Development of a Nonhuman Primate Model for Spontaneous Hypertension

Blood Pressures in First-Generation Offspring

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SUMMARY A breeding colony of two subspecies of African green monkeys has been established in an attempt to develop a strain of nonhuman primates with inherited spontaneous hypertension. Selective breeding of normotensive and hypertensive feral animals has produced over 300 first-generation, colony-born offspring of nine possible types, which were grouped according to parentage. Blood pressures were measured by indirect methods in 335 unanesthetized animals aged 0.5 to 6 years. Analysis of variance and covariance of mean blood pressures of animals aged 0.5 to 6 years indicated significant differences between control and experimental offspring groups (p<.001) both before and after adjusting for sex, subspecies, age, and body weight. Mean blood pressures of control and experimental (p<.02) offspring were significantly different from 0.5 to 5 years of age. The slopes of the regression of mean blood pressure on age were significantly different between the control and experimental groups (p<.001). Multiple linear regression analysis indicated significant differences among blood pressure, body weight, and age (p<.001) and also between age and weight (p<.001) between the groups. The relative contributions of age and weight to determining the mean blood pressure differed, however. The results indicate that elevated blood pressures can be detected in offspring with even one parent having elevated blood pressure from as early as 1 year of age and that the tendency to develop elevated blood pressure is transmitted from parent to offspring in this species, thereby providing a strong indication that a hypertensive strain of monkeys can be developed through selective breeding. (Hypertension 9 [Suppl III]: III-57-III-63, 1987)

KEY WORDS • hypertension • genetic hypertension • selective breeding • blood pressure • African green monkeys

GENETIC factors are generally accepted to exert a significant role in determining the level of arterial blood pressure. Much evidence in support of the hereditary aspects of blood pressure has come from studies of adopted and biological children as well as dizygotic and monozygotic twins.1-3 In addition, studies in animals have demonstrated an inherited component to blood pressure.4-8 Although essential hypertension is generally not recognized as a disease entity until adulthood, it is likely that the process leading to chronically elevated blood pressures begins during childhood, or perhaps even infancy, as a result of as yet undetermined genetic factors. Recent studies in adolescents with a family history of hypertension lend further support to genetically determined traits for hypertension.9-12

Studies aimed at a more complete understanding of the pathogenesis, prevention, and control of essential hypertension have been conducted using animal models developed through selective breeding of animals with spontaneously elevated blood pressures.13-16 Among these various models, the spontaneously hypertensive rat strain (SHR), developed by Okamoto and Aoki,17 is currently the animal model of choice. Although the SHR has provided valuable information concerning hypertension, an animal model for essential hypertension that more closely resembles humans with respect to cardiovascular dynamics, upright posture, developmental physiology, and behavioral patterns might provide new information regarding the
factors underlying the development of essential hypertension. In particular, such an animal model could be used for a wide spectrum of longitudinal studies aimed at identifying causative factors in hypertension and their genetic basis. The long life span of nonhuman primates would also enable studies of hypertension in the aged. Ultimately, this model could be utilized to study new approaches for the treatment and prevention of human essential hypertension.

Over the past 10 years, efforts in this laboratory have been directed toward developing a nonhuman primate model for spontaneous hypertension through selective breeding. For this model, animals with spontaneously elevated blood pressures, as well as normotensive controls, are being selectively bred ultimately to develop a strain of monkeys with spontaneous hypertension that is genetically transmitted to succeeding generations. We previously reported on the selection of feral animals for the original breeding colony and some preliminary results. In this paper, we report the data pertaining to the blood pressures of the first generation of colony-born offspring.

**Methods**

The primate species utilized for the development of the animal model was *Cercopithecus aethiops*, commonly known as the African green monkey. As reported previously, the original breeding colony comprised two subspecies, vervet and grivet, which were imported from East Africa between 1976 and 1981. The two subspecies, which have an average expected longevity of 20 to 25 years in captivity, are similar in overall appearance and behavior. Furthermore, the blood pressures of the two subspecies are not significantly different. The original breeding colony included 162 feral male and female animals at its maximum. Animals included in the breeding colony were selected as having normal or spontaneously elevated blood pressures on the basis of multiple unanesthetized blood pressure evaluations over a period of 5 years. Animals with known causes of elevated blood pressure were excluded from the breeding colony. Breeding groups were established with one male and 6 to 8 female monkeys housed as a group in large animal runs (770 cu ft). Details of the breeding plan and housing conditions have been previously described.

