Renin-Angiotensin and Sympathetic Nervous System Activity in Grade School Children

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SUMMARY Renin-angiotensin and sympathetic nervous system activity were evaluated in grade school children selected from the upper 0.26% and lower 5% of the blood pressure distribution constructed from a survey in the Minneapolis Public Schools. Eleven children from the upper 0.26% group and 19 children from the lower 5% group were admitted to the Clinical Research Center for 5 days and maintained on a 110 mEq sodium and 75 mEq potassium diet. On the fifth hospital day blood samples were obtained supine, after 2 hours of upright posture and after treadmill exercise. Mean sodium and potassium excretion and serum sodium and potassium were similar between the two groups. Plasma norepinephrine was not significantly different between the two groups at any of the three sampling times. Plasma renin activity was significantly lower in the upper 0.26% group in the supine and 2-hour upright samples. Mean plasma aldosterone (measured only in the supine blood samples) was not significantly different between groups. Plasma aldosterone values were significantly correlated with plasma renin activity only in the lower 5% group \( r = 0.67, p < 0.005 \). This study suggests that in grade school children sympathetic nervous system activity is similar between children with high and low blood pressure but that plasma renin activity is lower and an apparent dissociation between plasma aldosterone and renin activity exists in the high blood pressure group. These findings should be confirmed in studies with larger numbers of subjects selected from the entire distribution of blood pressure.

Key Words • renin-angiotensin system • childhood blood pressure

During the past decade, a growing interest in childhood hypertension has culminated in the publication of a number of epidemiologic surveys of blood pressure in children and adolescents. These studies and others strongly suggest that factors influencing the development of essential hypertension extend into the first decade of life, even to infant years, and that levels of blood pressure associated with significant risk for cardiovascular morbidity in adults are also found within the pediatric age group. Thus, considerable support exists to warrant investigation of patients within this age group in an attempt to define determinants of essential hypertension.

The sympathetic nervous and renin-angiotensin systems are known to play a prominent role in the maintenance of blood pressure homeostasis. Because of this relationship, attempts have been made to correlate activity within these systems to the development of essential hypertension. In the spontaneously hypertensive rat model of genetic hypertension, plasma and kidney renin activity and plasma and tissue levels of norepinephrine (NE) appear to be age-dependent and to differ significantly from levels in control normotensive rats. In humans it has been suggested that increased levels of plasma catecholamines or increased sensitivity to NE may be associated with the presence of essential hypertension and that patterns of plasma renin activity (PRA) correlate with sympathetic nervous system activity and may affect the development of essential hypertension. Recently, it has been reported that differences in plasma NE between normotensive and hypertensive subjects can be detected as early as the second decade of life.

In the present investigation, renin-angiotensin-aldosterone and sympathetic nervous system activity were
evaluated in grade school children with high and low blood pressure. The children participating in this study were selected from the extreme ends of a normal distribution curve constructed from an ongoing survey of blood pressure within a large metropolitan public school district. This method of selection provided an opportunity to evaluate children considered to be from an average population of healthy subjects.

**Patients and Methods**

**Selection of Children with High Blood Pressure**

Beginning in January, 1978, approximately 10,000 children enrolled in grades 1, 2 and 3 of the Minneapolis public school system (representing greater than 98% of all children enrolled in those grades) had their blood pressure recorded in school while in a supine position (as previously described in detail for The Minneapolis Children's Blood Pressure Study). Two blood pressure measurements separated by at least 30 seconds were obtained by trained personnel using a Hawksley Random-Zero Sphygmomanometer. The distribution of systolic and fourth phase diastolic blood pressure for this group of children is represented in figure 1.

