Pressor Hormones in Elderly Hypertensive Persons
Racial Differences

Michael Bursztyn, Margaret Bresnahan, Irene Gavras, and Haralambos Gavras

The purpose of this study was to examine pressor hormones and platelet α1-adrenergic receptors in elderly unmedicated free-living subjects. Eighty-seven subjects, 70±5 years old (mean±SD), hypertensive or normotensive (blood pressure <160/90 mm Hg) were recruited for measurement of blood levels of norepinephrine, epinephrine, and vasopressin, as well as density and affinity of α1-adrenergic receptors from platelet membranes, assessed by maximal binding (Bmax) and dissociation constant (Kd) of rauwolscine. They were separated into white hypertensive (n=22) or normotensive (n=41), and black hypertensive (n=11) or normotensive (n=13) groups, with similar age distribution throughout and similar blood pressure levels in the hypertensive and normotensive groups. Vasopressin was higher in the black hypertensive than white hypertensive group (1.5±1.0 vs. 0.7±0.5 pg/ml, respectively, p<0.005), whereas epinephrine correlated inversely with diastolic blood pressure (r= -0.7, p<0.02, in the black hypertensive group). Kd was higher in the black normotensive group than in the other groups (1.6±0.6 vs. 1.0±0.2, 1.1±0.3, or 1.0±0.3 nM in the white normotensive, black hypertensive, or white hypertensive group, respectively, p<0.002). Bmax was no different among groups but was significantly correlated with vasopressin levels for the whole group (r=0.4, p<0.0004) although no such correlation existed within the black hypertensive group. The data suggest that various vasoconstrictor systems participate to different extents in the mechanisms generating and sustaining hypertension in elderly white and black subjects. (Hypertension 1990;15(suppl I):I-88–I-92)

As hypertension in the elderly becomes a greater fraction of hypertension in the general population, it is apparent that relatively little is known of its possible mechanisms.

Plasma norepinephrine rises with age but, although it is positively correlated with blood pressure (BP) in young hypertensive subjects, the correlation becomes inverse in older subjects. On the other hand, arm vascular resistance is greater in older as compared with younger hypertensive subjects with similar norepinephrine levels. This is probably important because increase in total peripheral resistance is the hallmark of hypertension in general as well as in the elderly. There seems to be reduced β-adrenergic receptor responsiveness in the elderly, and unopposed constrictor α1-adrenergic receptor activity could elevate the peripheral resistance. There are conflicting data, however, regarding the state of the α1-adrenergic receptors as studied in blood platelets, that is, some studies suggest no age-related differences, whereas others suggest a decrease in number of receptors or diminished function with age. Plasma renin activity is known to decrease normally with age and is suppressed in elderly hypertensive subjects. Hypertension in this age group is less likely to be renin-dependent notwithstanding the good clinical response to converting enzyme inhibitors.

If both the sympathetic nervous system and the renin angiotensin system are attenuated in old age, then hypertension can be sustained by another pressor mechanism. The third major pressor hormone is vasopressin with levels and response to hyperosmolar stimuli that can be increased in the elderly. The contribution of increased vasopressin to hypertension is less well understood but maintenance of high or normal BP has been shown to be more dependent on vasopressin when the sympathetic function is attenuated. Thus, the elderly hypertensive person can be the patient in whom vasopressin plays a greater pressor role.

In this study, we have further investigated the possible relations of the adrenergic receptor and vaso-
pressin systems in elderly hypertensive subjects. More specifically, we measured levels of plasma norepinephrine, epinephrine, and vasopressin, as well as maximal binding (Bmax) and dissociation constant (Kd) of platelet α2-receptors in black and white elderly hypertensive subjects in comparison with normotensive subjects of the same age range.

Methods

Elderly subjects (over 65 years of age) were recruited from senior citizen day centers in greater Boston and by advertisement in a local senior citizens' newsletter. The study was approved by our Institutional Review Board and subjects signed an informed consent form.

All subjects were required to be free of other cardiovascular diseases (except for hypertension), as defined by history and physical examination, and no long-term medication. Volunteers were excluded if they were being treated with cardiovascular or psychoactive medication, sex steroids, or nonsteroidal anti-inflammatory drugs. They abstained from food and cigarettes for at least 2 hours before blood sampling.

