Relation Between Blood Pressure and Renin, Renin Substrate, Angiotensin II, Aldosterone and Urinary Sodium and Potassium in 574 Ambulatory Subjects

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SUMMARY Five hundred seventy-four ambulatory subjects with blood pressures ranging from 94/58 to 250/145 mm Hg were studied on their usual dietary and sodium intake. Renin, renin substrate, angiotensin II, aldosterone and urinary sodium and potassium were compared with blood pressure to assess the contribution of these variables to the blood pressure variance. Our analyses revealed that renin substrate was highly and positively correlated with diastolic blood pressure ($r = +0.39; p < 0.00001$) but all other components of the renin-aldosterone system exhibited a significant negative correlation with blood pressure. A highly significant relationship between potassium, the renin-aldosterone system and blood pressure was found but no such relationship could be demonstrated for sodium. Subjects with higher blood pressures had lower urinary potassium concentrations and lower potassium/creatinine ratios. These findings raised the possibility of a significant pathogenetic relationship between potassium and high blood pressure. Multiple linear regression reveals that influences of the renin-angiotensin-aldosterone system can only account for less than 20% of the variance exhibited by the blood pressure in these subjects. (Hypertension 1: 287-291, 1979)

KEY WORDS • blood pressure • renin-angiotensin-aldosterone system • urinary electrolytes • multiple linear regression • renin substrate • log-normal distribution • urinary potassium • urinary sodium

The definition of mild hypertension poses genuine problems. Frequency distribution data on the blood pressure recorded in large numbers of individuals provide no evidence for a bimodal distribution.1, 2 Most epidemiologic data support the suggestion that any definition of hypertension is an arbitrary one and hence prevalence of hypertension is a comparably arbitrary figure. Unambiguous discussion of mild hypertension and risk factors and attributes of the mildly hypertensive patient is thus also difficult. The studies by Hamilton et al.3, 4 and Miall et al.5, 6 demonstrated that arterial pressure exhibited a distribution in population studies that was unimodal and almost certainly influenced extensively by both genetic and environmental factors. We have used their technique of treating the blood pressure as the variable of primary interest and have examined its relationship to endocrine and environmental factors such as the renin-angiotensin-aldosterone system and sodium and potassium excretion. This avoids the necessity of precisely defining mild hypertension but allows for examination of humoral and environmental factors that may play a role in elevating blood pressure.

We report here preliminary findings in 574 subjects, including a highly significant positive correlation between renin substrate and blood pressure and a negative correlation, also highly significant, between blood pressure and urinary potassium.

Methods

Study Population

This report is based upon the study of 574 subjects obtained from two sources. Subjects with elevated blood pressure were obtained by a previously reported screening mechanism.6 All subjects who were receiving drugs or who had evidence of other diseases were excluded from further study.
In addition to subjects obtained from this source, a large number of Air Force Reserve Personnel were studied in a similar manner after they had given their informed consent to serve as controls.

All subjects at the time of study were ambulatory and on an unrestricted diet. Thus, every effort was made to study them with as little dietary change as was possible. All studies were performed between 9:00 and 11:00 a.m. and urine specimens were obtained between 9:00 and 10:00 a.m. Written consent was obtained from all patients after the nature and hazards of the study were explained.

Collection of Blood Samples and Assay Techniques

Subjects were ambulatory for at least 2 hours before collection of venous blood for plasma renin activity, aldosterone, renin substrate, and immunoreactive angiotensin II. Blood for renin, angiotensin II, and aldosterone was drawn and assayed as previously described.*

Plasma renin substrate was measured by a modification of Poulsen's® antibody-trapping microradioimmunoassay. To 5 µl of a 1:30 dilution of the plasma were added 10 µl of human renin (0.0025 Goldblatt units, courtesy of Dr. E. Haas). The incubation was carried out in a total volume of 150 µl in the presence of angiotensin I antibody. All renin substrate was consumed within 3 hours and recoveries of added angiotensin established that no destruction of angiotensin I occurred during periods of incubation extending to 18 hours.

Sodium, potassium and creatinine were measured on the morning urine samples. Sodium intake was assessed from the sodium/creatinine (Na/Cr) ratio measured on urine collected between 9:00 a.m. and 9:30 a.m. to minimize fluctuations in the ratio.* Reliability of this index of sodium excretion was evaluated by comparison of a spot collection to a simultaneously obtained 24-hour specimen from 18 normal individuals on varying salt intake (r = +0.62; p < 0.025) and 37 patients on varying salt intake (r = +0.56; p < 0.005). In this validation study, spot collections obtained at 6:30 a.m. and at 10:30 a.m. yielded virtually identical correlation coefficients when tested against the 24-hour sodium/creatinine ratio. The use of the Na/Cr ratio was further validated by comparing Na/Cr ratios and plasma renin activities in a group of 22 subjects studied both on low and high salt diets (r = -0.42; p < 0.025).