First-generation, colony-born offspring were raised by their mothers until weaned at 6 months of age. All offspring were housed with animals of similar age, but not according to blood pressure, until sexually mature at 3 to 4 years of age. Mature animals were housed in smaller cohort groups or in individual cages until being placed in breeding groups. After weaning, all monkeys received the same diet of Purina Monkey Chow has a low sodium content (0.22 g/100 g), which is consistent with sodium content in the natural primate diet. In addition, the animals were exposed to natural lighting conditions as well as artificial light on a 12-hour cycle.

**Offspring Evaluation**

Blood pressures were evaluated in the offspring beginning at 6 months of age. Evaluations in young animals consisted of three separate blood pressure determinations. Each determination consisted of at least five individual blood pressure measurements, which were averaged. The determinations were conducted on three different days within a 2-month period. However, once the offspring were sexually mature and were also being considered for inclusion in the breeding colony, blood pressure evaluations consisted of a single determination conducted 2 to 3 times each year. Therefore, each animal underwent at least three blood pressure determinations, with many of the older animals having undergone as many as 15 determinations.

Blood pressures were measured in the offspring by indirect methods using a Doppler ultrasound stethoscope (Model BF5A; Medasonics, Mountain View, CA, USA) or Infrasonde blood pressure recorder (Model SR-2; Puritan Bennett, Linthicum Heights, MD, USA). Previous studies have shown that blood pressures measured with these indirect methods correlate closely with direct measurements and also with each other.

All blood pressures were measured in unanesthetized animals who had been given a low dose (1.5 mg/kg, i.m.) of ketamine hydrochloride five minutes before measurement. This dose of ketamine, an agent commonly used to immobilize nonhuman primates, does not alter the blood pressure but relaxes the animals sufficiently for the blood pressure to be measured within 15 to 20 minutes. The unanesthetized animals were seated in primate chairs for a maximum of 3 to 4 hours for each blood pressure determination. (All animals in the colony are fully adapted to sitting in primate chairs on a routine basis. This adaptation began prior to the selection of the animals for inclusion in the breeding colony and continued throughout the period that the animals were in the colony, extending up to 9 years.)

Initial blood pressure measurements were obtained with the Doppler stethoscope positioned over the brachial artery, a standard blood pressure cuff of appropriate size, and a sphygmomanometer. However, since we were able to consistently detect only the systolic blood pressure with this method, subsequent blood pressure measurements were obtained from the brachial artery using the Infrasonde blood pressure recorder. With this instrument, both the systolic and diastolic blood pressures can be measured reliably and accurately. With both indirect methods, each determination consisted of the average of at least five individual measurements. All studies were conducted in accordance with institutional and governmental guidelines.

**Data Analysis**

Mean blood pressures (MBPs) were used for all statistical analyses. MBPs were calculated for systolic (SBP) and diastolic (DBP) blood pressures measured with the Infrasonde instrument according to the stan-
standard formula $MBP = \frac{1}{3} SBP + \frac{2}{3} DBP$. MBPs were calculated for SBPs measured with the Doppler stethoscope by utilizing the regression of Infrasonde-measured SBP on Infrasonde-calculated MBPs. Previous studies in this laboratory have indicated a close relationship between SBPs measured with the Doppler stethoscope and the Infrasonde recorder. The data are expressed in terms of MBPs because the MBP is less influenced by the method used to measure the blood pressure and is more reproducible than either the SBP or DBP.

The data were analyzed by analysis of variance comparing unadjusted means; analysis of covariance comparing means adjusted for sex, subspecies, body weight, and age differences; and the chi-square test. In addition, a multiple linear regression analysis was used to compare the blood pressure data of offspring types for which body weight and age were covariates and sex and subspecies were factors. BMDP (Biomedical Data Package) statistical software (Los Angeles, CA, USA) was used for all analyses.

