Hypertension

Clinical Studies

Hormonal Responses and Blood Pressure Maintenance in Normal and Hypertensive Subjects During Acute Blood Loss

Manuel T. Velasquez, Jay E. Menitove, Meredith M. Skelton, and Allen W. Cowley, Jr.

SUMMARY Blood pressure (BP) and plasma indices of three major pressure control systems — plasma norepinephrine and epinephrine, plasma renin activity (PRA), and plasma arginine vasopressin — were measured simultaneously in 12 normal and 15 mildly essential hypertensive subjects before and after removal of 480 ml of blood by phlebotomy, to determine if there were differences in the compensatory response to acute blood loss. Responses to postural stress (change from supine to sitting position) following phlebotomy were also compared in a second group of subjects. Before phlebotomy, supine plasma hormone levels did not differ in the two groups. After phlebotomy, both groups exhibited only slight decreases (5 mm Hg) in systolic BP and a transient rise in heart rate. Only plasma norepinephrine increased significantly in both groups (35% above control in normal and 43% in hypertensive subjects). Similar results were obtained in a second group of normal and hypertensive subjects, who were also subjected to a 10-minute postural challenge after phlebotomy. After 10 minutes in a sitting position, BP in these subjects remained unchanged but heart rate and plasma norepinephrine increased further to levels almost twice that produced by phlebotomy alone. Plasma epinephrine levels and PRA also increased with this additional stress, but plasma vasopressin remained unchanged. Changes in BP, heart rate, plasma norepinephrine and epinephrine, and PRA did not differ significantly between the two groups. These data indicate that 1) hypertensive subjects are as capable as normal subjects of maintaining BP when subjected to standard phlebotomy, 2) the sympathetic nervous system appears to be the predominant pressor mechanism activated following an acute, nonhypotensive blood loss in both groups of subjects, and 3) the addition of postural stress further accentuates the sympathetic response and increases PRA. (Hypertension 9: 423-428, 1987)

Key Words: blood pressure • plasma catecholamines • renin activity • arginine vasopressin • phlebotomy • essential hypertension

The sympathetic nervous system and the renin-angiotensin axis play important roles in the control of arterial blood pressure (BP). These two systems, for example, are known to be activated in situations that provoke a decrease in BP, such as during hemorrhage or after a postural change. More recently, arginine vasopressin (AVP) also has been shown to play a contributory role in the maintenance of BP during hypotensive and volume-depleted states. In addition, this hormone has been implicated in some hypertensive models, including human essential hypertension.

Although each of these pressor systems is capable of contributing to maintenance of BP in conscious animals, it is still uncertain which of these systems are activated in human subjects in hypovolemic states. Whether the response of subjects with essential hypertension to hypovolemia differs from that of normotensive subjects is also unknown. Recently, it has been reported that rats with spontaneous hypertension are less capable than normotensive control rats of maintaining BP when subjected to graded hemorrhage. Whether such an abnormality also exists in human subjects with essential hypertension has not been examined.
The present study, therefore, was designed to evaluate simultaneously indices of three pressure control systems in response to acute blood volume reduction and to determine whether the response of hypertensive subjects differs from that of normal subjects. Changes in BP and heart rate (HR) were related to changes of plasma catecholamines, renin, and AVP during removal of 480 ml of whole blood over an 8- to 15-minute period.

Subjects and Methods

Study 1 Subjects

Twelve normal subjects (7 men and 5 women; 7 white, 5 black; aged 31-50 years; mean age, 35 ± 2 years) and 15 hypertensive subjects (10 men and 5 women; 5 white, 10 black; aged 31-56 years; mean age, 38 ± 2 years) participated in the first study. The average weight of the normotensive group was 73.6 ± 3.8 kg (range, 58-98 kg), while the average weight of the hypertensive group was 92 ± 5 kg (range, 69-126 kg).

In both studies, the normal subjects were healthy volunteers who had no prior history of hypertension and whose BP was found to be below 140/90 mm Hg in three separate prestudy measurements. The hypertensive subjects were patients with mild essential hypertension with untreated diastolic BP between 90 and 104 mm Hg. All antihypertensive medications were withdrawn for at least 2 weeks before entry into the study. Secondary causes of hypertension were excluded in all subjects by history, physical examination, and routine laboratory analysis (urine and plasma electrolytes, and creatinine). None of the subjects recruited for these studies appeared to require further evaluation. None had a history of or findings of stroke, heart failure, myocardial infarction, chronic pulmonary disease, liver disease, renal disease, diabetes, peripheral neuropathy, anemia or any other illness requiring drug therapy. All subjects were thoroughly screened for blood donation and fulfilled the donor acceptance criteria established by the American Association of Blood Banks. The study was approved by the Human Research Review Committee of the Medical College of Wisconsin and the Blood Center of Southeastern Wisconsin, and informed consent was obtained from each participating subject. Before the study, the subjects were instructed to continue their usual diet with no salt restriction and to avoid taking alcohol or any over-the-counter medications. They were also encouraged to have at least 6 hours of sleep the night before the study. All subjects abstained from smoking or drinking coffee or tea for at least 8 hours before the study.

