Effect of stress and food restriction on blood pressure and lifespan of Dahl salt-sensitive rats

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Objective: To evaluate the long-term consequences of stress in rats with genetic hypertension.

Design: Rapp–Dahl salt-sensitive rats, maintained on a low-salt diet, were stressed periodically over 8 weeks during which time their blood pressures were measured. In experiment 1 both stressed and unstressed control rats were given *ad libitum* access to food. Because of significant differences in body weights, in experiment 2 the unstressed controls were pair-fed to maintain their food intake at a level similar to that of the stressed rats.

Methods: Rats were subjected to 2-h sessions of supine immobilization stress 5 days a week every other week for 8 weeks. Blood pressures were measured during non-stress weeks, at least 4 days after the last exposure to the stressor and at monthly intervals thereafter. Survival curves were also established.

Results: In experiment 1 stressed rats developed hypertension at a slower rate than controls and lived significantly longer, but also weighed significantly less than controls, presumably because of diminished food intake. In experiment 2, in which food intake was controlled, body weights were similar in the two groups of rats, and hypertension developed at the same rate in both groups. Survival curves were not significantly different. Food restriction extended life compared with free feeding.

Conclusions: Stress need not have long-term, deleterious health consequences in rats with genetically inherited hypertension, whereas caloric restriction is protective.

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Introduction

In 1983 Natelson [1] hypothesized that stress did not have serious pathological consequences unless it occurred concomitantly with disease or a predisposition to disease. This formulation appears to have held in the case of stress and hypertension. In early experiments in which healthy animals were stressed, the most frequent result was transient hypertension (for review [2,3]). However, when rats with a genetic predisposition to hypertension were stressed, more-permanent hypertension developed; that is, the hypertension that was seen contiguous to stress continued for the previously stressed group as a whole in one study [4] and for a subset of rats in another study [5]. A critical question involves the health consequences of such behaviorally induced hypertension. If the hypertension was pathologically significant, the disease might be expected to affect life expectancy adversely. Natelson's hypothesis would predict that stressed rats with a genetic predisposition to hypertension would develop hypertension both earlier and of a more severe grade, and that these changes would produce a significant shortening of the rat's life.

The purpose of this experiment was twofold. First, we wanted to examine further the relation between stress and the development of hypertension in rats with a genetic predisposition to that disease. We chose as our subjects the Rapp–Dahl salt-sensitive rat maintained on a low-salt diet; this was one of the two rat models of borderline hypertension used to establish the relation between stress and hypertension. When fed on low-salt diets containing 1% NaCl, blood pressures in Rapp–Dahl rats become higher than in

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controls, but frank hypertension does not develop until months later; however, when high-salt diets containing 8% NaCl are provided, hypertension develops within weeks [6]. Although much is known about the early stages of hypertension in these rats [6], little is known about the full course and outcome of their disease. Thus, the second reason for these studies was to monitor blood pressure throughout the rats' lives in order to enable a better understanding of the relationship between elevated blood pressure and its effect on life expectancy to be obtained.

Methods

Seventy-two male Rapp–Dahl salt-sensitive rats (SS/JR) were obtained from Harlan Sprague–Dawley, Indianapolis, Indiana, USA. The rats were 6 weeks old and weighed 150–200 g on arrival. They were individually housed in shoebox cages and maintained on a 12 h : 12 h light : dark cycle with the onset of light at 0700 h. They had free access to tap water and Purina Rodent Laboratory Chow (Purina, St Louis, Missouri, USA) containing 0.56% NaCl by dry weight. Two weeks after arrival, the rats were habituated to the blood pressure apparatus for two 1-h periods within a week. Habituation sessions were separated by 3 days. In these sessions, rats were placed into clear plexiglass restraint tubes (7.6 cm in diameter) and a photoelectrically sensitive sensor in an inflatable cuff (B60; IITC Life Science, Inc., Woodland Hills, California, USA) was placed on their tails. To assure vasodilation during blood pressure measurement, the temperature was maintained between 27 and 29°C in the room in which blood pressure was measured.

Blood pressures were measured in the week following habituation (i.e. the fourth week after arrival). To measure blood pressures, rats were transferred to the blood pressure room and placed in the apparatus with a cuff placed on their tail. After a 10- to 15-min acclimation period, multiple blood pressure measurements were made for each determination, in order to obtain reliable physiological records. When artifacts were not evident on the records, only three readings were taken, but when artifacts were seen as many as seven readings were taken. From the multiple records, outliers were discarded if systolic blood pressure values deviated by more than 4 SD from the mean of the other readings in the same session. Mean blood pressures were also recorded and were used to calculate diastolic and pulse pressures [7]. The measurements for a session were averaged and used for subsequent analyses. Since analysis of mean blood pressure and the derived data provided no additional insights beyond analysis of average systolic pressures alone, only average systolic pressures are presented here.

