Beneficial Effects of a Potassium- and Magnesium-Enriched Salt Alternative

Eero M.A. Mervaala, Jaakko-Juhani Himberg, Juha Laakso, Päivi Tuomainen, and Heikki Karppanen

The effects on blood pressure and the development of cardiac hypertrophy of sodium chloride (regular salt) and a novel potassium-, magnesium-, and l-lysine-enriched salt alternative, which in a previous study prolonged the life span of hypertensive rats nearly threefold as compared with the animals receiving regular salt, were compared both in spontaneously hypertensive rats and their hypertension-resistant genetic controls. In particular, the possible protective effect of increased intakes of potassium, magnesium, and l-lysine during a high intake of sodium chloride was examined. Therefore, the salt alternative was added at 1.75 times higher levels to produce the same dietary levels of sodium chloride in the regular salt and the salt alternative groups. Regular salt produced a remarkable left ventricular hypertrophy in both rat strains, but as compared with the respective control groups, it induced an increase of blood pressure only in the spontaneously hypertensive rats. The salt alternative did not induce a rise in blood pressure in either of the rat strains, nor did it produce left ventricular hypertrophy in the hypertension-resistant rats and, in the spontaneously hypertensive animals, significantly less hypertrophy than regular salt. The salt alternative appeared to prevent the sodium chloride–induced volume load since plasma levels of atrial natriuretic peptide were increased in the regular salt groups but remained normal in the salt alternative groups. Therefore, potassium, magnesium, and/or l-lysine of the salt alternative produced a powerful protection against the harmful effects of sodium chloride. (Hypertension 1992;19:535–540)

KEY WORDS • essential hypertension • hypertrophy • sodium • potassium • magnesium • lysine • atrial natriuretic peptides • norepinephrine

High blood pressure is one of the main risk factors of heart attack, stroke, and congestive heart failure. Long-term arterial hypertension causes left ventricular hypertrophy (LVH), which is thought to represent an adaptive process of the heart, serving to compensate for the increased hemodynamic burden in hypertension. However, LVH has been identified as a powerful independent risk factor for cardiovascular diseases and sudden death. Reversal of LVH has become one of the main therapeutic goals in the treatment of essential hypertension.

Recent investigations have demonstrated the important role of high sodium chloride intake in arterial hypertension. Recently Law and coworkers provided further evidence that salt reduction lowers elevated blood pressure. High intake of dietary sodium chloride increases heart weight in experimental studies even without elevation in blood pressure and also may be an independent risk factor of LVH in humans. On the other hand, restriction of the dietary sodium chloride intake was able to produce regression of LVH. The mechanisms of salt-induced LVH remain unclear.

It has been estimated that even a moderate reduction of sodium chloride intake by 3 g per person each day, by a whole Western population, would reduce the incidence of stroke and ischemic heart disease in a population more effectively than the treatment of high blood pressure with drugs. Moreover, recent evidence indicates that potassium supplementation lowers blood pressure both in experimental animals and in humans. Increased intake of magnesium may also produce various beneficial cardiovascular and metabolic effects, at least in magnesium-deficient patients.

Even though there is a lot of evidence for the public health benefits of reduced intake of sodium chloride and increased intakes of potassium and magnesium, no practical, economical, and safe means have been available for the production of such changes in a population. The salt substitutes available so far have not been satisfactory with regard to taste and the technological requirements of the food industry whose products are largely responsible for the present high intakes of sodium chloride. Moreover, the salt substitutes with high potassium contents are, in general use, potentially hazardous due to the risk of producing hyperkalemia.

A recent innovation has described favorable taste and technological and health interactions of potassium, magnesium, and l-lysine with sodium chloride. Based on this innovation, a salt alternative with reduced content of sodium chloride and moderate concentrations of potas-
sium, magnesium, and L-lysine has recently been introduced into general use in Finland and Japan.30

In view of the recent findings and claims of the role of sodium chloride, potassium, and magnesium in cardiovascular diseases and the increasing use of the novel salt alternative, it appeared warranted to compare the cardiovascular effects of the salt alternative with those of regular salt. Therefore, we examined its effects on blood pressure and the development of cardiac hypertrophy in both spontaneously hypertensive rats and their hypertension-resistant control animals. In particular, the ability of increased intakes of potassium and magnesium to counteract the harmful effects of high intakes of sodium chloride was examined. We also tried to elucidate the mechanisms of the observed effects by measuring the plasma levels of atrial natriuretic peptide and the myocardial content of norepinephrine.

