THE ROLE OF RENAL PROSTAGLANDIN E AND KALLIKREIN IN PATHOGENESIS OF ESSENTIAL HYPERTENSION

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The present study was done to investigate the interrelationships between renal kallikrein-kinin, renal prostaglandin E and renin-angiotensin-aldosterone systems in human and the possibility that renal kallikrein-kinin and renal prostaglandin E may participate in the pathogenesis of essential hypertension by means of measuring urinary excretion of kallikrein and prostaglandin E, plasma renin activity and plasma aldosterone concentration before and after stimulation or inhibition of the renin-angiotensin-aldosterone system and inhibition of renal prostaglandin E generation. Urinary kallikrein excretion was increased after the stimulation of the renin-angiotensin-aldosterone system by low Na diet or the administration of furosemide and upright posture, while it tended to decrease after the inhibition of the renin-angiotensin-aldosterone system by the administration of 1-sarcosine-8-isoleucine angiotensin II under sodium depletion or spironolactone. These data showed that the changes in urinary kallikrein excretion paralleled with those of the renin-angiotensin-aldosterone system following various stimuli, suggesting that renal kallikrein-kinin system may regulate blood pressure by opposing the action of the renin-angiotensin-aldosterone system. Urinary PGE excretion was decreased after sodium depletion and increased after the administration of furosemide in spite of the augmentation of the renin-angiotensin-aldosterone system. The changes in urinary PGE excretion was closely related to those in urinary Na output after various stimuli and a significant positive correlation was found between basal levels of urinary PGE and those of urinary Na, suggesting that renal prostaglandin E may be involved in the regulation of blood pressure by affecting renal sodium handling. The present data showed that basal level of urinary excretion of PGE and kallikrein was lower in essential hypertension than in normal subjects and that the release of renal kallikrein and PGE after furosemide administration was also suppressed in essential hypertension compared with that in normal subjects, suggesting that there exists an impaired defense mechanism against the renin-angiotensin-aldosterone system resulting in sodium retention.

Key Words:
Renal prostaglandin
Renal kallikrein
Renin-angiotensin-aldosterone system
Essential hypertension
Pathogenesis

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PROSTAGLANDIN E and kallikrein are vasodilating substances naturally generated in the kidney. Recently, Margoliou and his coworkers\textsuperscript{1,2} have reported that renal generation of kallikrein is regulated by aldosterone or other sodium-retaining steroid hormones. McGiff and his coworkers have also reported that there were close interrelationships between renal PGE and renin-angiotensin system\textsuperscript{3} or renal PGE and renal kallikrein-kinin system\textsuperscript{4} in animal experiments. It has been reported that urinary excretion of kallikrein or PGE was significantly decreased in essential hypertension\textsuperscript{5-9}. Thus, possibilities exist that renal PGE and kallikrein-kinin system may regulate blood pressure by opposing the action of renin-angiotensin-aldosterone system in human subjects and that the impairment of production of renal PGE and kallikrein in essential hypertension may play some role in the pathogenesis of this disease. To investigate these possibilities, we examined interrelationships among renal PGE, renal kallikrein-kinin system and the renin-angiotensin-aldosterone system in normal volunteers and in patients with essential hypertension by means of measuring urinary excretion of PGE and kallikrein, plasma renin activity (PRA) and plasma aldosterone concentration (PAC) before and after stimulation or inhibition of the renin-angiotensin-aldosterone system and inhibition of renal PGE generation.

MATERIALS AND METHODS

Subjects

Eighty-four healthy subjects and 55 patients with essential hypertension were included in this study. Normal subjects were 61 men and 23 women ranging in age from 18 to 66 years with an average and SEM of 40.0 \( \pm 1.6 \). They were normal in physical examination and routine laboratory tests. Essential hypertensives were 35 men and 20 women ranging in age from 15 to 63 years with an average of 37.1 \( \pm 1.8 \). All subjects were hospitalized during this study. The diagnosis of essential hypertension was made by history, physical examination, radioisotope renography, renoscintigraphy, angiography and determination of 11-OHCS, aldosterone and urinary catecholamines or vanillyl mandelic acid. They had blood pressures of 150 mmHg in systolic and 90 mmHg in diastolic or higher on repeated observations with average mean blood pressure of 117 \( \pm 3 \) mmHg. They were allowed to be on unrestricted Na diet and antihypertensive medications had been discontinued at least 2 weeks before study. Sampling of blood was done in all fasting subjects kept in recumbent position for 1 hr in the morning, and urine was collected for 24 hrs in a bottle kept in a refrigerator. Basal levels of PRA, PAC and urinary excretion of PGE and kallikrein were measured.

