Genetic influence on brain catecholamines: High brain norepinephrine in salt-sensitive rats

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Brain catecholamines and salt sensitivity

Key words: salt sensitivity, catecholamines, hypothalamus, brain stem.

Abbreviations: S = Dahl salt-sensitive rats; R = Dahl salt-resistant rats; E = epinephrine; NE = norepinephrine

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Summary

1. Rats genetically sensitive to salt-induced hypertension evinced higher levels of plasma norepinephrine and epinephrine than rats genetically resistant to hypertension.

2. The hypertension-sensitive rats showed higher hypothalamic norepinephrine and lower epinephrine than resistant rats.

3. In response to a high salt diet brain stem norepinephrine increased in sensitive rats while resistant rats exhibited a decrease on the same diet.

Introduction

Dahl salt-sensitive (S) rats develop severe hypertension on a high salt diet but remain normotensive on a low salt diet while Dahl salt-resistant (R) rats remain normotensive on either diet (Dahl, Heine, & Tassinari, 1962). Studies using the techniques of parabiosis and renal homografts (Dahl & Heine, 1975) as well as studies indicating reduced natriuretic capacity of the kidneys of S rats viz-a-viz R rats (Tobian, Lange, Azar, Iwai, Koop & Coffee, 1977) have suggested that renal factors may be primarily responsible for the differential susceptibility to hypertension. However, recent evidence has accumulated which implicates both peripheral (Takeshita & Mark, 1978; Friedman, Tassinari, Heine & Iwai, 1979) and central (Ikeda, Tobian, Iwai, & Goossens, 1978; Saavedra, Del Carmine, Iwai, & Alexander, 1979) nervous system factors as being at least partially responsible for the development of hypertension in S rats.

All of the studies, concerning nervous system involvement in the Dahl model, to date, have examined mature S and R rats following a fixed period on either a high or low salt diet. The purpose of the present study was an examination of some central and peripheral catecholamine levels accompanying changes in blood pressure on graded amounts of dietary salt and at different time intervals following excessive salt intake.

Methods

The first study examined circulating norepinephrine (NE) and epinephrine (E) levels in response to diets differing in sodium content. Weanling (21 days of age) S and R rats were fed a diet containing either; 0.3%; 1.0%; 4.0%; or 8.0% NaCl by weight. After 5 weeks, each rat was lightly anesthetized with ether and the blood pressure was measured indirectly by tail plethysmography. Immediately following blood pressure determination, a mid-line incision was made

and a 10 ml blood sample was obtained from the abdominal aorta of each subject. The heparinized blood samples were treated and subsequently assayed for catecholamines according to Anton and Sayre (1962).

The second experiment examined hypothalamic and brain stem catecholamine levels at different intervals after the initiation of a high salt diet. S and R rats were placed on a diet containing 8.0% NaCl by weight at two weeks post-weaning (35 days of age) and sacrificed either 0, 2, 6, 8, 10 or 12 weeks later. In all cases, indirect blood pressures were obtained 24 hours prior to sacrifice. Subjects were sacrificed by decapitation at which time the brain was quickly excised, chilled in ice, dissected along natural demarcation lines into cortex, hypothalamus and brain stem (Glowinsky & Iversen, 1966) and frozen. Simultaneously, the heart and adrenals were quickly removed, rinsed, weighed and immediately frozen. Tissues were subsequently assayed for NE and E. The present study presents only data obtained from hypothalamic and brain stem tissue.

Results

In the first study, the anticipated blood pressure pattern emerged. As indicated in the upper portion of Table I, S rats exhibited significantly higher blood pressures than R rats. The significant strain x diet interaction was due to the increased blood pressure of S rats on the 4.0% and 8.0% NaCl diets and the absence of such an effect in R rats. The blood pressure results were not closely paralleled by changes in either plasma NE or E. As can be seen in Table I, S rats had higher plasma NE levels in every dietary condition resulting in a statistically significant effect. However, the absence of a significant strain x diet interaction indicates that the magnitude of this difference remained fairly constant. Similarly, although there appeared to be more variability, S rats had significantly higher E levels than R but the strain x week interaction was not significant.

Table I

The lower portion of Table I presents the results of the second experiment. For clarity of presentation, only the data obtained on weeks 0, 2, 6 and 12 are presented. The statistical analysis, however, included the data from weeks 4, 8 and 10 as well. Once again, the blood pressure data were generally as expected although the hypertension evinced by S rats following 12 weeks exposure to the 8.0% NaCl diet was less than anticipated. S rats had higher concentrations of NE in the hypothalamus than R rats each week. The rather dramatic rise in hypothalamic NE in S rats at 2 weeks was not maintained nor was the less dramatic fall in R rats at the same interval. Hence, although there was a very significant strain effect, the strain x week interaction failed to reach statistical significance. The results of the hypothalamic E concentrations were in the opposite directions. Overall, S rats had significantly lower levels than R rats. Once again, there was no significant strain x weeks interaction. Brain stem NE was the same in S and R rats at week 0. However, in response to the high salt diet, S rats evinced an increase whereas R rats showed a decrease. S rats tended to maintain relatively high levels while R rats tended to maintain relatively low levels. This resulted in a statistically significant strain effect and a significant strain x weeks interaction. As was the case with hypothalamic tissue, the direction of the strain difference was opposite for NE and E. Overall, S rats had significantly lower E levels in brain stem than R rats although considerably more week to week variability existed. There was a definite trend toward lower brain stem E levels in both lines, but the strain x week interaction was not significant.

