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# Hypotensive and Cerebrovascular Protective Effects of Whey Mineral Concentrate in Spontaneously Hypertensive Rats

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ABSTRACT

Whey mineral concentrate (WM) contains high concentrations of beneficial electrolytes in natural balance along with low molecular weight milk proteins. The contribution of WM to the regulation of blood pressure in spontaneously hypertensive rats (SHR) and the protection against cerebrovascular damage in stroke-prone spontaneously hypertensive rats (SHRSP) were evaluated. This study was designed to separate the preventive effects of WM from its K effects on salt-induced high blood pressure and cerebral stroke in SHRs. Male SHR at weaning were divided into 4 groups, The normal (N) group received regular rat chow while the other 3 groups received rat chow containing 5.7% NaCl. The control (C) group received rat chow containing only 5.7% NaCl; WM-salt (M-salt) group received 10% WM–salt mixture (WM containing 57% NaCl and 19.2% KCl); KCl-salt (K-salt) group received the same amounts of KCl and NaCl as the WM group. The hypertensive effects of NaCl and hypotensive effects of WM were noticeable at 2 weeks. Blood pressure at 8 weeks was 53 mm Hg higher by NaCl loading; this increase was only 15 mm Hg in M-salt, and 35 mm Hg in K-salt group. A 34% increase of water retention due to NaCl loading over N-group was also reduced to 17% in M-salt, while the Na excretion in M-salt group was increased over C-group. However, the K-diet neither changed the water retention nor enhanced the Na excretion. When Na-loaded erythrocytes were incubated in plasma from each group, the Na efflux rate in C plasma was 43% less than in N plasma. This reduction was only 14% and 24% in the M-salt and K-salt group, respectively. The urinary excretion of dopamine was 42% higher in C-group, and 59% higher in M-salt than that in N-group, suggesting the relationship of dopamine excretion to natriuresis in M-salt. However, the K-diet did not affect dopamine excretion. Endothelium-dependent relaxation in the aorta was markedly attenuated in C-group, and this attenuation was significantly alleviated in M-salt. In the second experiment, male SHRSP were maintained from 8 weeks of age on the 4 same diets as above, and the protective effects of WM on development of stroke were examined. The onset of stroke was remarkably hastened by NaCl loading. The M-salt diet significantly prolonged the onset time shortened by NaCl. However, K-salt diet has no obvious effects. The M-salt diet partly or completely corrected these adverse effects resulting from excessive salt intake while the effect of K-salt diet was either less efficient or ineffective. These results suggest that other factors besides K in WM possibly mediate its hypotensive and vascular protective effects.

## INTRODUCTION

Hypertension develops through the interplay of genetic and environmental factors such as salt ingestion. The connection between dietary salt intake and hypertension has been revealed by epidemiological (Dahl and Love, 1954; Gleiberman, 1973) and experimental studies (Meneely and Dahl, 1961). The highest levels of sodium intake and the greatest prevalence of hypertension in the world are found among the Japanese, especially those residing in northern Japan. Thus, the curtailing of dietary salt intake has been one of the social goals to improve the health of the people. In contrast to salt, intake of some dietary electrolytes such as K, Ca, and Mg seems to facilitate a decrease in blood pressure. Whey mineral concentrate (WM) contains high concentrations of beneficial electrolytes in natural balance along with low molecular weight milk proteins. Therefore, we undertook this study to evaluate the potential contribution of WM to the regulation of blood pressure in spontaneously hypertensive rats (SHR) and the protection against cerebrovascular damage in stroke-prone spontaneously hypertensive rats (SHRSP). We previously demonstrated that a diet containing WM significantly prevented the

