Low-salt diet downregulates plasma but not tissue kallikrein-kinin system

J. Hilgenfeldt, U. Puschner, U. Riester, J. Finsterle, U. Hilgenfeldt, and E. Ritz. Low-salt diet downregulates plasma but not tissue kallikrein-kinin system. Am. J. Physiol. 275 (Renal Physiol. 44): F88–F93, 1998.—The kallikrein-kinin system (KKS) is involved in the regulation of blood pressure and in the sodium and water excretion. In humans, the KKS is divided functionally into a plasma KKS (pKKS) generating the biologically active peptide bradykinin and into the tissue (glandular) KKS (tKKS) generating the active peptide kallidin. The objective of this study was to examine the effect of a low-NaCl diet on the concentration of both pKKS and tKKS in plasma and urine in 10 healthy volunteers. After a 4-day low-NaCl diet, the urinary sodium and chloride excretions had decreased from 234 to 21.2 mmol/24 h and from 198 to 14.6 mmol/24 h, respectively. The plasma levels of ANG I, aldosterone, and angiotensin converting enzyme (ACE) significantly increased from 50.4 to 82.8 pg/ml, from 129 to 315 pg/ml, and from 46.4 to 59.8 U/ml, respectively, demonstrating the physiological adjustment to the low-salt diet. In plasma, the levels of bradykinin and plasma kallikrein had significantly decreased from 13.7 to 7.57 pg/ml and 14.4 to 7.13 U/ml, respectively. However, the levels of high-molecular-weight kininogen (HMW kininogen) remain unchanged (101 vs. 112 µg/ml, not significant). Contrary to plasma kallikrein, the plasma levels of tissue kallikrein increased (0.345 vs. 0.500 U/ml; P < 0.05). The plasma kallidin levels, however, did not change (64.7 vs. 68.6 pg/ml, not significant). This can be explained by a simultaneous decrease in the plasma low-molecular-weight kininogen (LMW kininogen) levels (89.9 vs. 44.4 µg/ml; P < 0.05). As in plasma, we find increased urinary concentrations of renal (tissue) kallikrein (23.3 to 42.8 U/24 h; P < 0.05) that contrast with, and are presumably counterbalanced by, urinary LMW kininogen levels (77.0 vs. 51.8 µg/24 h; P < 0.05). Consequently, in urine low-NaCl diet caused no significant change in either bradykinin or kallidin (9.2 vs. 10.8 µg/24 h, and 10.9 vs. 10.3 µg/24 h). It is concluded that the stimulation of the renin-angiotensin system on a low-NaCl diet is associated with a decrease in pKKS (bradykinin and plasma kallikrein) but not in tissue and renal KKS. Although tissue kallikrein is increased, there is no change in kallidin, as LMW kininogen in plasma and urine is decreased. These data suggest a difference in the regulation of pKKS and tKKS by low-salt diet.

Blood pressure, local blood flow, and salt-water homeostasis are closely linked to two different endocrine systems, namely, the renin-angiotensin-aldosterone system (RAAS) (8) and the kallikrein-kinin system (KKS) (5). The RAAS stimulates blood pressure and increasing salt and water reuptake in the kidney via ANG II and aldosterone. By contrast, the KKS reduces peripheral vascular resistance and lowers the blood pressure, although this concept is still controversial. With respect to functional aspects, the KKS can be divided into a plasma KKS (pKKS) and tissue (glandular) KKS (tKKS). It is well accepted that kallidin is released from low-molecular-weight (LMW) kininogen by the action of tissue kallikrein and bradykinin from high-molecular-weight (HMW) kininogen by the action of plasma kallikrein. It is generally assumed that the pKKS participates in the activation of coagulation, inflammation, and circulatory shock (7). The tKKS plays a role in many excretory functions and the regulation of local blood flow in different organs. All the components of the tKKS are present in the kidney, and their distribution is consistent with a local action within the kidney (9).

