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The Effect of Dietary Sodium and Potassium Upon Blood Pressure and Catecholamine Excretion in the Rat (40483)

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Clinical, epidemiological, and animal studies have shown that excess dietary sodium is in some way causally related to essential hypertension. Extensive reviews of the evidence for this effect of sodium have recently been conducted (1, 2). Meneely (3) and Dahl (4) were among the first to show that the common laboratory rat could be induced to develop hypertension when fed sodium in amounts comparable on a caloric basis to that found in "acculturated" societies. Dahl further showed that there was a genetic substrate for sodium dependent hypertension in these animals (4).

Meneely later showed that small increases in dietary potassium conferred some degree of protection against the effects of excess sodium in animals. In his studies, blood pressures in a group of rats receiving a moderately high sodium diet and a dietary potassium supplement did not differ significantly from that of a group fed a similar high sodium ration without potassium, but the treatment did lead to greater longevity (2, 5, 6). Louis (7) revealed a blood pressure lowering effect of supplemental dietary potassium in the Okamoto-Aoki SHR model fed excess sodium. There is also evidence that dietary potassium may be antihypertensive in man (8, 9) and that it might protect against the hypertensigenic effects of excess dietary sodium (10–12). The mechanism of these effects of potassium remain unknown.

DeChamplain *et al.* (13) observed in rats consuming excess sodium that the capacity of rat neural tissue to bind and store catecholamines, was greatly influenced by sodium intake. Catecholamine uptake across axonal membranes was normal but binding or retention was greatly reduced leading to increased turnover and decreased endogenous norepinephrine levels. These changes occurred before the appearance of hypertension and therefore could have participated in the pathogenesis of the disorder and could be returned to normal by a low sodium intake.

The present study was conducted to ascertain whether or not changes in the handling of catecholamines could be demonstrated in Sprague-Dawley derived rats made hypertensive by dietary sodium. In addition, consideration was given to the demonstrated protective role of a small dietary supplement of potassium and how this relates to blood pressure and catecholamine excretion.

Materials and methods. Care of animals. Ninety male Sprague-Dawley derived rats purchased from Holtzman Co. and initially weighing 94.3 ± 0.8 g (SD) were housed three to a cage in a climate controlled room (23 ± 2° and 70 ± 4% relative humidity) with 9.5 hr of light from 8:30 AM to 6:00 PM. Animals were divided into three groups of 30 animals per group upon arrival and placed on the special diets consisting of Purina Lab Chow with the following total sodium and potassium content: 0.21 mEq Na/g and 0.23 mEq K/g (Control Diet), 0.91 mEq Na/g and 0.24 mEq K/g (high sodium diet), and 0.98 mEq Na/g and 0.35 mEq K/g (high sodium with potassium supplement diet).[1] Deionized water and feed were provided *ad libitum.* The animals' weights and systolic blood pressures were recorded periodically. Blood pressures were measured using a programmed electrosphygmomanometer[2] and cuff over the caudal artery. After 12 months on the diets, animals were selected from the high sodium diet group such that all animals with very high blood pressures (BP > 163) were represented (High Na* group), and a group of animals with blood pressures clustered near the mean for that dietary group (High Na group) were included. Animals in the high

[1] Formulated by Bioserve, Inc., Frenchtown, New Jersey.

[2] Narco Biosystems, Houston, Texas.

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salt with potassium supplement group (High Na + K) that had blood pressures near the mean for the group were also selected and a group from the control diet group was selected using a system of random numbers. These selected animals were then housed individually in Wahman LC 176 metabolic cage units and allowed 5 days to become accustomed to their new surroundings before the collection of 24 hr urine specimens.

Collection of urine specimens. Twenty-four-hr urine specimens for the assay of norepinephrine and epinephrine content were collected at room temperature in brown glass medicine bottles containing 10 ml 1 *N* acetic acid and 2 mg of ascorbic acid as preservatives. Several open beakers containing 1 *N* acetic acid were placed in the animal room 48 hr prior to the period of sample collection to acclimate the animals to the odor. At the end of the collection period, the urinary volume was recorded, the pH adjusted to 3.0 with 1 *N* HCl, and the sample was frozen and stored at −20°.