TABLE 1

Mineral contents in the experimental diets. Normal: CE-2 (Clea), Control: 5.7% NaCl equivalent to M-salt diet and 4.3% lactose in CE-2, K-salt: 5.7% NaCl, 1.92% KCl and 2.38% lactose in CE-2, M-salt: 10% M-salt (WM containing 57% NaCl, 19.2% KCl) in CE-2

| Diets | Na (%) | K (%) | Ca (%) | P (%) | Mg (%) |
|---|---|---|---|---|---|
| Normal | 0.28 | 1.00 | 1.09 | 1.05 | 0.360 |
| Control | 2.62 | 0.93 | 0.97 | 0.95 | 0.321 |
| K-salt | 2.52 | 2.11 | 0.97 | 0.93 | 0.315 |
| M-salt | 2.62 | 2.13 | 1.07 | 0.94 | 0.300 |

development of hypertension in SHR (Mitsubori et al., 1990), and the inhibitory effects of hypertension development were marked in a high salt diet (Mitsubori et al., 1989). As the hypotensive effects of potassium are usually marked in high salt diets (Ullian and Linas, 1987), the study was designed to separate the preventive effects of WM from the K effects on salt-induced high blood pressure in SHR, and cerebral stroke in SHRSP.

## MATERIALS AND METHODS

### Preparation of whey mineral concentrate

Whey mineral concentrate was prepared by removal of whey protein (MW > 50,000) through ultrafiltration, crystallizing out of the lactose in the filtrate, and spray-drying of the resulting supernatant. Whey low molecular weight protein (molecular weight: 5,000–50,000), when used, was separated from whey mineral concentrate by ultrafiltration.

### Experimental animals and animal treatment

SHR and SHRSP, originally provided by Professor K. Okamoto and maintained by brother–sister breeding in our laboratory, were used. In the first experiment, male SHR at weaning were divided into 4 groups. The normal (N) group received a regular rat chow diet. The other 3 groups all received rat chow containing an equal amount of NaCl (5.7%). The M-salt group received 10% WM–salt mixture (WM containing 57% NaCl, 19.2% KCl); WM–salt mixture was replaced with a NaCl (57%)–lactose mixture in control (C) group, and with a KCl (19.2%), NaCl (57%)–lactose mixture in KCl–salt group. The Ca content was similar in all 4 diets (0.97–1.07%). They were maintained for 8 weeks on this diet regimen. During the last week, they were housed individually in metabolic cages so that urinal samples could be collected, and the consumption of food and water were also measured. In the second experiment, male stroke-prone SHR at 8 weeks of age were divided into 4 groups and were fed a normal, control, K-salt and M-salt diet. The onset of stroke and life span were compared between groups.

### Blood pressure measurement

Blood pressure was measured by the tail cuff method once a week in unanesthetized rats by means of a photoelectric detector (UR 1000, Ueda, Tokyo).

## RESULTS

### Composition of the experimental diets

The mineral contents of the 4 experimental diets were determined by atomic absorption spectrophotometry (Table 1). Na content in 3 NaCl-loaded diets was 10 times as much as that in N-diets; K content in K- and M-salt diets was two times as much as that in N- and C-diets. The ratio of Na to K were 0.28 (N-diet), 2.82 (C-diet), 1.19 (K-salt diet) and 1.23 (M-salt diet).

*Development of high blood pressure*

The blood pressure of the 4 groups was approximately 140 mm Hg at the start of the experiments (4 weeks of age), and progressive increase of blood pressure in each group was shown in Fig. 1. NaCl loading greatly accelerated the development of hypertension; blood pressure in C-group was 21 (2 weeks), 34 (4 weeks) and 53 mm Hg (8 weeks) higher on the average than that in N-group. The M-salt diet, however, prevented the elevation of blood pressure seen in the C-group. The blood pressure in M-salt group

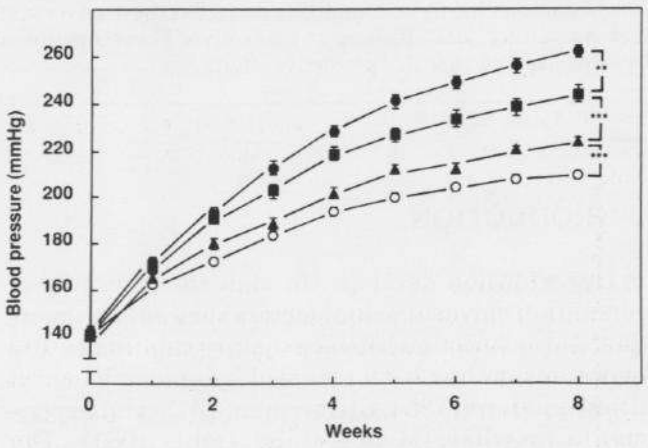


Fig. 1. Development of blood pressure in the experimental groups. Blood pressure was measured once a week by tail-cuff plethysmography. Each point and vertical bar indicates mean ± S.E. for the number of rats used (n). ○ Normal (20), ● control (23), ■ K-salt (24), ▲ M-salt (25). ** Significance: $P < 0.01$ by ANOVA. ***Significance: $P < 0.001$ by ANOVA.