There is accumulating evidence that the kidney plays an important role in the long-term blood pressure regulation and in the pathogenesis of hypertension (18). This evidence has been supplied by data obtained with ANG II converting enzyme (ACE) inhibitors and the development of kinin receptor antagonists (4). It has been shown that kinins inhibit water permeability and sodium absorption in the collecting duct of the kidney (25). Data obtained with kininogen-deficient Brown-Norway Katholiek rats indicate that kinins may play a protective role in the development of hypertension by decreasing the intracellular sodium chloride content (15).

The components of both pKKS and tKKS are found in plasma. Tissue kallikrein can generate kallidin in the circulation. No reliable data are available as to the contribution of bradykinin and kallidin in various physiological responses in humans. Part of the confusion has been caused by technical problems associated with measurement of true levels of kinins in blood. Activation of the pKKS may cause a dramatic increase in the bradykinin levels, as shown in patients undergoing hemodialysis (23). This activation can be reduced or even avoided by the technique of venipuncture. In addition, the commercially available bradykinin assays cannot distinguish between bradykinin and kallidin, as the antibodies are directed against the identical COOH terminus of both peptides (16). To overcome these problems, we have recently developed a sensitive and specific radioimmunoassay for bradykinin as well as for kallidin (12).

The obvious importance of the KKS in the maintenance of salt-water homeostasis was examined in the present study. It was our aim to characterize the change and possible involvement of the pKKS and tKKS under a low-salt diet. By using specific assays for bradykinin and kallidin, we were able to measure the...
change of the components of the pKKS and tKKS separately in plasma and urine.

MATERIALS AND METHODS

Volunteers. Ten healthy volunteers entered the study [3 male, 32.7 ± 2.2 (SD) yr, 73.3 ± 2.05 kg, 181 ± 4.5 cm height; and 7 females, 27.7 ± 4.0 yr, 63.4 ± 32 kg, 171 ± 5.9 cm height]. Before enrollment and at the end of the study, the subjects were checked medically by physical examination, clinical laboratory tests, and vital sign parameters. All subjects received a detailed information on the trial and study procedure. In addition, they were informed about the aims and the possible risks of the study and gave their written informed consent in presence of a witness.

Experimental protocol. The study started at Monday morning at 0800 and was finished on the following Friday at 1200. During this 4-day period, the volunteers obtained an NaCl-free diet. This diet consists of food like fruits and vegetables, e.g., apples, bananas, tomatoes, cauliflower, paprika, and unsalted nuts. Mineral water (containing 1.9 mg sodium and 4.0 mg chloride per liter), coffee, and tea were permitted ad libitum. Food containing salt as well as meat or processed food like cheese, milk, hamburger, chips, and bread, was not permitted.

Supine blood pressure and heart rate were measured at the beginning and at the end of the study by using an automatic sphygmomanometer (Boso compact; Bosch and Sohn, J uningen, Germany).

Blood (5 ml/tube) was obtained at the beginning and at the end of the study by venipuncture without aspiration by a silanized hypodermic needle (1.8 mm, 15 gauge) within 3–5 s. Four tubes were collected, containing 20 ml ethanol (tube 1), 2 ml citrate (tube 2), inhibitor cocktail (2 ml; 10 µl o-phenanthroline, 16.7 µl polypropylene, 0.0625% EDTA, 40 µl/mI blood; tube 3), and again ethanol (20 ml; tube 4).

The total 24-h urine volume was collected at the day before the beginning of the diet and at the day before the end of the study. Two aliquots (5 ml) were from each urine passage, one with 20 ml ethanol and one without ethanol, were obtained and stored at −20°C until use.

Adverse effects. All participants complained of transient but pricking headache at the third day of the study.

Assays. The ethanol extracts were evaporated to dryness after centrifugation and dissolved in 0.75 ml PBS containing 0.1% BSA.

Bradykinin and kallidin were measured in these samples without further extraction as recently described (12).

Plasma ANG I levels were estimated with the radioimmunoassay (13). HMW and LMW kininogen were monitored according to the method of Uchida and Katori (30).