Extraction and purification of urinary catecholamines. Each urine sample was thawed and filtered through three thicknesses of Whatman #1 filter paper. The volume of each was again recorded, and the sample was diluted to 120 ml with distilled water. Each specimen was divided into three equal aliquots and two were refrozen. Five hundred mg of ethylenediaminetetraacetate (EDTA) were added to the remaining sample along with approximately 100,000 dpm of ^{3}H norepinephrine (1.2 ng) for the later determination of column recovery. A 1 cm diameter chromatographic column was prepared by packing with approximately 1.5 g (dry weight) of acid washed, fine free aluminum oxide in water. The column was used within 30 min to minimize packing. The urine sample was adjusted to a pH of 8.2–8.5 with NaOH (2 *N*, 1 *N*, and 0.1 *N*) and immediately poured onto the column and chromatographed using a modification of the method of Von Euler and Lishajko (14). Catecholamines were eluted with 0.25 *N* acetic acid and column recovery of norepinephrine was determined by counting a small aliquot of the eluate for ^{3}H norepinephrine. Samples having recoveries of less than 60% were rerun. The mean recovery of all samples was 69.8 ± 11.8%.

Assay technique. Urinary epinephrine and norepinephrine were quantitated by the automated method of Viktora, Baukal and Wolfe (15). An aliquot of each alumina column eluate was added to a scintillation cocktail (Ready Solv HP, Beckman Instruments), and the amount of tritiated norepinephrine was measured (Beckman LS/250 liquid scintillation counter) so that column recovery of catecholamines could be determined. The samples were then assayed fluorometrically (Aminco-Bowman model H spectrophotofluorometer). Standard curves for norepinephrine, epinephrine and mixtures of the two amines prepared in acetic acid were run before and after each six urine samples. In addition to the urine samples and the catecholamines used for standard curves, norepinephrine standards and aliquots of pooled urines to which known amounts of epinephrine and/or norepinephrine had been added were carried through the column procedure and assayed to assure the precision and accuracy of the method.

Results. Dietary group and body weight. Analysis of variance (ANOVA) of the body weights of the various treatment groups revealed slight but significant differences among the groups. The control group's weight was significantly higher ($p < .05$) than that of the High Na group and High Na + K group by the 2nd dietary month and remained different for the duration of the study. The body weights of the High Na and High Na + K groups did not differ (Fig. 1). A

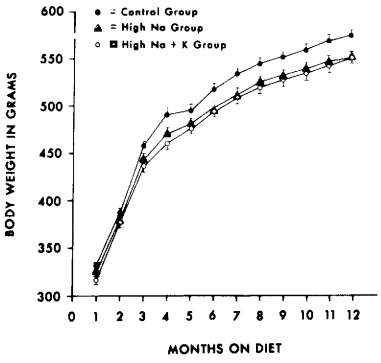

FIG. 1. Differences in body weight among dietary groups containing differing amounts of sodium and potassium are depicted. The control diet group differed from each of the remaining groups by the second dietary month ($p < .05$) and remained different for the duration of the study.

comparison of the body weights of the animals selected for catecholamine excretion studies revealed similar results. The mean body weights ± SEM of these animals were: 559 ± 13 g, 533 ± 12 g, 527 ± 7 g, 541 ± 6 g for the Control group, High Na group, High Na* group, and High Na + K group respectively at the time urine samples were collected.

