Hypertension in the Recently Weaned Dahl Salt-Sensitive Rat Despite a Diet Deficient in Sodium Chloride

Abstract. *The Dahl rat is used as a model of hypertension that is "sensitive" to dietary salt (sodium chloride, NaCl). When dietary salt is supplemented in the Dahl rat, the arterial blood pressure of the "salt-sensitive" strain (S) becomes much greater than that of the "salt-resistant" strain (R). It has been widely reported that arterial blood pressure of the young Dahl S rat is not greater than that of the young Dahl R rat before dietary salt is supplemented. In the present study, however, mean arterial pressure directly measured in unanesthetized, unrestrained S rats was greater than in R rats, both when they had been recently weaned and for at least 10 weeks thereafter, despite their having been fed a diet frankly deficient in salt. In weanling S rats, the ratio of heart weight to body weight was also significantly greater than that in weanling R rats, suggesting that the greater blood pressure in the S rat causes cardiac hypertrophy. Thus, biologic differences demonstrated between the S rat and the R rat after weaning, including the phenomenon of salt-sensitivity, could be a consequence of, or be dependent on, an already extant difference in arterial blood pressure between the two strains.*

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In 1962, Lewis Dahl and his co-workers at Brookhaven National Laboratory reported their classic studies in which they selectively bred Sprague-Dawley rats to be either resistant or sensitive to the hypertensinogenic effects of large amounts of dietary salt (sodium chloride, NaCl) (*1*). Since Dahl's original report, many workers have studied salt-sensitive (S) and salt-resistant (R) rats from the Brookhaven colony in order to investigate biologic determinants of NaCl-induced hypertension (*2, 3*). In most studies of the Dahl rat, dietary NaCl is supplemented in amounts that induce a large increase in blood pressure in the S rat but no detectable increase in the R rat. It has been widely reported that blood pressure in the young S rat is not greater than that in the young R rat before dietary NaCl is supplemented (*4–8*). Accordingly, biologic differences between the young S rat and the young R rat demonstrated before dietary NaCl is supplemented have been inferred to precede the occurrence of a difference in blood pressure between the two strains. Among such biologic differences, those in prostaglandin metabolism (*4*) and in the renal handling of sodium (*7*) have been held to precede and possibly mediate differences in blood pressure that obtain between the S and R rat when dietary NaCl is supplemented.

Yet, in the S rat that has been given a "low" or "normal" amount of dietary NaCl for a few weeks or months, blood pressure has been found to be greater than that in the R rat (*6, 9, 10–12*). This observation suggests the possibility that the greater blood pressure in the S rat can be sustained and even initiated through a mechanism that is largely independent of the dietary intake of NaCl. With respect to the NaCl content of rat diets, however, the terms "low" and "normal" have been used in a somewhat arbitrary fashion. Dahl considered a diet that contained 0.3 to 0.4 percent NaCl (51 to 68 mmol/kg) to be a "low" salt diet (*3*) while others have considered this to be a "normal" salt diet (*5*). In fact, the NaCl content of a "normal" rat diet is reported to range from approximately 0.3 percent (51 mmol/kg) (*5*) to 1.2 percent (205 mmol/kg) (*13*). The lower end of this range could be considered a still excessive amount of dietary NaCl since it constitutes more than twice the amount required for optimal weight gain in weanling rats (*14*). Thus, it remains to be determined whether the greater blood pressure in the S rat can be sustained, or initiated, through a mechanism that is largely independent of the dietary intake of NaCl. We have tested this hypothesis by directly measuring blood pressure in unanesthetized, unrestrained, recently weaned S rats and R rats given a diet frankly deficient in NaCl, that is, a diet in which the amount of NaCl is insufficient to support optimal weight gain (*14*). Our findings demonstrate that the blood pressure of the S rat is not only greater than that of the R rat despite an NaCl-deficient diet, but also that the blood pressure of the S rat is greater from weaning. Furthermore, in the weanling S rat, the ratio of heart weight to body weight is significantly greater than that in the R rat, suggesting that cardiac hypertrophy is a consequence of the greater blood pressure.

Weanling female Dahl S and R rats were obtained from Brookhaven Laboratories. Until the beginning of 1985, the Dahl rats were routinely shipped from Brookhaven with a diet that contained approximately 1 percent NaCl. Since the beginning of 1985, Brookhaven has shipped rats with a diet that contains approximately 0.35 percent NaCl. In the present study, we measured blood pressure in rats shipped with the 1 percent NaCl diet and in rats shipped with the 0.35 percent NaCl diet.

