HYPERTENSION INDUCTION IN DAHL RATS

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There is experimental and epidemiologic evidence that some minerals and trace elements play a role in hypertension. We designed an experiment in which salt and water sources were manipulated to examine the possible impact of this relationship.

A strain of rats (Dahl rats) known to become hypertensive with sodium chloride ingestion was used to study the effect of salt source and water source on the induction of hypertension. The group on tap water and table salt had blood pressures (184 mmHg ± 19) significantly higher than every other group in the experiment. The experimental animals receiving tap water plus table salt had the highest blood pressure levels, although they consumed the lowest quantity of sodium. Analysis of the tap water samples showed "soft water" by analysis of calcium and magnesium concentration. This could adversely affect blood pressure. The relatively high magnesium concentration in sun evaporated sea salt may play a protective role in hypertension induction. The zinc and copper present in tap water may play an exacerbating role.

Key words • hypertension • sodium chloride • trace minerals • rat model

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There has been much research into the etiology of hypertension. Family history and stress are known to be contributing variables. A great deal of scientific literature is available regarding the relationship between salt and hypertension.[1-3] Recently, epidemiologic data have indicated a positive correlation between soft and corrosive tap water and mortalities from hypertensive heart disease.[4-6]

Very little research has been done on the source of sodium chloride as a factor in the development of hypertension.[7] Several studies have examined the relationship between water hardness, mineral and trace metal content, and hypertensive heart disease. These data have suggested a protective role for calcium, magnesium, lithium, and vanadium. In general, they have demonstrated a contributing role for cadmium, lead, copper, and zinc.[4,5,8-11] The concentration of these substances differs in relation to the water source and in relation to the salt source.[8]

Table salt is produced in two markedly different ways: solar evaporation and vacuum pan evaporation.[12] Salt produced by solar evaporation starts with the collection of sea water in shallow ponds. The water is evaporated by the sun and wind. The saturated brine is then pumped into a crystallizing pond, which has a deep salt floor that has precipitated over years. This evaporation process takes place over several months and is continuous. As the solution is saturated with salt, several inches of new salt is crystallized on the floor of the pond. The pond is drained, and the salt is harvested mechanically.

Salt produced by the evaporation of salt brine in large commercial evaporators begins with solution mining. Once a large underground salt table is located, a well is sunk into it. A second well is sunk approximately 1000

TABLE 1. SODIUM COMPOSITION OF DIET*

Rat No.	Regimen	Group No.	Period 1	Period 2	Period 3	Overall Mean
1–8	Control	1	2.9 ± 0.5	2.6 ± 0.3	3.2 ± 0.3	2.9 ± 0.42
10–16 (#9 died)	Tap water Table salt	2	5.3 ± 1.5	4.8 ± 0.6	6.3 ± 0.4	5.5 ± 1.1
17–24	Tap water Sea salt	3	6.0 ± 1.0	5.9 ± 1.5	6.9 ± 0.6	6.3 ± 1.1
25–32	Distilled water Table salt	4	5.5 ± 0.8	6.9 ± 0.7	6.3 ± 0.6	6.2 ± 0.9
34–40 (#33 died)	Distilled water Sea salt	5	6.6 ± 1.4	5.2 ± 0.9	6.6 ± 0.2	6.1 ± 1.1

ANOVA† (Significance $P < .05$)

Group 2 vs 3 $P = .00694$ — Group 3 vs 4 $P = 1.000$
Group 2 vs 4 $P = .01084$ — Group 3 vs 5 $P = 1.000$
Group 2 vs 5 $P = .02642$ — Group 4 vs 5 $P = 1.000$

*(mEq Na/rat/day)
†Average sodium consumption per day for each rat in a given group was compared with the same data for the second group.

feet away into the same vein. The first well is then flooded. The water dissolves the salt and the solution comes up through the second well; the brine then is stored in tanks. Subsequently, the brine is pumped into a series of vacuum pans where it is boiled by steam heat. A slurry (mixture of brine and salt) is collected and passed to a filter dryer that removes the moisture, leaving a residue of salt crystals. One salt company adds a coat of sodium silica aluminate to keep the salt free flowing in damp weather. Salt produced in this manner is commonly referred to as table salt.

