Plasma Renin Activity in Psychosocial Hypertension of CBA Mice

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SUMMARY We studied plasma renin activity (PRA) in male full-color brown Agouti (CBA) mice subjected to varying degrees of psychosocial stress induced by manipulation of their housing patterns. Blood samples were obtained from unanesthetized mice by retro-orbital puncture; blood pressure (BP) was measured by tail plethysmography. At 4 months of age, PRA was lower in mice isolated since weaning (isolates) than in mice housed together in standard cages (boxed sibs). Isolation of boxed sibs for 7 days also decreased PRA. PRA did not change in isolates at 20 years; the details of husbandry have been described previously.1 This young remained with their parents until they were weaned at 18–21 days. After weaning, all mice (except as noted below) were fed a standard commercial diet containing 1% NaCl (Purina Lab Chow). In other observations, it was found that food intake was the same not been studied previously and is the subject of this investigation. We hypothesized that the activity of this pressor system might be increased, since renin secretion is stimulated by the sympathetic nervous system5–8 and since plasma renin activity is elevated by psychosocial stress in rats,9 baboons,10 and man.11 Methods

The strain of CBA Agouti mice used in these experiments has been bred in the U.S.C. Laboratories for 20 years; the details of husbandry have been described previously.1,2 This strain has a hypothalamic lesion of the ventromedial hypothalamic area and a renin-angiotensin system.13 The possible participation of the renin-angiotensin system in the initiation and maintenance of the hypertension has

SUSTAINED hypertension can be induced in CBA mice by placing them in complex population cages which facilitate social interactions and competition for territory.1,2 This psychosocially induced hypertension may result in part from repeated activation of the sympathetic nervous system and release of pituitary-adrenocortical hormones.3,4 The possible participation of the renin-angiotensin system in the initiation and maintenance of the hypertension has...
under all the housing conditions described below. After they were weaned, the mice were placed in two different housing situations where they remained until at least 4 months of age: (1) "boxed siblings"—a total of eight male or female mice were maintained in a standard plastic "shoebox" cage, 23 x 11 x 11 cm; (2) "isolates"—mice were placed in individual 0.5-liter glass jars.

At 4 months or later, the specific protocols described below were performed. All data to be reported are for male mice only. All mice were weighed at least once a month when their blood pressures were measured in the conscious state by the method of tail plethysmography described previously. The blood pressure of all males in any particular cage was measured in a single session; measurements were not made while a mouse was adapting to the restraining tube. Blood (400 μl) for plasma renin activity (PRA) and hematocrit was obtained by retro-orbital puncture in conscious animals; the mice had been previously conditioned to the handling procedures, and the collection was completed within 30 seconds after puncture. All blood samples were drawn between 8:30 a.m. and 10:30 a.m. in all experiments.

As Ely and Henry have described, the mice can be coded to determine social rank. The rump and tail became nicked and scarred as a result of bites received in conflicts for dominance. An inverse association exists between the score and the animals’ status; the dominant mice have few subordinated mice usually have many. Observations on tail scarring were made during the course of the routine animal husbandry and weighing, bleeding, and blood pressure determinations.

Specific Protocols

Comparison of Isolates and Boxed Siblings

At 6 months, blood samples were obtained from isolates and boxed siblings. Another group of boxed siblings of similar age was placed in isolation in the standard 23 x 11 x 11-cm shoebox cages for an additional 9 days or 1 month, and blood samples were obtained.

Nonlongitudinal Population-Cage Study

At 4 months, isolated males were placed in special "population cages" which greatly enhance social interactions and prevent stable territory formation. These cages have been described previously: they consist of a system of six standard boxes with narrow 3.2-cm connecting tubes forming a circle. Additional tubes ("spokes") connected each box to a seventh hexagonal central feeding and watering place. A group of 16 males (formerly "isolates") and 16 females (formerly "boxed siblings") together constituted one experimental colony (although samples were not always obtained on all 16 males). Under such conditions, the males are particularly aggressive, fail to establish a stable social hierarchy, and develop marked persistent hypertension within the first week. Each mouse was bled only once, and all in a single population cage were bled on the same day; thus each population cage provided data for only one point in time after the 4-month-old mice were placed in the cages—2 or 7 days, and 1, 2.5, or 5 months. Samples also were collected from control groups (males left in isolation for similar lengths of time) so that comparisons could be made between isolates and PC mice of the same age; as for the PC mice, only a single blood sample was obtained from each control-isolate mouse.