All studies were performed between 9:00 AM and 12:00 noon. BPs were recorded by a mercury sphygmomanometer for at least three times over several minutes of quiet sitting, and pulse rate was taken manually by counting radial pulse for 1 minute. Hypertension was defined as systolic BP greater than 160 mm Hg or diastolic BP greater than 90 mm Hg of the mean of the three readings.

A needle was inserted into the antecubital vein and was kept open with repeated flushes of saline (total, 5 ml). A 65-ml blood sample was collected after 15 minutes of quiet sitting, distributed in appropriate tubes for each test, and transported immediately to the laboratory for further processing.

Platelet Receptor Binding

Platelet membrane preparation. Blood (50 ml) was prepared 8:1 (v:v) in ACD (0.8% citric acid, 2.2% trisodium citrate, 2.45% dextrose). It was centrifuged at 200g for 10 minutes at room temperature. The plasma was treated with 10 μl 0.1 M EGTA/ml and recentrifuged to move residual red blood cells. The plasma was transferred to another tube and centrifuged at 16,000g for 10 minutes at room temperature. The pellet was washed twice by centrifugation in a glass tissue grinder with a motor-driven moderately tight Teflon pestle, five strokes at high speed. The homogenate was set in ice for 1 minute and rehomogenized, before centrifuging in the cold at 39,000g for 10 minutes. This step was repeated with two more homogenizations in fresh lysing buffer.

Receptor binding. Specific binding of [3H]rauwolscine (73.5-77 Ci/mmol) (New England Nuclear, DuPont, Boston, Massachusetts) was measured by a modification of the method established in our laboratory for brain.10 Membranes (generally 120-150 μg protein) were incubated with [3H]rauwolscine at six concentrations (range, 0.15-10.0 nM) in a final volume of 500 μl in 50 mM Tris-HCl, 1 mM EDTA (2 Na), pH 7.2, at 4°C and overnight. Nonspecific binding was determined in the presence of 10 μM phentolamine (Regitine mesylate, CIBA-GEIGY, Somerville, New Jersey) at each level of radioligand. The incubation was terminated by vacuum filtration and washing on Whatman GF/F filters with 3×5 ml ice-cold incubation buffer. The dried filters were extracted overnight in Liquiscint (National Diagnostics, Somerville, New Jersey) and counted in a Beckman liquid scintillation counter (Beckman Innstrs., Inc., Wakefield, Massachusetts).

The saturation isotherms were transformed by the method of Rosenthal,11 and Kd and Bmax were calculated with unweighted linear regression analysis. Bound receptor per liter was expressed as femtomoles of radioligand bound per milligram of protein.

To test whether guanosine 5'-triphosphate (GTP) would change the binding characteristics with high agonist concentrations, samples of five elderly subjects with high norepinephrine were studied with and without 10 μM 5′ guanylylimidodiphosphate (Gpp[NH]P [GTPase resistant analogue]) and 200 mM NaCl.

Catecholamines. Catecholamines were assayed by high-performance liquid chromatography with electrochemical detection.

Vasopressin. Arginine vasopressin was measured by radioimmunoassay,2 with a highly specific antibody (kindly supplied by Dr. Leonard Share, University of Tennessee Center for the Health Sciences, Memphis, Tennessee). Results are reported as mean±SD. Data were analyzed by analysis of variance with the Bonferroni method for multiple comparisons and linear regression analysis. A p value less than 0.05 was considered significant.

Results

Hypertensive individuals had higher SBP and DBP by definition than their normotensive counterparts. Isolated systolic hypertension was present in seven of 21 white hypertensive and four of 11 black hypertensive subjects. There were no significant differences between the four groups in terms of age, heart rate, or male-female ratio (Table 1). Patients with systolic hypertension alone did not differ from the others in any of these characteristics. Likewise, there were no significant differences in terms of plasma norepinephrine, epinephrine, and Bmax, which reflects the number of α2-adrenergic recep-
In our present study, we document a number of hormonal differences between normotensive and hypertensive black and white elderly subjects. Black hypertensive subjects had significantly higher vasopressin levels as compared with white subjects of the same age and BP level, but vasopressin did not correlate with BP. This extends the findings of Crofton et al, 12 who found elevated vasopressin in the younger black normotensive group. On the other hand, Cowley et al 13 found that age in men was one of the major determinants of vasopressin levels (in patients excreting more than 250 meq Na+/day) but not race. Nevertheless, in that study, blacks were overrepresented in the hypertensive group as compared with the normotensive one. Our findings in the elderly hypertensive group (two thirds of whom were women in this age group) cannot be explained by sexual dimorphism 12 because both sexes were similarly distributed in all groups (Table 1). It should be noted, however, that both increased age and black race are reportedly associated with sodium sensitivity of BP, 15 and vasopressin was reportedly higher in older hypertensive men excreting high concentrations of sodium. 13 That black normotensive subjects did not have elevated vasopressin as did black hypertensive subjects suggests that vasopressin might be relevant to the hypertension of our subjects. Thus, our results appear to be consistent