Blood pressure measurements were recorded by three different individuals, each of whom recorded a supine and standing blood pressure. Data obtained on the same individual by different observers correlated well (r = +0.9). For statistical analysis, each individual subject had six blood pressures recorded before blood samples were collected. Statistical analysis employed standard statistical methods.**

*Results

The initial approach used to identify relationships between the several variables measured in this group was to search for correlations between the variables. A partial listing of these is shown in table 1. Initially the relationship between recumbent diastolic blood pressure and the other recorded attributes was examined. The first eight correlation coefficients shown in table 1 are a product of this effort. Significant correlations were noted between diastolic blood pressure and plasma aldosterone, plasma renin activity and urinary potassium. Interestingly, no significant correlation was identified between the diastolic blood pressure and either urinary sodium concentration or the sodium/creatinine ratio.

The most remarkable finding in this correlation series is the strong positive correlation between plasma renin substrate and recumbent diastolic blood pressure (r = +0.39; p < 0.000001). The expected relationships between blood pressure and weight and blood pressure and age were identified.

An important and previously unrecognized association was that between plasma aldosterone and urinary potassium. This highly significant correlation (r = +0.24; p < 0.00001) was virtually identical whether aldosterone was tested against the urinary potassium concentration or against the urinary potassium/creatinine ratio as an index of potassium excretion. In contrast, the relationship between aldosterone and urinary sodium was borderline at best (r = -0.08; p = 0.06).

Thus, the most impressive findings in this series of correlations were: 1) Plasma renin substrate exhibited a much closer positive correlation to the recumbent diastolic blood pressure than did any of the other attributes measured; 2) The highly significant negative correlation between urinary potassium and blood pressure, though not apparently as strong as the relationship between substrate and blood pressure, was a new and unexpected finding; and 3) A negative correlation of a similar magnitude was demonstrated between plasma aldosterone and blood pressure, and a negative correlation of lesser magnitude was demonstrated between plasma renin activity and diastolic blood pressure. A strong positive correlation was demonstrated between plasma aldosterone and urinary potassium, although no correlation between aldosterone and urinary sodium could be found.

Many of the variables exhibiting significant correlation with blood pressure are also correlated (table 1). In some instances, such as potassium concentration in the urine versus blood pressure, the association is strengthened when the partial correlation coefficient is calculated. Because of the number of variables examined in this study, it is more instructive to use the technique of multiple linear regression as a means of assessing the influence of these variables upon the observed variance of the blood pressure.* Taking recumbent diastolic blood pressure (RDBP) as the dependent variable and testing as independent variables aldosterone (A), plasma renin activity (RA),
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urinary potassium (K), and plasma renin substrate (RS) yields the following equation:

\[ \text{RDBP} = 83.7 - 0.14(A) - 0.6(RA) - 0.025(K) + 0.005(\text{RS}). \]

The multiple correlation is 0.431 \((p < 0.00001)\) and the variance \(R^2\) is 0.19, indicating that these four independent variables together only account for 19% of the total observed variance of the diastolic blood pressure. The mean arterial blood pressure, aldosterone, plasma renin activity and renin substrate values in this study all exhibited a log-normal frequency distribution. The multiple linear regression of these variables on mean blood pressure was calculated after logarithmic transformation but this transformation did not significantly alter the relationship. This calculation yielded a multiple correlation coefficient of 0.42 \((p < 0.00001)\), with \(R^2 = 0.18\). Thus, 80% of the observed variance of the blood pressure in this study is unaccounted for by the variables tested. Successive elimination of the independent variables, one at a time, and recalculation reveals that the renin substrate alone accounts for more than one-half of the fraction of the variance of the blood pressure that is associated with these variables.

The relatively strong correlations, both positive and negative, identified in the preceding sections indicate significant differences in the values of these attributes over different ranges of the blood pressure above and below the mean. When these differences were examined by dividing the group at the diastolic blood pressure of 90 mm Hg, placing all subjects with a diastolic blood pressure of 90 mm Hg or less in one group and the remainder in a second group, highly significant differences emerge (table 2). The most remarkable differences are a very marked increase in the renin substrate in patients with diastolic blood pressures greater than 90 mm Hg and a significantly lower value for urinary potassium in those individuals with the higher diastolic blood pressure. The significantly lower mean values for plasma renin activity, angiotensin II and aldosterone, despite nearly identical values for urinary sodium and the Na/Cr ratio is consistent with suppression of the renin angiotensin system by some means other than an increased sodium intake.