was significantly lower than that of C-group, but a significant difference was still observed between M-salt and N-groups. On the other hand, the preventive effect on the development of hypertension of K-salt diet was significant, but it was obviously smaller than that of M-salt diet. Blood pressure was in the order of N > M-salt > K-salt > C-group with a highly significant difference between each group. These results suggest that WM contains hypotensive components besides K and/or hypotensive factors which exert their effects by acting synergistically with K.

### Body weights and relative weights of the organs

Body weights of 3 NaCl-loaded groups were approximately 12% less compared with those of N-groups while no significant difference was observed between the 3 NaCl-loaded groups. Figure 2 shows the relative weights of the heart to body weights. A significant increase in the values due to NaCl loading was greatly prevented by WM feeding whereas the effect of K was insignificant.

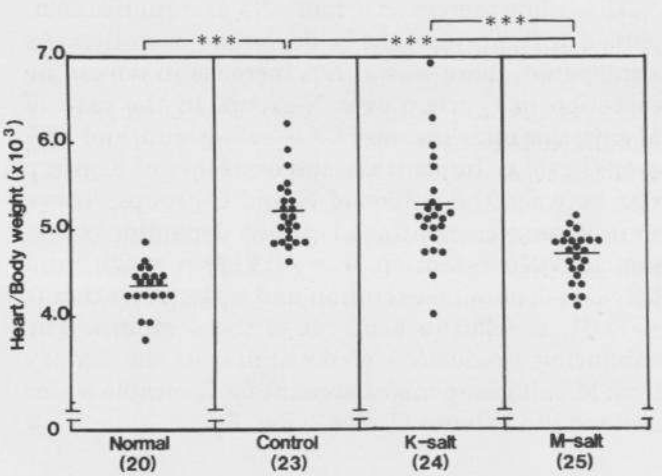


Fig. 2. Heart to body weight ratios of the 4 experimental groups. Bars indicate the means. ***Significance: P < 0.001 by Student's *t*-test.

### Water balance

Table 2 shows the water intake, urinary volume and water retention during the last one week. The water intake in NaCl-loaded group was twice as much as that in N-group. Although both the water intake and urinary volume were larger in M-salt group than in C-group, water retention estimated by subtracting urinary volume from water intake was 17% lower in M-salt group than C- and K-salt groups. The K-salt diet did not influence water retention.

TABLE 2

Water intake, urinary volume and water retention in the experimental groups. During the last week of the experimental period, rats were housed individually in metabolic cages. Water retention was calculated by subtracting urinary volume from water intake

| Groups | Number of rats | Water intake (ml) | Urinary volume (ml) | Water retention (ml) |
|---|---|---|---|---|
| Normal | 19 | 34±2.9 | 11.6±2.1 | 22.8±2.9 |
| Control | 21 | 70±9.4*** | 39.5±7.9*** | 30.5±3.9*** |
| K-salt | 24 | 75±12 | 44.3±12 | 30.6±4.4 |
| M-salt | 25 | 78±10## | 51.6±9.3### | 26.6±3.4### |

***Significance: P<0.001 vs. normal by Student's *t*-test.
##Significance: P<0.01 vs. control by Student's *t*-test.
###Significance: P<0.001 vs. control by Student's *t*-test.