Distilled water is chemically pure water collected as steam. Tap water, on the other hand, has been shown to be contaminated with various minerals and trace elements.[13]

MATERIALS AND METHODS

Forty female weanling Dahl rats, a strain of rat known to be salt sensitive, were obtained from the Brookhaven National Laboratory in Long Island, New York. These rats were randomly distributed into five groups of eight rats each. All groups were placed on a special salt- and sucrose-free diet (manufactured by ICN Nutritional Biochemicals), which was available ad libitum.

The control diet consisted of 20% casein purified high nitrogen, 0.3% dl-methionine, 20.0% corn starch, 35.0% soybean protein, 10.0% maple sugar, 5.0% alphacel, non-nutritive bulk, 5.0% corn oil, 3.5% AIN mineral mixture modified, 1.0% AIN vitamin mixture (modified), and 0.2% choline bitartrate.

The modified AIN mineral mixture contained no sucrose or sodium chloride. The composition was as follows: 500.0 g/kg calcium phosphate, dibasic ($CaHPO_4$), 220.0 g/kg potassium citrate, monohydrate ($HOC(COOK)(CH_4COOK)_4 \cdot H_2O$, 52.0 g/kg potassium sulfate (K_2SO_4), 24.0 g/kg magnesium oxide (MgO), 3.5 g/kg manganous carbonate (43–48% Mn), 6.0 g/kg ferric citrate (16–17% Fe), 1.6 g/kg zinc carbonate (70% ZnO), 0.3 g/kg cupric carbonate (53–55% Cu), 0.01 g/kg potassium iodate ($KI0_3$), 0.01 g/kg sodium selenite ($Na_2SoO_3 \cdot 5H_2O$), and 0.55 g/kg chromium potassium sulfate ($CrK(SO_4)_2$-$12H_2O$) with soybean protein.

The modified AIN vitamin mixture contained no sucrose or sodium chloride and had the following composition: thiamine hydrochloride 600 mg, riboflavin 600 mg, pyridoxine HCl 700 mg, nicotinic acid 3 g, D-calcium pantothenate 1.6 g, folic acid 200 mg, D-biotin 20 mg, cyanocobalamin (vitamin B_{12}) 1 mg, retinyl palmitate (vitamin A) pre-mix (250 000 μ/g) 800 mg, dl-alpha-tocopheryl acetate (vitamin E) pre-mix (250 μ/g) 20 g, cholecalciferol (vitamin D_3) (400 000 μ/g) 2.5 mg, and menoquinone (vitamin K) 5.0 mg OS with soybean protein.

The salt regimen was delivered in the water bottles which were available on demand. The sodium consumption of the rats is listed in Table 1.

Initially, the rats were screened for marked differences in blood pressures and weights between groups. None were found. The rats were then started on the experimental protocol. One month later, the first set of blood pressure determinations were made. Six weeks later, the second set was taken. Six weeks after that, the third and final set was taken. These data are listed in Table 2.

TABLE 2. SYSTOLIC BLOOD PRESSURE

Rat No.	Regimen	Group No.	Period 1*	Period 2*	Period 3*	Overall Mean
1–8	Control	1	157 ± 15	169 ± 11	169 ± 10	165 ± 13
10–16 (#9 died)	Tap water Table salt	2	171 ± 16	184 ± 9	196 ± 23	184 ± 19
17–24	Tap water Sea salt	3	166 ± 16	166 ± 16	184 ± 17	172 ± 18
25–32	Distilled water Table salt	4	163 ± 21	174 ± 18	167 ± 18	168 ± 19
34–40 (#33 died)	Distilled water Sea salt	5	157 ± 20	173 ± 9	188 ± 20	173 ± 21

ANOVA† (Significance $P < .05$)

Group 1 vs 2 $P = .00014$
Group 1 vs 3 $P = .13577$
Group 1 vs 4 $P = .45605$
Group 1 vs 5 $P = .10065$
Group 2 vs 3 $P = .01496$
Group 2 vs 4 $P = .00159$
Group 2 vs 5 $P = .02735$
Group 3 vs 4 $P = .44682$
Group 3 vs 5 $P = 1.000$
Group 4 vs 5 $P = .35241$

*(mmHg) Value represents mean of individual values at close of that period; at least three measurements were taken.
†The average of the multiple blood pressure readings at the end of each period for each rat in a given group was compared with the same data for the second group.

Group 1 was the control. They were given distilled water containing a maintenance dose of salt (half sea salt and half table salt). The concentration of the salt solution in group 1 was one half that of the other groups. Group 2 was given tap water and table salt. Group 3 received tap water and sea salt. Group 4 was given distilled water and table salt. Group 5 received distilled water and sea salt.

