Effects of Chronic Excess Salt Ingestion

MODIFICATION OF EXPERIMENTAL HYPERTENSION IN THE RAT BY VARIATIONS IN THE DIET

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ABSTRACT
A strain of rats that will predictably develop experimental hypertension by means of different techniques was used to study NaCl-induced hypertension. Observations were continued for 1 year after weaning unless death intervened. Among groups of rats on 0.4, 1, 2, 4, and 8% NaCl chow, respectively, blood pressures generally rose as dietary NaCl increased. Average blood pressures ranged from 146.8 mm Hg in the group on the lowest, to 210.2 mm Hg in the group with the highest NaCl intakes. Morbidity and mortality also increased. Even transient high NaCl diets were capable of inducing permanent hypertension; 4 of 34 rats on 8% NaCl chow for only 2 weeks after weaning had pressures of 180 to 206 mm Hg, although most rats did not become significantly more hypertensive than those on the low (0.4%) NaCl diet. When this same diet was continued for a total of 6 weeks in a group of 29 animals, the blood pressure averaged 198.6 mm Hg. The age at which the high NaCl intake began also influenced the course of the hypertension. Weanling rats rapidly developed fulminating hypertension on the high NaCl diet. After 3 months on this regimen, the average pressure of 40 rats exceeded 200 mm Hg, and 35 animals were dead or terminally ill. In rats that were older when high NaCl diets were started, hypertension developed more slowly and was less fulminant. Among 38 rats in which NaCl was not begun until 3 or 6 months past weaning, blood pressures averaged 175 to 180 mm Hg after 3 months on NaCl; 31 appeared in good health but none survived 8 months.

ADDITIONAL KEY WORDS
salt hypertension
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genetic influence on hypertension
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Evidence that NaCl will induce experimental hypertension has been reported (1-6) and reviewed (7, 8). Over the first decade that high salt diets were used in this laboratory to induce hypertension in rats, the animals were obtained as weanlings from different suppliers and were then fed various concentrations of NaCl in chow for 12 to 15 months. Yet, even among rats on the highest NaCl intakes, about one-fourth remained normotensive. Furthermore, the degree of hypertension varied in the others, including some rats that died with fulminating hypertension within a few months (9). These disparities in response suggested that differences in genetic substrate might be responsible and led to selective inbreeding experiments. Two strains of rats were ultimately evolved, one of which readily developed hypertension from NaCl, whereas the other did not (10-11). Later it was found that the two strains had dissimilar...
blood pressure responses to DOCA-NaCl, unilateral renal artery compression without NaCl, cortisone, and to adrenal regeneration (12, 13).

The present study was designed to extend these observations by varying the NaCl content of the diet given to hypertension-prone rats. We wanted to know whether there is a correlation between the average amount of NaCl ingested and the blood pressures; whether even brief periods of high NaCl intake early in life might result in permanent hypertension; and, finally, whether the age at which high NaCl ingestion starts influences the development of hypertension.

**Materials and Methods**

The rats used in these studies were derived originally from a Sprague-Dawley strain by selective inbreeding, based on their blood pressure responses, individuals were selected from successive generations that were the least, or most, responsive to NaCl ingestion. Because of opposite genetic predispositions to this effect of NaCl, and their response to other techniques for inducing hypertension (12, 13), the two strains are called the Resistant (R) and Sensitive (S).

In the experiments reported in this paper, only rats with a constitutional predilection for hypertension (S-strain) were used. Details on the care, feeding, and technique of measuring blood pressure were published earlier (14).

All animals were weaned at 21 to 23 days (weanlings) and housed in air-conditioned rooms, one to six animals per cage depending upon the size of the rats. They received tap water ad libitum (0.5 to 0.7 mEq of sodium/liter on repeated analyses) and chow. Purina chow specially prepared for these experiments contained the factory to our specifications, and all shipments were analyzed for their NaCl concentrations. Systolic blood pressure was measured by the microphonic method of Friedman and Freed (15). Measurements were made under light ether anesthesia induced by a flowing oxygen-ether mixture; this procedure was carried out in a special box with temperature controlled at 35°. Two of the authors made all the blood pressure measurements. Subjective factors in estimating blood pressures were minimized by using two precautions routinely: (1) individual rats were identified (metallic numbered clips attached to the back of the neck) only after blood pressures were recorded; (2) groups of rats were rotated so that the same person did not measure the pressure on a rat at successive sittings. Although the variation among the readings at any one session was negligible, at least four systolic readings were recorded with each measurement, and the average was used for that day. Disparities between successive measurements in rats with stable pressures were small; differences within ±10% of the previously recorded pressure were considered acceptable within the range of physiological variation, while greater differences were rechecked at 2-day intervals alternately by the two authors until satisfactory agreement was reached. The number of the latter instances was less than 1 in 20, and they were randomly distributed among the rats. Because of the reproducibility of the blood pressure estimates in rats with stable blood pressures, the frequent great differences between successive measurements in rats with rapidly advancing hypertension were accepted as valid.

