Non-Modulation as an Intermediate Phenotype in Essential Hypertension

Gordon H. Williams, Robert G. Dluhy, Richard P. Lifton, Thomas J. Moore, Ray Gleason, Roger Williams, Steven C. Hunt, Paul N. Hopkins, and Norman K. Hollenberg

Non-modulation is a trait characterized by abnormal angiotensin-mediated control of aldosterone release and the renal blood supply. To determine whether non-modulation defines a specific subgroup of the hypertensive population and its utility as an intermediate phenotype, we have studied the distribution of this quantitative trait, whether its features are reproducible on repeated testing, and whether there is concordance of its multiple features. Essential hypertensive patients (224) and normotensive subjects (119) received an infusion of angiotensin II (Ang II) at 3 ng·kg⁻¹·min⁻¹ for 30–45 minutes. p-Aminohippurate (PAH) clearance was assessed as an index of renal plasma flow while the subjects were on a 200 meq sodium diet; plasma aldosterone levels were measured while the subjects were on a 10 meq sodium diet. In 54 subjects, diuretic-induced volume depletion superimposed on a low salt diet was substituted for the Ang II infusion. The results of each study were submitted to maximum likelihood analysis to assess bimodality. In response to both diuretic-induced volume depletion (p<0.000023) and Ang II infusion (p<0.0009), aldosterone responses were bimodally distributed in the essential hypertensive but not in the normotensive subjects, suggesting that this trait identifies a discrete subgroup. In the 59 subjects who had both an adrenal and renal study, 50 (85%) were concordant. Finally, in 27 subjects studied two to six times over a span of 1–60 months, the intraclass correlations of the adrenal, PAH, or aldosterone responses were highly significant (p values between 0.001 and 0.00007), indicating high reproducibility of results on repeated testing. Thus, this analysis supports the hypothesis that non-modulators are a discrete subset of the essential hypertensive population, suggesting that this trait may be useful as an intermediate phenotype in human hypertension. (Hypertension 1992;20:788–796)

Key Words • hypertension, non-modulating • genetics • renal circulation • angiotensin II • aldosterone

Since a wide array of regulatory systems that influence blood pressure also respond to changes in blood pressure, it is not surprising that abnormalities in a host of physiological systems have been described in hypertensive subjects. The major difficulty has been distinguishing between primary contributors to the disorder and secondary consequences of the hypertensive process. This problem may well prove insoluble from physiological analyses alone: despite exhaustive physiological investigation, the primary abnormalities that lead to the development of hypertension remain unknown.

The study of genetics provides a complementary approach to physiological analysis. Identification of mutations contributing to the pathogenesis of hypertension would permit identification of individuals at risk in the preclinical stages, thereby providing insights into the physiological changes and the natural history of the disorder and perhaps even affording early therapeutic intervention. Epidemiological studies have established that a substantial proportion of the observed interindividual variation in blood pressure is genetically determined. This conclusion derives from population studies controlling for confounding physical and environmental variables, as well as from studies of monozygotic and dizygotic twins, including twins raised in separate environments. From such studies, the estimates of variation attributable to inheritance range from 20% to 40%. The continuous and unimodal distribution of blood pressure in the general population, as well as in the offspring of hypertensive parents, suggests that the inheritance of blood pressure variation is multifactorial, thereby complicating the search for blood pressure–determining genes. It is readily apparent that the chance of success of this search could be increased substantially if the heterogeneous general hypertensive population could be divided into more genetically homogeneous subsets. The physiology of hypertension suggests that this can be accom-
plished via the use of "intermediate phenotypes."8 The most appropriate intermediate phenotypes would use indexes that are not merely secondary consequences of the hypertensive process but instead appear to define unique physiological or genetic subsets of the hypertensive population. If the intermediate phenotype is determined at least in part by inheritance, then these phenotypic subsets will identify populations in which specific genetic factors are more prevalent. The result will be increased power to detect linkage to specific genes and stronger evidence in favor of linkage.