Methods

Experimental Animals and Diets

Twenty-nine male spontaneously hypertensive rats (SHR) of the Okamoto strain and thirty-one male rats of the Wistar-Kyoto (WKY) strain were purchased from Mollegaards Breeding Centre, L.I. Skensved, Denmark. At the beginning of the study, the 9-week-old SHR and WKY rats were randomly assigned to three subgroups to receive different diets. The high sodium chloride diet was produced by adding sodium chloride (Merck, Darmstadt, Germany) to the standard rat pellets (Finnewos Aqua, Helsinki, Finland). The high sodium chloride diet with potassium and magnesium supplements was produced by adding a commercially available salt alternative (Pansalt, Oriola Oy, Espoo, Finland) with the following composition (%): NaCl 57, KCl 28, MgSO4·7H2O 12, L-lysine hydrochloride 2, and antiacaking agents (MgCO3, SiO2) 1, to the standard rat pellets. The high sodium chloride diet with potassium and magnesium supplements was produced by adding a commercially available salt alternative (Pansalt, Oriola Oy, Espoo, Finland) with the following composition (%): NaCl 57, KCl 28, MgSO4·7H2O 12, L-lysine hydrochloride 2, and antiacaking agents (MgCO3, SiO2) 1, to the standard rat pellets. The contents of sodium, potassium, and magnesium in the different diets used in our experiment are presented in Table 1. The rats had free access to tap water and food pellets during the follow-up time. Measurement of body weight at 1-week intervals was used as an indicator of any marked difference in food or water consumption among the groups.

Measurement of Blood Pressure and Sample Preparation

Systolic blood pressure was measured weekly by using the tail-cuff method and a blood pressure recorder (model 8002e, W+W Electronics Inc., Basel, Switzerland). Before the measurement the rats were warmed for 30 minutes at 32°C to make the pulsations of the tail artery detectable. Five to six consecutive measurements of the systolic blood pressure were performed on each rat openly by the same technician, and the arithmetic mean of the three last readings was recorded as the systolic blood pressure. After the 8-week experimental period, the rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.), the carotid artery was cannulated, and blood samples were drawn into chilled tubes on ice using EDTA as anticoagulant. The hearts were excised; great vessels, atria, and the free wall of the right ventricle were dissected; and the left ventricular mass (LVM) was estimated as left ventricular wet weight-to-body weight ratio.

<table>
<thead>
<tr>
<th>Mineral element</th>
<th>Normal sodium chloride diet</th>
<th>High sodium chloride diet with potassium and magnesium supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>0.4*</td>
<td>2.7*</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.9*</td>
<td>0.9*</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.2*</td>
<td>0.2*</td>
</tr>
</tbody>
</table>

Other nutrients (contents common for all diets)

<table>
<thead>
<tr>
<th>Mineral element</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Iron</th>
<th>Zinc</th>
<th>Manganese</th>
<th>Copper</th>
<th>Iodine</th>
<th>Cobalt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0*</td>
<td>0.75*</td>
<td>178 mg/kg</td>
<td>115 mg/kg</td>
<td>89 mg/kg</td>
<td>18 mg/kg</td>
<td>2 mg/kg</td>
<td>1 mg/kg</td>
</tr>
</tbody>
</table>

Vitamins

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>D3</th>
<th>E</th>
<th>K3</th>
<th>B1</th>
<th>B2</th>
<th>B5</th>
<th>B6</th>
<th>B12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12,000 IU/kg</td>
<td>1,500 IU/kg</td>
<td>63 mg/kg</td>
<td>0.25 mg/kg</td>
<td>10 mg/kg</td>
<td>3 mg/kg</td>
<td>12 mg/kg</td>
<td>5 mg/kg</td>
<td>0.02 mg/kg</td>
</tr>
<tr>
<td>Calcium pantothenate</td>
<td>10 mg/kg</td>
<td>Niacin</td>
<td>40 mg/kg</td>
<td>Choline chloride</td>
<td>1,000 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major constituents</td>
<td>Water</td>
<td>11.7*</td>
<td>Crude fat</td>
<td>5.3*</td>
<td>Crude protein</td>
<td>21.0*</td>
<td>Fiber</td>
<td>2.8*</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>Ash</td>
<td>7.0*</td>
<td>Metabolizable energy</td>
<td>13.0 MJ/kg</td>
<td></td>
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</tr>
</tbody>
</table>

*Values are expressed as % dry weight of the pellets.