Plasma and tissue kallikrein were measured amidolytically with the chromogenic substrates S-2302 and S-2266 (o-Val-Leu-Arg-p-nitroanilide), respectively (Haemochrom Diagnostica, Essen, Germany) (2), on a 96-well microplate. For tissue kallikrein determination, 40-µl samples, were incubated together with 50 µl Tris buffer, 0.2 mol/ml, pH 8.2, and substrate S-2266, 20 µl, for 30 min at 37°C. Nonspecific substrate conversion was determined by blocking other serine proteases with aprotinin (Trasylo; Bayer, Wuppertal, Germany), 50 µl of 20 U/ml on the same 96-well microplate. The enzymatic reaction was stopped with 10 µl of 50% saturated acetic acid. For measuring plasma kallikrein, a volume of 20 µl was incubated with 50 µl Tris buffer, 0.05 mol/ml, containing 0.113 mol/ml NaCl, and 20 µl substrate S-2302 for 10 min at 37°C. The reaction was stopped with 50% saturated acetic acid.

The samples were measured in an ELISA reader at 405 nm. Kallikrein standard was measured on every plate to calculate the kallikrein activity in units per liter.

ACE was measured with the enzymatic method (11), with slight modifications. Briefly, a 20-µl urine or plasma sample was incubated with 3-(2-furylacryloyl)-L-phenyl-alanyl-glycine (FAPGG; Sigma, Munich, Germany), 50 µl in 200 µl borate buffer, 8 mmol/ml containing 0.3 mol/ml NaCl. FAPGG is cleaved by ACE by forming FAP and GG. This cleavage causes a loss of absorbance at 340 nm. The kinetic was measured every 3 min at 37°C with an ELISA reader. The calculation of the enzymatic activity (ACE activity at 37°C in U/l) occurred according to the formula

\[
\text{activity} = - \frac{V_t \cdot 1.000 \cdot \Delta A}{\Delta e \cdot V_s \cdot d}
\]

where \(V_t\) = total volume; \(V_s\) = sample volume; \(d\) = cuvette diameter; \(\Delta e\) = −0.58 (maximum change of absorbance at 340 nm); and \(\Delta A\) = change in absorbance per minute.

Statistical analysis. The data are presented as means ± SD. The statistical significance was calculated with the Student's t-test in connection with the computer program EXCEL (Microsoft). A probability value of \(P \leq 0.05\) was considered as being significant.

RESULTS

Low-salt diet did not cause any change in blood pressure. The mean blood pressure (systolic over diastolic) was 116.8 ± 11.7/67.8 ± 6.7 mmHg before low-salt diet and 116.1 ± 14.1/69.6 ± 9.7 mmHg at the end of the study. Mean heart rate was 70.4 ± 9.9 before and 75.5 ± 18.1 beats/min at the end.

The recovery of bradykinin was more effective after ethanol extraction, which was 64 ± 6.5% than after acetone extraction (25.6 ± 8.6%). The activation of pKKS during venipuncture was assessed by comparing the bradykinin values in the early and late samples 1 and 4, respectively. The formation of bradykinin during venipuncture is negligible. There was no significant difference in bradykinin levels between sample 1 and 4 (14.4 ± 4.41 vs. 12.4 ± 3.5 pg/ml). Therefore, the mean of both values is presented.

As shown in Table 1 low-NaCl diet did not cause a significant change in the plasma concentration of Na⁺, K⁺, Cl⁻, and Ca²⁺. However, the urinary NaCl excretion was significantly lower (Na⁺, 234 ± 56.5 vs. 21.2 ± 16.0 mmol/24 h; and Cl⁻, 198 ± 76.1 vs. 14.6 ± 9.93 mmol/24 h; both \(P < 0.0001\)). In this context, Ca²⁺ excretion was significantly reduced (5.05 ± 2.07 vs. 1.67 ± 1.08), as Ca²⁺-containing food (milk, cheese) was not ingested during the diet period. Neither K⁺ nor creatinine excretion were significantly different from control.