Among the candidates proposed as intermediate phenotypes, a group of patients with essential hypertension whom we have called "non-modulators" represents an attractive possibility.6,10 Non-modulation is a trait characterized by abnormal angiotensin-mediated control of aldosterone release and the renal blood supply, abnormal sodium handling, and sodium-sensitive hypertension.11-13 These features are functional rather than fixed, because they are largely corrected by the administration of a converting enzyme inhibitor (CEI).11,12-16

A number of features of non-modulation have made it an attractive candidate as an intermediate phenotype, including a frequently reported positive family history of hypertension12,17; association with indexes known to be genetically determined, such as increased sodium-lithium countertransport;18 concordance of the renal vascular response to angiotensin II (Ang II) in hypertensive sibling pairs19; and abnormalities resembling non-modulation in the normotensive offspring of hypertensive subjects.17,20-22

An ideal intermediate phenotype would display patent Mendelian segregation, allowing completely unambiguous phenotypic classification. For quantitative traits, the key parameter is the ability to distinguish distinct modes in the distribution of the trait. The greater the separation of different modes (as reflected in the number of standard deviations separating mean values of different modes), the greater the accuracy in classification of the trait. Although such bimodality or trimodality does not ensure a genetic basis for a trait, the absence of such features would indicate that such an intermediate phenotype is unlikely to be useful for genetic studies. Such intermediate phenotypes can be used as the trait of interest for linkage studies or alternatively can be used to stratify the general population into more homogeneous subgroups for linkage using other traits.

This study was designed to address three features of non-modulation: 1) the quantitative distribution of various features of the trait; 2) the reproducibility of measurement of the trait; and 3) the concordance of different features of the trait.

Methods

The hypertensive patients described in the present report came from a pool of 449 patients admitted consecutively to the Clinical Research Centers at the Brigham and Women's Hospital, Boston, or the University of Utah Medical Center, Salt Lake City. Some of the findings in some of these subjects have been reported.6-12,17,18,22 Of these patients, 151 were excluded from further analysis because they had either secondary hypertension or low renin hypertension. A low renin hypertensive patient was defined as an individual in balance on a 10 meq sodium intake who had a plasma renin activity less than 2.4 ng angiotensin I (Ang

1) • ml⁻¹ • hr⁻¹ after 90-120 minutes of upright posture.24 Patients with low renin levels were excluded for two reasons: they have another form of salt-sensitive hypertension and, if anything, they have been reported to have increased aldosterone responses to Ang II—the opposite of the non-modulating trait.25-27 An additional 55 patients (16%) had incomplete studies resulting from failure to achieve the appropriate sodium balance, a plasma cortisol rise greater than 7 μg/dl during the course of the Ang II infusion, or the premature termination of the Ang II infusion because of an excessive rise in diastolic blood pressure. In sum, studies in 243 hypertensive patients were available for analysis. The responses in these patients were compared with 119 normotensive control subjects studied over the same time interval as the hypertensive patients, with equivalent distributions between Boston and Salt Lake City. Because few black subjects were studied (7% in the hypertensive group and 4% in the normotensive group), they also were excluded from further analysis. Urinalysis, serum potassium and renal function tests (glomerular filtration rate greater than 80 cm³/min or serum creatinine less than 1.5 mg/dl), 24-hour urine cortisol and catecholamines, thyroid function tests, and renal vascular studies (hypertensive intravenous pyelogram, digital subtraction angiogram, or arteriogram where indicated) were all normal in the hypertensive subjects. All antihypertensive agents were discontinued at least 2 weeks before the study began. The protocols were approved by the Human Subjects Committee of either the Brigham and Women's Hospital or the University of Utah Medical Center, and written informed consent was obtained from each patient.

Entry criteria for the normotensive patients were freedom from any known disease and the subject's not being aware of siblings or parents with hypertension before age 65.

In all patients, the specific tests were performed in an in-patient setting. In some cases, dietary control was established in an ambulatory clinical center before admission. Patients were placed on a constant isocaloric diet containing either 200 or 10 meq sodium and 100 meq potassium. During the day of study, the subjects' activity was controlled. When sodium balance was achieved (as determined by urine sodium and potassium content), specific provocative tests were performed.

Statistical Analysis

Group means were computed with the SEM serving as the index of dispersion. Statistical significance was determined by Student's t test for data that were normally distributed (paired or unpaired) and the Fisher exact test or Wilcoxon signed rank test for non-normally distributed data. To determine whether the observed distribution is best described by a single, continuous distribution or commingling of multiple distributions, maximum likelihood analysis was performed.28 Equal SDs as well as unequal SDs in the two modes were modeled. Day28 recognized four critical parameters for the statistical evaluation of a data base for bimodality: the number of observations, the separation of the mean values for each of the subpopulations, their relative size, and the SD of the distribution (or distributions). If one of the modes is separated from the other by 3 SDs and one represents 30% of the total
were considered significant at p<0.05, unless otherwise indicated.

