Increased Plasma Norepinephrine Response to Yohimbine in Elderly Men

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Background. The effects of aging on sympathetic nervous system and adrenomedullary outflow were estimated by the measurement of plasma norepinephrine (NE) and epinephrine (EPI) responses to yohimbine and clonidine in healthy young and healthy older subjects.

Methods. Yohimbine (0.65 mg/kg), clonidine (5 μg/kg), and placebo were administered on separate days in random order to 5 healthy older men (age 74 ± 1 years) and 18 healthy young men (age 26 ± 1 years). NE and EPI were measured by radioenzymatic assay in plasma samples obtained before and 30, 60, and 90 minutes after drug administration.

Results. Plasma NE increases after yohimbine were greater in older men than in young men, but plasma NE decreases following clonidine did not differ between groups. Plasma NE and systolic blood pressure were higher in older men than in young men at baseline but no longer differed 90 minutes after clonidine. Plasma EPI increases after yohimbine and decreases after clonidine did not differ between groups.

Conclusions. These results suggest increased sympathetic nervous system outflow in human aging that is not a function of reduced responsiveness of alpha-2 adrenergic receptor-mediated feedback inhibition.

RESTING plasma norepinephrine (NE) concentrations are higher in older persons than in young persons (1–3). That increased plasma NE in human aging represents predominantly increased sympathetic nervous system (SNS) outflow is supported by NE kinetic studies (4,5,6) and by direct measurements of SNS nerve activity (7). The mechanisms responsible for increased SNS outflow with aging remain unclear. One possible mechanism is decreased alpha-2 adrenergic inhibition of SNS outflow. Stimulation of alpha-2 adrenergic autoreceptors by NE released from SNS noradrenergic terminals produces feedback inhibition of subsequent NE release from SNS neurons (8). However, we have demonstrated comparable reductions of plasma NE concentrations following the alpha-2 adrenergic agonist clonidine in healthy older and healthy young subjects (9,10). These findings are consistent with increased SNS drive, independent of decreased alpha-2 feedback inhibition, with human aging.

To assess more directly the presence of increased SNS drive in human aging, we measured the response of plasma NE to the removal of alpha-2 adrenergic inhibition by the alpha-2 adrenergic receptor antagonist drug yohimbine in groups of healthy older and healthy young men. Plasma NE responses to the alpha-2 adrenergic receptor agonist clonidine were also measured in these subjects. A greater increase of plasma NE following yohimbine in older subjects than young subjects, if accompanied by similar degrees of plasma NE reduction following clonidine, would provide further support for increased SNS drive that is independent of alpha-2 inhibitory mechanisms in human aging.

Methods

Subjects

This study was approved by the University of Washington Human Subjects Review Committee and informed consent was obtained from all subjects. Subjects were 18 normal young men (26.4 ± 0.9 years [mean ± standard error of the mean {SEM}] of age) and 5 cognitively normal elderly males (74 ± 1.4 years of age).

Subjects were nonsmokers in good general health who had been free of medications except for occasional laxatives or nonopiate analgesics for at least 1 month before the study. They were normotensive (systolic pressure <135 mmHg and diastolic pressure <90 mmHg) and were within 125% of their ideal body weight (Metropolitan Life Insurance Tables, 1983). Subjects underwent a medical history, semistructured psychiatric interview, physical examination, electrocardiogram, and laboratory evaluation of serum electrolyte and glucose levels, renal, hepatic and thyroid function, and complete blood cell count. Subjects were free of all past or present major psychiatric disorders, neurologic disorders, renal or hepatic disease, diabetes mellitus, thyroid disease, congestive heart failure, and cardiac arrhythmias. All subjects had Mini-Mental State Examination scores of 29 or 30 and no history or evidence of cognitive decline.
Procedures

Studies were performed in a clinical research unit at the Veterans Affairs Puget Sound Health Care System, Seattle Division. The following three treatment conditions were administered in random order, separated by at least 1 week: placebo, clonidine hydrochloride 5 μg/kg by mouth, and yohimbine hydrochloride 0.65 mg/kg by mouth. Subjects were blind to the treatment condition. Clinical investigators were aware of the treatment conditions for reasons of subject safety. Laboratory personnel performing the catecholamine assays were blind to subject group and treatment condition. Subjects fasted and were prohibited from the use of caffeine or known stimulants of catecholamine release after midnight before the start of each study day. At approximately 9:00 a.m., subjects assumed a recumbent position, and a 19-gauge plastic catheter was inserted into an antecubital vein. After 30 minutes were allowed for plasma NE and epinephrine (EPI) to return to resting levels, two blood samples 5 minutes apart were taken through the catheter to provide estimates of baseline levels. After the baseline samples had been obtained, drug was administered. Additional blood samples were obtained at 30, 60, and 90 minutes after drug administration. Blood pressure and pulse rate were measured automatically (Dinamap, Critikon Inc., Tampa, FL) immediately after each blood sample was drawn. Mean arterial pressure was calculated as (diastolic + 1/3 [systolic–diastolic]).