Protocol

1) Low Na diet: Ten patients received first a diet containing 200 mEq of Na per day for a week and then 100 mEq and finally 30 mEq of Na each for 3 days, and the 24 hours' urine was collected in the last day in each period and the blood sampling was done in the next morning after overnight fasting.

2) Furosemide iv and an upright posture: Nineteen normal subjects and 16 patients with essential hypertension were allowed to take an unrestricted diet for 2 weeks. They were fasted overnight, and kept supine for 1 hr in the next morning, then sampling of blood and urine were done. Then, furosemide (1 mg/kg) was injected intravenously and the subjects were asked to assume an upright posture for 120 minutes. At the end of the standing, blood sample was taken, and urine was also collected.

3) Administration of angiotensin II antagonist: Ten patients with essential hypertension were allowed to take an unrestricted diet for 2 weeks, and then received a diet containing 50 mEq of Na per day for 3 days. In the next morning of the last day of each Na status, the angiotensin II antagonist, 1-sarcosine-8-isoleucine angiotensin II, was infused intravenously at a rate of 300 – 600 ng/kg/min for 1 hr. The sampling of blood and urine was done before and at the end of the infusion.

4) Administration of spironolactone: Three patients with essential hypertension were allowed to take an unrestricted diet for 2 weeks and then, spironolactone (100 mg/day) was given for a week. Sampling of blood and urine was done before and at the end of the spironolactone administration.

5) Administration of indomethacin: Eleven hypertensive patients were given a diet containing 90 mEq/day of Na with the oral administration of furosemide (80 mg/day) for 3 days, and then, the oral administration of indomethacin (150 mg/day) was added for additional 3 days. Sampling of blood and urine was done before and at the end of each 3 day period.

Measurement of urinary PGE: Urinary PGE was measured radioimmunologically by the method already described\textsuperscript{8} A urine sample (5 – 10 ml)
was lyophilized. After the residue had been dissolved in 1 ml of 0.05 M phosphate buffer, pH 7.4, urinary PGE was converted to PGB by alkaline treatment according to Zusman’s method. Then, the sample was acidified to pH 3 to 4 with hydrochloric acid and extracted with ethyl acetate. The organic phase was dried and the residue was applied to a silicic acid column: PGB was eluted by a mixture of benzene-ethyl acetate (60:40) according to the method of Jaffe. The PGB fraction was dried and measured radioimmunologically using PGB antiserum (CAS501, Clinical Assay) which does not distinguish PGB1 from PGB2. The pre-existing PGB was also measured by the same procedure without alkaline treatment. The urinary PGB value was calculated by subtracting the PGB value before alkaline treatment from that after alkaline treatment. The amount of naturally occurring PGB to PGE was 8 – 25%. Prior conversion of PGE to PGB precluded dehydration of PGE to PGA during the extraction procedure. The overall recovery rate of added PGE (1 to 3 ng) was 54.8 ± 0.7% (mean ± SE, n = 15). The estimated value was corrected for this loss.

Measurement of urinary kallikrein: Urinary kallikrein activity was measured as kininogenase activity using radioimmunoassay of kinin and by TAME esterolytic activity.

Kininogenase activity: The urine sample (0.05 – 0.1 ml) was incubated with 4 μg of bovine serum low molecular weight kininogen in 0.1 M phosphate buffer, pH 8.4, containing 0.1% neomycin, 3 mM 8-hydroxy-quinoline and 30 mM disodium ethylenediaminetetraacetic acid at 37°C for 20 minutes. After the incubation, the mixture was diluted 5-fold with cold water, heated at 80°C for 15 minutes to stop the enzymatic reaction, and the generated kinin was measured radioimmunologically using the kallidin antibody. With the present method, the separation of kinin from kininogen was not necessary, because bovine serum low molecular weight kininogen did not cross-react with the kallidin antibody. The kinin present in the urine before incubation was also measured and urinary kallikrein activity was calculated by subtracting the preincubation kinin value from that obtained postincubation. In the present study, kallikrein activity was expressed as total kinin generated during an incubation of 20 minutes. The values of urinary kallikrein determined by the present method in 31 subjects showed a highly significant correlation with the values determined by the TAME esterolytic activity (r = 0.78, p < 0.001).