Dietary groups' heart rates and blood pressure. The mean of the systolic blood pressures or each of the entire treatment groups, including animals selected for urinary catecholamine assays did not vary significantly early in the study, but by the 6th dietary month the mean systolic pressure of the High Na group (151 ± 3 mmHg) was significantly different from that of the Control group (136 ± 2 mmHg, $p < .001$) and that of the High Na + K group (141 ± 2, $p < .001$), which itself varied significantly from Control ($p < .05$). Significant differences in the blood pressure between the groups persisted through the 12th dietary month at which time animals were selected from each of the groups for catecholamine studies. The mean systolic pressures at the 12th month were 133 ± 2 mmHg, 151 ± 4 mmHg, and 143 ± 3 mmHg for the Control, High Na, and High Na + K groups respectively. The blood pressures of groups of animals selected at the 12th dietary month for catecholamine studies are depicted in Fig. 2 along with a small table of p values. The mean systolic pressures were: 134.3 ± 0.4 mmHg, 155.5 ± 2.1 mmHg, 169.8 ± 3.9 mmHg and 139.8 ± 2.2 mmHg for the Control, High Na, High Na*, and High Na + K groups respectively. Each group consisted of 10 animals with the exception of the High Na* group which had five animals. No significant differences in the heart rates among the groups of the animals selected for catecholamine studies were demonstrated although the mean heart rates did vary considerably. The mean heart rates were 372 ± 9, 390 ± 11, 366 ± 6, and 369 ± 8 for the Control, High Na, High Na*, and High Na + K groups respectively.

Dietary groups and catecholamine excretion. The urinary excretion of norepinephrine differed greatly among the dietary groups. The mean excretion of norepinephrine in the High Na group was 4.3 ± 0.3 μg/kg/24 hr which was significantly greater than the 3.2 ± 0.3 μg/kg/24 hr excreted by the Control group ($p < .01$) and the 3.6 ± 0.3 μg/kg/24 hr found in the High Na + K group ($p < .05$). No other significant differences in norepi-

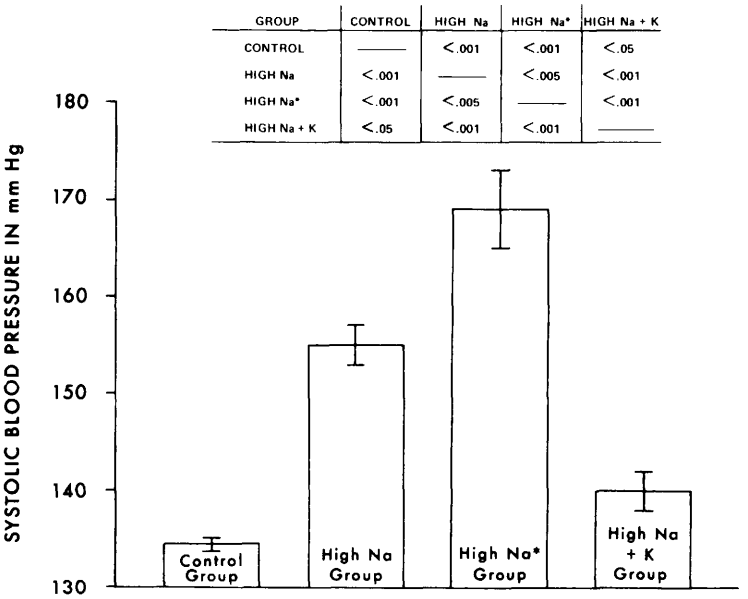

FIG. 2. Dietary treatment groups and the systolic blood pressures of groups of animals selected for catecholamine studies at the end of the twelfth dietary month are shown along with a table of p values.

nephrine excretion were found among the groups. The mean excretion of norepinephrine for the High Na* group was 3.5 ± 0.3 μg/kg/24 hr. See Fig. 3.

Epinephrine excretion was similarly altered among the dietary groups. The 2.8 ± 0.3 μg/kg/24 hr epinephrine found in the urine of the High Na group was significantly higher than the value of 1.3 ± 0.1 μg/kg/24 hr found in the Control group ($p < .001$), as was the 2.2 ± 0.4 μg/kg/24 hr found in the High Na* group ($p < .02$) and the 2.1 ± 0.2 μg/kg/24 hr found in the High Na + K group ($p < .01$). ANOVA excluding the Control group revealed no further significant differences in epinephrine excretion among groups. See Fig. 4.