Results

Blood pressures were evaluated in 335 first-generation, colony-born offspring aged 0.5 to 6 years. Of these, 249 offspring were evaluated at least twice, and 86 offspring were evaluated once. However, these evaluations represent a minimum of three separate blood pressure determinations for each animal (see Methods). The data presented include one value per monkey per year. When an animal was evaluated more than once at any given year, the average of all evaluations in that year was used. In all, 709 measurements were obtained, with a range of one to five measurements per monkey.

The offspring were identified based on their parents' blood pressure classifications of normotensive control (C), hypertensive (H), or borderline hypertensive (B). There were nine possible types, which were divided into a pure control group (CC), whose parents were both normotensive, and two experimental groups: the HH group, whose parents were both hypertensive, and a group of offspring of mixed parentage, which included the following offspring types: HB, BH, HC, CH, BC, CB, BB (each two-letter combination indicates the blood pressure classification of the father and mother, respectively).

The MBPs of offspring aged 0.5 to 6 years were analyzed for the three offspring groups using analysis of variance and covariance. The data indicated statistically significant differences between CC and HH ($t = 6.95, p < .001$) as well as between CC and mixed offspring ($t = 5.67, p < .001$). Blood pressures of HH and mixed offspring were also significantly different ($t = 3.81, p < .001$). These differences were seen both before and after adjusting for differences among the groups with respect to subspecies, sex, age, and body weight (Table 1). Clearly, there is a distinct difference in the group means, with the blood pressures of the HH group being greater than those of the mixed group, and the blood pressures of the mixed group being greater than those of the CC group.

The regression of MBP on age was examined in the three groups (Figure 1). In all groups, MBP and age were significantly correlated ($p < .001$), although the relationship became progressively stronger from the CC to the mixed and to the HH groups. Comparison of the slopes of the regression of MBP on age for the three groups yielded significant differences between the CC and HH and also the CC and mixed ($p < .001$) groups. The slopes in the HH and mixed groups were not significantly different.

The regression of MBP on body weight was also examined for the three offspring groups (Figure 2). MBP and weight were significantly correlated ($p < .001$) in all three groups, indicating a close relationship between blood pressure and weight. As with MBP and age, the relationship became progressively stronger from the CC to the mixed and to the HH groups. However, the slopes of the regressions among the three groups were not significantly different.

Analysis of the body weights measured at the time of each blood pressure determination revealed no significant differences in the group means of the three groups over the ages of 0.5 to 6 years or at any of the age intervals tested. However, body weights of male animals were significantly higher ($p < .001$) than weights of females from 2 to 6 years of age. These differences were greatest at 4 to 6 years of age, when the males weighed an average of 2 kg more than the females. Sex-related differences in blood pressure were also examined. Blood pressures of male and female animals were not significantly different over the ages of 0.5 to 6 years. In addition, there were no

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of measurements</th>
<th>Number of monkeys</th>
<th>Unadjusted MBP (mm Hg)</th>
<th>Adjusted MBP (mm Hg)</th>
<th>SE of adjusted MBP</th>
<th>Species*</th>
<th>Sex†</th>
<th>Age (yr)</th>
<th>Body weight (kg)</th>
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</thead>
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<tr>
<td>CC</td>
<td>211</td>
<td>96</td>
<td>80</td>
<td>80</td>
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<td>2.6</td>
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<tr>
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<td>86</td>
<td>0.47</td>
<td>1.4</td>
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<td>2.2</td>
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<td>91</td>
<td>90</td>
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<td>1.4</td>
<td>1.7</td>
<td>2.1</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*Vervet = 1; grivet = 2. A value of 1.5 indicates equal numbers of measurements from vervet and grivet animals.
†Male = 1; female = 2. A value of 1.5 indicates equal numbers of measurements from male and female animals.
significant differences between the blood pressures of male and female animals within the three offspring groups at any age.