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Feasibility of measurements in the same subject was assessed by intraclass correlation. Differences were considered significant at p<0.05, unless otherwise indicated.

Features of Non-Modulation
Ten features have been reported to be associated with the non-modulation trait (Table 1). In a preliminary survey of which of these features should be subjected to maximum likelihood analysis, several appeared unlikely because there were too few observations in relation to the difference between the mean levels of the feature in modulators versus non-modulators (see "Statistical Analysis" section and Reference 28). Two features, the renal plasma flow response to an Ang II infusion (89 patients) and changes in renal plasma flow in response to a shift from a high salt to a low salt diet (48 patients), involved too few studies for an adequate assessment of bimodality, although the data were subjected to analysis. In the case of the change in renal plasma flow with change in salt intake, a statistically significant bimodal distribution was identified (p<0.013). Statistical significance for the renal plasma flow response to Ang II infusion was approached (p=0.11). A rigorous assessment with adequate power to accept or reject the null hypothesis could be performed only on the adrenal response to either a true volume deficit induced by a low salt diet plus furosemide or the adrenal response to Ang II, aldosterone, and cortisol were determined by radio-immunoassay procedures, as previously reported. Aldosterone secretion rate. The aldosterone secretion rate was determined by modification of the Kliman-Peterson method. Five to 10 μCi of 1-2-tritiated d-aldo
tsterone were given intravenously in 15 ml sterile isotonic glucose solution between 8 and 9 AM, and the urine was collected for the next 24 hours. The 18-glucuronide conjugate of aldosterone was hydrolyzed at pH 1 for 24 hours at 25°C and the liberated aldosterone quantified after chromatographic isolation, as previously described.

Results
Do the Features of Non-Modulation Suggest a Discrete Group?
To assess whether the abnormal physiological features in non-modulators were part of a continuum of responsiveness or rather reflected a discrete abnormality that occurred in a specific subset of patients, the available data were examined for evidence of bimodality, which would indicate distinct subgroups or a unimodal distribution, which would suggest a continuum. Two features, the adrenal response to Ang II infusion on a low salt diet and the adrenal secretory response to diuretic-induced volume contraction after a low salt diet, were studied in a sufficient number of patients and showed sufficient separation that the hypothesis of bimodality could be assessed adequately.

Responsiveness of aldosterone secretory rate. All subjects were studied after sodium and potassium balance had been achieved on a 10 meq sodium, 100 meq potassium diet (Table 2). An aldosterone secretion rate was obtained, and then furosemide was administered in divided doses over 24 hours to produce a 2-3% reduction in weight. Over the next 24 hours, a repeat aldosterone secretion rate was determined, and the change in the secretion rate was calculated. The aldosterone secretion rate response to volume depletion was 198±41 μg/24 hr in the hypertensive subjects and 713±59 μg/24 hr in the normotensive subjects, a highly significant difference (p<0.001). The strong suggestion of bimodality in the hypertensive but not normotensive subjects was confirmed by maximum likelihood analysis (Figure 1) (p<0.0000023). Of the hypertensive patients, 23 belonged to the first mode, with a mean value of 45±10 μg/24 hr, whereas the remainder belonged to the second mode, with a mean value of 769±52 μg/24 hr, a level indistinguishable from that observed in the normotensive subjects (Figure 1). There were no differences between the two subgroups of hypertensive patients in terms of age, blood pressure at the time the diuretic was administered, total diuretic dose given, body weight, or body surface area.