Comparison of total urinary catecholamine excretion also revealed some significant differences among the dietary treatment groups. The mean total catecholamine excretion for the High Na group was 7.2 ± 0.5 μg/kg/24 hr which was significantly different from the 4.5 ± 0.3 μg/kg/24 hr excreted by the Control group ($p < .001$) and the 5.8 ± 0.4 μg/kg/24 hr excreted by the High Na + K group ($p < .02$). The total catecholamine excretion of the High Na + K group also differed from that of the Control group ($p < .02$). No other significant differences in total urinary catecholamine excretion were found among the groups. The High Na* group had a mean excretion rate of 5.8 ± 0.6 μg/kg/24 hr. See Fig. 5.

Catecholamine assay variance. Between assay variance for norepinephrine was evaluated by including a 1.0 μg standard of norepinephrine in each assay. The mean assayed norepinephrine value for 6 such standards was 1.12 ± 0.07 μg and considered acceptable. Since the epinephrine concentration was determined by taking the difference between the total catecholamine concentration and the norepinephrine concentration, epinephrine assays were also run on each of the above norepinephrine standards to determine the degree of overlap between the two types of measurements. The mean epinephrine concentration determined from the norepinephrine standards was 0.16 ± 0.02 μg and considered acceptable.

Discussion. In this study, the effect of a moderately high sodium diet upon blood

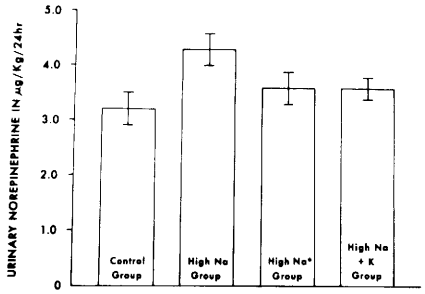

FIG. 3. The twenty-four-hr urinary excretion of norepinephrine among the four groups of animals selected for catecholamine evaluation is depicted. The mean excretion of norepinephrine in the High Na group was significantly greater than the control group's value ($p < .01$) and that of the High Na + K group ($p < .05$).

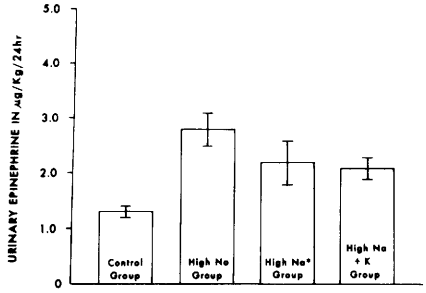

FIG. 4. The twenty-four-hr urinary epinephrine excretion rate among the four groups of animals selected for catecholamine studies is shown. Epinephrine excretion was significantly greater in the High Na group when compared to controls ($p < .001$) as was that of the High Na* group ($p < .02$) and the High Na + K group ($p < .01$).

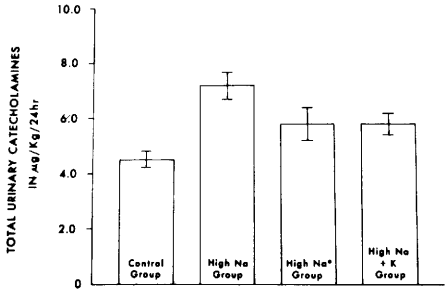

FIG. 5. Twenty-four-hr urinary excretion rate of total catecholamines for the four groups of animals selected for assay is illustrated. The mean excretion of total catecholamines in the Control diet group was significantly less than that of the High Na group ($p < .001$) and the High Na + K group ($p < .02$). The excretory rate of the High Na group was also significantly greater than the High Na + K group ($p < .02$).

pressure and the protective effects of small supplements of dietary potassium in the common Sprague-Dawley derived laboratory rat were reexamined. Meneely (5) had earlier reported that the life shortening effects of a moderately high sodium diet could be prevented by small supplements of dietary potassium, but, since there was no significant difference between the blood pressures of the two test groups, the increased longevity could not be attributed to any antihypertensive effect of the additional dietary potassium. This lack of a demonstrable difference in blood pressure could have been the result of convergence toward the mean in that animals with the highest blood pressure, the animals that would affect the mean of the group the most, experience the greatest mortality. This has the effect of reducing the mean of the group and increasing its variance due to the smaller number of surviving animals. If the blood pressures in this earlier study are examined at the 12th dietary month before any significant mortality occurred, the data suggest that there were differences in the mean systolic blood pressures of the two groups that were perhaps later obscured. The present data demonstrated that significant differences in blood pressure between animals eating a moderately high sodium diet and animals eating a similar diet but including a small potassium supplement appeared by the 6th dietary month and persisted until the termination of the study at the end of the 12th dietary month.