To confirm the physiological response to the low-salt diet, we followed the plasma levels of ANG I, angiotensinogen, aldosterone, and ACE. As shown in Fig. 1, the plasma aldosterone level was significantly increased on the low-salt diet (129 ± 70.7 vs. 315 ± 192 pg/ml; \(P < 0.01\)) as well as the plasma ANG I levels (50.4 ± 13.2 vs. 82.8 ± 22.8 pg/ml). In this connection, the plasma angiotensinogen levels were decreased (5.4 ± 3.95 vs. 3.33 ± 1.95 µg ANG I·equivalents⁻¹·ml⁻¹; not signifi-
Table 1. Plasma and urinary electrolyte content before and after low-salt diet in healthy volunteers

<table>
<thead>
<tr>
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<th>Plasma, mmol/l</th>
<th>Urine, mmol/24 h</th>
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<tr>
<td></td>
<td>Na⁺</td>
<td>K⁺</td>
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<tr>
<td>Control</td>
<td>145 ± 1.42</td>
<td>4.8 ± 0.43</td>
</tr>
<tr>
<td>Low-salt diet</td>
<td>144 ± 1.49</td>
<td>4.4 ± 0.43</td>
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<td>P</td>
<td>0.06*</td>
<td>0.10*</td>
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Values are means ± SD; n = 10 experiments. *Not significant. ND, not done. Note that plasma values are in mmol/l, urine values are in mmol/24 h, and creatinine values are in g/24 h.

Discussion

On the basis of biochemical and functional considerations, the KKS in humans is divided into a plasma system (pKKS) and a tissue system (tKKS). It is well accepted that by cleaving plasma HMW kininogen, plasma kallikrein (EC 3.4.21.34; 88 kDa) releases bradykinin. Tissue kallikrein (EC 3.4.21.35; 32 kDa) by cleaving HMW and LMW kininogen releases kallidin (Lys-bradykinin) (20). In this context, the main question addressed in this study concerns the physiological relationship and importance of pKKS and tKKS in humans under conditions of a low-NaCl diet.

Blood pressure regulation and salt-water homeostasis is under the control of the RAAS (8). In addition, they are influenced by the KKS action on two distinct systems. In the blood vessels, kinins directly lower blood pressure by releasing NO and indirectly by generating prostacyclins from the endothelial cells (29). The second main system is the kidney, where all components required to generate kinins have been localized (9). There are many reports dealing with the importance of kinins in regulating electrolyte and water excretion (6). These findings support the hypothesis that kinins modulate blood pressure through effects on renal vascular resistance and perhaps by modulating tubular ion and water transport. Recent data obtained with mutant mice lacking the kinin B₂-receptor gene indicate that kinins via the B₂-receptor play an important role in preventing salt-sensitive hypertension, possibly by maintaining renal blood flow (1). In line with this hypothesis is the finding that the overexpression of human tissue kallikrein in transgenic mice caused hypertension that was reversed by aprotinin, a kallikrein inhibitor (32). Taken together, there is strong evidence that the physiological action of the RAAS in blood pressure regulation and salt-water homeostasis is counterbalanced by the KKS.

Although there are obviously two different KKS involved in the blood-pressure and salt-water homeostasis, a clear distinction between the contributions of kallidin and bradykinin in human biological fluids so far could not be made because reliable measurement were not available. A recently published bradykinin...
The plasma bradykinin levels are significantly decreased on a low-NaCl diet, possibly as a consequence of a highly significant decrease in the plasma kallikrein levels. In contrast, the concentration of plasma kallikrein substrate, HMW kininogen, remained unchanged (Fig. 2). In agreement with many other reports (22, 31), we found significantly increased tissue kallikrein levels in plasma on a low-NaCl diet (Fig. 2). Based on these studies, one can propose the hypothesis that tissue kallikrein synthesis and release into the circulation is stimulated by mineralocorticoids, similar to what is seen for renal kallikrein (21). Nevertheless, we failed to find any alteration in plasma kallidin levels. This may be the consequence of the simultaneous decrease of the tissue kallikrein substrate, i.e., plasma LMW kininogen.

In this context, we have to admit that it is presently unknown whether e.g., the decrease in LMW kininogen plasma levels on a low-NaCl diet results from a decreased synthesis and secretion or from an increased consumption by the stimulated tissue kallikrein levels. To the best of our knowledge, the regulation of HMW and LMW kininogen synthesis in the liver, e.g., under low-salt diet is still an open question. As the volunteers were healthy normal volunteers and able to maintain their blood pressure under this condition, a change in blood pressure was not observed. This data support recent data obtained by a meta-analysis (19).