Rats were stratified on their prestress blood pressures, then randomly assigned to either the stressed (n = 14) or the unstressed control (n = 13) group. Initial body weights were equivalent for rats to be stressed (mean 306.8 g) and unstressed controls (mean 306.1 g).

Stress consisted of 2 h supine restraint with limbs extended and body movement further limited by a cloth strap snugly fitted over the abdomen. Restraint began at 1400 h on five consecutive days each week and was administered on alternate weeks for a total of 4 weeks. We have previously shown that non-hypertensive rats exposed to a stressor regimen similar to that used here continue to show stress responses to each restraint session for 4 weeks, as assessed by the plasma corticosterone level [8].

Blood pressures were measured the week after each stress week, 3 weeks after the last stress week and at monthly intervals thereafter, and were measured at least 4 days after the end of each week of stress.

After nine blood pressure samplings, approximately 3 months after the last stressor session and when rats were 8 months old, significant numbers of deaths made further statistical analysis of the systolic pressure problematic. This was because the data were being differentially censored after this point as a direct result of the treatment effect being studied. Thus, only the first nine blood pressure measurements are reported. Body weights were also measured throughout the rats' lives on the same days that blood pressure measurements were made.

A second experiment was conducted in a similar manner to the first except that a yoke-control feeding procedure was implemented. Following assignment to groups based on initial blood pressures, stressed (n = 13) and unstressed (n = 13) rats were paired in such as way that the unstressed rat in each pair was allowed only the number of food pellets eaten on the previous day by the stressed rat in the pair. These pellets were provided at the same time as the stressed rats were returned to their cages after stress. As in the first experiment, body weights were equivalent at the beginning of the experiment between rats assigned to the stress group (mean 296.6 g) and those assigned to the control group (mean 293.4 g).

Results

Experiment 1

The systolic blood pressures were analyzed with a 2 × 9 (stress × measurement day) mixed analysis of variance. The main effects of stress [$F(1,21) = 8.60$, $P < 0.01$] and measurement day [$F(8,188) = 71.31$, $P < 0.0001$] were significant. These were qualified by the significant stress × measurement day interaction, [$F(8,188) = 2.75$, $P < 0.01$; Fig. 1]. The unstressed rats had an initial systolic blood pressure of 137.5 mmHg,

which rose over the next four samplings (approximately 2 months) to an interim peak of 200.1 mmHg. After a plateau period, which included the next two samplings (approximately 1.5 months), the systolic blood pressure peaked at 225.3 mmHg before declining to 213.1 mmHg for the last reported measurement, which shortly preceded the death of some of the rats. F-tests for simple effects indicated that repeated stressor exposures slowed the development of hypertension ($P<0.05$ for each). The initial systolic blood pressure of the stressed rats was 135.5 mmHg, which rose more gradually to an interim peak of 181.0 mmHg. After a plateau period coincident with the plateau period for the unstressed rats, the systolic blood pressures of the stressed rats peaked at 229.6 mmHg for the last reported measurement. Dunnett's test for *a priori* comparisons involving a control mean indicated that the last reported systolic blood pressure of the stressed rats was significantly higher than that of the unstressed rats ($P<0.05$). Stressor exposure delayed the rise in systolic blood pressure and reduced the amplitude of the initial peak.

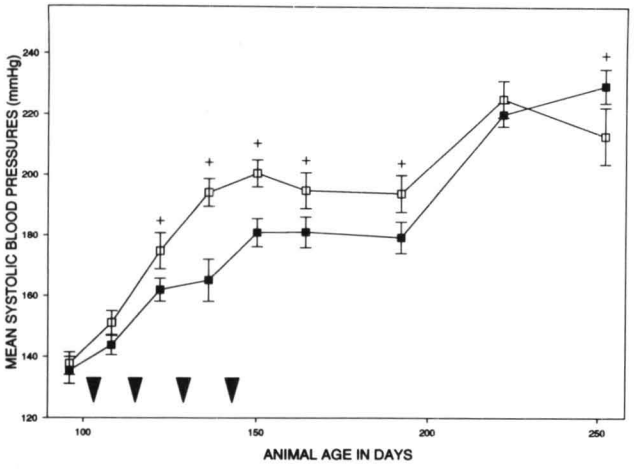

Fig. 1. Effects of repeated stressor exposure on average systolic blood pressures in free-feeding rats. Stressed rats exhibited a slower development of hypertension. ■, Stress; □, control; ▼, stressor exposure. $^{+}P<0.05$, versus stress.