Studies were performed at the Blood Center of Southeastern Wisconsin. On the morning of arrival at the center following an overnight fast, the subjects were familiarized with the study protocol and prepared for blood donation according to standard donation procedures. In both studies, an indwelling needle was inserted into an antecubital vein and kept open with a microdrip infusion of 5% dextrose in water for blood sampling. After the subject had rested supine for 30 minutes (20 minutes after venipuncture), a blood sample was withdrawn from the arm vein and three sets of BP recordings were immediately taken in the same area by means of a standard mercury sphygmomanometer. The first and fifth Korotkoff sounds were designated as the systolic and diastolic pressures, respectively. HR was counted for 1 minute from the radial pulse after each pressure determination.

Study 1 Protocol

Following control measurements, a phlebotomy needle was inserted into the antecubital vein of the opposite arm and blood was withdrawn by gravity into the donor bag for collection of 1 unit (480 ml) of blood. Blood samples and three sets of BP and HR measurements were obtained successively at the following three times: 1) after 240 ml of blood (50% of total) was collected in the donor bag; 2) immediately after a total of 480 ml was collected (the donation usually took 8-15 minutes to complete); 3) 10 minutes after phlebotomy. After completion of the study, the donor was observed and allowed to recover according to postdonation procedures.

Study 2 Protocol

Study 2 was identical to Study 1, except that after phlebotomy was completed, the subjects changed from a supine to a sitting position to provide one more level of hemodynamic stimulus. Measurements and blood sampling were repeated after 10 minutes of sitting in a chair.

Sample and Data Analysis

Blood samples for determination of plasma catecholamine levels, plasma renin activity (PRA), plasma AVP, plasma sodium and potassium, and plasma osmolality were processed within 1 hour after collection, and plasma samples for hormonal analysis were frozen immediately.

Plasma levels of norepinephrine (NE) and epinephrine were determined by a radioenzymatic assay, using the CAT-A-KIT (Upjohn Diagnostics, Kalamazoo, MI, USA). Interassay and intrassay coefficient of variation averaged less than 8%.

Plasma AVP levels were determined with a radioimmunoassay procedure developed in our laboratory and described previously. Plasm was extracted within 60 days of collection. The midrange of the assay aver-
Blood loss responses in normal and hypertensive humans

Overview

The intrasassay coefficient of variation averaged less than 5%, and the interassay coefficient of variation averaged less than 10%.

The PRA was also determined by radioimmunoassay of generated angiotensin I using a modification of the procedure described by Sealey and Laragh. Intrasassay and interassay variability always averaged less than 5 and 9.5%, respectively. Angiotensin I antibodies were kindly provided by Dr. Jean Sealey.

Plasma sodium and potassium concentrations were determined by flame photometry (Model 443; Instrumentation Laboratory, Lexington, MA, USA). Plasma osmolality was determined within 10 days of collection using a Wescor Vapor Pressure Osmometer (Model 5100C; Logan, UT, USA). Coefficient of variation of multiple determinations was less than 1%.

All data are expressed as means ± SEM. Statistical analysis was performed using a two-way analysis of variance and Dunnett's test for repeated measurements. Between-group comparisons of the different variables were made using Student's t test for unpaired data. Results were considered significant if the p value was less than 0.05.

Results

Study 1

Figure 1 and Table 1 summarize the results of serial measurements of BP, HR, plasma NE, PRA, and plasma AVP in the two groups of subjects before, during, and after blood donation. Removal of 480 ml of blood resulted in only a small decrease of systolic BP (5 mm Hg) in the two groups of subjects. HR, however, increased slightly (2-3 beats/min) during phlebotomy and promptly returned to baseline values 10 minutes after completion of phlebotomy. Plasma NE increased progressively in both groups, increasing 35% above control in normotensive subjects and 43% in hypertensive subjects. It remained elevated 10 minutes after completion of phlebotomy. PRA, plasma AVP, and