TAME esterolytic activity: TAME esterolytic activity was measured by the method modified by Matsuda and coworkers. Urine sample, 8 ml, was dialysed against running tap water for 16 hrs at 4°C, and then concentrated to 1/2 – 1/3 volume with polyethylene glycol at 4°C. The esterolytic activity was determined as follows. The concentrated urine, 0.1 ml, was incubated with 5 mM of p-tosyl-arginine-methyl-ester (TAME) dissolved in 0.1 M phosphate buffer, pH 8.0, at 37°C for 30 minutes, and 0.2 ml of 10% HClO4 solution was added to terminate the reaction. To oxidize methanol formed by the enzymatic reaction, 0.2 ml of 0.1% K2MnO4 solution was added, and then 0.1 ml of 0.1% NH2OH-HCL solution and 3.0 ml of 0.2% acetylacetone in 0.5 M ammonium malate solution, pH 6.0 were added. After the mixture was kept in a water bath at 56°C for 20 minutes, the fluorescence was measured at 410 nm excitation and at 510 nm emission. Esterolytic activity was expressed in terms of esterase unit which is defined as the amount of kallikrein hydrolyzing 1 μM of TAME per minutes per ml. The values of urinary kallikrein determined by TAME esterase activity in 24 subjects showed a highly significant correlation with the values determined by a bioassay using the autoperfused dog femoral arterial blood flow (r = 0.91, p < 0.001).

Measurement of urinary kinin: Urinary kinin was measured by Carretero’s method. The incubation system consisted of 125I-8-tyrosine-bradykinin, 3000 cpm (specific radiological activity, 800 – 1000 mCi/μM, Daiichi Radioisotope Corp), 0.01 – 0.02 ml of urine, and 0.1 ml of antiserum (1:16,000) adjusted to a final volume of 0.8 ml with 0.1 M Tris buffer, pH 7.4, containing 0.2% of gelatin and 0.1% of neomycin. The mixture was incubated for 24 hrs at 4°C and free kinin was separated with dextrancoated charcoal. After counting radioactivity, kinin content was calculated. This method is sensitive to measure 10 pg of kallidin. The recovery rate of added kallidin (50 – 500 pg) was 97 ± 4% (mean ± SE, n = 15). The metabolic fragments which are produced by incubating bradykinin, kallidin and methionyl-lysyl-bradykinin with chymotrypsin showed 0.5% cross-reaction with kallidin antiserum. The values of urinary kinin determined by the present method in 32 subjects showed a highly significant cor-
TABLE I  CHARACTERISTICS OF THE NORMAL SUBJECTS AND PATIENTS WITH ESSENTIAL HYPERTENSION UNDER AD LIBITUM Na DIET

<table>
<thead>
<tr>
<th></th>
<th>Normal Subjects</th>
<th>Essential Hypertension</th>
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<tbody>
<tr>
<td>No. of subjects</td>
<td>84</td>
<td>55</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>40.0 ± 1.6</td>
<td>37.1 ± 1.8</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>94 ± 1</td>
<td>117 ± 3***</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>1.48 ± 0.15</td>
<td>2.37 ± 0.41*</td>
</tr>
<tr>
<td>PAC (ng/100ml)</td>
<td>6.2 ± 0.5</td>
<td>6.7 ± 1.0</td>
</tr>
<tr>
<td>UV (ml/day)</td>
<td>1878 ± 67</td>
<td>1493 ± 69***</td>
</tr>
<tr>
<td>Urinary NaV (mEq/day)</td>
<td>281 ± 11</td>
<td>217 ± 14***</td>
</tr>
<tr>
<td>Urinary KV (mEq/day)</td>
<td>52 ± 3</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>Urinary PGEV (ng/day)</td>
<td>736 ± 32</td>
<td>397 ± 29***</td>
</tr>
<tr>
<td>Urinary KAV (μg/day)</td>
<td>34.5 ± 4.0</td>
<td>18.3 ± 2.8***</td>
</tr>
</tbody>
</table>

Values are expressed mean ± SEM. PRA = plasma renin activity; PAC = plasma aldosterone concentration; UV = urine flow; Urinary NaV = urinary excretion of Na; Urinary KV = urinary excretion of K; Urinary PGEV = urinary excretion of prostaglandin E; Urinary KAV = urinary excretion of kaliurex. *Values significantly different from normotensive values (P < 0.05); ***P < 0.005; ****P < 0.001.

Relation with values determined by a bioassay of extracted kinin using the autoperfused dog femoral arterial blood flow (r = 0.71, p < 0.01). Measurement of plasma renin activity: PRA was determined by means of radioimmunoassay of angiotensin I. Plasma, 1.0 ml, was adjusted to pH 5.5 and incubated at 37°C for 6 hrs with EDTA and disopropyl fluorophosphate. After the incubation, the sample was diluted 10-fold with physiological saline and heated in a boiling water bath for 5 minutes. After centrifugation, angiotensin I in the supernatant was assayed radioimmunologically. PRA was expressed in terms of nanograms of generated angiotensin I per milliliter of plasma per hour of incubation. This method was approximately 4 times more sensitive than Haber’s method.6

Measurement of aldosterone: PAC and urinary aldosterone were measured with a commercial radioimmunoassay kit (Cer Ire Sorin). This method is sensitive to 10 pg of aldosterone.