Immediately upon arrival at our laboratory, the rats were given distilled, deionized water and a NaCl-deficient, natural ingredient whole-grain diet (Teklad) that contained, per kilogram, less than 12 mmol of NaCl (determined by an independent laboratory). This corresponds to an NaCl content of less than 0.07 percent or a sodium content of less than 0.03 percent. This amount of dietary sodium is insufficient to support optimal weight gain in weanling rats (*14*). In the first study, the rats were individually housed in metabolic cages in order to control food and fluid intake and to collect urine for analysis of sodium. The daily amount of food and fluid provided to the S rats was determined by the daily amount of food and fluid consumed by the R rats. Blood pressure was measured 1 week and 6 weeks after the rats were shipped with a 1 percent NaCl diet from Brookhaven. In a second study, the rats were housed in standard rat cages, three to a cage, and given free access to the distilled water and the NaCl-deficient diet. In this study, blood pressure was measured 1 week and 10 weeks after the rats were shipped with a 0.35 percent NaCl diet from Brookhaven.

Measurements of blood pressure were performed in the unanesthetized, unrestrained state through indwelling catheters (polyethylene tubing No. 10 or No. 50) implanted in the femoral artery. The catheters were tunneled subcutaneously to an exit in the nape of the neck and filled with a heparinized solution of 5 percent dextrose. In the first study, the rats were allowed to recover from the surgery and the anesthetic (ether) for 4 hours before their blood pressure was measured. In the second study, blood pressure was measured after a recovery period of 48 hours. At the time blood pressure was measured, the arterial catheter was connected to a low-volume pressure transducer (Statham p23Gb) and the pressure tracing was recorded on a Gould recorder. Mean arterial pressure

was measured 20 to 30 minutes after the arterial catheter was connected to the transducer. Blood pressure was calculated from the average of six readings obtained while each animal was resting and motionless but alert. Blood pressures were measured in S rats and R rats at the same time of day. The initial blood pressure measurement, determined after 1 week of the NaCl-deficient diet, was obtained through a catheter implanted in the right femoral artery. Immediately after this measurement, the catheter was removed and the feeding protocol continued. At the end of each study, blood pressure was remeasured through a catheter implanted in the left femoral artery. Statistical analysis was performed with Student's one-tailed *t*-test. Statistical significance was defined as $P < 0.025$ after the Bonferroni correction for multiple comparisons (two per experiment).

In each study, mean arterial pressure in the recently weaned S rats was significantly greater than that in the R rats (Fig. 1). The greater blood pressure in the S rats persisted for the duration of each study. Over the first week of the initial study, we could detect no significant differences between the S rats and the R rats with respect to food intake or body weight. Over the 6-week period of the study, the average food intake of the S rats, 66.0 ± 0.9 g/week, was slightly less than that of the R rats, 69.6 ± 1.2 g/week ($P < 0.025$). Therefore, in the S rats, dietary intake of NaCl was less than that in the R rats. After 6 weeks, the mean body weight of the S rats, 113.4 ± 2.3 g, was slightly less than that of the R rats, 123.3 ± 2.5 g ($P < 0.01$), consistent with the lower food intake of the S rats. Throughout the study, sodium could not be detected in the urine of either the S rats or the R rats.

In another study in female S and R rats shipped with a 1 percent NaCl diet from Brookhaven, we measured blood pressure after pair-feeding the rats an NaCl-deficient semipurified liquid diet for 4 weeks. This liquid diet (Osmolite, Ross Laboratories), which was completely different from the natural ingredient diet used in the first two studies, was diluted with distilled, deionized water to contain just slightly less than 0.05 percent sodium. In this study, blood pressures in the S rats measured 24 hours after insertion of the femoral artery catheters were also significantly greater than in the R rats (Fig. 2A).

To investigate whether the greater blood pressure in the recently weaned S rat has important biologic consequences, we measured heart weight in five weanling female S rats and five weanling female R rats on the same day the rats were received from Brookhaven (shipped with a 0.35 percent NaCl diet). With the rats under ether anesthesia, the hearts were removed, trimmed of their atrial appendages, pressed between gauze pads to remove blood in the chambers, and weighed on a Mettler balance. In the S rats, the ratio of heart weight to body weight was significantly greater than that in the R rats (Fig. 2B). The mean body weight of the S rats, 61.2 ± 5 g, was not significantly different than that of the R rats, 61.6 ± 4 g.

