Influences of Low Sodium Diets on Vascular Effects of Bradykinin and on Bradykinin Receptors in the Uterine Smooth Muscle in the Rats

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A low sodium diet for 7 days in the rat induced an enhancement of the vascular effects of bradykinin, determined as the blood pressure response, by 56%. However, this enhancement reverted after 28 days of a low sodium diet. A sustained increase in the number of uterine smooth muscle bradykinin receptors during low sodium diets was observed, 1.3 times of the control on the 7th day and 1.7 times on the 28th day. No change in binding affinity was found in any of the studies.

These results suggest that the vascular effects of bradykinin after low sodium diets may be regulated by homeostatic mechanisms via the change in the number of vascular smooth muscle bradykinin receptors at subcellular levels, and that the number of uterine smooth muscle bradykinin receptors may be affected by sodium status per se.

The kallikrein-kinin system is a peptide-generating enzyme system that participates in blood pressure regulation and sodium homeostasis. Kinins including bradykinin are potent vasodilator peptides which act directly on vascular smooth muscle. The actions of the system is mediated, at least in smooth muscle cell, by plasma membrane-located hormone receptors. However, little has been documented about the factors regulating the vascular effects of kinins and their mechanism.

Variations in sodium balance exert several effects on the kallikrein-kinin system. The administration of a low sodium diet stimulated the renal kallikrein-kinin system, followed by an increase in urinary excretion of kallikrein.

In earlier studies, we characterized bradykinin receptors in the rat uterus smooth muscle as a model of vascular smooth muscle and examined the action of bradykinin at the subcellular level.

Therefore, the present study was designed to characterize the influence of sodium depletion on the vascular effects of bradykinin, assessed by its blood pressure effect, and to explore the role of changes in bradykinin receptors on these effects.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing from 150 to 200 g were used. They were maintained in metabolic cages on a normal sodium diet (0.30 mmol/day) or on a low sodium diet (0.05 mmol/
TABLE I  EFFECTS OF LOW SODIUM DIETS ON URINARY EXCRETION OF SODIUM AND KALLIKREIN, AND BLOOD BRADYKININ

<table>
<thead>
<tr>
<th></th>
<th>U Na V (mmol/day)</th>
<th>U Kall V (mg BK/hr/day)</th>
<th>Blood BK (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days Low sodium</td>
<td>0.06 ± 0.03*</td>
<td>3.38 ± 0.57*</td>
<td>0.96 ± 0.50</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>Normal sodium</td>
<td>0.25 ± 0.05</td>
<td>2.23 ± 0.11</td>
</tr>
<tr>
<td>28 days Low sodium</td>
<td>0.02 ± 0.01*</td>
<td>3.77 ± 0.64*</td>
<td>1.00 ± 0.30</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>Normal sodium</td>
<td>0.30 ± 0.05</td>
<td>2.33 ± 0.22</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. * = p < 0.05 compared to normal sodium

TABLE II  EFFECT OF LOW SODIUM DIETS ON THE MEAN OVERALL VASODEPRESSOR RESPONSE TO EXOGENOUS BRADYKININ

<table>
<thead>
<tr>
<th></th>
<th>Mean overall vasodepressor response (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal sodium</td>
<td>12.9 ± 2.2</td>
</tr>
<tr>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>7 days Low sodium</td>
<td>23.1 ± 1.8*</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
</tr>
<tr>
<td>28 days Low sodium</td>
<td>17.0 ± 2.6</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM. * = p < 0.01 compared to normal sodium

by the method of Hunter and Greenwood. The preparation of a subcellular fraction enriched in myometrial plasma membranes, and the determination of specific $^{125}$I-Tyr$^8$-bradykinin binding were done as detailed previously. The apparent affinity and the number of binding sites were determined by Scatchard analysis of concentration dependent binding at initial concentrations from $2 \times 10^{-10}$ M to $1.2 \times 10^{-9}$ M of $^{125}$I-Tyr$^8$-bradykinin. Protein content was determined by the method of Lowry et al.

To confirm the availability of uterine smooth muscle as a model of vascular smooth muscle, we compared the binding characteristics of myometrium to $^{125}$I-Tyr$^8$-bradykinin with those of smooth muscle from mesenteric artery. Plasma membrane enriched subcellular fraction from rat mesenteric artery was prepared by modifications of the method of Wei et al.