Longitudinal Population-Cage Study

This study was similar to the nonlongitudinal study described above, in that formerly isolated males were placed, along with boxed-sibling females, in population cages at 4 months of age. The difference was that the mice from this single colony were studied longitudinally over the next 4 months. Blood samples were not drawn from every mouse at each sample interval, particularly during the first month when sampling was performed at 1, 7, and 21 days, but all mice sampled belonged to this single colony. A group of isolate controls was left in isolation throughout the same period and was sampled on the same days as were the PC mice.

Population-Cage Study with Previously Socialized Males

This study was analogous to the nonlongitudinal study described above, except that the males placed in the population cages at 4 months were not previous isolates but were boxed siblings.

Analytical Techniques

Blood samples were collected in 200-μl Natelson micro-pipettes; prior to their use, tetra-sodium EDTA (7.5%, pH 6.5) was drawn to the blue line in the pipettes, then blown out twice, leaving approximately 2.5 μl of solution. Hematocrit was measured in a micropipette after centrifugation. PRA was determined on 50 μl of plasma, using a modification of the technique previously described.
Angiotensin I was generated in vitro during a 1-hour incubation at pH 6.5 and 37°C; the incubation mixture contained 50 μl of test plasma, 10 μl of 2 M maleic acid buffer (pH adjusted to 6.5 with NH₄OH), 1 μl of British anti-lewisite (BAL) (1.7 g/100 ml), and 1 μl of 8-hydroxyquinoline (6.6 g/100 ml). Angiotensin I generated was then measured with radioimmunoassay kits (New England Nuclear). Plasma renin substrate (PRS) was determined by adding an excess of semipurified mouse renin to 10 μl of test plasma. Maleic acid (199 μl), BAL (10 μl), 8-hydroxyquinoline (19 μl), and saline (390 μl) were added, and incubation and radioimmunoassay were performed. In a pilot study, we verified that we could detect physiological changes in PRA; PRA in samples obtained from CBA mice maintained on lab chow containing 8% NaCl or 0.3% NaCl were (mean ± SE) 1.4 ± 0.2 and 7.9 ± 2.6, respectively (P < 0.04). Blood urea was estimated by use of Azostix.

Results

Figure 1 summarizes the effects of isolation on PRA (Protocol 1). For mice maintained continuously in isolation since weaning, PRA was significantly lower than in boxed siblings. Isolation of normal boxed siblings at 6 months reduced PRA but not to the level exhibited by mice maintained in isolation since weaning. Body weight, hematocrit, systolic blood pressure, and PRS were not different in the various groups.
Figure 2 summarizes data for PRA and blood pressure in the nonlongitudinal study (Protocol 2). In the isolates, PRA remained low and relatively constant for as long as 15 months; blood pressure was stable for 6 months and then increased by 10 mm Hg (\(P < 0.01\)) between 6 and 15 months. In comparison to the age-matched isolate controls, PC mice always showed significantly elevated values of PRA (except at 7 days) and blood pressure (blood pressure was not measured prior to 2.5 months, but it has been shown previously\(^2\) to be elevated as early as 2 days). No significant correlation was observed between blood pressure and PRA at 2.5 and 5 months.

Figure 3 summarizes the data for PRA and blood pressure as a function of time in the longitudinal PC study (Protocol 3). Again, PRA and blood pressure in the isolates showed little change with time. In contrast, PRA increased markedly the first day after the mice were placed in the population cage, decreased over the next 3 weeks, and then rose progressively during the next 5–6 months; mean PRA was significantly higher at all times than in the control isolates group. Blood pressure was significantly elevated at 1 week and rose slowly for the remainder of the study.

At 1 week and at 2 months, the PC mice could be divided into two categories according to degree of tail scarring; the data for PRA and blood pressure in these two groups are summarized in Table 1. At 1 week, the unscarred (probably more dominant) mice were normotensive, and their PRA was not significantly higher than that of isolate controls. In contrast, blood pressure was significantly elevated in those mice exhibiting tail scarring, and PRA also tended to be higher, although not significantly so. At 2 months there were no unscarred mice, and the degree of scarring had increased; those mice with ± scarring had essentially the same degree of elevation of blood pressure and PRA as did mice similarly scarred at 3 weeks. They had a significantly lower blood pressure than did their more heavily scarred (probably more subdominant) cage mates, but in contrast to the PRA relationship observed at 7 days, these less scarred mice now had significantly higher values of PRA than did the heavily scarred mice. After 2 months, scarring was too uniform to permit categorization.