Children having an average systolic blood pressure of 130 mm Hg or greater, or diastolic blood pressure of 90 mm Hg or greater (approximately 1% of the survey) were selected for reevaluation within 1 week based on previously published data showing that these levels represent the highest percentiles of blood pressure in children within this age range. At the time of reevaluation, similar levels of pressure were confirmed in 26 children (0.26% of the survey), 11 of whom agreed to participate in this study (upper 0.26% group). The study was explained in detail to each parent and child and written informed consent obtained. Particular emphasis was placed on assuring the children and their parents that the children were not hypertensive and that selection for this study was based solely on the fact that their blood pressure was at the upper end of normal distribution for their age.

Each of the children in this group had normal physical examination, including assessment of abdominal bruits, femoral pulses, and leg blood pressure. In addition, the following laboratory tests were normal: serum sodium, potassium, chloride, bicarbonate, creatinine, and calcium; urinalysis; and 24-hour creatinine clearance and catecholamine excretion. An intravenous pyelogram was performed in four children because of both systolic and diastolic elevation at the time of admission to the Clinical Research Center, but was normal in each case.

**Selection of Control Low Blood Pressure Children**

Letters describing this study were mailed to 150 parents and children randomly selected from the school survey population with blood pressure in the lowest five percentiles of blood pressure distribution. Parents and children interested in participating were asked to respond by mail, following which an orientation meeting was conducted and written informed consent for participation obtained from 40 children. These children were then reevaluated and confirmed to have blood pressures within the lowest five percentiles.

An attempt was made to match children from the control group (lower 5%) to the upper 0.26% group according to age, race, and sex to provide a ratio of 2:1. However, because of space constraints it was possible to admit only 19 control children to the Clinical Research Center. In addition, it was necessary to eliminate three control children because of technical problems that prevented blood samples from being obtained. This resulted in a final study population consisting of 11 children from the upper 0.26% group (8 male, 3 female) and 16 children from the lower 5% group (12 male, 4 female) who completed the Clinical Research Center protocol (table 1). The mean age of

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Upper 0.26%*</th>
<th>Lower 5%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of children</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>5</td>
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<tr>
<td>Black</td>
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<td>2</td>
</tr>
<tr>
<td>White</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>8.7 ± 0.2</td>
<td>8.6 ± 0.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>134.9 ± 2.5†</td>
<td>122.9 ± 1.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>43.4 ± 2.8†</td>
<td>27.8 ± 0.9</td>
</tr>
</tbody>
</table>

*Percent of distribution in Minneapolis Children's Blood Pressure Study.
†Height: p < 0.0025; weight, p < 0.0005.
Values are means ± SEM.
the groups was similar. However, the mean height and weight of the upper 0.26% group were significantly greater than the lower 5% group ($p < 0.0002$ and $p < 0.0005$, respectively). None of the children from either group was involved in an organized physical fitness or other exercise program.

To determine whether the children admitted to the Clinical Research Center were representative of the cohort of children from each respective percentile group, physical characteristics of study participants and nonparticipants were compared. Neither the height nor weight (mean ± SEM values) of the 11 study children from the upper 0.26% were significantly different from the remaining children in that group (height = $134.9 ± 2.5$ (SEM) cm vs $132.6 ± 2$ cm, $p = 0.47$; weight = $43.4 ± 2.8$ kg vs $37.8 ± 2.8$ kg, $p = 0.18$). Similarly, the height and weight of the children admitted to the Clinical Research Center from the lower 5% were not significantly different from the other children in their group (height = $122.9 ± 1.5$ cm vs $122.6 ± 0.3$ cm, $p = 0.78$; weight = $27.8 ± 2.2$ kg vs $24.4 ± 0.3$ kg, $p = 0.07$).

Clinical Research Center Protocol

The same protocol was used for all children in the upper 0.26% and lower 5% groups. The children were admitted to the Clinical Research Center of the University of Minnesota Hospitals for 5 days. They were maintained on a diet consisting of 110 mEq sodium and 75 mEq potassium, with caloric content determined from a food record supplied by the parents. Urine was collected each 24-hour period (8:00 p.m. to 8:00 p.m.) and analyzed for sodium, potassium, and creatinine.