The body weight data were analyzed with a similar analysis of variance. The main effects of stress and measurement day were significant [$F(1,195) = 6.8$, $P<0.01$; $F(8,195) = 570.1$, $P<0.0001$]. These were qualified by the stress × measurement day interaction [$F(8,195) = 16.5$, $P<0.001$; Fig. 2]. For the first three stress weeks the stressed rats gained weight more slowly than unstressed rats. Between the third and fourth stress weeks the stressed rats gained weight faster than the unstressed rats. This reduced the difference in body weight between the two groups, although significant differences between the two groups still remained. The diminution in difference in body weights between the two groups suggests that there may have been some stressor habituation to the repeated exposures of supine restraint. When rats were 164 days old the body weights of the stressed rats were equivalent to those of the unstressed rats and remained at that level until the final measurement day.

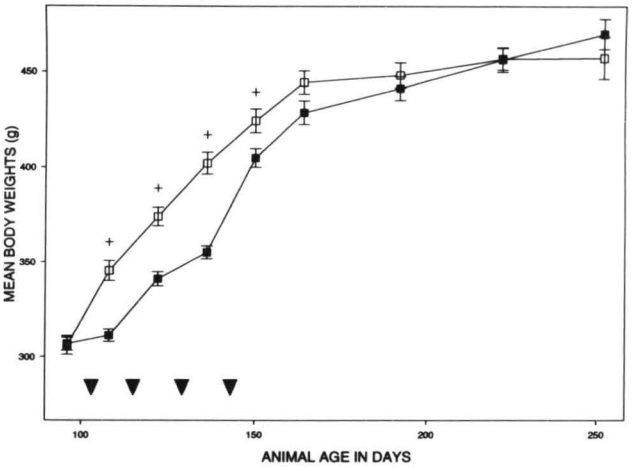

Fig. 2. Effects of repeated stressor exposure on body weight in free-feeding rats. Stressed rats gained weight more slowly than unstressed rats. ■, Stress; □, control; ▼, stressor exposure. $^{+}P<0.05$, versus stress.

Kaplan–Meier estimates of the conditional probability of survival were used to compare longevity between the stressed and unstressed groups. This technique takes into account the change in an individual animal's probability of death as others in the study die. Statistical significance was determined on these probabilities by log-rank tests [9]. The median death ages of the unstressed and stressed rats were 268 and 324 days, respectively. The stressed rats lived approximately 21% longer than the unstressed rats, and the difference in survival was statistically significant ($\chi^2 = 14.2$, $P<0.0001$; Fig. 3).

Fig. 3. Effects of repeated stressor exposures on lifespan in free-feeding rats. Stressed rats lived longer than unstressed rats. —, Control; – · –, stress.

Experiment 2

The systolic blood pressures were analyzed as above. Only the main effect of measurement day was significant [$F(7,183) = 79.16$, $P<0.0001$; Fig. 4]. Although initial blood pressures were slightly higher in experiment 2 for both stressed and unstressed groups than in experiment 1, the peak levels attained were the same. As the blood pressure curves for the stressed and unstressed groups are generally the same, the feeding regimen administered to the unstressed group in experiment 2 appeared to delay the relatively rapid rise in systolic blood pressure observed in the unstressed group in experiment 1. However, even compared with food-restricted unstressed rats, Dunnett tests indicated that stressed rats developed hypertension more slowly, as shown by lower systolic blood pressures immediately after the last stressor period ($P<0.05$). Body weights were not statistically different between the two groups at any measurement point during this experiment (data not shown).