Aldosterone response to angiotensin II infusion. The subjects received an Ang II infusion after achieving
TABLE 2. Characteristics of Patients Studied on Each Protocol

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Subjects (No.)</th>
<th>Male (%)</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone secretory response to acute diuretic-induced volume depletion on low salt (10 meq) diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive</td>
<td>19</td>
<td>80</td>
<td>22–48</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>36</td>
<td>75</td>
<td>21–56</td>
</tr>
<tr>
<td>Plasma aldosterone response to Ang II on a low salt (10 meq) diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive</td>
<td>61</td>
<td>79</td>
<td>20–57</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>150</td>
<td>73</td>
<td>17–65</td>
</tr>
<tr>
<td>Renal plasma flow response to Ang II on a high salt (200 meq) diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive</td>
<td>59</td>
<td>65</td>
<td>20–58</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>89</td>
<td>79</td>
<td>21–65</td>
</tr>
</tbody>
</table>

Ang II, angiotensin II.

balance on a 10 meq sodium intake (Table 2). The normotensive subjects were slightly but significantly (p<0.025) younger (34.5±1.4 years) than the hypertensive subjects (42.3±1.1 years).

The hypertensive patients had a significantly lower basal plasma aldosterone level (21.1±1.2 ng/dl) than did the normotensive subjects (25.5±2.2 ng/dl) (p<0.007). However, there was no significant difference in the mean absolute plasma aldosterone response to Ang II (50.4 ng/dl in the hypertensive and 57.4 ng/dl in the normotensive subjects) or in the mean rise in plasma aldosterone (24 and 25 ng/dl, respectively). The individual responses in the hypertensive but not the normotensive group demonstrated a clear bimodal distribution (p<0.00009, maximum likelihood analysis) (Figure 2A). The dominant mode had a mean±SD of 22.6±10.4 ng/dl. The mean±SD value of the lower mode, which comprised 23% of the population, was 7.7±2.7. There was even a suggestion that in the hypertensive patients, the data were trimodal (p<0.01 versus bimodal): a small mode (8%) with a mean±SD of 76.2±15.4 ng/dl may also be present. The lowest mode was the same, whether the distribution was assumed to be trimodal or bimodal. The responses of the normotensive subjects primarily fit a unimodal distribution, although there was a slight suggestion (p=0.05) of bimodality, with the modes equivalent to the middle and high mode of the trimodal hypertensive patient distribution (Figure 2B).

The upper limit of values for the non-modulating subgroup was defined as 3 SD (i.e., 99% confidence limit) above the mean value of the low mode, i.e., 15 ng/dl. By this definition, non-modulators composed 39.3% of the normal and high renin hypertensive patients studied. The characteristics of the patients in this group (n=59) were compared with a smaller group (n=39), with a more restricted definition of non-modulation (mean±1 SD or increments <10 ng/dl) to determine whether a more restricted criterion (which presumably would include fewer modulators) would change the demographics of the non-modulating group. Both age (46.9±1.7 versus 49.4±2.0 years) and mean basal aldosterone levels (15.9±1.2 versus 14.7±1.4 ng/dl) were similar to each other but significantly different (p<0.001) from values in the modulating hypertensive subjects (age, 39.5±1.4 years; basal aldosterone, 25.0±1.8 ng/dl).

Although the normotensive subjects had basal aldosterone levels indistinguishable from those of patients with modulation, they were still slightly younger (p<0.025). Finally, to determine the influence of gender on the bimodality of this trait, the responses in the 107 male hypertensive subjects were separately subjected to maximum likelihood analysis. Again, bimodality was documented (p<0.0018). Too few female subjects were studied to permit separate analysis.

Renal plasma flow response to angiotensin II on a high salt diet. Renal plasma flow on a high salt diet was determined in 89 hypertensive patients and 59 normotensive subjects before and after Ang II infusion (Table 2). The hypertensive subjects were older (43.9±1.2 years) than the normotensive subjects (39.2±1.3 years, p<0.001). The mean basal renal plasma flow levels were highly significantly less (p<0.0001) in the hypertensive subjects (498.8±9.9 ml/min per 1.73 m²) compared with the normotensive subjects (596.0±13.7 ml/min per 1.73 m²). At the time of study, there were no significant differences in the 24-hour urine sodium and potassium, serum sodium and potassium, blood urea nitrogen, creatinine, plasma aldosterone, renin activity, Ang II, or cortisol levels. Similarly, glomerular filtration rates, as determined in some cases by creatinine clearance (54%) and in others by inulin (46%), were not significantly different. One third of the hypertensive subjects had a basal renal plasma flow less than any normotensive subject.