In an attempt to gain some insight into hypertension brought about by dietary sodium and the role of potassium in this process, urinary catecholamines were collected and assayed. The authors are cognizant of the many caveats of the measurement of urinary catechols as an index of sympathetic nervous system activity. Many of these also apply to other commonly used indices such as plasma levels, tissue turnover studies, etc. (16). No single method seems sufficient as such an index. Never-the-less, with the controls that can be exerted over experimental hypertensive models, urinary samples do afford a number of advantages over other methods that made the method seem suitable for a preliminary study. Samples can be collected from unstressed, unanesthesized animals in their natural habitat that represent the cumulative excretion of catecholamines over the period of an entire day and the animals may be further utilized in a continuing study, as was the case in this investigation. However, careful attention must be given to some of the secondary effects of hypertension such as impaired renal function. Ikoma (17) has shown that the urinary excretion rate of catecholamines in human essential hypertension was significantly related to blood pressure as long as renal function was normal, but there was no relationship between the two in subjects with renal damage. In general, as renal impairment progressed, catecholamine excretion decreased. Our results tend to support these observations in that animals in our study that developed severe hypertension manifest diminished norepinephrine and epinephrine excretion when compared to moderately hypertensive animals. All of the severely hypertensive animals, with the exception of one, succumbed to a fulminating hypertension within a few weeks after urinary catecholamines were collected suggesting that the animals had probably sustained some renal damage by the time of the collection. There were no deaths in the other groups. This rapidly progressing malignant hypertension-like syndrome accompanied by severe nodular glomerulosclerosis has been repeatedly observed in our rat colony over the past several years. As animals' systolic blood pressure approach approximately 170 mmHg, the hypertensive processes appear to accelerate and the animal usually dies within a few weeks with pressures in excess of 200 mmHg (unpublished observations). In addition to the renal factor, it is possible that as hypertension becomes more severe, sympathetic activity diminishes due to the hypertrophic effects of increased pressure upon the structure of resistance vessels (18) and inhibitory feedback effects by buffer nerves (19).

Further consideration of the catecholamine excretion of the treatment groups revealed that animals with modest elevations in blood pressure excreted 34% more norepinephrine than did normotensive animals on a control diet. The inclusion of a small amount of additional potassium in the high sodium diet resulted in a norepinephrine excretion rate that was 19% less than that of the moderately

hypertensive group and indistinguishable from normotensive controls. Epinephrine excretion was similarly affected. The moderately hypertensive animals excreted 115% more epinephrine than normotensive controls and 33% more epinephrine than animals fed a high sodium diet with supplemental potassium. The epinephrine excretion of the potassium supplement group was also greater than that of the normotensive control group being elevated 62%.

Although these observations are themselves suggestive and interesting, it is difficult to understand their exact role in the pathogenesis and maintenance of hypertension without additional more definitive studies. It is clear that in the hypertensive animals the excretion of both norepinephrine and epinephrine were elevated and that this elevation in catecholamine excretion was moderated by small dietary supplements of potassium, as was the hypertension. When normal cardiovascular reflexes are taken into account, the excretion of catecholamines in hypertensive animals appears inappropriately high for the level of blood pressure observed. The mechanism of the antihypertensive effect of dietary potassium remains unknown.

Summary. Male Sprague-Dawley derived rats fed a moderately high sodium diet became hypertensive by the 6th dietary month. After the 12th dietary month, the urinary excretion of epinephrine and norepinephrine was moderately increased in this group. The inclusion of a small dietary supplement of potassium in the diet had an ameliorating effect upon the development of hypertension and resulted in lower excretion rate for catecholamines.

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