Table 1. Effects of Phlebotomy of 480 ml of Blood in Normotensive and Essential Hypertensive Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n = 12)</th>
<th>Hypertensive (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Phlebotomy</td>
</tr>
<tr>
<td>BP (mm Hg)</td>
<td>Control</td>
<td>Phlebotomy</td>
</tr>
<tr>
<td>Systolic</td>
<td>120 ± 3</td>
<td>116 ± 3*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>80 ± 2</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>64 ± 2</td>
<td>67 ± 3*</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (pg/ml)</td>
<td>294 ± 51</td>
<td>398 ± 90*</td>
</tr>
<tr>
<td>Epi (pg/ml)</td>
<td>29 ± 6</td>
<td>37 ± 9</td>
</tr>
<tr>
<td>AVP (pg/ml)</td>
<td>2.4 ± 0.4</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>Osmolarity (mosm/kg)</td>
<td>283 ± 2</td>
<td>284 ± 2</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>140 ± 1</td>
<td>140 ± 1</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>4.5 ± 0.1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>PRA (ng ANG 1/ml/hr)</td>
<td>2.2 ± 0.3</td>
<td>2.3 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SEM. HR = heart rate; NE = norepinephrine; Epi = epinephrine; AVP = arginine vasopressin; ANG 1 = angiotensin I.

* p < 0.05, compared with control values.

† p < 0.05, compared with corresponding values in the normotensive group.
plasma epinephrine levels were not changed significantly in either group by the decrease of blood volume. PRA increased slightly but significantly 10 minutes after phlebotomy in hypertensive subjects only. Plasma sodium, potassium, and osmolality remained unchanged throughout the study.

**Figure 2.** Comparison of changes in BP, heart rate (HR), plasma norepinephrine (PNE) PRA, and plasma vasopressin (PVP) during a control period (Con), after full donation, and 10 minutes after a postural challenge (sitting) in nine normotensive (solid bars and open circles) and eight hypertensive subjects (hatched bars and closed circles). Asterisk indicates significant change (p<0.05) from control values. Dagger indicates significant difference between groups (p<0.05). AI = angiotensin I.

**Study 2**

Since the results indicated that phlebotomy had no apparent effect on PRA and plasma AVP, additional studies were performed in nine normotensive subjects and eight subjects with mild essential hypertension to evaluate their responses to a postural challenge following blood donation.

The results of the serial measurements are summarized in Figure 2 and Table 2. As in Study 1, little or no changes were observed in PRA, plasma AVP, or plasma epinephrine with phlebotomy. Systolic and diastolic pressures also were not measurably altered in either group. HR and plasma NE were again increased significantly by phlebotomy. Following 10 minutes of quiet sitting, HR and plasma NE rose further and PRA increased to levels significantly above control values, while plasma AVP levels remained unchanged. BP also remained unchanged in both groups after the postural challenge.

Plasma epinephrine and plasma potassium also increased slightly in both groups of subjects, but the increase was significant only in the normotensive group. The average increases in HR, plasma NE, plasma epinephrine, and PRA after phlebotomy alone and after phlebotomy and sitting did not differ between the two groups.

**Discussion**

The removal of 1 unit (about 480 ml) of whole blood represents a decrease of approximately 10% of blood volume in normal humans. This amount of blood loss usually does not cause a significant fall of BP in normal supine subjects, as was clearly demonstrated in the present studies. To our knowledge, these studies are the first to examine these responses in subjects with mild uncomplicated essential hypertension. We found that the BP response of hypertensive subjects was simi-

### Table 2. Effects of Phlebotomy (480 ml) and Postural Challenge (Sitting) in Normotensive and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n = 9)</th>
<th>Hypertensive (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Phlebotomy</td>
</tr>
<tr>
<td>BP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>115 ± 3</td>
<td>112 ± 3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>78 ± 3</td>
<td>78 ± 3</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>60 ± 3</td>
<td>65 ± 3†</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (pg/ml)</td>
<td>215 ± 26</td>
<td>345 ± 40†</td>
</tr>
<tr>
<td>Epi (pg/ml)</td>
<td>22 ± 5</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>AVP (pg/ml)</td>
<td>2.7 ± 0.3</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>Osmolality (mosm/kg)</td>
<td>287 ± 3</td>
<td>—</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>143 ± 1</td>
<td>—</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>4.5 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>PRA (ng ANG I/ml/hr)</td>
<td>1.7 ± 0.3</td>
<td>1.9 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SEM. See Table 1 for key to abbreviations.

* p < 0.05, compared with corresponding values in the normotensive group.