The decrement in renal plasma flow in response to Ang II was significantly greater (p<0.001) in the normotensive (143.0±5.8 ml/min per 1.73 m²) than in the hypertensive subjects (96.3±4.4 ml/min per 1.73 m²). Likewise, the RPFs at the end of the Ang II infusion were significantly different (p<0.001) (normotensive, 453.0±8.6 [mean±SEM] and hypertensive, 402.5±9.2 ml/min per 1.73 m²). Twenty-nine hypertensive subjects (33%) had decrements smaller than any normotensive subject (i.e., less than 78 ml/min per 1.73 m²). Forty-nine hypertensive subjects (55%) but only 10% of the normotensive subjects had a decrement less than 100 ml/min per 1.73 m². Hypertensive subjects with decrements in RPF less than any normotensive subject (RPF decrements <78 ml/min per 1.73 m²) were 47.7±1.8 years old and had a basal RPF of 437.4±13.9 ml/min per 1.73 m².

Correlation Between Adrenal and Renal Plasma Flow Responses to Angiotensin II: Definition of Non-Modulation

On the basis of the data presented above, non-modulating subjects were defined as individuals with changes in aldosterone in response to Ang II on a low salt diet of less than 15 ng/dl. Forty hypertensive patients (age, 43.1±2.2 years) and 19 normotensive subjects (age, 34.8±2.2 years) who had valid assessment of both renal plasma flow response to Ang II on a high salt diet and adrenal response to Ang II on a low salt diet were evaluated. The correlations of these responses were highly significant (p<0.00009, r=0.50 for reciprocal of change in aldosterone versus change in PAH) (Figure 3). In 50 of the 59 subjects (84.7%), both criteria agreed if the RPF cutoff for non-modulation was assumed to be less than 100 ml/min.
Hypertensives

Normotensives

FIGURE 1. Bar graphs show increment in aldosterone secretion in normotensive and hypertensive subjects in balance on a 10 meq sodium intake after 24 hours of volume depletion induced by furosemide (normotensive subjects, n=18; hypertensive subjects, first mode, n=14; second mode, n=12).

per 1.73 m² (Figure 3). Of the remaining nine, five were non-modulators only by the RPF criterion, and four were non-modulators only by the adrenal criterion. Using this cutoff for RPF, the age (45.5±1.5 years) and basal RPF (454.0±10.4 ml/min per 1.73 m²) of the non-modulating group were no different from those observed in hypertensive patients whose RPF was less than that of any normotensive subject (i.e., less than 78 ml/min per 1.73 m², see above).

Reproducibility of the Features

The reproducibility of the aldosterone response to sodium restriction and Ang II infusion was assessed in 27 subjects. Seventy Ang II infusions were performed in these subjects; the interval between the first and the last infusion ranged from 1 to 60 months (mean, 9.6±2.2 months). The intraclass correlations are highly significant for all parameters: baseline aldosterone, 0.563, \( p=0.000014 \); aldosterone response to the 3 ng Ang II infusion, 0.534 (\( p=0.00004 \)); baseline PAH on a high salt diet, 0.675 (\( p=0.001 \)); PAH responses to a 3 ng Ang II infusion, 0.887 (\( p=0.000007 \)). The duration between the first and last PAH infusion in these subjects was 1–25 months; mean, 5.6±1.6 months.

If non-modulators are classified as those with an aldosterone increment in response to 3 ng Ang II infusion of less than 12 ng/dl, no subjects change classification on repeat study. If the cut point is estab-
Hypertensives

Normotensives

Figure 2. Bar graphs show rise in plasma aldosterone concentration in response to an infusion of 3 ng Ang II · kg⁻¹ · min⁻¹ in subjects in balance on a 10 meq sodium intake. Panel A: Responses of 150 normal renin or high renin essential hypertensive patients. Note the highly significant bimodal distribution (p < 0.00009). Panel B: The responses of 61 normotensive subjects free of known disease or family history of hypertension.

lished at 15 ng/ml, two of the 27 subjects changed classification. For PAH clearance, using the cut point of 100 ml/min per 1.73 m², one subject changed classification.

Finally, we have studied one subject five times over a 5-year period. All five times, his change in plasma aldosterone was less than 15 ng/dl. At the time when the first three studies were performed, he was normotensive (blood pressure < 125/82 mm Hg). He was restudied 2 and 3 years later. On both occasions, his blood pressure had risen to the hypertensive range (> 140/95 mm Hg).