At 7:00 a.m. of the final study day, either a 21-gauge scalp vein needle or a 22-gauge angiocath was placed into a peripheral vein while the children remained in a supine position from the previous night’s sleep. Blood was obtained immediately for analysis of PRA, plasma NE, and plasma aldosterone. A heparin lock was attached after this blood sampling and the needle or catheter left in place. The child then received breakfast (with the exclusion of caffeine-containing beverages) and maintained an upright posture for 2 hours, at which time a second blood sample was obtained for PRA and NE determination. Shortly thereafter, the child underwent maximal exercise treadmill testing using a modified Balke protocol in which the treadmill speed was constant at 3 miles per hour and the treadmill elevation was increased by 2.5% every 2 minutes. Exercise was continued for a maximum of 20 minutes, until the heart rate reached 200 beats/min or until a state of exhaustion was reached. At the completion of this exercise, a third blood sample was obtained for PRA and NE determination.

Analytical Procedures

Plasma Renin Activity

Blood samples were collected in tubes containing EDTA, immediately centrifuged at 4°C, and the plasma stored at −20°C. After thawing at 4°C, plasma (1.0 ml) was incubated with 2.0 ml maleate buffer (pH 6), 0.01 ml 2,3-dimercapto-1-propanol (BAL) (1.7%), and 0.01 ml 8-OH-quinoline (6.6%) at 37°C for 3 hours. PRA was determined by radio-immunoassay for angiotensin I (AI) by the method of Haber et al., with results expressed as nanograms of AI/ml plasma/hr (ng AI/ml plasma/hr). The intra-assay and interassay reproducibility, as determined by coefficient of variation, are 7.2% and 13.3%.

Plasma Norepinephrine

Blood was collected in tubes containing heparin and placed immediately into ice water. After centrifugation at 4°C, the plasma was separated and sodium bisulfate added. The plasma was stored at −80°C until assayed.

NE was determined using a modification of a previously described method incorporating high-pressure liquid chromatography and electrochemical detection. Plasma (1 ml) was combined with 0.5 ml Tris buffer (1 M, pH 12) and 10 mg acid washed alumina, and 2.5 ng methyladrenamine was added as an internal standard. The mixture was agitated for 10 minutes, and the supernatant discarded. The alumina was then washed 2 times and the NE eluted from the alumina with 0.05 ml perchloric acid (0.1 M). The detection threshold of this assay is 50 pg norepinephrine. The intraassay and interassay reproducibility, as determined by coefficient of variation, are 7.9% and 6.9%, respectively.

Plasma Aldosterone

After centrifugation of blood samples, the plasma was separated and stored at −20°C. The assay for aldosterone was performed by Bio-Science Laboratories using radioimmunoassay. The interassay coefficient of variation is 7.5% for the low value pool and 7.8% for the high pool.

Statistical Analysis

Data were analyzed using Student’s t test for paired or unpaired groups where appropriate; by non-parametric analysis using the Mann-Whitney U test; and by linear regression analysis with Student’s t test used for comparison of regression data between groups. A $p$ value of less than 0.05 was considered to be statistically significant.

Results

Blood Pressure and Heart Rate

On the day of admission to the Clinical Research Center, four children in the upper 0.26% group had a systolic blood pressure of 130 mm Hg or greater, three had a diastolic blood pressure of 90 mm Hg or greater, and four had both systolic and diastolic elevation (group mean, $130 ± 3/87 ± 3$ mm Hg). By the fifth day of admission, both systolic and diastolic mean values had fallen significantly ($p < 0.001$) to $112 ±$
3/75 ± 3 mm Hg, and individual levels, with the exception of two children whose diastolic blood pressures were 90 and 94 mm Hg, all were less than 130 mm Hg systolic and 90 mm Hg diastolic.

In the lower 5% group, the mean blood pressure on admission to the Clinical Research Center was 96 ± 2/62 ± 2 mm Hg. The reduction in blood pressure during hospitalization was very small and not statistically significant (95 ± 2/59 ± 2 mm Hg, p = 0.30).