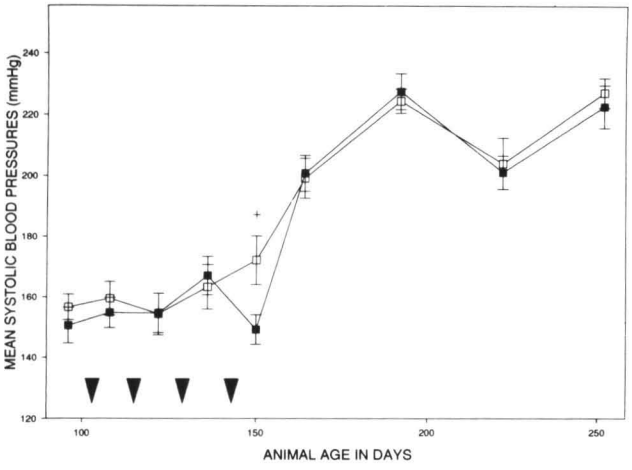

Fig. 4. Comparison of the effects of repeated stressor exposures on average systolic blood pressure in free-feeding rats and food-restricted controls. Except for the fourth measurement, the systolic blood pressures of free-feeding stressed rats were not different from those of restricted-feeding controls. ■, Stress; □, control; ▼, stressor exposure. $^{+}P<0.05$, versus stress.

Survival data were analyzed as above. Kaplan–Meier estimates indicate that the groups did not differ in their conditional probability of survival ($\chi^2 = 3.5$, $P>0.05$; Fig. 5). The median death ages for the food-restricted unstressed and the stressed rats were 293 and 302 days, respectively. A comparison of the survival curves for the unstressed rats from the two experiments revealed that the food-restricted rats in experiment 2 lived significantly longer than the free-feeding rats in experiment 1 ($\chi^2 = 3.9$, $P<0.05$).

Discussion

The purpose of these experiments was to evaluate the effects of repeated presentation of an intense stressor

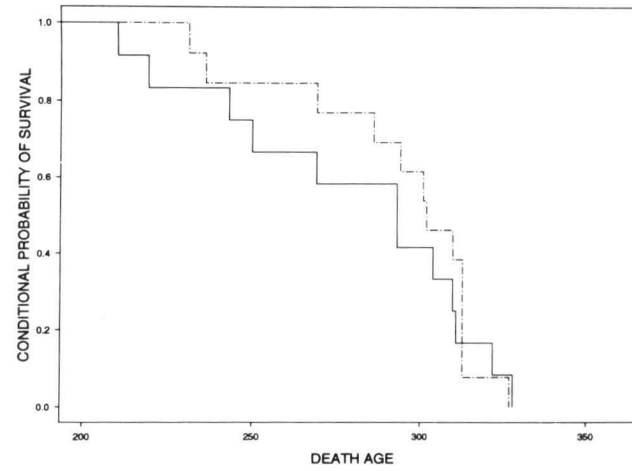

Fig. 5. Comparison of the effects of repeated stressor exposures on the lifespans of free-feeding and food-restricted rats. Repeated stressor exposures did not alter lifespan compared with unstressed rats which ate the same amount of food as the stressed rats. —, Control; –·–, stress.

on the course and outcome of hypertensive cardiovascular disease in Dahl salt-sensitive rats. Our first experiment produced unexpected results. Stress protected the rats from the development of hypertension and delayed the lethal consequences of the hypertension. An evaluation of body weights in the two groups suggested a reason for this finding. Stressed rats gained less weight, probably because of stress-induced decreases in food intake, an effect that we have noted previously [10]. Whether the hypertension was delayed by the reduction in calories or quantity of salt ingested is unclear, since our manipulation probably decreased both caloric and salt intake. Nevertheless, this experiment suggested that differences in food intake might be the reason for delay in the development of hypertension in the stressed group. The consequences of the difference were dramatic: the stressed group lived significantly longer than the unstressed free-feeding group.

Our hypothesis that food ingestion was responsible for the differences in hypertension and survival was supported by the second experiment. In that experiment food intake was monitored, and unstressed controls were allowed only the same amount of food as the stressed rats. That treatment produced superimposable patterns of blood pressure over time, except for one blood pressure determination at the end of the 4-week stress period, when stressed rats had lower blood pressures than unstressed controls. We do not know the significance of this transient difference.

Thus, when similar caloric and salt intakes were maintained in the stressed and unstressed groups, stress did not alter the development of hypertension. A comparison of Figs 1 and 4 makes it clear that this was not because of a ceiling effect. In contrast to blood pressures of approximately 160 mmHg in the 150-day-old food-restricted rats (Fig. 4), free-feeding un-

stressed rats (Fig. 1) of the same age had blood pressures of approximately 200 mmHg. Moreover, in both experiments blood pressures increased even further to mean levels of approximately 220 mmHg.