Measurement of Myocardial Norepinephrine

The content of myocardial norepinephrine was analyzed by high-performance liquid chromatography (HPLC) using Ultratechsphere 5 ODS column and electrochemical detection (HPLC Technology, Cheshire, UK) based on the method of Männistö et al31 with the following modifications for the myocardial tissue. Samples from left ventricle were homogenized on ice with Ultraturrax (Ika-Labortechnik, Staufen, Germany) for 2 minutes (50,000 rpm, 1 min+1 min) in 1.0 ml (0.5 ml+0.5 ml) of cold 0.4 M perchloric acid containing 0.1% Na2S2O3, 0.15% EDTA, and 125 ng/ml 3,4-dihydroxybenzylamine as an internal standard. After centrifugation the clear supernatant was filtered through 0.45 μm HPLC grade syringe filter and 20 μl of the filtrate was injected into the HPLC system. The standard was obtained by diluting the stock solution of norepinephrine (1.0 mg/ml) with perchloric acid. A linear regression was obtained at a concentration range of 25–500 ng/ml. Detection limit was 20 pg per injection for norepinephrine. The content of myocardial norepinephrine was calculated by plotting the peak areas against the concentrations of norepinephrine standards.
nephrine was estimated as nanograms per gram wt weight.

Measurement of Atrial Natriuretic Peptide

Plasma atrial natriuretic peptide (ANP) was measured with a specific radioimmunoassay kit for rat atrial natriuretic factor-(99–126) (Peninsula Laboratories, Belmont, Calif.) according to the protocol of the manufacturer. The sensitivity for ANP was 10 pg/tub (tubes).

Statistical Analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) supported by Bonferroni test. Data for multiple observations over time were analyzed by two-way ANOVA with repeated measures for overall treatment effect, and the Bonferroni test was used for multiple pairwise comparisons of treatment groups at different times. The area under the curve (AUC) (blood pressure versus time) of each individual in different treatment groups was also calculated mathematically by the methods outlined by Matthews et al. The AUCs were then tested by ANOVA supported by Bonferroni test. Differences between means that had \( p < 0.05 \) were considered significant. The data were analyzed using BMDP statistical software (Los Angeles, Calif.). The results are expressed as mean±SEM.

Results

Blood Pressure, Left Ventricular Mass, and Body Weight in Spontaneously Hypertensive Rats

In SHR, a high intake of regular salt during the 8-week period produced a marked increase in systolic blood pressure (Figure 1). At 1 and 2 weeks, the age-related rise of blood pressure in the salt alternative group was significantly less than that in the controls (Figure 1). Therefore, no further rise of blood pressure took place in the salt alternative group, whereas the blood pressure continued to rise in the controls during the whole follow-up period. At the end of the follow-up period, the systolic blood pressure of the salt alternative group was even slightly lower than that of the controls in pairwise comparison. Overall, during the whole 8-week experimental period the blood pressure of SHR receiving the salt alternative did not differ from that of the control SHR as indicated by similar AUCs of blood pressures (Figure 1). LVM was clearly higher in control SHR than in control WKY rats (Figure 2). The high intake of the salt alternative also induced a significant increase in LVM of SHR as compared with control SHR. However, the salt alternative produced a significantly smaller increase in LVM than did regular salt (Figure 2).

No differences in the right ventricular mass could be detected among the groups (ANOVA, \( p = 0.4812 \)). There was not any significant difference in the body weight increase among the control group, regular salt group, and salt alternative group (body weight gains 57.8±4.1%, 48.7±2.0%, and 51.5±3.5%, respectively; ANOVA, \( p = 0.1666 \)). Two SHR were lost during the follow-up time. One rat of the control group died at 6 weeks and one rat of the regular salt group died at 4 weeks after the start of the experiment.

Blood Pressure, Left Ventricular Mass, and Body Weight in Wistar-Kyoto Rats

In WKY rats, regular salt during the 8-week period produced a significant increase in LVM (Figure 2) with a minor rise in systolic blood pressure (Figure 3). The salt alternative did not produce any rise in blood pressure or any increase in LVM of WKY rats (Figures 2 and 3).

No differences in the right ventricular mass could be detected between the groups (ANOVA, \( p = 0.2884 \)). Rats in the salt alternative group gained weight slightly slower than those in the control group (body weight gains, 46.6±1.3% and 55.9±2.5%, respectively; ANOVA, \( p = 0.0074 \); SA versus control, \( p < 0.01 \), but there was not any difference in the body weight increase between salt alternative and regular salt groups (46.6±1.3% versus 53.4±1.8%, \( p = \text{NS} \)).

Myocardial Norepinephrine

The content of myocardial norepinephrine was reduced in WKY rats receiving regular salt. A similar trend was seen in SHR, but the difference was not statistically significant. The levels of myocardial norepinephrine in the rats receiving the salt alternative were not different from those of the controls (Figure 4).
Plasma Atrial Natriuretic Peptide

The level of plasma ANP was elevated in both SHR and WKY rats receiving regular salt. In the salt alternative group there was no increase in the plasma ANP levels (Figure 5).