The admission mean heart rate of the upper 0.26% group was higher than but not significantly different from the lower 5% group (83.5 ± 5.0 vs 75.7 ± 2.2, respectively; p = 0.11). On the fifth hospital day, mean heart rate was 75.9 ± 4.9 in the upper 0.26% group and 74.1 ± 1.5 in the lower 5% group (p = 0.68). In addition, the reduction in heart rate between admission and Day 5 was not significant for either group (upper 0.26%, p = 0.21; lower 5%, p = 0.53).

Electrolyte Balance

Neither the serum sodium nor serum potassium measured in the 7:00 a.m. blood specimens were significantly different between the two groups of children. The serum sodium was 139 ± 0.5 mEq/liter in the upper 0.26% group vs 138 ± 0.4 mEq/liter in the lower 5% group (p = 0.09); the serum potassium was 4.1 ± 0.29 mEq/liter in the upper 0.26% group vs 4.1 ± 0.06 mEq/liter in the lower 5% group (p = 0.62).

Sodium excretion in the initial urine collection was 117 ± 14 mEq in the upper 0.26% group and 128 ± 9 mEq in the lower 5% group (p = 0.46). Sodium balance on the 110 mEq sodium, 75 mEq potassium diet was achieved in all children by 3-4 days. The 24-hour sodium excretion on the fifth day had decreased to 72 ± 4 mEq in the upper 0.26% group and 71 ± 4 mEq in the lower 5% group (p = 0.87).

Potassium excretion in the upper 0.26% group and lower 5% group was 54 ± 3 and 45 ± 3 (p < 0.05) respectively, in the first 24-hour urine collection and 50 ± 2 and 49 ± 3 (p = 0.77), respectively, in the fifth day collection.

Plasma Norepinephrine

Supine norepinephrine levels were determined in blood samples obtained at the time of venipuncture (fig. 2). Because the experience of venipuncture itself has been reported to cause an elevation in circulating levels of NE, the influence of venipuncture on plasma NE was assessed in 10 children in whom plasma samples were obtained at the time of venipuncture and 30 minutes after placement of a heparin lock. The difference between the mean plasma NE at the time of and 30 minutes after venipuncture was not statistically significant (217 ± 24 pg/ml vs 175 ± 17 pg/ml, respectively; p = 0.18). Similar results have been reported by others.22

Due to a laboratory accident, plasma NE measurements were determined in only eight of the patients in the upper 0.26% group. Supine plasma NE was higher, but not significantly different in the lower 5% group than the upper 0.26% group (228 ± 20 pg/ml vs 189 ± 32 pg/ml; p = 0.28) and remained higher in this group after 2 hours in the upright position (273 ± 28 pg/ml vs 199 ± 39 pg/ml; p = 0.18). A significant increase in plasma NE occurred in response to treadmill exercise in both groups (upper 0.26%, p < 0.01; lower 5%, p < 0.001). The levels at the end of the exercise period were higher in the upper 0.26% group than the lower 5% group (1265 ± 304 pg/ml vs 1068 ± 142 pg/ml, respectively), but this difference was not statistically significant (p = 0.85).

The duration of treadmill exercise was significantly longer in the lower 5% group than the upper 0.26% group (19.5 ± 1 minutes vs 15.6 ± 0.7 minutes, respectively; p < 0.02). However, the maximum heart rate achieved was not significantly different between groups (199 ± 3 beats/min vs 198 ± 3 beats/min, respectively; p = 0.31).
respectively; \( p = 0.72 \), suggesting that the degree of exercise was similar.

Post-exercise response was also analyzed by determining the percent increase in NE from the level obtained just prior to exercise (2 hours upright) to the post-exercise level. Although the mean percent increase in the upper 0.26% group (610% ± 160%) was higher than that in the lower 5% group (379% ± 75%), this difference was not statistically significant \( (p = 0.14) \).