Sample Collection and Measurement

Blood samples for NE and EPI determinations were collected into prechilled tubes that contained egtazic acid (ethylene glycol-bis-[β-aminoethyl ether]-N, N-tetraacetic acid) and reduced glutathione. Blood samples were placed on ice and cold centrifuged within 1 hour of collection; plasma samples were stored at −70°C until assay. The plasma NE and EPI levels were determined by a sensitive single-isotope radioenzymatic assay as previously described (11). The coefficient of variation within assays was 11% and between assays was 15%.

Statistical Analyses

Data are expressed as mean ± SEM. The significance of differences in endocrine and cardiovascular responses between groups and over time was evaluated by a two-way analysis of variance (ANOVA) with repeated measures. Post hoc comparisons of baseline values with those at 30, 60, and 90 minutes were performed with Dunnett’s test. Results with a probability of 5% or less (p ≤ .05) were considered statistically significant. Portions of these data (i.e., for results with a probability of 5% or less (p ≤ .05), because of greater plasma NE levels in the older subjects at 30 and 90 minutes (F[1,21] = 5.61 and 7.52, respectively, p < .05).

Plasma NE concentrations in the clonidine condition are presented in Figure 2. Plasma NE decreased over time (time effect: F[3,42] = 18.12, p < .001). The group by time interaction was not significant. However, one-way ANOVAs showed that plasma NE levels were greater in the older subjects at baseline and at 30 minutes (p < .05). Because baseline plasma NE concentrations differed between groups, the data also were analyzed as percentage of change from baseline. In this analysis, the strong time effect was again present (F[3,42] = 11.24, p < .001) and the group by time interaction was again not significant. Post hoc Dunnett’s tests showed that plasma NE levels were lower than baseline at 60 and 90 minutes in both groups of subjects (p < .05).

Plasma NE concentrations in the yohimbine condition are presented in Figure 3. There was a significant group by time interaction (F[3,63] = 9.95, p < .001), with greater increases of plasma NE following yohimbine in older subjects than in young subjects. Plasma NE levels at baseline were also higher in the older subjects (F[1,21] = 30.10, p < .001). When the data were analyzed as percentage of change from baseline, there continued to be a significant group by time interaction (F[3,63] = 2.89, p < .05). Post hoc Dunnett’s tests showed that plasma NE levels were elevated above baseline at 30, 60, and 90 minutes in both groups of subjects (p < .05). One-way ANOVAs at each time point showed that plasma NE levels were greater in the older subjects at 60 and 90 minutes following yohimbine administration (F[1,21] = 5.85 and 5.69, respectively, p < .05).

Results

Plasma NE

Plasma NE concentrations in the placebo condition are presented in Figure 1. Plasma NE differed between groups (F[1,21] = 5.79, p < .05), with significantly higher concentrations in older subjects than in younger subjects. Plasma NE levels did not change significantly over time. However, there was a significant group by time interaction (F[3,63] = 3.00, p < .05), because of greater plasma NE levels in the older subjects at 30 and 90 minutes (F[1,21] = 5.61 and 7.52, respectively, p < .05).

Figure 1. Plasma NE levels (pmol/L) over time following placebo in normal young males (open circles) and normal older males (solid circles). Bars indicate SEM. A steriks indicate a significant difference between groups at the same time point (p < .05 by one-way ANOVA).
Plasma EPI

Plasma EPI concentrations are presented in Table 1. EPI concentrations in the placebo condition did not differ between groups and did not change over time. EPI increased following yohimbine and decreased following clonidine in both older and young subjects. The EPI responses to clonidine and to yohimbine did not differ between groups.

Blood pressure and heart rate.— Mean arterial blood pressure, systolic blood pressure, and heart rate are presented in Table 2. Mean arterial blood pressure was higher in older subjects at baseline in the placebo and yohimbine conditions. Mean arterial pressure and systolic blood pressure decreased to the same degree in both older and young subjects following clonidine administration. In the yohimbine condition, mean arterial pressure and systolic blood pressure increased over time and to the same degree in both subject groups. Because both blood pressure measurements were higher in older subjects at baseline in the yohimbine condition, these data were also analyzed as a percentage of baseline values. The percentages of baseline mean arterial and systolic blood pressure increased over time and to the same degree in both subject groups following yohimbine.

Heart rate at baseline did not differ between groups during any of the treatment conditions. There was no change in heart rate over time in the placebo and clonidine conditions. However, heart rate increased to the same degree in both subject groups following yohimbine administration ($F[3,63] = 15.17, p < .001$).

**Discussion**

Plasma EPI decreases following clonidine did not differ between normal young and normal older subjects in the present study, confirming previous reports from our laboratory (10,13). These findings indicate that alpha-2 adrenergic autoreceptor sensitivity is unaltered in aged adults. They also suggest that elevated resting plasma NE levels in elderly people arise from an increase in SNS drive rather than a decrease in autonomic variance.