### Table 1: Blood Pressure, Heart Rate, and Age in 87 Elderly Hypertensive and Normotensive Subjects

<table>
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<tr>
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<th>Hypertensive</th>
<th>Normotensive</th>
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<tbody>
<tr>
<td></td>
<td>White (n=22)</td>
<td>Black (n=11)</td>
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<tr>
<td></td>
<td>White (n=41)</td>
<td>Black (n=13)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>72.3±5.9</td>
<td>70.1±6.6</td>
</tr>
<tr>
<td>Male/female</td>
<td>5/17</td>
<td>3/8</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>164.6±14.3</td>
<td>166.7±14.4</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>90.9±11.4</td>
<td>96.0±13.5</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>76.0±13.3</td>
<td>76.0±8.0</td>
</tr>
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Values are mean±SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. 

•p<0.01 vs. white hypertensive subjects; tp<0.01 vs. black hypertensive subjects.

### Table 2: Plasma Norepinephrine, Epinephrine, Vasopressin, and Platelet α2-Adrenergic Receptor Levels in 87 Elderly Hypertensive and Normotensive Subjects

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<tr>
<td></td>
<td>White (n=41)</td>
<td>Black (n=13)</td>
</tr>
<tr>
<td>NE (pg/ml)</td>
<td>438±195</td>
<td>480±232</td>
</tr>
<tr>
<td>EP (pg/ml)</td>
<td>80±50</td>
<td>50±15</td>
</tr>
<tr>
<td>AVP (pg/ml)</td>
<td>0.7±0.5</td>
<td>1.5±1.0*</td>
</tr>
<tr>
<td>Kd (nM)</td>
<td>1.0±0.3</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>Bmax (fmol/mg protein)</td>
<td>288±62</td>
<td>299±46</td>
</tr>
</tbody>
</table>

Values are mean±SD. NE, norepinephrine; EP, epinephrine; AVP, vasopressin; Kd, platelet α2 dissociation constant; Bmax, platelet α2 maximal binding.

* p<0.005 vs. white hypertensive subjects.

† p<0.002 vs. black hypertensive subjects.

‡ p<0.0002 vs. white normotensive subjects.

Epinephrine, however, tended to be lower among black hypertensive subjects at 50±15 pg/ml when compared with white hypertensive subjects at 80±50 pg/ml although the difference did not achieve statistical significance. Moreover, there was an inverse correlation (r = −0.7, n = 11, p < 0.02) between epinephrine and DBP in the black hypertensive group. Plasma vasopressin was not different between hypertensive and normotensive subjects within each racial group; however, it was significantly higher in the black hypertensive when compared with the white normotensive subjects (Table 2). The elevated vasopressin did not correlate with SBP, DBP, norepinephrine, epinephrine, Kd, or Bmax of α2-adrenergic receptors in this group. Black normotensive subjects had a significantly higher platelet–α2-adrenergic receptor Kd in comparison with the other groups (Table 2). Among the white hypertensive subjects, there was an inverse correlation between Bmax of platelet α2-adrenergic receptors and DBP (r = −0.45, n = 22, p < 0.05). For all the participants, there was a highly significant correlation between Bmax and plasma vasopressin levels (r = 0.4, n = 87, p < 0.0004) (Figure 1).

No relation was found between vasopressin, norepinephrine, and Kd with either SBP or DBP.

The inclusion of Gpp(NH)P (10 μM) and NaCl (200 mM) did not change Bmax (from 219±64 before to 209±46 fmols/mg after; not significant).

