Effect of Methionine on the Blood Hypertension of Rats Induced by a High Salt Diet

By

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Koyanagi et al.\(^1,2,3\) have reported that the hypertension of rats induced by a high sodium chloride diet consisting of rice and soybean meal can be prevented by an inclusion of vitamin A, riboflavin, choline or pantothenic acid in the diet. The present study was designed to determine whether methionine with or without a liberal supply of vitamin A would be effective in prevention of elevation of blood pressure in rats.

EXPERIMENTAL

Experiment 1. Albino rats (Wister strain), weighing approximately 40 g, were placed in raised-bottom wire cages individually and fed a stock diet consisting of dried rice (washed) powder 76, soybean meal 10, skim milk powder 8, horse liver powder 2, salt mixture\(^4\) 4 (g), thiamine 0.3 (mg) and 10 i.u. of vitamin A with drops of fish liver oil for 3 weeks. After this pre-experimental period they were divided in 6 groups of 6 each.

The experimental diets included a basal diet, high salt diet, high salt diet supplemented with 0.2% of dl-methionine, and corresponding series of 3 diets containing 1000 i.u. of vitamin A instead of 10 i.u. of that in the basal diet (Table I). The basal diet had the same composition as the stock diet except that in the basal diet, skim milk powder and liver powder was replaced by the same amount of soybean meal, thus the meal amounted to 20 g per 100 g of the basal diet. The diet made into a paste by heating with 140 ml of water per 100 g was given, and drinking water was not given. The amount of food was restricted to 10, 12 and 15 g during the initial 3, next 7 and final 4 weeks respectively. The rats were weighed initially and weekly thereafter.

At the end of 14 weeks the systolic blood pressure was determined by the method of Williams et al.\(^5\) Then all animals were killed by decapitation and the vitamin A content in the liver was determined. After the liver was saponified
alcoholic potash the unsaponifiable fraction was extracted with diethyl ether. The extract was evaporated to dryness in vacuo and residue was immediately taken up in chloroform. Vitamin A in the chloroform solution was determined by the method of \( \alpha\alpha\alpha\)-glycerol dichlorohydrine with the use of a photoelectric absorption-meter.\(^6\)

**Experiment 2.** This experiment was conducted to determine the effect of methionine and vitamin A on the excretion of sodium and potassium in urine. The diets were the same as those in Experiment 1.

In the eleventh week of experiment, 4 randomly selected rats were placed in metabolism cages. During 3 successive days 24-hour urine was collected and sodium and potassium in it were determined flamephotometrically.\(^7\)

**RESULTS**

**Experiment 1.** The growth of rats retarded with the increase of sodium chloride content in the diet. At the end of 7 weeks body weight of rats in group 3 receiving methionine was comparable to that of group receiving methionine and vitamin A, while at the end of 14 weeks the growth rate of rats in group 3 was retarded significantly in comparison with that of rats in group 6. And it was presumably caused by the restricted supply of vitamin A in group 3 as seen by the small storage of vitamin A in the liver (Table I).

<p>| Table I. Effect of Vitamin A and Methionine on the Blood Pressure of Rats |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Vitamin A (i.u.)</th>
<th>Sodium chloride (mg)</th>
<th>Methionine (mg)</th>
<th>Per 100 g of diet</th>
<th>Body weight at 7 weeks (g)</th>
<th>Body weight at 14 weeks (g)</th>
<th>Vitamin A per 100 g of liver (i.u.)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.83*</td>
<td></td>
<td></td>
<td></td>
<td>136.7±5.7</td>
<td>190.3±16.1</td>
<td>59±24</td>
<td>117.4±3.1</td>
</tr>
<tr>
<td>2</td>
<td>1.83</td>
<td>1.83</td>
<td>0.2</td>
<td></td>
<td>128.7±4.3</td>
<td>174.5±18.9</td>
<td>113±68</td>
<td>130.6±1.8</td>
</tr>
<tr>
<td>3</td>
<td>1.83</td>
<td>1.83</td>
<td>0.2</td>
<td>131.6±17.1</td>
<td>173.3±14.2</td>
<td>215±28</td>
<td>109.4±1.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>1.83</td>
<td>0.2</td>
<td>141.7±9.4</td>
<td>211.7±26.6</td>
<td>703±363</td>
<td>114.8±4.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>1.83</td>
<td>0.2</td>
<td>138.5±9.2</td>
<td>173.8±25.5</td>
<td>810±70</td>
<td>132.6±1.3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1000</td>
<td>1.83</td>
<td>0.2</td>
<td>133.8±2.5</td>
<td>165.9±7.9</td>
<td>1153±86</td>
<td>108.0±4.7</td>
<td></td>
</tr>
</tbody>
</table>

* Amounts to 2.5 g when accounts for sodium chloride in salt mixture.  
** Mean value ± standard deviation.  
*** Number of rats.

The blood pressure measured at the end of 14 weeks was elevated in rats fed the high salt diet as compared to those fed the normal salt diet and supplement of methionine to the diet largely counteracted the elevation of blood pressure induced by the high salt diet. And the effect was much the same regardless of whether vitamin A was supplied liberally or not.