The current findings demonstrate that in the recently weaned, unanesthetized, unrestrained Dahl S rat, directly measured mean arterial pressure is greater than that in the Dahl R rat despite an NaCl-deficient diet. The greater blood pressure in the S rat persists despite sustained administration of the NaCl-deficient diet and occurs with administration of either a natural ingredient diet or semipurified liquid diet. Thus, the greater blood pressure in the S rat is not the consequence of a particular NaCl-deficient diet. The present findings are consistent with the hypothesis that greater blood pressure in the recently weaned S rat can occur and persist through a mechanism that is largely independent of the dietary intake of NaCl. These findings raise the possibility that in the S rat, blood pressure might be greater than that in the R rat from birth. It could be argued that the occurrence of greater blood pressure in the weanling S rat requires a certain dietary intake of NaCl during the neonatal period (through the agency of the mother's milk). This possibility remains to be investigated. Conceivably, greater blood pressure might occur in recently weaned female S rats but not in recently weaned male S rats. It appears, however, that hypertension occurs more frequently in male S rats than in female S rats (*10*).

In previous studies in young Dahl rats given "low" or "normal" amounts of dietary NaCl, failure to consistently detect a difference in blood pressure between the S and R strains may have been due to the use of insufficiently sensitive

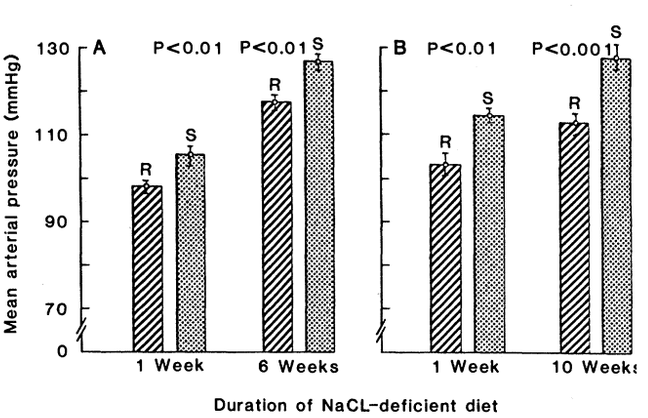

Fig. 1. Mean arterial pressures of recently weaned, unanesthetized, unrestrained Dahl R rats (denoted by the crosshatched bars) and S rats (denoted by the stippled bars) given a NaCl-deficient, whole-grain natural ingredient diet. (A) Blood pressures measured after 1 week and 6 weeks in eight R rats and in seven S rats individually housed in metabolic cages and pair-fed. (B) Blood pressures measured after 1 week and 10 weeks in six R rats and in six S rats housed three to a cage and fed ad libitum. Vertical bars and their brackets indicate group means ± standard errors. *P* values indicate statistical differences between group means by Student's one-tailed *t*-test. Statistical significance was defined as $P < 0.025$ after the Bonferroni correction for multiple comparisons (two per study).

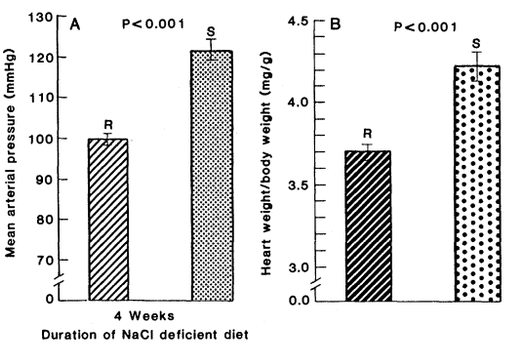

Fig. 2. (A) Mean arterial pressures of six unanesthetized, unrestrained Dahl R rats (denoted by the crosshatched bars) and six S rats (denoted by the stippled bars) pair-fed an NaCl-deficient semipurified liquid diet for 4 weeks after weaning. Vertical bars and their brackets indicate group means ± standard errors. *P* values indicate statistical differences between group means by Student's one-tailed *t*-test. Statistical significance was defined as $P < 0.05$. (B) Ratios of heart weight to body weight in five weanling Dahl R rats (denoted by the crosshatched bars) and five S rats (denoted by the stippled bars) determined immediately after delivery of the rats from Brookhaven Laboratories. Vertical bars and their brackets indicate group means ± standard errors. *P* values indicate statistical differences between group means by Student's one-tailed *t*-test. Statistical significance was defined as $P < 0.05$.