Urinary Na⁺ and K⁺ were measured with an autoanalyzer. All results were expressed as mean ± SEM. The significance of differences between mean values were evaluated by Student’s t-test.

RESULTS

Urinary excretion of PGE and kaliurex under ad libitum Na diet: Basal levels of PRA, PAC, urine flow, urinary excretion of PGE, that of kaliurex and that of Na in 84 normal subjects and in 55 patients with essential hypertension under unrestricted diet were shown in Table I. The urinary excretion of PGE and kaliurex was significantly lower in essential hypertensives than in control subjects (PGE; p < 0.001, kaliurex; p < 0.005). Urine flow and urinary Na excretion were also significantly decreased in essential hypertensives than in control subjects, while PRA was significantly higher in the former than in the latter (Urine flow; p < 0.001, Urinary Na; p < 0.001, PRA; p < 0.05). Among these parameters, significant correlations were observed between urinary excretion of PGE and that of Na (normal; r = 0.39, hypertensives; r = 0.62) or urine flow (normal; r = 0.30, hypertensives; r = 0.38) between urinary excretion of K and that of kaliurex (normal; r = 0.36, hypertensives; r = 0.33) and between urinary excretion of Na and that of K (normal; r = 0.52, hypertensives; 0.58) in normal subjects and in the patients with essential hypertension. There were also significant correlations between urinary excretion of PGE and that of K (r = 0.33) or between urinary kaliurex excretion and urine flow (r = 0.30) in normal subjects and between urinary excretion of kaliurex and PAC (r = 0.55) in the patients with essential hypertension. On the contrary, there were no significant correlations between urinary excretion of PGE and PAC or PAC, between urinary excretion of kaliurex and that of PGE or PAC, and between mean blood pressure and urinary excretion of PGE or kaliurex in normal subjects and in the patients with essential hypertension. No significant correlations were also found between PAC and urinary

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Fig. 1. Changes of urinary excretion of Na (U Na V), prostaglandin E (U PGE V) and aldosterone (U ALDO V), and plasma renin activity (PRA) after the dietary sodium depletion in 10 patients with essential hypertension. Urinary excretion of Na was decreased from 168 ± 14 mEq/day on the last day of the control period to 93 ± 9 mEq/day (p < 0.001) on that day of Na intake of 100 mEq/day and to 26 ± 4 mEq/day (p < 0.001) on that day of Na intake of 30 mEq/day. Similar change was found in urinary PGE excretion. The excretion rate was decreased from 529 ± 26 ng/day to 451 ± 40 (n.s) and to 305 ± 33 (p < 0.001), respectively. On the contrary, urinary excretion of kallikrein, that of aldosterone and PRA were augmented after sodium depletion. Urinary kallikrein output was increased from 3.1 ± 0.4 EU/day to 3.2 ± 0.4 (n.s) and to 4.8 ± 0.6 (p < 0.05), respectively. Urinary aldosterone excretion was also increased from 2.1 ± 0.4 μg/day to 3.4 ± 0.6 (n.s) and to 5.8 ± 1.2 (p < 0.02), respectively. PRA was increased from 4.0 ± 0.9 ng/ml/hr on the last day of the control period to 9.4 ± 1.4 ng/ml/hr (p < 0.01) on the last day of 30 mEq/day of Na intake.

Effect of furosemide and upright posture: The changes of urine flow, urinary excretion of Na, kallikrein and PGE, PRA and PAC after the administration of furosemide and upright posture in 19 normal subjects and in 16 patients with essential hypertension were shown in Table II. After furosemide administration each parameter was increased in both groups. The increment of PRA and PAC following furosemide administration was similar in normal subjects and in essential hypertensives. On the contrary, the increment of urinary PGE and kallikrein after furosemide administration was lower in

kallikrein excretion in normal subjects, between urinary excretion of PGE and that of K, and between urinary excretion of kallikrein and urine flow in the patients with essential hypertension. Urinary excretion of PGE in the patients with essential hypertension was compared with that in normal subjects with matched urinary Na excretion. In 32 normal subjects with the mean values of urinary Na excretion of 205 ± 5.5 mEq/day, the urinary excretion of PGE was 644 ± 36 ng/day and this value was significantly higher than that in essential hypertensives with mean values of urinary Na excretion of 217 ± 14 mEq/day (p < 0.001). There was obvious sexual difference in urinary PGE output. The excretion rates were 792 ± 28 ng/day in 61 healthy men and 592 ± 53 ng/day in 23 healthy women, and 454 ± 36 ng/day in 35 hypertensive men and 288 ± 48 ng/day in 20 hypertensive women, respectively. In healthy women and in hypertensive women, lower excretion of urinary PGE was found compared with healthy men and hypertensive men (healthy; p < 0.005, hypertensive; p < 0.02). Higher excretion of urinary PGE was found in healthy men than in hypertensive men (p < 0.001) and in healthy women than in hypertensive women (p < 0.001).