Clinical Characteristics

Using the criteria defined above, patients were classified as non-modulators on the basis of the results of one of two tests. In all patients in whom a low salt study was performed, the aldosterone response to 3 ng · kg⁻¹ · min⁻¹ infusion of Ang II was used. Patients whose increment in plasma aldosterone was less than 15 ng/dl in response to an Ang II infusion or whose increment in aldosterone secretion was less than 150 µg/24 hr in response to volume depletion were classified as non-modulators, unless the basal plasma aldosterone
was greater than 35 ng/dl. Such patients \((n=6)\) were excluded. If results were not available from a valid low salt study, then the RPF response to the same dose of infused Ang II in subjects on a high salt diet was used. In this case, a decrement of less than 100 cm\(^3\) \(\cdot\) min per 1.73 m\(^2\) defined the non-modulator. One hundred two subjects were defined as non-modulators, with 122 having normal modulating status; however, the percentage of the normal and high renin Caucasian hypertensive population that were non-modulators ranged from 24% (Figure 2) to 67% (Figure 1), depending on which criterion was used.

The non-modulators were slightly but significantly \((p<0.02)\) older than the hypertensive patients with intact modulation and had a lower proportion of women. For several other characteristics (blood pressure and electrolyte and cortisol levels), there were no significant differences between the two groups (Table 3). Although there were no differences in the supine or upright plasma renin activity obtained on a low salt diet, the increment was 21% greater in the non-modulators \((p<0.05)\). Despite this tendency toward greater plasma renin activity levels, plasma aldosterone levels both supine \((p<0.001)\) and upright \((p<0.0001)\) on the low salt diet were highly significantly lower in the non-modulators. However, there was no difference in the high salt supine plasma aldosterone level. As anticipated, the high salt PAH clearance was also significantly lower in the non-modulators (Table 3). In the 150 patients who had a low salt Ang II infusion performed, Pearson correlation coefficients were calculated between changes in aldosterone and admission weight, age, change in Ang II, and change in cortisol. The change in aldosterone was not significantly affected by any of these factors except for age, which however, accounted for less than 8% of the variation in aldosterone response.

Discussion

During the past decade, at least two important changes have occurred in the approach to determining the mechanisms responsible for hypertension. The first reflects the growing realization that hypertension is a syndrome with a number of different underlying mechanisms producing a common end point—an elevated blood pressure. The second is that there is growing interest in pursuing the genetic factors that may play a role in the development of hypertension in some patients as the potential of this approach has grown. Mechanisms by which we might identify patient subsets have been proposed, including sensitivity to salt intake (salt-sensitive and non-salt-sensitive), renin status (low, normal, and high renin), and sympathetic nervous system activity (adrenergic and nonadrenergic). This approach will identify the most useful intermediate phenotypes if at least three criteria are met. These include a logical explanation for how the phenotype could produce an elevated blood pressure, evidence that the phenotype is discrete (bimodally distributed) rather than part of a continuum, and evidence of familial aggregation of the phenotype.

In 1983, we described a crucial feature of a subgroup of patients with hypertension: sodium intake did not modulate adrenal and renal vascular responsiveness to Ang II. The present report extends that observation on "non-modulators" to indicate that this group is indeed a distinct entity; bimodality is evident, occurring in several features that distinguish non-modulators from the rest of the hypertensive population. Furthermore, there is concordance among the abnormalities that define non-modulation, and the abnormalities are re-
The strongest evidence for bimodality came from an assessment of the aldosterone secretory response to volume depletion. For this feature, noise was considerably reduced, because adrenal function was assessed over a 24-hour period, the spread of values ranged more than 140-fold (10–1,400 μg/24 hr), and the stress of the procedure produced a clear separation of the two subgroups with a relatively small sample size of 36 subjects. In contrast, 89 subjects participated in the high salt RPF study, yet bimodality was not statistically verified \( (p=0.11) \). The power calculation suggests that more than 400 patients would be needed to definitely prove or disprove bimodality of this feature. Although the adrenal response to Ang II infusion is clearly distributed bimodally, it falls in between in statistical power, range of response, degree of stress on the system being measured, and precision of the technique.