Multiple linear regression analysis was performed using age, body weight, subspecies, and sex as covariates. This analysis indicated statistically significant correlations \((p < 0.001)\) among blood pressure, body weight, and age, as well as between age and body weight \((p < 0.001)\) in all three offspring groups (for CC, \(r = 0.84\); for HH, \(r = 0.87\); for mixed, \(r = 0.87\)). The regression equations resulting from this analysis are

\[
\text{CC: } \text{MBP} = 3.83 \text{ species} + 3.86 \text{ sex} - 0.46 \text{ age} + 4.70 \text{ weight} + 58
\]

\[
\text{Mixed: } \text{MBP} = 3.34 \text{ species} + 2.84 \text{ sex} + 1.05 \text{ age} + 3.67 \text{ weight} + 65
\]

\[
\text{HH: } \text{MBP} = 2.33 \text{ species} + 0.22 \text{ sex} + 4.03 \text{ age} + 0.90 \text{ weight} + 76
\]

The analysis indicated that for the CC and mixed groups, subspecies, sex, and body weight are statistically significant correlates of MBP \((p < 0.01)\), whereas age plays no independent role in regression. In fact, the coefficient of age in the regression is near zero in the CC group. In contrast, for the HH group, although the overall regression model with all four covariates is significant \((p < 0.001)\), no individual covariate is at a statistically significant level. Age shows the largest covariance, however \((t = 1.93, \ p = 0.058)\). The lack of statistical significance of the individual covariates may be due to the small number of animals in the HH group at each age level. Nevertheless, the difference in the magnitude of the covariates of age and weight between the CC and HH groups is striking. It is also noteworthy that sex is a much smaller determinant of blood pressure in the HH than in the CC group.

The results indicate that the relative contributions of age and weight to determining the MBP are distinctly different among the groups. In the CC group, body weight is the dominant covariate, while age contributes relatively little. In the mixed group, weight is also the dominant covariate, with age playing a greater role than in the CC group. In the HH group, in contrast, age is the major determinant of the blood pressure, and weight contributes very little. The different contributions of weight and age to determining the MBP in each group are depicted in Figure 3.

Unlike the unadjusted regression of MBP on age or weight, the adjusted regression on age clearly depicts the difference between the influence of weight and age on blood pressure in the three groups. More specifically, when adjustments are made for the variances of body weight, sex, and subspecies, leaving age as the only remaining variable, the increase in blood pressure with age is absent in the CC group, greatly diminished in the mixed group, and largely unaffected in the HH group. Clearly, factors besides weight contribute to the increase in blood pressure observed in the HH group as these monkeys grow from 6 months to 5 years of age.

The blood pressure data were also analyzed at selected age intervals beginning at 0.5 to 1 year of age and at yearly age intervals thereafter (Table 2). Analysis of the blood pressure data by analysis of covariance with adjustment for subspecies and sex yielded significant differences between the CC and HH groups at each age interval from 0.5 to 5 years of age. At 5 to 6 years of age, the MBPs of the HH group were 10 mm Hg higher than those of the CC group, but the differ-
ence was not statistically significant, probably because of the small number of monkeys in the HH group. Analysis of the CC and mixed groups also yielded significant differences in MBP from 1 year of age to 5 years. After 5 years of age, there were small differences in the blood pressures of the two groups, but the number of monkeys in each group was greatly decreased. Comparison of the blood pressures of the HH and mixed groups indicated significant differences between the two groups only at 2 to 3 years of age. At all other age intervals tested, however, the blood pressures of the HH groups were consistently higher than those of the mixed group.

The blood pressure data of the seven subgroups included in the mixed group were also examined. In the seven subgroups of the mixed group, the group means of monkeys aged 0.5 to 6 years were all higher by at least 3 mm Hg than means of the CC group. The differences between the CC group and mixed subgroups were statistically significant (p < .01) with the exception of the CH and CB subgroups.

The overall results from 249 offspring who had at least two blood pressure evaluations were also analyzed using an average MBP of 100 mm Hg (two standard deviations above the average MBP of the control offspring group) as the criterion for classification into the hypertensive and normotensive groups. With this criterion, only 1% of the CC group had high blood pressure. In other words, a MBP of 100 mm Hg or more reflected only 1% of the normal population of MBPs (99th percentile). In contrast, 19% of the offspring with two hypertensive parents (HH) and 8% of offspring in the mixed group had MBPs of 100 mm Hg or greater. Within the mixed group, offspring from one hypertensive and one borderline hypertensive parent exhibited the greatest incidence of elevated blood pressure. The difference between the CC and HH group is statistically significant (p < .01).

Although the offspring from the HH and mixed groups exhibited a greater incidence of high blood pressure than the CC group, there was no evidence of tracking of blood pressures of individual animals within these two experimental groups; that is, the animals exhibiting the highest average blood pressures did not consistently exhibit the highest blood pressures at each of the age intervals tested. Therefore, the blood pressures of hypertensive animals showed some variation from evaluation to evaluation, with the average of all evaluations being 100 mm Hg or greater.