Blood pressure measurements were recorded on all rats on the same day to minimize variation. The equipment used is marketed by IITC (Innovators in Instrumentation, Inc, Lansing, New Jersey), including a heating chamber that accommodates three rats simultaneously, a PE-300 automatic programmed electrosphygmomanometer and baumanometer system, a model 663 channel preamplifier, and a three-channel chart recorder. The blood pressure measurements were recorded from the tails of the individual rats using a previously described technique.[7] All measurements were taken in triplicate on each animal.

All salt and water specimens were analyzed by atomic absorption spectrophotometry. Constituents (magnesium, calcium, sodium, potassium) and certain trace metals (copper, zinc, and lithium) were measured by direct aspiration of solutions. Flameless atomic absorption techniques using a heated graphite atomizer (HGA-2100 with Perkin-Elmer Model 503) were used for the determination of vanadium and cadmium.

In all cases blanks and quality control specimens were analyzed together with these samples. The data obtained from the analysis of these specimens is listed in Table 3. The sodium concentrations were chosen so as to give the control group the basic sodium requirement for these animals (3–4 mEq/kg/day) and to give the experimental groups approximately three times this amount. In reality, as feeding was provided on demand, the control group received approximately twice the sodium requirement and the experimental groups received approximately four times the basic requirement.

TABLE 3. TRACE METAL CONCENTRATION IN SEA SALT, TABLE SALT, TAP WATER, AND DISTILLED WATER

	Sea Salt (ppm)	Table Salt (ppm)	Tap Water (μg/dL)	Distilled Water (μg/dL)
Copper	2	2	6	0
Zinc	2	2	10	0
Magnesium	2050	4.5	213	0
Calcium	9	237	375	0
Potassium	5	5	56	0
Sodium	3.75×10^5	3.75×10^5	625	0
Cadmium	0.13	0.08	0	0
Vanadium	1.0	1.2	1.2	0.5
Lithium	2.5	2.0	0	0

PPM = μg/g salt

The data were evaluated by analysis of variance.

RESULTS

The experiment was conducted over 4 months. Blood

pressure determinations were made three times during this period. In general, blood pressure levels rose with each successive determination. The control group had the lowest mean blood pressures (165 ± 13 mmHg), whereas group 2 on tap water and table salt had the highest levels (184 ± 18 mmHg).

These data were examined by analysis of variance (ANOVA). The group on tap water and table salt had blood pressure levels significantly higher than those of the other groups in the experiment.

Blood pressure levels of group 2 were higher than those of the control group and the other experimental groups (Table 2). All other groups were analyzed relative to each other, but no statistically significant difference was found.

An analysis of variance was used to compare the experimental groups. It was striking that the sodium consumption of group 2 varied significantly from all of the other experimental groups—in group 2 sodium ingestion was markedly lower. If we compare group 2 with group 3, sodium consumption was 5.5 vs 6.3 mEq/rat/day; if we compare group 3 with group 4, sodium consumption was 5.5 vs 6.2 mEq/rat/day; and if we compare group 2 with group 5, sodium consumption was 5.5 vs 6.1 mEq/rat/day. This result further heightens the significance of the blood pressure differences.

The values resulting from atomic absorption spectrophotometry are listed in Table 3. The most striking feature with regard to the salt specimens was the difference in the magnesium concentrations. The difference was greater than 4×10^2. The sodium concentration was equal in both types of salt.

The New Jersey tap water samples were found to be "soft" by calcium and magnesium concentration. Copper was found in a concentration of 6 μg/dL and zinc was found in a concentration of 10 μg/dL.

DISCUSSION

The enhanced hypertensinogenic effect of table salt and tap water was especially notable, as this effect was demonstrable despite a significantly lower consumption of salt and water. Soft water with zinc and copper present may also enhance the hypertensinogenic effect. The spectrophotometric data were useful in analyzing our results. The high concentration of magnesium in sea salt was a significant finding.

Several studies demonstrate the blood pressure-lowering effect of magnesium.[9,14] Recent data indicate that magnesium affects vascular tone, reactivity, and total peripheral resistance.[15,16]

Also, epidemiologic data indicate that populations exposed to soft water (low in calcium and magnesium) have a relatively higher incidence of hypertension and hypertensive cardiovascular disease mortality.[4-6,8,13,17] Soft water, copper, and zinc have been shown to be related to hypertension and hypertensive cardiovascular disease.[10,11]

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