<table>
<thead>
<tr>
<th>Time in population cage</th>
<th>Systolic BP (mm Hg)</th>
<th>PRA (ng/ml per hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 scarring (3)</td>
<td>125.3 ± 2.7</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>± scarring (7)</td>
<td>144.0 ± 4.8</td>
<td>5.8 ± 0.8</td>
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<tr>
<td></td>
<td>(P &lt; 0.05)</td>
<td>(P &gt; 0.05)</td>
</tr>
<tr>
<td>2 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>± scarring (8)</td>
<td>140.5 ± 6.2</td>
<td>5.2 ± 0.8</td>
</tr>
<tr>
<td>± 2 and + 3 (6)</td>
<td>158.7 ± 2.5</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.04)</td>
<td>(P &lt; 0.03)</td>
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</tbody>
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* These mice are from the longitudinal population-cage study (Protocol 3).

Figure 4 Relationship between PRA and systolic blood pressure after 7 days (A), 2 months (B), 3 or 4 months (C), 5 months (D), and 6 months (E) in population cages. Hypertension was defined as systolic blood pressure >130 mm Hg (determined by 95% confidence limits for blood pressure in isolate controls), and the linear regressions were calculated for hypertensive mice only (those excluded are enclosed by loops in A and B). Scarring was too uniform beyond 2 months to permit categorization.
The relationship between PRA and blood pressure in these 7-day and 2-month PC mice is shown in Figure 4, A and B. All three of the mice that were unscarred at 7 days failed to develop hypertension; for the hypertensive mice, there was an inverse correlation of borderline significance between blood pressure and PRA. This inverse correlation still existed at 2 months, but the slope of the regression line was not as steep. When the data at 7 days and 2 months were pooled, $r^2 = 0.36$ and $P < 0.01$. In contrast, at 3-6 months, no significant correlation existed between blood pressure and PRA.

Table 2 summarizes data concerning body weight, hematocrit, and plasma renin substrate (PRS) in isolate controls and population-cage mice in Protocol 3 (longitudinal study).

![Table 2: Comparison of Body Weights, Hematocrits, and Plasma Renin Substrate (PRS) in Isolate Controls and Population-Cage Mice in Protocol 3 (Longitudinal Study)](image)

All data are mean ± se. A dash (—) indicates that no measurements were made.

$^* = 0.1 > P > 0.05$.

$^t = P < 0.05$.

The discussion of the data shows that enhanced psychosocial interactions induced by manipulation of caging conditions cause an increase in PRA in CBA mice. The fact that PRS was unchanged indicates that the increased PRA reflects an increase in plasma renin concentration. A

![Figure 5: Effects on PRA and systolic blood pressure of placing previously socialized mice in population cages for 3-10 weeks](image)

This nonlongitudinal study, and each bar represents a different group of mice at one point in time. Both groups of PC mice were compared to the 6-month-old, control boxed siblings for calculation of $P$ values.
17% difference was observed at 6 months, but this was much too small to account for the many-fold increase in PRA seen at this same time, and was due entirely to the large increases that occurred in the uremic mice. This finding possibly is analogous to the increased substrate reported in malignant hypertension or after nephrectomy. Renin secretion was not measured in these experiments, so that we cannot be certain that the increased plasma renin was due solely to increased renin secretion rather than decreased removal of renin from the circulation by the liver. However, it is very unlikely, given the magnitude and duration of some of the changes, that the latter is a major factor. One basis for this belief is the existence of the highly sensitive feedback inhibition which angiotensin II exerts on renin secretion; any significant elevation of plasma renin (and, therefore, plasma angiotensin) due mainly to decreased hepatic inactivation should result in inhibition of renin secretion and a return of renin toward its original value. Second, such large steady state elevations would require the existence of severe hepatic malfunction, and there was no evidence for this in these mice or those previously studied under similar conditions.