### Urinary excretion of electrolytes and their correlation to blood pressure

Na excretion in M-salt group was significantly higher than in C- and K-salt groups. Na retention calculated by subtracting the excretion from the intake was raised to on the average 132 mg/day in C-group from 19.8 mg/day by NaCl loading. The ingestion of the M-salt diet reduced this increase of sodium retention to 68.6 mg/day; the K-salt diet tended to decrease it, but it was insignificant (Fig. 3). There was a correlation between individual Na retention and blood pressure (r = 0.762, n = 85). In spite of similar amounts of Ca contained in the 4 experimental diets, Ca excretion was increased by NaCl loading [0.432±0.089 mg/day in N-group (n =

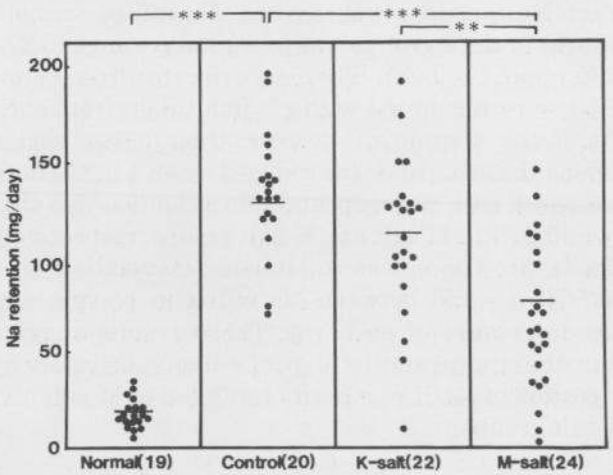


Fig. 3. Na retention of the 4 experimental groups. Na retention was calculated by subtracting urinary Na excretion from Na intake. Bars indicate the means. **Significance: P < 0.01 by Student's *t*-test. ***Significance: P < 0.001 by Student's *t*-test.

TABLE 3

Na Efflux from erythrocytes incubated in plasma from each rat of the experimental groups

| Group | Number of rats | Na efflux (mmol/l cells/h) |
|---|---|---|
| Normal | 20 | 5.859±1.51 |
| Control | 24 | 3.359±1.36*** |
| K-salt | 13 | 4.447±1.1# |
| M-salt | 12 | 5.028±1.22## |

Data are shown as mean±S.E.
#Significance: P<0.05 vs. control by Student's *t*-test.
##Significance: P<0.01 vs. control by Student's *t*-test.
***Significance: P<0.001 vs. normal by Student's *t*-test.

20), and 3.37±0.28 mg/day in C-group (n = 22)]. This increased Ca excretion was prevented by 39% in the M-salt group, while only a tendency of the decrease was observed in K-salt group. There was a significant correlation between the individual ratios of Ca to P in urine and blood pressure (r = 0.638, n = 82). These results strongly indicate that WM exerts greater effects on the alleviation of Na/water and Ca balance altered by ingestion of NaCl. Thus, the hypotensive effects of WM are closely associated with the transport of these electrolytes in the kidney.

*Sodium efflux in erythrocytes*

Na content in the erythrocytes from C group was higher than that from N-group (4.87±0.91 vs. 4.36 ±0.17 mmol/l cells). The increase due to NaCl loading was completely prevented in M-salt group (P<0.05 vs. C-group); the decrease was insignificant in K-salt group. Ouabain-sensitive Na, K-ATPase activity in the erythrocytes was measured through Na efflux from Na-loaded erythrocytes. Na-efflux (mmol/l cells/h) in the 4 groups was in a similar range (5.27–5.80 mmol/l cells/h). When erythrocytes from donor SHR were incubated with plasma taken from each rat of the 4 groups, however, their efflux varied among the groups; it was reduced by 43% in C-group compared with N-group, and this reduction was only 14 and 24% in M-salt and K-salt groups, respectively (Table 3). There was an inverse correlation (r = –0.561, n = 69) between Na efflux in plasma and blood pressure of each rat. These results suggest that the appearance of digitalis-like substances by ingestion of NaCl was partly inhibited in M-salt and K-salt groups.

## Excretion of catecholamines

Dopamine in the kidney reportedly regulates Na excretion by inhibiting Na, K-ATPase in the proximal tubule cells (Aperia et al., 1987; Seri et al., 1988). Therefore, the urinary dopamine excretion which reflects the production in the kidney (Stephenson et al., 1982), was analyzed by HPLC with an electrochemical detector (Table 4). Hegde et al. (1989) and Ball et al. (1978) demonstrated that NaCl loading increased urinary Na excretion accompanied with an increase in dopamine excretion. As anticipated, there was a 29% increase in dopamine excretion in C-group over N-group. In the case of M-salt, this increase was 59% over N-group, and 18% over C-group. In contrast, the excretion of K-group was between the values of N and C groups. There were inverse correlations between dopamine excretion and Na retention (r = –0.612, n = 26), and between dopamine excretion and water retention (r = –0.61, n = 26) in each rat of the 4 groups. The enhancing production of dopamine in the kidney from M-salt group might account for favorable water and sodium balance (Table 2, Fig. 2).