Discussion

Although none of the associations or correlations presented above permit any inferences about causality, several of the findings warrant careful and critical examination. The fact that the strongest correlation was identified between renin substrate and blood pressure is surprising. The value of 1754 ± 58.5 ng Angio I equivalents/ml plasma for those subjects with diastolic blood pressures of 90 mm Hg or less (table 2) agrees very well with normal values of 1657 ng reported by Kotchen et al.\(^9\) and 1517 ng reported by Sealey et al.\(^11\) and Eggena et al.\(^12\) This agreement furnishes additional validation of the methodology. The possibility that sampling bias produced this apparent association between blood pressure and substrate was also examined. Any influence of the well-recognized relationship between estrogens and substrate levels was excluded by examining the relation between blood pressure and substrate in males only. Thus, for white males the mean substrate value for those with diastolic blood pressure ≤ 90 mm Hg was

| Table 1. Bivariate Correlations in 674 Subjects |
|-----------------|-----------------|-----------------|
| Variables tested for correlation | Correlation coefficient \(r\) | \(p\) value |
| Recumbent DBP vs plasma renin substrate | +0.39 | <0.000001 |
| Recumbent DBP vs urinary K | -0.23 | <0.00001 |
| Recumbent DBP vs aldosterone | -0.20 | <0.00001 |
| Recumbent DBP vs PRA | -0.13 | <0.005 |
| Recumbent DBP vs urinary Na | -0.01 | NS |
| Recumbent DBP vs weight | +0.13 | <0.005 |
| Recumbent DBP vs age | +0.12 | <0.01 |
| Aldosterone vs urinary K | +0.24 | <0.00001 |
| Aldosterone vs urinary Na | -0.08 | -0.06 |
| PRA vs aldosterone | +0.14 | <0.002 |
| PRA vs urinary K | +0.14 | <0.002 |
| PRA vs urinary Na | -0.13 | <0.005 |

Abbreviations: DBP = diastolic blood pressure; K = potassium; PRA = plasma renin activity; Na = sodium.

| Table 2. Comparison of Data on Subjects Grouped According to Blood Pressure |
|-----------------|-----------------|-----------------|
| Attribute | Diastolic BP ≤ 90 mm Hg | Diastolic BP > 90 mm Hg | \(p\) value |
| Age (yrs) | 31.3 ± 0.4 | 32.1 ± 0.4 | <0.0001 |
| Height (inches) | 69.1 ± 0.2 | 67.5 ± 0.2 | <0.0001 |
| Weight (lbs) | 173.0 ± 1.4 | 177.0 ± 2.3 | <0.0001 |
| PRA | 0.62 ± 0.03 | 0.40 ± 0.04 | <0.0001 |
| Aldosterone | 14.5 ± 0.61 | 11.1 ± 0.61 | <0.0001 |
| Renin substrate* | 1754 ± 58.5 | 2076 ± 54.5 | <0.0001 |
| Venous angio II† | 14.1 ± 1.7 | 4.1 ± 0.47 | <0.0001 |
| Urinary potassium | 66.6 ± 1.74 | 53.9 ± 1.74 | <0.0001 |
| Urinary sodium | 129.0 ± 2.91 | 128 ± 3.16 | NS |
| Urinary creatinine | 165.0 ± 5.03 | 164 ± 4.74 | NS |
| no. of subjects | 274 | 300 | |

All labeled \(p\) represents probability that observed differences are chance variations due to sampling error. BP = blood pressure; PRA = plasma renin activity; NS = not significant.

*For substrate \(n = 187\) for normals and \(108\) for hypertensives.
†For venous angio II \(n = 112\) for normals and \(186\) for hypertensives.
1585 ± 25 ng and for blood pressure > 90 mm Hg, 2344 ± 103 ng Angio I equivalent/ml (p < 0.001). The corresponding values for black males were 1701 ± 71 and 2372 ± 91 ng, respectively (p < 0.001). The relationship between higher substrate levels and higher blood pressure can thus be demonstrated in men making it unlikely that our results have been influenced by higher levels of substrate that result from an estrogen effect. As noted above, great care was taken to exclude subjects who were taking oral contraceptives.