Influence of low Na diet: Figure 1 illustrates the changes of urinary excretion of Na, PGE, kallikrein and aldosterone and PRA after the dietary sodium depletion in 10 patients with essential hypertension. Urinary excretion of Na was decreased from 168 ± 14 mEq/day on the last day of the control period to 93 ± 9 mEq/day (p < 0.001) on that day of Na intake of 100 mEq/day and to 26 ± 4 mEq/day (p < 0.001) on that day of Na intake of 30 mEq/day. Similar change was found in urinary PGE excretion. The excretion rate was decreased from 529 ± 26 ng/day to 451 ± 40 (n.s) and to 305 ± 33 (p < 0.001), respectively. On the contrary, urinary excretion of kallikrein, that of aldosterone and PRA were augmented after sodium depletion. Urinary kallikrein output was increased from 3.1 ± 0.4 EU/day to 3.2 ± 0.4 (n.s) and to 4.8 ± 0.6 (p < 0.05), respectively. Urinary aldosterone excretion was also increased from 2.1 ± 0.4 μg/day to 3.4 ± 0.6 (n.s) and to 5.8 ± 1.2 (p < 0.02), respectively. PRA was increased from 4.0 ± 0.9 ng/ml/hr on the last day of the control period to 9.4 ± 1.4 ng/ml/hr (p < 0.01) on the last day of 30 mEq/day of Na intake.

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TABLE II  EFFECT OF FUROSEMIDE AND UPRIGHT POSTURE ON URINE FLOW (UV), PLASMA RENIN ACTIVITY (PRA), PLASMA ALDOSTERONE CONCENTRATION (PAC) AND URINARY EXCRETION OF PROSTAGLANDIN E (UPGEV), Na (UNaV) AND KALLIKREIN (UKAV) IN 19 NORMAL SUBJECTS AND 16 PATIENTS WITH ESSENTIAL HYPERTENSION

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
<th>Essential hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Furosemide</td>
</tr>
<tr>
<td>UV (ml/hr)</td>
<td>61.8 ± 5.7</td>
<td>510 ± 33***</td>
</tr>
<tr>
<td>UNaV (mEq/hr)</td>
<td>10.4 ± 0.8</td>
<td>69.1 ± 4.3***</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>1.6 ± 0.5</td>
<td>7.5 ± 0.7***</td>
</tr>
<tr>
<td>PAC (ng/100ml)</td>
<td>4.8 ± 0.6</td>
<td>18.2 ± 2.6***</td>
</tr>
<tr>
<td>UKAV (μg/hr)</td>
<td>2.8 ± 0.4</td>
<td>5.3 ± 0.9*</td>
</tr>
<tr>
<td>UPGEV (ng/hr)</td>
<td>26.2 ± 3.0</td>
<td>64.5 ± 11.3*</td>
</tr>
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</table>

*Values are expressed as mean ± SEM. **Values significantly different from control values (P < 0.05); ***P < 0.005; ****P < 0.001.

Fig. 2. Effect of angiotensin II antagonist on mean blood pressure (MBP), plasma renin activity (PRA), plasma aldosterone concentration (PAO), and urinary excretion of Na (UNaV), prostaglandin E (UPGEV) and kallikrein (UKAV) in 10 patients with essential hypertension (mean ± SEM). **p < 0.05, ***p < 0.01, ****p < 0.005.

essential hypertensives than in healthy subjects.

Effect of angiotensin II antagonist: To elucidate whether angiotensin II itself participates in the regulation of renal release of PGE and kallikrein, angiotensin II antagonist was infused. The changes of mean blood pressure, PRA, PAC, urinary Na excretion, urinary PGE excretion and urinary kallikrein excretion after the administration of 1-sarcosine-8-isoleucine-angiotensin II were illustrated in Figure 2. On the normal Na diet, mean blood pressure rose from 112 ± 4 to 126 ± 4 mmHg (p < 0.05) and PRA tended to decrease from 4.5 ± 1.3 to 2.3 ± 0.8 ng/ml/hr (n.s.). These changes might be explained by the agonistic action of this analogue. PAC and urinary kallikrein excretion tended to increase from 6.8 ± 0.8 ng/100 ml and 16.7 ± 4.3 ng/min to 11.4 ± 2.1 ng/100 ml (n.s) and 24.8 ± 5.5 ng/min (n.s), respectively. On the contrary, urinary excretion of Na, K and PGE tended to reduce from 188.3 ± 31.7 μEq/min, 43.3 ± 5.0 μEq/min and 0.40 ± 0.05 ng/min to 140 ± 16.7 μEq/min (n.s), 36.7 ± 3.3 μg/min (n.s) and 0.29 ± 0.04 ng/min (n.s), respectively. After sodium depletion, mean blood pressure, urinary Na output and urinary PGE excretion were reduced from 112 ± 4 mmHg, 188.3 ± 31.7 μEq/min and 0.40 ± 0.05 ng/min to 105 ± 3 mmHg (n.s), 30.0 ± 5.0 μEq/min (p < 0.01) and 0.25 ± 0.05 ng/min (p < 0.05), while PRA, PAC and urinary kallikrein excretion were increased from 4.5 ± 1.3 ng/ml/hr, 6.4 ± 0.8 ng/100 ml and 16.7 ± 4.3 ng/min to 10.8 ± 1.7 ng/ml/hr (p < 0.01), 16.2 ± 3.5 ng/100 ml (p < 0.05) and 36.8 ± 4.5 ng/min (p < 0.005), respectively. Under this sodium status mean blood pressure tended to reduce.