TABLE 4

The urinary excretion of dopamine in the experimental groups. Urine samples were collected in metabolic cage and acidified with 6 N HCl. Dopamine level was analyzed by HPLC with electrochemical detector

| | Number of rats | Urinary excretion of dopamine (μg/day) |
|---|---|---|
| Normal | 9 | 7.76±2.5 |
| Control | 8 | 10.4±1.7* |
| K-salt | 9 | 9.04±3.4# |
| M-salt | 9 | 12.3±1.9# |

Data are shown as mean±S.E.
*Significance: P<0.05 vs. normal by Student's *t*-test.
#Significance: P<0.05 vs. control by Student's *t*-test.

## Endothelium-dependent relaxation of aortic rings

Since the finding of endothelium-relaxing factors by Furchgott (1980), it has been commonly assumed that the endothelium plays important regulatory roles of vascular tension. Ring segments (4 mm) of the thoracic aorta were mounted in organ baths which contained normal Tyrode solution (pH 7.35, 37°C) aerated with $O_2/CO_2$ gas (95:5). Acetylcholine was applied cumulatively on aorta rings precontracted with phenylephrine ($10^{-6}$ M) to measure endothelium-dependent relaxation. Figure 4 shows acetylcholine-induced endothelium-dependent relaxation curves of the 4 groups. The relaxation was markedly attenuated by NaCl loading (in C group) in comparison with N-group, and this attenuation was

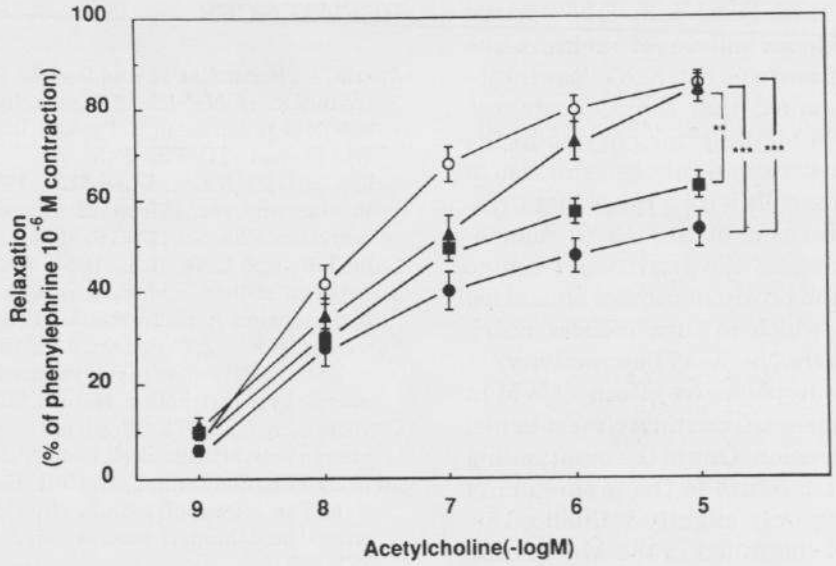


Fig. 4. Acetylcholine-induced endothelium-dependent relaxation in thoracic aortas from SHR fed the experimental diets. Each point and vertical bar indicates mean ± S.E. for the number of rats (n). ○ Normal (17), ● control (13), ■ K-salt (14), ▲ M-salt (13). **Significance: P < 0.01 by ANOVA. ***Significance: P < 0.001 by ANOVA.