Discussion

The SHR of the Okamoto strain are generally considered to be an excellent model of human essential hypertension. In agreement with the previous findings by Tobian, Oparil et al., and ourselves, an increased intake of sodium chloride produced in the present study a remarkable rise of blood pressure in SHR. On the contrary, in the genetic normotensive controls of SHR, the WKY rat, the increased intake of sodium chloride produced only a minor if any hypertensive effect. The resistance of WKY rats and one substrain of SHR to the hypertensive effect of sodium chloride has previously been reported by Oparil et al. and Chen et al.

There was a marked difference in LVM between WKY rats and SHR during the control diet with relatively low sodium chloride content. This cardiac hypertrophy in SHR was probably due to the bigger pressure load of the myocardium. The further increase in the left ventricular mass of SHR after the increased intake of sodium chloride might have been, at least in part, due to the salt-induced elevation in blood pressure. However, the increased intake of sodium chloride also produced a marked increase in the LVM in WKY rats, despite only minor effect on blood pressure. Thus, the previous reports suggesting that high intake of sodium chloride could cause cardiac hypertrophy without elevation in blood pressure are supported by our findings. In fact, in spite of the markedly lower blood pressure levels, the LVM of the WKY rats after the increased intake of sodium chloride was enlarged to the same level found in control SHR. A high intake of sodium chloride also appears to be an independent risk factor of LVH in humans.

The mechanisms of the pressure-independent myocardial hypertrophy, induced by sodium chloride, are still unknown. An increased volume load may have been involved since the plasma ANP levels were elevated in both WKY rats and SHR with increased intakes of regular salt. A change in hemodynamic situation, leading to structurally redesigned left ventricle in the direction of a smaller lumen and a relatively thicker wall without a concomitant elevation in blood pressure in normotensive WKY rats with high sodium chloride intake has previously been described by Friberg et al. Interestingly, the development of LVH is associated with increased levels of ANP even in the myocardial tissue.

Previous studies have suggested that salt loading may increase the activity of the sympathetic nervous system. Increased adrenergic tone might also have partly accounted for the salt-induced myocardial hyper-
tropy. The decreased myocardial levels of norepinephrine in our study might have resulted from increased release or decreased reuptake, or both, of the neurotransmitter into the sympathetic nerve endings. Fields et al. have suggested that sodium chloride-induced cardiac hypertrophy is not mediated through increased cardiac neuronal sympathetic activity since they did not find any increase in norepinephrine turnover or in tyrosine hydroxylase activity. However, the mechanism of the decrease in myocardial norepinephrine levels also seen in their study remained unexplained.

The marked hypertensive effect of sodium chloride in SHR and the small effect seen in WKY rats were abolished when sodium chloride was derived from the salt alternative. The content of sodium chloride in the diet was adjusted to the same level in the rats receiving the salt alternative as in the rats receiving regular salt. Therefore, due to this experimental setting the beneficial effects of the salt alternative in the present study appear to be due to the potassium, magnesium, and l-lysine components. However, other possibilities should also be considered. Since we did not directly measure food intake or urinary excretion of electrolytes, a decreased intake of food, and thus sodium chloride, in the salt alternative group might explain the observed effects in this group. However, in the present study as well as in one previous study and in our very recent unpublished study, the rats receiving the salt alternative and regular salt gained weight similarly, suggesting unaltered food consumption. Another possibility for the effects of the salt alternative might be a toxic effect. In a previous long-term study, using the same dietary levels of regular salt and the salt alternative as in the present study, the average life span of SHR receiving regular salt was shortened to approximately one third that of the control SHR. On the contrary, in spite of the high intake of sodium chloride, the life span of the SHR receiving the salt alternative did not differ from that of the SHR receiving a normal sodium diet. These findings indicate that the observed effects of the salt alternative were indeed beneficial and by no means toxic in nature. Thus, neither of these factors appears to be involved.

Therefore, the increased intakes of potassium and magnesium would be the main reasons for the absence of the hypertensive effect of the salt alternative, as has been previously suggested. l-Lysine, which is crucial for avoiding the unpleasant aftertaste characteristic of potassium- and/or magnesium-containing salt alternatives, may also partly account for the absence of the hypertensive effect of the salt alternative.

Unlike the rats receiving regular salt, plasma ANP was not elevated in the animals receiving the salt alternative. This finding would suggest that some agents in the salt alternative were able to prevent the increase in the extracellular fluid volume. In fact, a potent natriuretic effect of potassium has been demonstrated. Moreover, recent evidence indicates that potassium supplementation lowers blood pressure in humans. The levels of myocardial norepinephrine were also unaffected by the administration of the salt alternative. Therefore, the complete absence of the sodium chloride-induced development of myocardial hypertrophy in WKY rats and the marked reduction of this effect in SHR receiving the salt alternative could be explained by normalization of sodium and water balance and sympa-
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