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TABLE III  EFFECT OF SPIRONOLACTONE ON URINARY EXCRETION OF Na (UNaV), PROSTAGLANDIN E (UpGEV) AND KALLIKREIN (UKAV) IN 3 PATIENTS WITH ESSENTIAL HYPERTENSION

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
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<tbody>
<tr>
<td>UNaV</td>
<td>UpGEV</td>
<td>UKAV</td>
</tr>
<tr>
<td>mEq/day</td>
<td>ng/day</td>
<td>µg/day</td>
</tr>
<tr>
<td>271</td>
<td>265</td>
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<tr>
<td>Control period</td>
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<td>147</td>
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<td>6 - 7th day</td>
<td>283</td>
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<td>217</td>
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<td>66</td>
<td>184</td>
<td>20</td>
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</table>
| Values are presented as an actual value or the mean value in each period.

from 10.8 ± 1.7 ng/ml/hr and 36.8 ± 4.5 ng/min to 8.7 ± 2.2 ng/ml/hr (n.s) and 31.2 ± 4.8 ng/min (n.s), respectively, after the antagonist administration, while PAC tended to increase from 16.2 ± 3.5 to 23.0 ± 3.3 ng/100 ml (n.s). On the other hand, urinary excretion of PGE, Na and K did not change following the administration of angiotensin II analogue during low Na diet.

**Effect of spironolactone:** The changes of urinary excretion of Na, kallikrein and PGE following spironolactone administration in 3 patients with essential hypertension were shown in Table III. Urinary excretion of kallikrein was gradually decreased after the administration of spironolactone, while urinary excretion of Na and PGE was increased immediately after the spironolactone administration and then decreased gradually to control level.

**Effect of indomethacin:** The changes of urinary excretion of Na, kallikrein and PGE, PRA and PAC after the administration of indomethacin in patients with essential hypertension were illustrated in Figure 3. After the indomethacin administration, each parameter was reduced. Urinary excretion of Na, kallikrein and PGE, PRA and PAC were decreased from 84 ± 8 mEq/day, 37.1 ± 16.7 µg/day, 321 ± 16 ng/day, 5.47 ± 1.7 ng/ml/hr and 19.0 ± 3.9 ng/100 ml to 64 ± 7 mEq/day (n.s), 24.5 ± 8.6 µg/day (n.s), 170 ± 10 ng/day (p < 0.001), 1.55 ± 0.20 ng/ml/hr (p < 0.05) and 4.7 ± 1.2 ng/100 ml (p < 0.005), respectively, following the indomethacin, while mean blood pressure tended to elevate from 101 ± 5 to 108 ± 6 mmHg (n.s).

Fig. 3. Effect of indomethacin on plasma renin activity (PRA), plasma aldosterone concentration (PAC), and urinary excretion of Na (UNaV), prostaglandin E (UpGEV) and kallikrein (UKAV) in 11 patients with essential hypertension (mean ± SEM). *p < 0.05, ***p < 0.005, ****p < 0.001.

from 105 ± 3 to 101 ± 2 mmHg (n.s) following 1-sarcosine-8-isoleucine-angiotensin II. PRA and urinary kallikrein excretion tended to decrease

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DISCUSSION

There are evidences that urinary kallikrein and PGE are originated in the kidney and their excretion rates reflect the biosynthesis of renal kallikrein and PGE or their release\textsuperscript{17–19}. Therefore, the urinary excretion of kallikrein and PGE was measured as an indicator of renal biosynthesis of these substances in the present experiment.