TABLE 5

Effects of the experimental diet on the onset of stroke in male stroke-prone SHR. Male stroke-prone SHR at 8 weeks of age were divided into 4 groups and maintained on the 4 experimental diet regimens

| Groups | No. of rats | Onset of stroke[1)] (days) | Survival[2)] (days) | Life span (days) |
|---|---|---|---|---|
| | | | mean±S.E. | |
| Normal | 17 | 53±1.9 | 74±2.3 | 130±2.3 |
| Control | 32 | 27±1.0*** | 34±1.3*** | 90±1.0*** |
| K-salt | 21 | 30±1.4 | 38±1.5 | 94±1.4 |
| M-salt | 23 | 39±1.9***### | 47±2.0***### | 102±1.7***### |

1) Onset days of stroke indicate days from the start of the experiment to the incidence of stroke.
2) Survival days are periods for which rats survived after the start of experimental regime.
***Significance: P<0.001 by Student's *t*-test vs. normal.
###Significance: P<0.001 by Student's *t*-test vs. control.
***Significance: P<0.001 by Student's *t*-test vs. K-salt.

significantly prevented by M-salt diet. Although K-salt diet slightly normalized the alteration, there was no significance against C-group. Correlation coefficient between the relaxation and blood pressure was 0.605 (n = 57).

### Lipid peroxide level

Lipid peroxides estimated as thiobarbituric acid reactive substances were increased in plasma (26%, P<0.001), in the kidney (40%, P<0.001) and the liver (86%, P<0.001) by NaCl loading. M-salt diet completely prevented the increase in plasma and the kidney, and partly in the liver while the effects of the K-diet was smaller than in M-salt diet. Elevation in serum lipid peroxide level due to salt ingestion might result in this dysfunction of endothelium.

### Influence of the development of stroke and life span

Male stroke-prone SHR at 8 weeks of age were divided into 4 groups and maintained with the same diet regimen as above. Their onset of stroke and life span were carefully observed (Table 5). Stroke occurred 27 days in C-group and 30 days in K-salt group after the start of experiment. M-salt group had stroke on 39th day, which was significantly later than in C- and K-salt groups, but 14 days earlier than N-group. Life span was also prolonged in M-salt group compared with C- and K-salt groups. These results indicate that NaCl loading causes serious cerebrovascular damage and WM has stronger protective effects than K itself.

## DISCUSSION

It is still not known how the NaCl signal is received in causing hypertension. One possible NaCl receptor is in the juxtaglomerular cells, and another may be in the central nervous system (Lee et al.,

1989). Halperin et al. (1985) and Kelly (1986) found that NaCl loading induces release of digitalis-like substances which are known as Na, K-ATPase inhibitors. It has been assumed that these substances induce natriuresis by inhibiting this enzyme in the epithelial cells of renal proximal tubules, and also in vascular smooth muscle cells with a consequent rise in blood pressure. Hayashi et al. (1990) demonstrated that NaCl increases the activity of L-amino acid decarboxylase in the proximal tubules enhancing dopamine production, which in turn induces natriuresis by inhibiting there Na, K-ATPase activity.

We investigated the hypotensive effects of WM in SHR thought to be salt sensitive and a closest model of spontaneous hypertension. One of the outstanding features of the M-salt mixture is the promotion of natriuresis, which was only slightly influenced by the same amount of K contained in the M-salt mixture. The M-salt diet did not change the level of atrial natriuretic peptides, but reduced digitalis-like substances in plasma, and increased urinary dopamine excretion reflective of its renal production. Another possibility associated with the hypotensive properties of WM is alleviation of impaired endothelium-dependent relaxation due to NaCl loading. NaCl-induced increase of plasma lipid peroxides, which are supposed to decompose endothelium-derived relaxing factors, might be involved in the endothelial damage.

Several possibilities relating to the favorable effects of WM have become clear, but it is still uncertain which factors besides K in the M-salt mixture contribute to the hypotensive and vascular protective effects of WM. The salty taste equivalence of M-salt to NaCl is found to be 0.85 in human test, so the same salty taste as 1 g of NaCl is obtained by 1.18 g of the mixture, in which only 0.67 g NaCl is present. In addition, M-salt mixture has a mild salty taste. Therefore, this mixture could be an acceptable salt substitute with potential health benefits.

In conclusion, M-salt diet prevented development of hypertension to a greater extent than K-salt diet in SHR through enhancing natriuresis and protecting the endothelium. In addition, M-salt significantly delayed onset of cerebral stroke compared to K-salt in stroke-prone SHR. These results suggested that WM contains hypotensive and vascular protective components besides potassium and/or factors which act synergistically with potassium.

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