The plausible explanation that this results from the suppressed renin and reflects accumulation of substrate as a result of decreased substrate consumption, is not supported by the statistical analysis. If the sequence of events is elevation of blood pressure followed by renin suppression followed by elevation of the plasma substrate, a much closer correlation would be expected between plasma renin activity and blood pressure than between blood pressure and substrate. As seen in table 1, the converse is true. This raises the question whether substrate elevation could not possibly be a very early event in essential hypertension. In view of the strength of the correlation, exploration of this possibility seems warranted. It is certainly well established that elevation of the plasma renin substrate can contribute to elevated blood pressure in some, but not all, individuals. 15

Previous studies on substrate in essential hypertension have failed to identify such marked elevations of substrate as are reported here. 16-17 The majority of these studies represent observations on relatively small numbers of subjects. The coefficient of variation associated with substrate measurement was 40% in our study population. This precludes recognition of differences when the determinations are made in only a relatively small number of subjects. To date no studies, other than the present one, have been reported on a large group of individuals who have not been on any type of medication. In any case it is noteworthy that the substrate measured in these studies is the only component of the renin-angiotensin-aldosterone system for which a positive correlation has been identified with blood pressure in a population study of this size, when cases of renovascular hypertension and primary renal disease are excluded.

The strong negative correlation between urinary potassium and blood pressure is even more remarkable. This finding, along with the positive correlation between plasma aldosterone and urinary potassium, serves to focus attention upon the possible influence of potassium in alterations in the blood pressure. The fact that the urinary potassium was 20% lower in the group with the higher blood pressure, despite identical values for creatinine and sodium in the urine of the two groups, seems most readily explained by differences in dietary intake of potassium. While urinary potassium levels may only grossly reflect the dietary intake of potassium, other explanations for the observed differences in urinary potassium seem less likely. For reasons outlined below, it seems improbable that this finding could reflect increased gastrointestinal loss of potassium in the group with higher blood pressure. The identical values for creatinine and sodium in the two groups excludes differences in water intake as an explanation.

One of the most remarkable aspects of this study was the ready demonstration of highly significant correlations between potassium and such parameters as aldosterone, blood pressure and renin despite the fact that we were able to demonstrate only a weak correlation between plasma renin activity and sodium, the element usually considered to be primarily responsible for setting the status of the renin-angiotensin system.

The early observations of Dahl et al. 18 and Meneely et al. 19 are relevant to the present observations. They demonstrated that increasing potassium intake in rats effectively counters the adverse effects of increased sodium intake. Whether a relative potassium deficit (inferred from the lowered concentrations of potassium and lowered potassium:creatinine ratios in the urine of subjects with the higher blood pressures in the present study) precedes any increase in blood pressure or whether this is a secondary phenomenon seen only in those patients who already have substantial elevations of blood pressure, cannot be inferred from these studies. Further studies are needed. The possibility that a relative potassium deficit could aggravate the untoward or adverse effects of elevated salt intake in humans in a manner comparable to that already demonstrated in the rat is a hypothesis that merits critical examination. Langford and Watson 20 have recently described an association between blood pressure and the urinary Na/K ratio.

This study provides adequate documentation for a close association between alterations in potassium metabolism (or potassium balance) and such parameters as the blood pressure, plasma aldosterone, and plasma renin activity. A more critical study of the possible role of potassium in the evolution of early elevations of blood pressure is certainly warranted.

The fact that the lowered potassium concentration in the urine and the lowered potassium excretion rate is found in that segment of the study population with the lowest levels of circulating aldosterone can be construed as presumptive evidence that the potassium abnormality is not induced by steroids. Although the possibility cannot be completely excluded that an unrecognized or unidentified steroid could have promoted potassium losses by way of the gastrointestinal tract and thus produced a potassium-deficient state that would lead to compensatory reduction in the urinary excretion, this seems unlikely since most steroids also increase renal K+ excretion. It is improbable that an unidentified steroid could exert such an effect without disturbing the correlation between urinary potassium excretion and plasma aldosterone concentration.

The fact that the segment of the study population with a diastolic pressure exceeding 90 mm Hg exhibited lower values for plasma renin activity, plasma aldosterone and plasma angiotensin II all point
toward progressive suppression of the renin-angiotensin system as the blood pressure increases. Furthermore, the coefficients for these variables in the multiple regression equations are all negative and it is thus exceedingly difficult to assign a primary pathogenetic role to this system in essential hypertension.

As stated at the outset, an important objective of this study was to identify that portion of the variance of the blood pressure that was associated with fluctuations in the behavior of the renin-angiotensin-aldosterone system. It is of interest that with one exception we have succeeded only in identifying an inverse relationship, with suppression of the system being associated with the higher levels of blood pressure. The multiple regression analysis indicates that, even so, this accounts for no more than about 15-20% of the variance observed in the blood pressure. The most striking positive association identified in these studies was the relationship between circulating renin substrate and blood pressure. This effect was essentially as great as that of all of the other elements of the renin-angiotensin system combined and since it is positively correlated it could possibly exert a causal influence. This is an observation that merits further study.

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