Plasma Renin Activity

PRA was significantly lower in the upper 0.26% group than the lower 5% group in the supine position \((1.65 \pm 0.36 \text{ ng Al/ml/hr vs 2.67} \pm 0.34 \text{ ng Al/ml/hr, respectively; } p < 0.05)\) and after 2 hours in the upright position \((2.55 \pm 0.43 \text{ ng Al/ml/hr vs 3.99} \pm 0.49 \text{ ng Al/ml/hr, respectively; } p < 0.035)\) (fig. 3). After exercise, mean PRA continued to be higher in the lower 5% group than upper 0.26% group, but this difference \((17.13 \pm 4.09 \text{ ng Al/ml/hr vs 12.51} \pm 3.17 \text{ ng Al/ml/hr, respectively})\) was no longer statistically significant \( (p = 0.41) \). PRA was not correlated with plasma NE levels in either group at any of the sampling times.

Plasma Aldosterone

Aldosterone was measured only in samples obtained at 7:00 a.m. in the supine position. Although the upper 0.26% group had a higher mean plasma aldosterone level than the lower 5% group \((32.3 \pm 5.5 \text{ ng/dl vs 21.9} \pm 3.3 \text{ ng/dl, respectively})\), there was overlap in the values from children in the two groups (fig. 4), and the difference in mean values was not significant \( (p = 0.08) \).

A significant correlation between PRA and plasma aldosterone was not identified when data from the upper 0.26% and lower 5% groups were combined \((r = 0.35, p > 0.05)\). However, when plasma aldosterone and PRA data were plotted separately for each group (fig. 5), a significant correlation was demonstrated for the lower 5% group \((r = 0.67, p < 0.05)\). A positive correlation was also noted for the upper 0.26% group \((r = 0.44)\), but it was not statistically significant.
Discussion

Studies in adult populations have suggested that the renin-angiotensin and sympathetic nervous systems may influence the establishment and course of essential hypertension. However, it may be difficult in individuals with established hypertension to distinguish whether abnormal findings are etiologically related to the development of the disease or occurred as a consequence of elevated blood pressure. Because it is now apparent that factors influencing the course of essential hypertension are operative prior to the second decade of life, the present study was designed to evaluate renin-angiotensin and sympathetic nervous system activity in grade-school children.

Average blood pressures of 130 mm Hg systolic and/or 90 mm Hg diastolic were arbitrarily used to select the high blood pressure group of children, and in the original cohort of 10,000 students from the Minneapolis Public Schools these levels were found to represent only the upper 0.26% of distribution. It was anticipated that comparing these children from the upper extreme of blood pressure distribution with children in the lowest percentiles (lower 5%) would provide the highest likelihood that potential differences, suggesting pathogenic mechanisms, might be uncovered.

Body weight was also found to be significantly greater in the upper 0.26% group of children, and, therefore, is potentially confounding to the data analysis. Weight is known to be positively correlated with blood pressure in children, a relationship that was confirmed in the Minneapolis Children's Blood Pressure Study. The influence in children of weight on the renin-angiotensin and adrenergic nervous systems has not been defined. However, in a study examining the effect of obesity on factors related to essential hypertension in adults, it was shown that PRA was not significantly different between lean and obese subjects, regardless of blood pressure groupings, i.e., normotensive, borderline hypertension, established hypertension. It was not possible to separate the independent effect of weight from that of blood pressure on the measurements reported herein because of the high degree of selectivity of the two groups. Future studies utilizing a random sample from the entire student blood pressure distribution may provide data permitting this type of analysis.

Mean plasma NE levels obtained in the supine position after 2 hours of upright posture or after strenuous treadmill exercise were not significantly different between children from the upper 0.26% and lower 5% groups. Thus, data from this study suggest that differences in plasma levels of adrenergic neurotransmitter are not correlated with levels of blood pressure during the first decade of life. While the relationship between circulating levels of catecholamines and blood pressure has not been previously reported for children, results from a number of studies conducted in adults have not been consistent, with a positive correlation between catecholamines and blood pressure noted by some but lack of such a correlation reported by others.