### Discussion

In our present study, we document a number of hormonal differences between normotensive and hypertensive black and white elderly subjects. Black hypertensive subjects had significantly higher vasopressin levels as compared with white subjects of the same age and BP level, but vasopressin did not correlate with BP. This extends the findings of Crofton et al, 12 who found elevated vasopressin in the younger black normotensive group. On the other hand, Cowley et al 13 found that age in men was one of the major determinants of vasopressin levels (in patients excreting more than 250 meq Na+/day) but not race. Nevertheless, in that study, blacks were overrepresented in the hypertensive group as compared with the normotensive one. Our findings in the elderly hypertensive group (two thirds of whom were women in this age group) cannot be explained by sexual dimorphism 12 because both sexes were similarly distributed in all groups (Table 1). It should be noted, however, that both increased age and black race are reportedly associated with sodium sensitivity of BP, 15 and vasopressin was reportedly higher in older hypertensive men excreting high concentrations of sodium. 13 That black normotensive subjects did not have elevated vasopressin as did black hypertensive subjects suggests that vasopressin might be relevant to the hypertension of our subjects. Thus, our results appear to be consistent.
with most of these previous findings, extending them to the elderly hypertensive population.

Epinephrine levels were not statistically different among the groups. This could be because of a type II error because the difference between the white hypertensive and black hypertensive groups (30 pg/ml) was close to the detection limits of our method (20 pg/ml), and the number in the black hypertensive group was smaller. Epinephrine levels, however, correlated inversely \((p<0.05)\) with DBP in the black hypertensive group. This is in accordance with the idea that \(\beta\)-blockers might be less effective as antihypertensive treatment in elderly black hypertensive patients.

An unexpected finding was the significantly lower affinity (i.e., higher \(K_d\)) of platelet \(\alpha_2\)-adrenergic receptors in the black normotensive group. In a study of a small group of young subjects,\(^1\) it was shown that hypertensive subjects do not reduce their platelet \(\alpha_2\)-adrenergic receptor affinity in response to use of exogenous or endogenous catecholamines; the race of the patients, however, was not reported. Although we have no information on \(\alpha_2\)-adrenergic receptor affinity of young black normotensive subjects, in white subjects we have shown that age and the subsequent norepinephrine increase can down-regulate \(B_{\text{max}}\) but has no effect on platelet \(\alpha_2\)-adrenergic receptor affinity; thus, there does not seem to be an age effect on \(K_d\).\(^7\) There is evidence that hypertension is more common in blacks and more likely to be associated with increased sympathetic activity and a higher salt intake.\(^5\) It is tempting to speculate that such a finding might represent a protective mechanism in those elderly blacks who remained normotensive if platelet adrenergic receptors reflect the status of receptors in other sites such as specific brain areas and vascular or renal nerves; however, there is no firm basis of support for such a projection.

In the white hypertensive group, there was an inverse correlation \((p<0.05)\) between DBP and \(B_{\text{max}}\). The significance of this finding is not known at present, but the absence of this finding in the white normotensive group \((r=0.2,\) not significant\) suggests that it might be relevant to the hypertension in elderly whites despite the absence of difference in the means of the two groups.

An interesting finding is the highly significant correlation between \(B_{\text{max}}\) of \(\alpha_2\)-adrenergic receptors in platelets and vasopressin in the whole study population \((r=0.4,\) \(n=87,\) \(p<0.0004)\) (Figure 1). Because vasopressin release can be dependent on \(\alpha_2\)-adrenergic receptor mechanisms,\(^9\) this finding suggests that the status of platelet adrenergic receptors might reflect the status of central nervous system receptors, especially those of the supraoptic and paraventricular nuclei. Curiously, the black hypertensive group had no such correlation \((r=0.05)\), whereas in the black normotensive group, the correlation was even stronger than in the whole group \((r=0.7,\) \(n=13,\) \(p<0.0005)\).

We describe a number of differences in hormone levels and status of \(\alpha_2\)-adrenergic receptors in elderly hypertensive and normotensive subjects. Our findings support the notion of heterogeneity of hypertension in the elderly with possibly different mechanisms of hypertension in white and black subjects. Further work is needed to evaluate the functional or pathogenetic significance of these differences.

References


**KEYWORDS** • vasopressin • catecholamines • norepinephrine
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