Blood pressures were measured in animals on NaCl from twice a week to once a month, depending on the rate at which the blood pressure was advancing. In some animals on high NaCl fulminating hypertension developed between successive examinations. In these, the last pressure recorded while the animal was in apparent good health sometimes was only mildly to moderately elevated. Although such values probably underestimated the peak pressure, they were used in the statistical analyses. Statistical significance for differences between groups was assessed by analysis of variance or chi-square, and P values of <0.05 were accepted as significant. Values for systolic blood pressure used in statistical analysis were the last obtained either when the experiment ended or, if an animal died earlier, when it was still in apparent good health. The average blood pressure of a group therefore represents the average of all such final pressures of individual rats whether they survived 6 weeks or 12 months on the indicated regimen. The earliest objective sign of ill health was weight loss. If this was in excess of 10 g and occurred between successive blood pressure measurements, it was assumed that the animal was ill—although at no time did the animals appear to be suffering. The previously recorded pressure was used in the final calculations if this illness proved terminal. Occasionally, intermittent respiratory infections were associated with transient weight loss followed by recovery and subsequent normal weight gain; in these instances, the final blood pressures recorded at the end of the observation period were used for statistical analysis. We considered that systolic pressures ≥ 140 mm Hg were indicative of "hypertension," since among unselected animals on low NaCl chow, as well as rats from the R-strain.
(9, 16), a blood pressure of 140 mm Hg is more than three standard deviations in excess of the average pressure of such groups. Weights were recorded with each measurement of the blood pressure, but since there was no significant weight difference among groups ($P > 0.05$), this will not be considered further. There was no statistically significant difference in blood pressure between males and females, and, accordingly, data on both were combined.

**Experiment 1: Effect of different levels of NaCl intake on blood pressure.**—Each group of weanling rats, containing an equal number of males and females, was fed the same basic chow in which the NaCl concentrations differed among groups as follows: 0.4% (low NaCl), 1, 2, 4, and 8% (high NaCl). Members of any group received only one chow. Unless death or terminal illness intervened, observations were continued for 12 months. Of the 130 animals studied, 30, 20, 20, 20, and 40 were on 0.4, 1, 2, 4, and 8% NaCl chow, respectively.

**Experiment 2: Effect of transient high NaCl intakes on blood pressure.**—Two groups of weanling rats, with the same number of each sex, were placed on high NaCl (8%) chow for either 2 or 6 weeks, after which they were permanently placed on low NaCl (0.4%) chow. Initially there were 35 animals in each group, but deaths prior to decreasing the NaCl allowed final analysis on 34 and 29 in the 2- and 6-weeks groups, respectively. Unless death occurred earlier, observations were terminated 1 year after weaning.

**Experiment 3: Effect of age at which high NaCl diet was begun.**—Forty weanling rats, 20 of each sex, were placed on low NaCl chow. Three months later they were randomly divided into two groups, each with equal distribution of the sexes; one group was fed 8% NaCl chow, and the other group continued as before for another 3 months, when it was also started on 8% NaCl chow. In the second group, one rat of each sex died before the high NaCl intake began, so the final analysis was based on 18 animals, a total of 38 for both groups.