Discussion

The influence of genetic factors on blood pressure is now well established in both humans and several species of animals.28-31 We undertook this project to determine whether elevated blood pressure can be transmitted from one generation to the next in nonhuman primates, with the aim of developing a strain of primates with inherited spontaneous hypertension to be used as an animal model. The selective breeding program produced offspring that were classified by parentage as CC (two normotensive parents), HH (two hypertensive parents) or mixed (any other combination of parents).

The data obtained from 335 offspring aged 0.5 to 6 years indicate that the three groups of offspring exhibit distinctly different blood pressures. More specifically, the group averages of mean blood pressure were highest in the HH group and lowest in the CC group, with the blood pressures of the mixed group falling between those of the CC and HH groups. Even within the mixed group, none of the subgroups exhibited blood pressures higher than those of the HH group or lower than those of the CC group. Furthermore, individual offspring with a family history of elevated blood pressure exhibited a higher incidence of elevated blood pressures than individuals in the CC group. In this species,
as in others, there is apparently a close relationship between the blood pressures of the parents and those of the offspring.

In each of the three offspring groups, blood pressure increased with age, as would be expected from studies of the human population and the SHR animal model. In our study, however, the rate of change in blood pressure with age differed significantly among the three groups. The greatest slope (rate of change) was observed in the HH group, the least slope was observed in the CC group, and the slope of the mixed group fell between the two extremes. This difference was also reflected in the blood pressures of the three groups when examined at selected age intervals. The blood pressures of offspring of the experimental groups (HH and mixed) were significantly higher than those of the CC group as early as 0.5 years of age, when all were weaned from their mothers. These differences persisted to 6 years of age. The blood pressures of the HH and mixed groups not only appeared to be higher than those of the CC group at the age of 6 months, but also to increase more over time than those of the CC group, at least up to 6 years of age.

We examined some of the factors that might contribute to changes in blood pressure over time in the three groups of offspring, particularly age, body weight, sex, and subspecies. The regression models we developed using MBP as a function of these four variables indicated that body weight is a more dominant covariate of MBP in the CC and mixed groups than in the HH group. In contrast, age is the dominant covariate in the HH group. Sex and subspecies had the least effect on blood pressures in all three groups. The data suggest that both body weight and age contribute to determining the blood pressure in all three groups but that the relative contribution of the two variables differs. In the CC group, the increase in blood pressure with age is largely due to the strong relationship between blood pressure and increases in body weight as the animals grow, whereas in the HH group, the blood pressure increase with age is largely independent of body weight and appears to be influenced by unidentified inherited factors. The mixed group falls between the extremes of the CC and HH groups, with both body weight and age contributing to the level of blood pressure. The findings in the CC group are consistent with previous observations of the relationship among body weight, age, and blood pressure in children. Differences in the relationship between blood pressure and either body weight or age in individuals with two hypertensive parents have not been previously reported, however. The data reported here suggest that inherited factors other than body weight also contribute to determining blood pressure in the two experimental groups and that these factors are most significant in the HH group. Our analysis indicates that these unknown factors do not include either sex or factors peculiar to either of the two subspecies.

The results of our breeding program to date indicate that the tendency to develop elevated blood pressure is transmitted from parents to offspring in the African green monkey and that elevated blood pressure can be detected before the monkeys reach adulthood. More specifically, the data indicate that 1) offspring of even one parent with elevated blood pressure demonstrate significantly higher blood pressures than offspring of two normotensive parents from as early as 1 year of age and that 2) offspring of two hypertensive parents are especially prone to development of elevated blood pressures. In addition, these animals would be expected to exhibit higher blood pressures up to maturity (4–5 years) and beyond.

Information is currently available from only one generation, so it is not possible to determine whether the tendency to develop elevated blood pressure can be maintained or even enhanced in subsequent generations. However, the results obtained with the first generation provide a strong indication that it is possible to develop a hypertensive strain of African green monkeys through selective breeding.

References


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Hypertension. 1987;9:III57
doi: 10.1161/01.HYP.9.6_Pt_2.III57

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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