That psychosocial stress can stimulate renin release has been demonstrated in other studies for rats, baboons, and man. However, these previous studies all involved acute stresses and responses, and the present study is the first demonstration, to our knowledge, that psychosocial factors can influence renin for long periods of time. This study also strongly suggests that the degree of psychosocial interaction required to increase renin need not be very great; this is evidenced by the fact that the boxed siblings had higher values of PRA than isolated animals, despite the absence of any apparent aggressiveness between mice (no tail scarring or fighting) and the lack of arousal adequate to raise the systolic blood pressure (isolates and boxed siblings had similar blood pressures). This is the second difference in endocrine function observed between isolates and boxed siblings. It previously was shown that activity of the adrenomedullary enzymes, tyrosine hydroxylase and phenylethanolamine N-methyl-transferase, is approximately 50% lower in isolates. Thus, the data are consistent with the hypothesis that the differences in PRA may reflect differences in the degree of sympathetically-nervous-system stimulation of renin secretion. In this regard, it has been reported by others that renal renin secretion can be stimulated by the sympathetic nervous system in mice as in other species.

A major purpose of this study was to determine the relationship over time between renin and blood pressure in this model of psychosocially induced hypertension. The results of the longitudinal PC study (Fig. 3) are most important in this regard, but it should be noted that the time courses observed in the nonlongitudinal PC studies (Figs. 2 and 5) were similar; thus, the results observed for this former single colony are corroborated by data from seven other distinct colonies. It is clear that, after mice were placed in the population cages, PRA was markedly elevated during the first 1-2 days. Then, from 1 week to 2 months (as blood pressure rose progressively and tended to plateau), PRA decreased from the initially high values to levels that remained greater than those seen in isolates but not significantly different from those of nonhypertensive boxed siblings. Accordingly, in its earliest stage (previous studies have documented that blood pressure is elevated within 1-2 days), psychosocial hypertension in CBA mice is a “high-renin” hypertension, whereas in its middle stages it is essentially a “normal-renin” or mildly “high-renin” hypertension. This is quite analogous to the PRA pattern seen in experimental renovascular hypertension, in which it has been demonstrated, using blockers of the renin-angiotensin system, that the vasoconstrictor action of angiotensin II is directly involved in maintenance of the hypertension only at its onset, and that other factors are directly responsible for the long-term hypertension. That this latter phenomenon may have occurred in our experiments is indicated by the existence of an inverse correlation between PRA and blood pressure during the middle period, which suggests that, at this time, the elevated blood pressure was inhibiting renin release via intrarenal receptors and this inhibition was offsetting any continued stimulation of renin release via the sympathetic nervous system. The nature of the factor(s) maintaining the hypertension in chronic hypertension has been the subject of considerable speculation. One candidate—sodium retention and expansion of plasma volume — may be important in the present model if the reduced hematocrit which we found were due to an increased plasma volume. Clearly, further studies of sodium metabolism and the effects of blockers of the renin-angiotensin system will be required to evaluate the role of renin and sodium retention in the early and middle phases.

The fact that the inverse correlation between PRA and blood pressure is lost during the later stages in this model and that PRA again increases to levels greater than those seen in normotensive boxed siblings suggests that yet another factor influencing renin release must be coming into play. One factor may be parenchymal renal disease secondary to the hypertension or to atherosclerotic vessel damage. Previous studies have demonstrated that blood urea is elevated as early as 2 months after entry into the population cages, as is the presence of detectable renal interstitial fibrosis. Thus, during its late stages, this model may be analogous to certain forms of human renal parenchymal-disease hypertension or malignant hypertension in which there often is a direct correlation between blood pressure and PRA. Consistent with this is the fact that a direct correlation (although not statistically significant) was observed for the 5-month-old mice. However, that renal damage is unlikely to be the sole additional factor is indicated by three types of evidence from the 6-month-old mice (Fig. 4E): (1) a direct correlation between PRA and blood pressure was not seen in the 6-month-old mice; (2) neither blood pressure nor PRA correlated with blood urea; and (3) a significant number of mice manifested a “low-renin” hypertension.

* We have assumed that the renin measured in these experiments is mainly of renal origin since others have shown that submaxillary renin does not normally contribute to plasma renin.
A final important question raised by these studies is the relevance of this model of psychosocial hypertension for human essential hypertension, in which numerous investigators have hypothesized that psychosocial factors play an etiological role. Increased PRA and hyperactivity of the sympathetic nervous system are present in approximately 15–30% of young patients with mild essential hypertension and in a smaller fraction of older patients with established essential hypertension. The fact that PRA is normal in the majority of patients with established hypertension is also consistent with psychosocial stress being a causal factor, since most of our mice also manifested an essentially normal PRA during the middle phase of their hypertension. Thus, the PRA pattern exhibited in these experiments is additional evidence that this model of psychosocial hypertension in CBA mice may be highly relevant to human hypertension.

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