**Results**

**Experiment 1: Effect of different levels of NaCl intake on blood pressure.**—The effects of blood pressure are graphically summarized in Figure 1. Except for the response of the animals on 2% NaCl chow, the average blood pressure rose as the intake of NaCl increased. Many of the animals on low NaCl chow also developed mild hypertension ($\geq 140$ mm Hg—see Methods), so that the average pressure of the control group on low NaCl intake was elevated by the end of the tenth month. From the second through the eighth months,
Experiment 2: Effect of transient high NaCl intakes on blood pressure.—The effects on blood pressure are summarized in Figure 2, where they are compared with two groups as controls from experiment 1, one of which was on a constant low (0.4%) NaCl diet and the other on a constant high (8%) NaCl diet. After 1 year the mean blood pressure of the rats fed 8% NaCl for 6 weeks was significantly higher than that of the rats on salt for only 2 weeks (198.6 ± 6.49 vs. 149.1 ± 4.00 mm Hg, \( P < 0.01 \)). By contrast, there was no significant difference between the mean blood pressures of the control (low NaCl) group and the group on 8% NaCl for 2 weeks (146.8 ± 2.17 vs. 149.1 ± 4.00 mm Hg, \( P > 0.1 \)), although inspection of Figure 2 suggests that several individual animals became permanently hypertensive after only 2 weeks on the high NaCl regimen. The mean pressure of the group on 8% NaCl for 6 weeks did not differ significantly from that of the group permanently on 8% NaCl (198.6 ± 6.49 vs. 210.2 ± 4.71 mm Hg, \( P > 0.1 \)).

Transient high NaCl intake in early life influenced mortality as well as blood pressure, although the effect of NaCl on mortality was not necessarily related to an elevation in blood pressure, as is evident from inspection of Figure 2. By the chi-square test, final mortality was similar in the two groups transiently on high NaCl intake, but the animals on NaCl for 6 weeks died earlier in the experiment. Both of these groups had significantly higher mortality than the group on low NaCl intake permanently (\( P < 0.05 \)). As expected, animals permanently on high NaCl intake had
Effect on blood pressure of age at which high NaCl diet started. Results are 12 months after weaning. All rats were weanlings at the beginning of the experiments. Data on groups are marked “high NaCl at weaning” and “low NaCl only” from experiment 1. The two other groups were maintained on low NaCl diet until 3 and 6 months after weaning, respectively. See text.

a very significantly higher mortality than any of the other three groups (P < 0.01).

Experiment 3: Effect of age at which high NaCl diet was begun.—The primary data are summarized in Figure 3, which includes the data from animals started on high NaCl intake at weaning or maintained on a low intake permanently from experiment 1. These data show that among rats with a genetic predisposition to hypertension, a high NaCl intake started when the animal was mature still led to the development of hypertension and death. The average of the final blood pressures was not significantly different in the two groups in experiment 3 (182.0 ± 3.84 vs. 188.7 ± 3.85 mm Hg); both of these averages were lower (P < 0.01) than the group started on NaCl as weanlings (210.2 ± 4.71) and higher (P < 0.01) than the group maintained on low NaCl intake permanently (146.8 ± 2.2). This graph does not give a complete picture, however, for more detailed analysis indicates that delaying the high NaCl intake resulted in a more slowly developing, less fulminant type of hypertension. Nevertheless, no animal survived 8 months after commencing the high NaCl diet, although they developed blood pressures which averaged well below those of the group started on NaCl as weanlings. Figure 4 analyzes the

Average systolic blood pressure among healthy survivors of groups of rats started on high NaCl diets at different ages. Data derived from experiments 1 and 3. Average blood pressure for healthy survivors only. Among the three groups on high NaCl (78 rats), only 4 did not appear terminally ill 6 months after starting NaCl, and all 78 animals were dead before the end of the eighth month on NaCl. The difference in initial average blood pressures shown at zero on abscissa is due to the difference in ages of the animals in these groups; those on constant low and high NaCl from weaning were only 21 days old, whereas the members of the other two groups were 3 and 6 months older, respectively.
response of these three groups on high NaCl intake on a month-by-month basis. The fulminant character of the hypertension developing when the weanlings were given high NaCl is seen; after only 6 weeks, a third of the animals were dead or dying, and the survivors had an average systolic pressure in excess of 200 mm Hg. Two weeks later 75% of the group was dead or dying. Among members of the two groups in which high NaCl diets were delayed until the rats were mature, hypertension developed more slowly. Nonetheless, it is clear that maturity was no absolute protection against excessive NaCl intake; 2 months after the intake of NaCl was increased, the average systolic blood pressure of healthy survivors in both groups was about 175 mm Hg, and at the end of 6 months on the high NaCl regimen, only three rats appeared well, although all had hypertension (165, 192, 184 mm Hg, respectively). When this experiment was concluded 1 year after weaning, 37 animals were dead, and the remaining one was dying.