These data seem discrepant from the important results of Friedman and Iwai [11] and Lawler *et al.* [4], who reported that conflict stress produced an almost immediate increase in blood pressure to hypertensive levels in normotensive and borderline hypertensive rats, respectively. However, a close evaluation of the earlier work suggests that the data are not as different as they might seem. Both groups measured blood pressures shortly after the end of each stress session, whereas we deliberately waited 3–5 days from the end of stress before measuring the blood pressure. We did this because there is no doubt that blood pressure increases reliably after acute stress. What is less clear, however, are the long-term consequences of stress; the reason for the work reported here. In the work of Friedman and Dahl [5], who used the Rapp–Dahl rat, most rats showed a return of blood pressure to control levels after the cessation of stress; only a few rats continued to show hypertension and even these showed recovery of blood pressure over time. In the study by Lawler *et al.* [4], who used the F1 generation of a cross between spontaneously hypertensive rats and non-hypertensive control rats (i.e. the borderline hypertensive rat), blood pressures remained elevated for 9 weeks. However, in a follow-up study [12], all groups including unstressed controls had blood pressures that diminished over the 12-week period following stress. Thus, even with the best interpretation, recovery of blood pressure towards control levels is the rule. The difference between outcomes in the Rapp–Dahl and borderline hypertensive rats suggests the importance of genetic factors that are not currently understood.

Another difference between the earlier studies and this one concerns the nature of the stressor used. The original work from both the Lawler and Friedman groups used response-contingent shock (i.e. conflict stress) [4,11], whereas we used supine restraint. However, follow-up studies from both groups using response-independent shock produced similar stress-induced increases in blood pressure [5,12]. These data suggest that psychological contingency is less important than the physical properties of the stressor itself in producing blood pressure elevations. This interpretation is supported by a recent paper showing that the relatively mild stressors of light restraint and air jet noise precipitated frank hypertension (blood pressures of >150 mmHg, using the same blood pressure detection technology as used here) in rats that are borderline hypertensive in the baseline state [13]. The stressor in these studies was an intense one which produces stress-induced weight loss [10] as well as a glucocorticoid stress response which does not habituate but instead shows sensitization with tonic increases in glucocorticoids over time [14]. Thus, the fact that sustained increases in blood pressure did not develop here probably cannot be attributed to properties of the stressor. Our data strongly suggest that stress is not always a health danger, a conclusion in accord with the work of others [15].

We chose to study the salt-sensitive rat because we expected the process of hypertension to shorten its lifespan. Stress physiologists usually follow a parameter such as blood pressure and make inferences about health outcome based on differences in blood pressure. In general, experience with human hypertension makes it clear that hypertensive cardiovascular disease shortens life. The data from this experiment support this conclusion also for rats with genetic hypertension. The median lifespan of unstressed, salt-sensitive control rats was <1 year in both experiments, whereas non-hypertensive rats live more than twice as long [16]. However, the relatively short lifespan of the salt-sensitive hypertensive rat leads us to urge that lifespan be included as a variable in future studies so that researchers can be sure of the ultimate health consequences of their experimental manipulations.

The final point concerns the effects of food restriction, the one treatment that is known to extend life across species and in the presence and absence of disease [17]. Here too, the food-restricted group lived significantly longer than the free-feeding group. The life-extending effect of restricted access to food would appear to relate to the magnitude of hypertension developed over time. Free-feeding rats showed a rapid and robust increase in blood pressure, which appeared to be delayed by food restriction (compare lines with open squares in Figs 1 and 4). This observation is important because it indicates that food deprivation has a protective effect even in animals whose lives are greatly shortened by disease, and can exert this effect over the course of just a few months. Our data therefore extend earlier work showing that spontaneously hypertensive rats given 40% *ad libitum* food have lifespans similar to those of free-fed normotensive rats [18]. Although the applicability of these genetic animal models of hypertension to human hypertension may be somewhat limited, the absolute lack of similar outcome data in human hypertensive disease and the improbability of obtaining such data (Alderman MH, personal communication, 1992) lead us to suggest that the weight loss associated with limited food intake could also be protective in the human condition.

In conclusion, these experiments show that food intake is a critical variable to control in studies of the effect of stress on the development of hypertension. However, when food intake is controlled, under the conditions reported here, stress neither accelerated nor exacerbated hypertension. Food restriction, on the other hand, did delay the hypertensive process and was protective. Further work is needed to understand when stress increases the risk to a patient with hypertensive cardiovascular disease and whether

weight reduction can actually extend the lives of those with malignant forms of hypertensive disease.

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