techniques to measure blood pressure, lack of measurements of diastolic or mean pressures, failure to control intake of food and NaCl, use of anesthetics, or the use of an inadequate sample size. The tail-cuff technique, the method most commonly used to measure arterial blood pressure in the unanesthetized rat, requires restraint of the animal and either the deliberate or inadvertent induction of an increase in body temperature (*15*). Thus, hemodynamic effects of heat stress or immobilization stress may also have contributed to the variability in blood pressure results. Furthermore, this technique is generally used to measure only systolic pressure because tail-cuff measurements of diastolic or mean pressure are probably not as accurate or as reliable as those of systolic pressure (*15*).

In those studies in which blood pressure in young S rats has been found to be greater than in R rats despite a "low" NaCl diet, the amounts of sodium chloride provided were actually more than adequate for normal growth (*6, 9, 10–12*). Whether or not the stress of tail-cuff measurements or of anesthesia contributed to the occurrence of greater blood pressure in the S rats was also not addressed in those studies.

Our finding that blood pressure in the recently weaned S rat is greater than that in the R rat, despite a diet deficient in NaCl, has implications for studies of the pathogenetic determinants of salt-sensitivity and salt-resistance in the Dahl rat. Specifically, biologic differences demonstrated between the S and the R rat after weaning, including the phenomenon of salt-sensitivity, could be a consequence of, or be dependent on, an already extant difference in blood pressure between the two strains. The observation that, in the weanling S rat, heart weight is greater than in the weanling R rat suggests that the greater blood pressure in the young S rat has biologic consequences, for example, myocardial hypertrophy.

References and Notes

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A New HTLV-III/LAV Encoded Antigen Detected by Antibodies from AIDS Patients

Abstract. *A newly identified protein from HTLV-III/LAV, the virus implicated as the etiologic agent of the acquired immune deficiency syndrome, was studied. This protein, which has a molecular weight of 27,000 (p27), was shown by amino acid sequencing to have a coding origin 3' to the* env *gene on the HTLV-III genome. The presence of antibodies to p27 in virus-exposed individuals indicated that this gene is functional in the natural host.*

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Acquired immune deficiency syndrome (AIDS) is a disease that causes depletion of a subset of T lymphocytes and results in opportunistic infections and malignancies. A retrovirus, designated HTLV-III/LAV, has been isolated and implicated as the etiologic agent for AIDS (*1–6*). Proteins specific to HTLV-III/LAV have been described and include the *gag*-encoded products p55, p38, p24, and p17 and the *env*-encoded proteins gp160, gp120, and gp41 (*3, 4, 7–11*). Analysis of DNA provirus clones led to the identification of at least two open reading frames, in addition to *gag, pol*, and *env*, that could encode other protein products (*12–15*). A protein of approximately 27 kilodaltons (p27), which appeared to be unrelated to the known *gag*- and *env*-encoded products, was detected in an HTLV-III–infected cell line, H9/HTLV-III (*9*). This protein was analyzed to determine if it originated from one of the open reading frames.

The relative frequency of antibodies to p27 in the serum of patients with AIDS-related complex (ARC) or AIDS and healthy asymptomatic homosexuals was examined. Disease categories were determined according to the criteria for AIDS used by the Centers for Disease Control (*16*). The AIDS patients included those who had clinical manifestations of opportunistic infections or certain malignancies, and ARC patients included those with defined constitutional and laboratory abnormalities. Serum samples, obtained from 138 individuals, were selected at random from a bank of samples drawn from area hospitals and community clinics. These samples had previously been determined by radioimmunoprecipitation (RIP) and cell membrane immunofluorescence to contain antibodies reactive with HTLV-III/LAV proteins. Of the 138 samples tested by RIP analysis, 51 (37 percent) were positive for antibody to p27. The prevalence rates by disease category are given in Table 1. Approximately one-half of the samples from asymptomatic subjects who were positive for HTLV-III/LAV antibody also had antibodies to p27, but this frequency significantly declined with sever-