Margolius and his coworkers\textsuperscript{2} have reported that the release of kallikrein in the rat renal cortical cell suspension is increased by aldosterone and reduced by spironolactone and proposed a hypothesis that the biosynthesis of renal kallikrein is regulated by aldosterone or other sodium retaining steroid hormones. In the present study, urinary kallikrein excretion was decreased after the administration of spironolactone and this result supports their hypothesis. In 1976, Johnston and his coworkers\textsuperscript{20} reported that there was a close relationship between PRA and urinary kallikrein excretion in rats after the sodium loading or depletion. In the present experiment, urinary kallikrein excretion was increased after the stimulation of the renin-angiotensin-aldosterone system by low Na diet or the administration of furosemide and upright posture, while it tended to decrease after the inhibition of renin-angiotensin system by the administration of 1-sarcosine-8-isoleucine-angiotensin II under sodium depletion in spite of a mild increase in PAC value. These data suggest that angiotensin II itself may also directly regulate the biosynthesis of renal kallikrein though aldosterone is a major regulator of renal kallikrein generation. Recently, it has been reported that urinary excretion of kallikrein is dependent upon dietary potassium intake\textsuperscript{21}. In the present experiment, urinary kallikrein excretion was closely related to urinary K excretion in normal subjects and in the patients with essential hypertension. These data support that dietary potassium intake may participate in renal generation of kallikrein. However, a close relation between urinary excretion of K and that of kallikrein disappeared after the administration of 1-sarcosine-8-isoleucine-angiotensin II during normal Na diet or spironolactone. Thus, it seems that an effective levels of aldosterone regulated also by dietary potassium intake is more important regulator of renal kallikrein release than potassium itself. Our data showed that the changes in urinary kallikrein excretion paralleled with those of renin-angiotensin-aldosterone system following various stimuli, suggesting that renal kallikrein-kinin system may regulate blood pressure by opposing the action of renin-angiotensin-aldosterone system.

In 1970, McGiff and his coworkers\textsuperscript{3} reported that intrarenal arterial infusion of angiotensin II induced a marked release of PGE in venous effluent in the dog. Frolich and his coworkers\textsuperscript{19} also reported that urinary PGE\textsubscript{2} excretion was increased in the urine obtained from a ipsilateral ureter after the intrarenal arterial infusion of angiotensin II in the dog. In our experiment, however, urinary PGE excretion was decreased after sodium depletion in spite of an augmentation of the renin-angiotensin system. There was no correlation between urinary PGE excretion and PRA or PAC under normal sodium intake. Furthermore, urinary PGE excretion did not decrease after the inhibition of angiotensin II using antagonist during sodium depletion. On the other hand, urinary PGE excretion was concomitantly increased with PRA after the administration of furosemide and upright posture. However, the time course of PRA alteration following furosemide administration and upright posture was different from that of urinary PGE excretion in our recent paper\textsuperscript{22}. In addition, urinary excretion of PGE\textsubscript{2,α}-metabolite was decreased after the furosemide administration in spite of an increase in urinary PGE and PGE\textsubscript{2,α} excretion\textsuperscript{22}. The augmentation of urinary PGE excretion following furosemide administration seems to be independent of the enhanced renin-angiotensin system, but might be due to the inhibition of 15-hydroxy PG dehydrogenase induced by furosemide itself. Thus, the present data indicate that urinary PGE excretion may not be regulated by renin-angiotensin system in human subjects. In the present experiment, however, the urinary excretion of PGE was closely related to urinary Na output after low Na diet and after the administration of furosemide, 1-sarcosine-8-isoleucine-angiotensin II during normal Na diet or under spironolactone. A significant positive correlation was also found between basal levels of urinary PGE and those of urinary Na in normal volunteers and in the patients with essential hypertension. Recently, it has been reported that high potassium intake decreased urinary PGE excretion in rat\textsuperscript{23} and that hypokalemia caused an increased urinary excretion of PGE in dog\textsuperscript{24}. In our experiments, however, a positive correlation was found between urinary excretion of PGE and that of K in normal subjects. In the present data, there was a significant correlation

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between basal levels of urinary K and Na, and the relation between Na and urinary PGE is higher than that between K and PGE. Thus, the dietary Na intake seems to be more important regulator of urinary PGE excretion than potassium.

In our data, a significant correlation was also found between urinary PGE excretion and urine flow. A number of recent papers have described that PGE in the renal tubular compartment may participate in the feedback mechanism of antidiuretic action of this hormone is inhibited by PGE \(^2\) suggesting that renal PGE may also participate in the renal water regulation. These results suggest that renal PGE may be involved in the regulation of blood pressure by influencing the renal sodium and water handling.