Vasodepressor dose-response effects of bradykinin were measured in graded doses of synthetic bradykinin from 29 pmol/kg to 235 pmol/kg. These effects were examined in rats, anesthetized with the sodium salt of 5-ethyl-5-[1-methyl-propyl]-2-thio-barbituric acid [inactin 150 mg/kg, i.p.], in which the carotid artery was cannulated for bradykinin administration, and femoral artery was also cannulated for blood pressure measurement. Changes in blood pressure were recorded directly through the catheter in the femoral artery with Statham P23-Db pressure transducers on a Grass Model 7C polygraph recorder. Bradykinin was administered as a single bolus injection of 50 μl followed by washing through with 100 μl of 0.9% saline, a volume which did not affect the basal level of blood pressure in preliminary experiments. The absolute levels of changes in blood pressure in mmHg was used as the dependent variable. The mean overall responses were calculated as the average of all doses.

All data were expressed as mean ± standard
errors. Differences between groups were evaluated by Student's t-test.

RESULTS

The low sodium diet has a profound effect on urinary excretion of sodium which diminished from 0.25 ± 0.06 mmol/day to 0.06 ± 0.03 mmol/day on the 7th day and from 0.30 ± 0.05 mmol/day to 0.02 ± 0.01 mmol/day on the 28th day, respectively. The changes in urinary excretion of kallikrein and circulating levels of bradykinin after 7 days and 28 days on a low sodium diet are shown in Table I. The 24-hour kallikrein excretion was increased. On the other hand, circulating levels of bradykinin did not change significantly.

Vasodepressor dose-response effects of bradykinin measured by intra-arterial bolus injection into carotid artery was augmented after 7 days on the sodium restricted diet and tended to return to control after 28 days. The values of mean overall vasodepressor response after the dietary modification of sodium are shown in Table II.

The effect of sodium restricted diet on uterine smooth muscle bradykinin receptors are seen in Table III. Binding affinities for bradykinin-receptor interaction were not significantly altered by the sodium restricted diet. However, the number of uterine smooth muscle bradykinin receptors was significantly increased to 1.3 times of control on the 7th day and 1.7 times on the 28th day.

Binding characteristics for bradykinin-receptor interaction in myometrium and in mesenteric artery are shown in Table IV. There was no difference in binding affinities for bradykinin-receptor interaction between them, but significant difference in the number of bradykinin receptors.

DISCUSSION

The increased kallikrein excretion after low sodium diets and failure of circulating bradykinin levels to change significantly confirmed earlier observations. The production, origin and metabolic clearance for circulating blood kinins have not been well documented and it is controversial whether circulating levels of bradykinin reflect the levels at its effector sites or are influenced by tissue concentration of bradykinin.

Several possible explanations may be made on the transient alteration in the vasodepressor effect of bradykinin after low sodium diets. Sodium might have a direct effect on the vasodepressor effect of bradykinin. However, the present study showed that low sodium diets induced an increase in urinary excretion of kallikrein, suggesting that the local changes in

Japanese Circulation Journal Vol. 46, May 1982
kallikrein-kinin system occurred at the effector sites. So it is inappropriate in the present study to evaluate the direct action of sodium on vascular effects of bradykinin, and strong evidence against sodium hypothesis are the observations that the vasodepressor effect of bradykinin was enhanced transiently.

Sodium depletion has been reported to induce alterations in the activity of the renin angiotensin and sympathetic nervous systems which have an inverse effect on vascular tone. However, transient alteration in the vasodepressor effect of bradykinin could not be well explained by sustained changes in these vasopressor systems induced by low sodium diets.

In previous studies bradykinin was shown to bind to membrane receptors in rat myometrium which was down-regulated by the prevailing concentration of the endogenous hormone, similar to membrane receptors of other peptide hormones. Furthermore, the availability of uterine smooth muscle as a model of vascular smooth muscle contributing to the blood pressure regulation, was confirmed in the present study by the similarity in binding characteristics to bradykinin between myometrium and mesenteric artery. The evidence that a sustained increase in the number of smooth muscle bradykinin receptors occurred in the rat after low sodium diets, has made it more complicated to understand the relationship between the vascular effects of bradykinin and smooth muscle bradykinin receptors. The difference in the vascular effects of bradykinin between the rats after 7 days and those after 28 days of low sodium diets, was not explained by changes in the number of smooth muscle bradykinin receptors, but led to the hypothesis that the vascular effects of bradykinin after low sodium diets is mainly determined prior occupancy of endogenous bradykinin at its receptor sites and is regulated by homeostatic mechanisms via the change in the number of binding sites. A sustained increase in the number of uterine smooth muscle bradykinin in the present study is not due to changes in endogenous level of bradykinin, since local kallikrein-kinin system was observed to be enhanced by low sodium diets. This suggested the hypothesis that uterine smooth muscle bradykinin receptors are regulated in complicated manners by several factors including endogenous levels of bradykinin and sodium status per se.

Acknowledgements

We wish to acknowledge the excellent technical assistance of M.G. Straw and K. Sowards and the secretarial assistance of C. Onodera.

REFERENCES