In summary, these studies demonstrate that, compared with older normotensive subjects, older hypertensive subjects tended to have a further increase in systemic SNS activity and, despite equal or greater SNS activity, an increase in both platelet and vascular \( \alpha \)-adrenergic receptor responsiveness. These results suggest that heightened level of SNS activity in conjunction with enhanced \( \alpha \)-adrenergic receptor response may contribute toward the increase in peripheral vascular resistance and blood pressure in older hypertensive humans.

We thank Marla Smith and Eric Leendecker for technical assistance and the nursing staff of the Univ. of Michigan General Clinical Research Center for care of our subjects during this study. Angioten-

sin II was donated by Ciba-Geigy (Summit, NJ).

This work was supported in part by National Institutes of Health Grants RR-00042 (to the University of Michigan General Clinical Research Center), AG-08808 (to the Claude D. Pepper Geriatric Research and Training Center at the University of Michigan), and AG-00433 and AG-10053 (to M. A. Supiano); by the Geriatric Re-

search, Education, and Clinical Center and the Medical Research Service of the Ann Arbor Dept. of Veterans Affairs Medical Center (R. V. Higakiy and M. A. Supiano); and by a grant from the John A. Hartford Foundation (to J. L. Krueger).

Portions of this work were presented at the National Meeting of the American Federation for Clinical Research in 1993 and 1994 and the American Geriatrics Society in 1994.

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Received 27 Jan 1998; accepted in final form 10 November 1998.

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