† p < 0.05, compared with control values.
lar to that of normal subjects: only small decreases in systolic BP were observed, and no significant changes were seen in diastolic BP. Small increases in HR were noted during phlebotomy in both groups, but the changes were transient. These results also indicate that subjects with mild essential hypertension are as capable as normal subjects of maintaining BP when subjected to standard blood donation. Subjects with more severe established forms of hypertension were excluded from the study; such subjects may respond differently because of the presence of reductions in vascular compliance and blood volume.

The responses of three major pressure control systems were also evaluated, as represented by changes in catecholamines, PRA, and plasma AVP. Both normal and hypertensive subjects responded to the hypovolemic stimulus with significant increases in plasma NE, indicating an increase of sympathetic nerve activity. However, we did not detect any significant changes in plasma epinephrine, PRA, or plasma AVP levels with phlebotomy alone.

Goetz et al. also studied the effect of removal of 485 ml of blood by phlebotomy in seated, normal blood donors and found no significant changes in either PRA or plasma antidiuretic hormone (ADH) levels measured by bioassay after phlebotomy. However, these investigators did not measure plasma catecholamines. Similarly, Brown et al. have also shown that removal of 400 to 500 ml of blood from human subjects caused only small, insignificant changes of PRA. These findings, together with our results, indicate that in normotensive subjects only the sympathetic nervous system is activated in response to nonhypotensive decreases in blood volume and that the renin-angiotensin system and vasopressin system do not appear to be involved in the compensatory response to mild decreases of intravascular volume. Our studies thus further indicate that subjects with mild hypertension respond in a similar manner and do not appear to have altered responses to this hypovolemic stimulus.

Since the possibility existed that renin and AVP could be stimulated with more severe volume reduction, both groups were further tested with addition of a postural challenge to the hypovolemic stimulus. This additional maneuver caused further increases in HR and plasma NE levels in the two groups of subjects, indicating a greater sympathetic activation. Moreover, with the additional postural challenge, there was a significant rise in PRA while plasma AVP remained unchanged. Even with these two maneuvers, BP was well maintained in both normotensive and hypertensive subjects. Furthermore, we did not find any significant differences in the responses of HR, plasma NE, or PRA between the two groups of subjects.

A number of investigators have examined AVP responsiveness to a variety of stimuli in normal human subjects. Segar and Moore reported that plasma ADH measured by bioassay increased progressively in human subjects as they changed from supine to sitting and then to a standing position. Other investigators have reported either no change or increases in plasma AVP in response to upright posture. We, as well as others, have studied the role of body position as a maneuver that provides a more uniform stimulus whereby central venous pressure can be controlled independently of changes in arterial pressure. With the use of this maneuver, we have determined plasma AVP in two separate studies in normal subjects in which central venous pressure was decreased to either 3 or 7 cm H2O below control for 10 minutes, and we found no significant change of plasma AVP, while PRA and plasma NE significantly increased. In contrast, Rogge and Moore observed an increase in plasma ADH after 30 minutes of lower body negative pressure at 30 mm Hg, but not at 20 mm Hg. However, there was a noticeable decrease of about 10 mm Hg systolic BP in their study at both levels of lower body negative pressure. More recently, Egan et al. reported that thigh cuff inflation to 30–40 mm Hg, which caused a decrease in right atrial pressure and central blood volume with no change in mean arterial pressure, increased plasma AVP levels.

The present studies conform with our own previous studies in humans, which indicated that there is little cardiopulmonary reflex control of AVP secretion. This finding is in contrast to studies we have performed in normal conscious dogs in which nonhypotensive hemorrhage significantly increased plasma AVP. However, we and others have observed that a hypotensive hemorrhage consistently results in substantially greater increases of plasma AVP. The lack of an increase in plasma AVP levels in our subjects after phlebotomy and postural challenge may therefore be explained by the fact that BP was well maintained throughout the procedures, probably because of the compensatory responses of the sympathetic and renin-angiotensin systems during the hypovolemic stimulus. Therefore, there was no hypotensive stimulus to activate AVP release.

It seems apparent from the present studies that sympathetic nervous system activation is the predominant pressor mechanism for maintaining BP during acute nonhypotensive blood loss in normal and hypertensive subjects. When postural stress is superimposed after the volume loss, the renin-angiotensin system also appears to become activated.

Finally, our studies also show that hypertensive subjects controlled BP as well as normal subjects in response to acute nonhypotensive blood volume reduction. These observations have practical implications related to the BP criteria presently applied for accepting blood donors; that is, subjects with hypertension are often excluded from donating blood.

Acknowledgments

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