Discussion

These studies confirm and extend the evidence that NaCl induces experimental hypertension in rats. In conformity with the report of Meneely et al. (6), groups of rats chronically consuming different concentrations of NaCl in the diet had different average blood pressures; in general, as the dietary salt (NaCl) was increased, the blood pressure rose. An unexpected exception emerged when dietary NaCl was increased from 1 to 2%—the average blood pressure failed to increase. We have no explanation for this and will hazard no speculations at this juncture. Among these rats with a genetic predisposition to several “varieties” of experimental hypertension (10-13), one-third of those chronically consuming the highest concentration (8%) of NaCl developed such fulminating disease that they were dead or dying within 6 weeks after starting the NaCl. Two weeks later this held true for 75% of the 40 rats in this group. As noted earlier, some of the relatively low blood pressures recorded in this 8% NaCl group were probably artificial: frequent (twice a week) blood pressure measurements were not begun because the accelerated course of the disease was not recognized until the animal was terminally ill. In experiments on similar animals whose blood pressure was taken frequently during the early weeks on 8% NaCl, marked elevations were always found. Nonetheless, death was not directly correlated with blood pressure in any of the groups here. This was evident, for example, in experiment 1 in the two groups fed 1 and 2% NaCl; blood pressures were about the same, but 75% of the animals on 1% completed the 12-month study, whereas only 25% of those on 2% NaCl did so. Since fulminating disease did not occur among the rats in these two groups, it is highly unlikely that marked elevations escaped observation, as seems likely for the group on 8% NaCl. In many, perhaps most, cases it is not clear why these NaCl-fed animals died: gross renal failure (as measured by blood urea nitrogen) accounts for about one-third of the deaths, whereas cardiac failure and cerebrovascular accidents are uncommon. In the remainder, there seems to develop only an acceleration of the murine pneumonia that normally plagues many older rats.

Previously, self-sustaining hypertension was induced by NaCl feeding in rats that had no recognized predisposition to experimental hypertension (14). Therefore, it was not surprising to confirm this result in the present study, in which all of the animals were genetically predisposed to experimentally induced hypertension. However, in these hypertension-prone rats, even transient high salt intakes appeared capable of inducing permanent hypertension. Of 34 weanlings that received 8% NaCl chow for only 2 weeks, four had permanently elevated blood pressure ranging from 180 to 206 mm Hg; of 29 weanlings on the same high NaCl intake for 6 weeks, two-thirds maintained systolic pressures in excess of 180 mm Hg after NaCl was reduced to the lowest (0.4%) level. Therefore, whatever the mechanism by which NaCl acts, it need not be consumed continuously to maintain hypertension, once this has been induced.
SALT AND EXPERIMENTAL HYPERTENSION

Most of the rats of this strain, even those on the lowest NaCl intake used here, developed mild elevations (defined as systolic pressures ≥ 140 mm Hg—see Methods) ranging from 140 to 164 mm Hg. (In some unpublished work from our laboratory, a few rats developed mild hypertension during a 12-month period of observation while consuming an artificial diet containing only 0.15% NaCl.) This is not surprising since they readily develop severe "renal" hypertension following unilateral renal artery compression (only) while on a low NaCl diet (10). Thus, these animals do not require added dietary NaCl to develop hypertension. These rats might be compared to "prehypertensive" people in whom it seems likely that any one of several etiological factors, acting alone or together, may induce hypertension (13).

The greater sensitivity of young animals to the effects of NaCl observed here is compatible with many studies in which a variety of injurious agents is used to induce pathological states. Meneely (8) discussed a related experience in which rats nearly 2 years old gradually became hypertensive after they were placed on high salt diets, but the elevations were always less than in younger rats on the same ration. Our results are in accord with his; in our older rats, genetically predisposed to hypertension, NaCl induced hypertension less rapidly than in the weanlings, and the final blood pressure elevations were generally less. Nonetheless, after 8 months on a high salt intake, none of these older animals were alive.

These data may be pertinent to man. If so, they suggest that among those with a family history of hypertension, individuals with chronically "high" NaCl intakes will develop more severe hypertension than those on chronically "low" NaCl intakes; that relatively brief periods of high NaCl ingestion—at least early in life—may induce permanent hypertension; and, finally, that while age may moderate the injurious effects of a high NaCl diet, maturity confers no long-term protection against such effects.

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