Table 1. Plasma Epinephrine (pg/ml) Responses to Placebo, Clonidine (5μg/kg), and Yohimbine (0.65 mg/kg) in Young and Older Men (Mean and Standard Error of Mean)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Young Men, N = 18*</th>
<th>Older Men, N = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>30 min</td>
</tr>
<tr>
<td>Placebo</td>
<td>52 (8)</td>
<td>53 (7)</td>
</tr>
<tr>
<td>Clonidine†</td>
<td>42 (13)</td>
<td>44 (11)</td>
</tr>
<tr>
<td>Yohimbine‡</td>
<td>52 (9)</td>
<td>74 (11)</td>
</tr>
</tbody>
</table>

*N = 11 young subjects in clonidine condition.
†Decrease over time in both subject groups.
‡Increase over time in both subject groups.
Table 2. Mean Arterial Blood Pressure, Systolic Blood Pressure, and Heart Rate Responses to Placebo, Clonidine (5 μg/kg), and Yohimbine (0.65 mg/kg) in Young and Older Men (Mean and Standard Error of Mean)

<table>
<thead>
<tr>
<th>Data</th>
<th>Treatment</th>
<th>Young Men, N = 18*</th>
<th>Older Men, N = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>30 min</td>
<td>60 min</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>Placebo†</td>
<td>84 (1)</td>
<td>86 (1)</td>
</tr>
<tr>
<td></td>
<td>Clonidine‡</td>
<td>85 (2)</td>
<td>82 (2)</td>
</tr>
<tr>
<td></td>
<td>Yohimbine†§</td>
<td>82 (1)</td>
<td>90 (2)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>Placebo†</td>
<td>117 (2)</td>
<td>119 (2)</td>
</tr>
<tr>
<td></td>
<td>Clonidine‡</td>
<td>120 (2)</td>
<td>115 (2)</td>
</tr>
<tr>
<td></td>
<td>Yohimbine†§</td>
<td>116 (2)</td>
<td>124 (3)</td>
</tr>
<tr>
<td>HR (beats per minute)</td>
<td>Placebo</td>
<td>59 (2)</td>
<td>58 (2)</td>
</tr>
<tr>
<td></td>
<td>Clonidine</td>
<td>59 (2)</td>
<td>57 (2)</td>
</tr>
<tr>
<td></td>
<td>Yohimbine§</td>
<td>62 (2)</td>
<td>66 (3)</td>
</tr>
</tbody>
</table>

Notes: MAP = mean arterial blood pressure, SBP = systolic blood pressure, HR = heart rate.
*Higher in older subjects at all time points.
†Decrease over time in both subject groups.
‡Increase over time in both subject groups.
§Baseline value higher in older subjects.

It is unlikely that pharmacokinetic effects of aging on yohimbine disposition contributed to the enhanced effect of yohimbine on plasma NE in the older subjects. We have reported previously that aging does not affect concentrations of yohimbine or its active metabolite, 11-OH-yohimbine, in plasma or cerebrospinal fluid (21).

That plasma EPI increased following yohimbine and decreased following clonidine is consistent with other studies (19,22) demonstrating alpha-2 adrenergic regulation of EPI release from the adrenal medulla. In contrast to plasma NE, there was no effect of age on basal plasma EPI or EPI responses to yohimbine. That aging differentially affected the SNS and adrenomedullary components of the sympatho-chromaffin system confirms other reports (2).

SNS regulation is complex, and mechanisms contributing to increased SNS outflow in human aging could reside at a number of levels. In several animal species aging has been associated with attenuated baroreceptor regulation of SNS outflow (23,24). In a human study (25), baroreceptor regulation of SNS outflow was not affected by aging. However, no subject in that study was older than age 71 years. Multiple central nervous system sites provide stimulatory and inhibitory regulation of SNS outflow (26). The effects of aging-associated changes on the central nervous system’s regulation of SNS outflow remain unclear.

As has been reported previously (1,3,4,5,27,28), basal blood pressure was significantly higher in older subjects than in young subjects. Following the suppression of plasma NE by clonidine, systolic blood pressure and plasma NE concentrations achieved by older men became indistinguishable from those of the young men. These data confirm our previous observations made with NE kinetic techniques and suggest that the elevation of SNS outflow in older individuals makes an important contribution to the age-related increase in blood pressure in normotensive older individuals.

Increased SNS activity with aging may have important implications for both behavioral and cardiovascular health.
in later life. Increased SNS activity has been linked to cognitive impairment and to sleep disturbances in normal older persons (29,30). Increased SNS activity has also been associated with some forms of hypertension (31), with the progression of congestive heart failure (32,33), and with potentially fatal postmyocardial infarction events such as ventricular cardiac arrhythmia (34). Findings indicating that beta-adrenergic antagonists reduce the risk of recurrent myocardial infarction in older persons (35) and can improve survival in congestive heart failure (32) further support the hypothesis that increased SNS activity may be an important contributor to morbidity and mortality in the elderly.

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