The supine blood samples for plasma NE were obtained at the time of venipuncture, a procedure that has been shown by some investigators to stimulate catecholamine secretion. In a sample of children from the present study, plasma NE was slightly lower in blood samples obtained 30 minutes after placement of a heparin lock than in samples obtained at the time of venipuncture. However, the difference between these levels was not statistically significant, and similar findings have been reported previously.

The increase in plasma NE from the supine to upright position was small and does not appear to represent a true stimulated response, since maximum response to this stimulus would be expected to occur within minutes of standing. It seems likely that the 2 hours of upright posture provided a period of homeostatic readjustment resulting in a return of plasma NE to lower levels.

Treadmill exercise resulted in a significant increase in plasma NE in all children, and the difference in levels attained between the upper 0.26% and lower 5% groups was not significant. The fact that mean post-exercise NE was similar in the two groups despite a significantly shorter duration of exercise in the upper 0.26% may suggest that NE response was actually greater in this group; this suggestion may also be supported by the greater percentage increase in mean plasma NE in the upper 0.26% group.

To discriminate subtle differences between children with normal and elevated levels of adrenergic activity, it may be necessary to use specific controlled conditions to elicit NE secretion. In a study of 16- to 39-year-old patients with borderline hypertension, plasma NE levels were similar to those of a control.

![Graph showing relationship between plasma aldosterone and plasma renin activity in 7:00 a.m. supine blood samples.](http://hyper.ahajournals.org/)

**FIGURE 5.** Relationship between plasma aldosterone and plasma renin activity in 7:00 a.m. supine blood samples. The correlation coefficient for the lower 5% group is 0.67, *p* < 0.005; the correlation coefficient for the upper 0.26% group is 0.44, *p* > 0.05.
population at rest or after 3 hours in the upright posture. After maintenance on a low salt diet (10 mg/day) or 3 minutes of treadmill exercise, however, significantly higher plasma levels of NE were found in the hypertensive group. In the present study, more moderate stimuli to NE secretion, including a less severe form of exercise testing and the inclusion of larger number of subjects, may have uncovered differences between groups.

Mean PRA in the supine and 2-hour upright samples was significantly lower in the upper 0.26% group than lower 5% group. The explanation for these differences is not clear. Comparisons between normotensive and essential hypertensive adults have not demonstrated significant differences in either the mean values or range of PRA; similar data are not available for children. PRA has been shown to be dependent, in part, on the secretion of ACTH.

The sympathetic nervous system is intimately involved in modulation of renin secretion. In adults, plasma NE has been reported to be positively correlated with PRA and in particular, the increase in PRA occurring with exercise. A correlation between plasma NE and PRA was not noted in either of the two groups of children at rest, after 2 hours of upright posture, or after exercise. Although sympathetic input may play an important role in renin secretion, it is clear that other stimuli are of equal importance.

Plasma aldosterone levels in both groups of children are similar to the mean and range of levels reported for this age group. When these data were related to PRA, a positive and highly significant correlation was noted between plasma aldosterone and PRA in the lower 5% group whereas a significant correlation was not present in the upper 0.26% group. The dissociation between plasma aldosterone and PRA has been reported in adult patients with essential and renal vascular hypertension, but the explanation for this is not clear. As noted earlier, serum and urinary potassium and sodium levels were not significantly different between the two groups of children. Early morning recumbent plasma aldosterone levels are dependent, in part, on the secretion of ACTH. Therefore, studies designed to evaluate this axis completely may be warranted.

Our present study suggests that in grade school children sympathetic nervous system activity is similar between children with high and low blood pressure but that PRA is lower and an apparently dissociation between plasma aldosterone and PRA exists in the high blood pressure group. These findings are limited to a degree by the number of participants in the study and the potentially confounding factor of weight. Future studies should control for body weight and include larger numbers of subjects selected from the entire distribution of blood pressure.

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