**Experiment 2.** It will be noted that 2 groups receiving methionine exhibited a
significant increase in urine volume and sodium excretion (Table II). Addition of vitamin A together with methionine resulted in an increase of sodium excretion still more. The excretion of potassium, in contrast, showed no consistent result with the supplementation of methionine or vitamin A.

**DISCUSSION**

Koyanagi et al.\(^1,8\) found that the liberal supply of vitamin A in the high salt diet of rats increased the pantothenic acid content in the liver and prevented the elevation of blood pressure. It had not been expected as in the present experiment that even the 100 fold supplement of vitamin A to the basal diet could not prevent the elevation of blood pressure. Villaverde\(^9\) reported that the dosing of fish liver oil of high vitamin A content to human subjects had decreased their blood pressure. Taylor\(^10\) and Kats,\(^11\) however, could not confirm Villaverde’s results. On the other hand, Grollman et al.\(^12\) and Moss\(^13\) attributed the beneficial effect of fish liver oil on blood pressure to a constituent of the liver oil other than vitamin A. Thus the reports of the effect of fish liver oil on the hypertension are rather conflicting and it needs further investigation.

In all cases of high salt diet there was exhibited a rise in the systolic blood pressure of animal, which could be reversed by the addition of methionine to the diet. As shown in Table II, the excretion of sodium was increased by the supplementation of methionine. The effect of methionine on the sodium excretion can account for partly the preventive effect of methionine on the elevation of blood pressure. Concerning the urinary excretion of sodium, vitamin A acted synergistically with methionine.

The effect of methionine on the excretion of sodium and blood pressure may be in part attributed to that of choline which is derived from methionine in the

### Table II. Effect of Vitamin A and Methionine on the Excretion of Sodium and Potassium in Urine

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Weight (g)</th>
<th>Urine (ml)</th>
<th>Sodium mg/day</th>
<th>Sodium mg/ml</th>
<th>Potassium mg/day</th>
<th>Potassium mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>High salt</td>
<td>131.0 ± 7.0*</td>
<td>17.3 ± 3.5</td>
<td>35.9 ± 2.2</td>
<td>2.1 ± 0.3</td>
<td>114.9 ± 5.4</td>
<td>6.9 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>High salt, methionine</td>
<td>134.1 ± 8.1</td>
<td>16.0 ± 4.9</td>
<td>116.8 ± 7.7</td>
<td>8.1 ± 2.6</td>
<td>120.4 ± 8.3</td>
<td>7.6 ± 1.8</td>
</tr>
<tr>
<td>3</td>
<td>High salt, methionine</td>
<td>126.6 ± 10.0</td>
<td>17.2 ± 3.5</td>
<td>124.9 ± 8.8</td>
<td>7.6 ± 1.8</td>
<td>114.0 ± 6.9</td>
<td>6.8 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>VA</td>
<td>139.9 ± 12.0</td>
<td>15.8 ± 3.6</td>
<td>36.7 ± 2.2</td>
<td>2.4 ± 0.4</td>
<td>105.0 ± 7.9</td>
<td>6.9 ± 1.2</td>
</tr>
<tr>
<td>5</td>
<td>VA, high salt</td>
<td>133.4 ± 4.4</td>
<td>16.2 ± 3.7</td>
<td>120.7 ± 10.6</td>
<td>7.9 ± 2.2</td>
<td>117.5 ± 8.0</td>
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<td>6</td>
<td>VA, high salt, methionine</td>
<td>132.4 ± 11.9</td>
<td>19.5 ± 3.7</td>
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<td>7.0 ± 2.1</td>
<td>120.1 ± 7.3</td>
<td>6.4 ± 1.2</td>
</tr>
</tbody>
</table>

* Mean value of 4 animals, ± standard deviation.
body of rats. Koyanagi et al.3,8) have reported that the liberal supply of choline to the high salt diet was beneficial to the increase of the urinary excretion of sodium and effective in the prevention of the occurrence of hypertension.

The present author often encountered the difficulty of making rats hypertensive when he used casein in place of soybean meal as a principal source of protein in their diets. Data of present experiment showed that soybean meal when fed together with methionine effected as normotensive as casein. In this connection it is interesting that the successful production of atherosclerosis in monkeys by using soybean protein diet in contrast to the failure by using casein has been described by Mann et al.4) They observed that the addition of methionine to this choline containing soybean protein diet prevented the lesions of arteries. Thus methionine added to the diet acted in a different way to what choline did.

Studies concerning the comparison of the preventive effect of methionine on the occurrence of blood hypertension with that of choline are in progress.

SUMMARY

The effect of methionine supplementation on the elevation of blood pressure induced by high salt diet was investigated. The results of experiment showed that the addition of 0.2% dl-methionine to the high salt diet with or without liberal supply of vitamin A had a beneficial effect in prevention of occurrence of hypertension.

Acknowledgments

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References