In view of the fact that NaCl seems to play a crucial role in the blood pressure regulation and salt water homeostasis, the physiological interaction between RAAS and KKS is of great importance for the understanding of hypertension. Recently, Majima et al. (14) reported a 10-fold increase in the arteriolar response to ANG II after infusion of NaCl, 0.3 mol/l, for 4 days in mutant Brown-Norway-Katholiek rats. From these data, two levels of interaction between RAAS and KKS in salt balance and blood pressure regulation can be defined. The level relates to salt balance. ANG II causes

**Fig. 2.** Change in plasma levels of bradykinin and kallidin (A) high- (HMW) and low-molecular weight (LMW) kininogen (B), plasma kallikrein and tissue kallikrein (C) plasma levels after low-NaCl diet (control values, open bars; low-NaCl diet, solid bars). Values are means ± SE; n = 10. *P < 0.05. **P < 0.01. ***P < 0.0001.

Fig. 3. Change in urinary excretion of bradykinin, kallidin, LMW kininogen, and renal kallikrein after 4-day low-NaCl diet (control values, open bars; low-NaCl diet, solid bars). Values are means ± SE; n = 10. *P < 0.05.
sodium retention directly and via aldosterone. The KKS opposes this effect via B₂-receptors. Kinins are generated in the kidney, predominantly in the renal tubulus, and inhibit reabsorption of NaCl or accelerate its excretion. As the sodium load in the cells modulates the sensitivity of the arterial blood vessels to norepi-nephrine (14), an alteration in NaCl balance should cause a change in blood pressure. The second mechanism is the direct action of both peptides on the tonus of blood vessels, which has already been mentioned above.

Kinins have been implicated in the control of renal blood flow, glomerular filtration, and sodium excretion (25). Many studies found increased urinary kallikrein excretion during severe NaCl restriction. It is widely accepted that aldosterone increases renal synthesis and urinary excretion of kallikrein in humans and animals (17, 21). The renal KKS is located in the connecting tubule, and the collecting duct is involved in the regulation of NaCl and water excretion (10, 15). The increase in urinary kallikrein excretion on low-salt diet may counterbalance the increase in sodium reabsorp-tion induced by aldosterone (28). Nevertheless, despite stimulated urinary kallikrein levels (22, 31), we and others (24, 27) found unchanged urinary kinin excretion (Fig. 3). This can be explained by the decrease in urinary kininogen levels on a low-NaCl diet, consistent with downregulation of the renal synthesis of LMW kininogen.

Preliminary data (12) indicate that kallidin is the main kinin in the kidney, where it is generated by renal tissue kallikrein in the collecting duct. Urinary bradykinin is supposed to be a degradation product of renal aminopeptidases. Kallidin has a marked inhibitory effect on arginine vasopressin-stimulated water permeability in the kidney (26).

In summary, by measuring bradykinin and kallidin separately, we have demonstrated that a low-NaCl diet downregulated the pKKS system but not the tKKS. The decrease in plasma bradykinin is correlated with a decrease in plasma kallikrein, whereas the levels of HMW kininogen remain unchanged. Although tissue kallikrein in plasma is increased, we find no change in plasma kallikrein levels after low-NaCl diet, as this increase is compensated by a decrease in LMW kininogen levels. The regulation of renal KKS displays similarity to tKKS in plasma. As in plasma, we find significantly increased renal kallikrein levels and simultaneously decreased urinary LMW kininogen levels but no change in urinary kinin levels. As blood pressure regulation is most likely associated with NaCl load, which is regulated by tKKS, the data provide strong evidence that it is important to distinguish between the components of the pKKS and tKKS. Especially when analyzing the physiological and pathophysiological role of the KKS in different biological compartments, it seems reasonable to analyze bradykinin and kallidin separately. The physiological significance of the differences in the regulation of pKKS and tKKS after low-salt diet is an unanswered question and currently under investigation.

We are grateful for the excellent technical assistance of Susen Högenauer.

This work was supported by a Grant 34/1995 from the Science Support Program of the Medical Faculty of the University of Heidelberg.

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Received 18 September 1997; accepted in final form 9 March 1998.