The adrenal and renal vascular criteria identify the same patient as a non-modulator or modulator in 85% of the cases (Figure 3). Thus, one could reasonably use only one of the criteria to identify the non-modulator. This is particularly relevant when one considers the practical limitations imposed on a study when more than one criterion is used, including the amount of phlebotomy, the study duration, and costs. Of critical importance, however, is the accurate control of sodium balance prior to assessment of either adrenal or renal vascular responses to Ang II, since the level of sodium intake can dramatically influence both responses.\(^{33,36}\)

The reproducibility of the responses of either renal plasma flow or aldosterone to changes in sodium intake or Ang II infusion was remarkably good. With repeat measurements from 1 to 60 months apart, the intraclass correlation was excellent: in 90% of the cases, identification of the subject as a modulator or non-modulator was the same.

These data, as well as previous reports, are in accord with the possibility that non-modulation is an inherited trait. The non-modulating phenotype has been observed in normotensive subjects, primarily in the offspring of essential hypertensive subjects.\(^{17,20–22}\) Second, there is a high degree of concordance between non-modulation and a positive family history for hypertension.\(^{12,17}\) Eighty-four percent of non-modulators have a positive family history for hypertension, compared with only 25–30% in the rest of the hypertensive population.\(^{12,17}\) Third, when the renal vascular response to Ang II is assessed in hypertensive sib pairs, a high degree of concordance is also observed \( (p<0.001) \).\(^{19}\) Thus, the non-modulating phenotype aggregates in families, in accord with the possibility that it is an inherited abnormality.

In summary, the data presented herein provide strong support for three conclusions: 1) The two major criteria used to identify a non-modulator agree in 85% of the individuals identified by the criteria separately; 2) the reproducibility of the classification in subjects with repeated assessments over a 5-year period is better than 90%; and 3) most importantly, non-modulation is an identifiable subset of the hypertensive population and not part of a continuum. These individuals have a salt-sensitive component to their blood pressure elevation.\(^{12,23}\) However, in contrast to salt sensitivity in general,\(^{27}\) the non-modulation trait does show a bimodal distribution in the hypertensive population. The ethnic and geographic distribution of this defect is widespread. Evidence suggesting non-modulation has been reported

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**TABLE 3. Characteristics of Normal and High Renin Essential Hypertensive Subjects Divided Into Modulators and Non-Modulators**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-modulator</th>
<th>Modulator</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>102</td>
<td>122</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.4±1.2</td>
<td>40.1±1.3</td>
</tr>
<tr>
<td>Female (%)</td>
<td>26.1</td>
<td>35.5</td>
</tr>
<tr>
<td>Sitting admission blood pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>158.7±2.3</td>
<td>154.1±2.7</td>
</tr>
<tr>
<td>Diastolic</td>
<td>99.3±1.5</td>
<td>97.3±1.3</td>
</tr>
<tr>
<td>Serum sodium (meq/l)</td>
<td>139.8±0.4</td>
<td>138.9±0.4</td>
</tr>
<tr>
<td>Plasma cortisol (8:00 AM) (μg/dl)</td>
<td>4.09±0.06</td>
<td>4.19±0.03</td>
</tr>
<tr>
<td>Sodium restriction (10 meq)</td>
<td>13.7±0.6</td>
<td>14.2±0.6</td>
</tr>
<tr>
<td>Plasma renin activity (ng Ang I · ml⁻¹ · hr⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>3.52±0.26</td>
<td>3.97±0.24</td>
</tr>
<tr>
<td>Upright</td>
<td>9.82±0.79</td>
<td>9.19±0.63</td>
</tr>
<tr>
<td>Plasma aldosterone (ng/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>18.5±1.9</td>
<td>24.7±2.0</td>
</tr>
<tr>
<td>Upright</td>
<td>56.7±5.8</td>
<td>89.0±6.3</td>
</tr>
<tr>
<td>Sodium loaded (200 meq) PAH clearance (ml/min per 1.73 m²)</td>
<td>454±10</td>
<td>566±13</td>
</tr>
</tbody>
</table>

Ang I, angiotensin I; PAH, p-aminohippurate. Values are mean±SEM.
in individuals studied in Japan, Switzerland, Italy, Argentina, and in separate regions in the United States. Finally, because of previously published reports suggesting that the non-modulating trait aggregates in families and is present in normotensive subjects who have a positive family history for hypertension (a group not studied in this report), this trait may be very important in evaluating the genetics of human hypertension.

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