Discussion

The relationship between central and peripheral brain catecholamine

concentrations and salt-induced hypertension appears to be complicated. The results of the present study suggest that some central and peripheral catecholamine differences between S and R rats are dependent on genetic factors but do not closely parallel blood pressure changes. In the present study, for example, the difference in plasma NE and hypothalamic NE between S and R rats in the baseline state did not appreciably change during salt feeding and the development of hypertension. On the other hand, brain stem NE went up in S rats in response to the high salt diet while R rats showed a decrease. Hence, this difference appears to be due to differential responsiveness of the two strains to the hypertensinogenic stimulus. It is not yet possible to adequately determine the relevance of these peripheral and central catecholamine differences to salt-induced hypertension. Clearly, the longitudinal approach of the present study can be elucidating. However, more precise anatomical dissection may be more informative given the complex constellation of results reported by Saavedra, et al. (1979). Two additional theoretical issues bear mention. For those indices which differentially change in response excess salt ingestion in S and R rats, it becomes important to determine if the difference is actually due to differential responsiveness to salt per se. Changes occurring in S rats but not R rats may be secondary to elevations in blood pressure irrespective of the etiological stimulus. Another point concerns the nature of physiological and biochemical indices which correlate with the genetic predisposition to hypertension but that do not necessarily parallel the development of the high blood pressure. We have previously demonstrated that certain behavioral characteristics distinguish S rats from R rats in the normotensive state. This is analogous to some of the peripheral and central catecholamine differences reported here and by others (Saavedra, et al., 1979). A careful genetic cross study of the relationship between the behavioral and cardiovascular

phenotypes, however, indicated that the relationship between the two variables was not genetic but was fortuitous (Friedman; Haber & Iwai, 1979). Hence, the relevance of the correlations obtained between catecholamine levels and genetically determined blood pressure responsiveness to salt ingestion remains to be determined.

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Table I

Blood and Tissue Catecholamine Levels in S and R rats

	Percent NaCl in Diet				Statistical Significance		
	0.3	1.0	4.0	8.0			
	S/R	S/R	S/R	S/R	$F_{(S)}$	$F_{(D)}$	$F_{(SD)}$
Mean Systolic BP (mm Hg)	120/108	120/108	160/111	207/114	116.0*	52.9*	36.5*
Mean Plasma NE (µg/L)	2.91/2.17	3.45/1.55	3.60/2.17	3.77/2.04	35.6*	1.3	0.2
Mean Plasma E (µg/L)	10.72/10.92	11.12/9.02	10.22/8.83	11.66/10.76	4.2*	2.8	0.1

n in all cells = 5 samples individually assayed.

*Statistically significant, $P < 0.05$, 2-way analysis of variance

$F_{(S)}$ (Strain), $F_{(D)}$ (Diet), $F_{(SD)}$ (Strain x Diet interaction)

	Weeks on 8.0% NaCl Diet				Statistical Significance		
	0	2	6	12			
	S/R	S/R	S/R	S/R	$F_{(S)}$	$F_{(W)}$	$F_{(SW)}$
Mean Systolic BP (mm Hg)	123/99	129/101	172 /113	169/100	101.4*	3.3*	2.9*
Mean Hypothalamic NE (µg/g)	3.16/2.57	4.05/2.03	3.52/2.36	3.46/2.47	31.8*	0.1	1.0
Mean Hypothalamic E (µg/g)	2.27/2.69	2.35/4.04	1.34/2.28	1.60/1.60	17.6*	4.9*	1.2
Mean Brain Stem NE (µg/g)	0.83/0.83	1.06/0.69	0.93/0.67	1.07/0.64	70.7*	0.4	2.5*
Mean Brain Stem E (µg/g)	0.65/0.66	0.53/0.52	0.24/0.34	0.21/0.27	8.0*	13.1*	0.5

n in all cells = 3 samples individually assayed.

*Statistically significant, $P < 0.05$, 2-way analysis of variance

$F_{(S)}$ (Strain), $F_{(W)}$ (Weeks), $F_{(SW)}$ (Strain x Weeks interaction)

BP = blood pressure, NE = norepinephrine, E = epinephrine