It is now generally accepted that the kidney has both pressor and depressor mechanisms, and hypertension occurs when the former is stimulated or the latter is suppressed. As mentioned above, renal depressor substances, kallikrein and PGE generated in the kidney are considered to be involved in the regulation of blood pressure through the interaction with renin-angiotensin-aldosterone system and through influencing the renal sodium handling. In 1934, Elliot and Nuzum \(^5\) found that the excretion of urinary kallikrein was decreased in the patients with essential hypertension. In 1971, similar result was reported by Margolius and his coworkers. \(^6\) The present data that urinary output of kallikrein and PGE was decreased in essential hypertension indicate that the biosynthesis or the release of these vasodilating substances in the kidney is impaired in this disease. In addition, the data that the increase in urinary excretion of kallikrein and PGE after the furosemide administration and upright posture was less in essential hypertension than in normal volunteers in spite of the similar degrees of augmentation in renin-angiotensin-aldosterone system in both groups, support that the responses of renal kallikrein and PGE to these stimuli are also suppressed in essential hypertension when compared with that in normal volunteers.

What is the reason for the suppression of renal kallikrein and renal PGE in essential hypertension? It is very important to know whether the reduced renal kallikrein-kinin system and PGE in essential hypertension is one of the causes of hypertension or a secondary phenomenon in resulting from the development of essential hypertension. In the epidemiological study by Zinner and his coworkers \(^27\) in which urinary kallikrein concentration was measured in large population of children and their mothers, there was a significant familial clustering of high or low urinary kallikrein excretion. Families with lower kallikrein concentrations tended to have high blood pressures than did families with higher kallikrein concentrations. Their results suggest that the decreased urinary kallikrein excretion in essential hypertension may be a constitutional factor. Recently, Carretero and his coworkers \(^28\) have reported that urinary kallikrein excretion in the Dahl “S” rats is much lower than in the Dahl “R” rats and discussed the possibility that low kallikrein production in the S rats is the primary alteration in the kidney and perhaps the cause of hypertension. There is no epidemiological study regarding renal PGE and blood pressure. In 1976, Papanicolaou and his coworkers \(^29\) reported that there was a significant inverse relationship between mean blood pressure and urinary excretion of PGE in patients with essential hypertension, suggesting a causal relationship between raised blood pressure and the decreased levels of PGE in the urine. The recent report regarding orally active angiotensin I converting enzyme inhibitor, SQ 14225 indicates that the major antihypertensive effect of this drug is dependent upon the inhibition of renin-angiotensin-aldosterone system \(^30\). However, previous paper by Romero and Strong \(^31\) revealed that renin-angiotensin-aldosterone system was also inhibited by the administration of indomethacin in experimental hypertensive rabbits, but nevertheless hypertension advanced progressively in the animals with the impaired renal function. In the present experiment, blood pressure elevated slightly after the administration of indomethacin whereas the renin-angiotensin-aldosterone system was inhibited. Why does not the blood pressure reduce inspite of the suppression of renin-angiotensin-aldosterone system after the administration of indomethacin? This is a big puzzle in which a key may be in the depressor system. Suppression of renal PGE and renal kallikrein-kinin system in essential hypertension, an important finding in the present study, suggests that there is an impairment in defense mechanism against renin-angiotensin-aldosterone system and sodium retention in these patients.

There are several studies that the prostaglan- din system participates in the release of renin. Weber and his coworkers \(^32,33\) have reported that renin release can be stimulated by the PG precursor, arachidonic acid, or PG endoperoxides...
in the animals. Recently, Gerber and his coworkers\(^4\) have reported that PG\(_1\) and PGE\(_2\) can also stimulate the renin release in the dog kidney though the former is more potent than the latter. On the other hand, a number of observations indicate that PG synthetase inhibitor, indomethacin, inhibits the increase of renin secretion following furosemide injection or other stimuli\(^35\)–\(^37\). In the present experiment, an augmented PRA induced by the furosemide administration with low Na diet was also suppressed by the addition of indomethacin and this result supports the intrarenal mechanism of PG-mediated renin release. Recently, McGiff and his coworkers\(^4\) have found that PGI\(_2\) is generated in the renal cortical vascular compartment. The present data that urinary excretion of PGE was decreased after dietary Na depletion whereas PRA was increased and that a positive correlation was found between urinary excretion of PGE and Na suggest that renal PG, not PGE\(_2\) in renal tubular compartment, possibly PGI\(_2\) in renal vascular compartment may participate in the mechanism of the renin release.

McGiff and his coworkers\(^4\) have reported that there is some coupling between the kallikrein-kinin system and PGE within the kidney. In the present experiment, urinary excretions of PGE and kallikrein were increased after the administration of furosemide, and decreased after the administration of indomethacin. However, a dissociation between urinary kallikrein and PGE was found after dietary Na depletion and after the administration of the angiotensin II analogue during normal Na diet or under spironolactone. An opposite alteration occurred also after these stimuli in urinary Na excretion and in the renin-angiotensin-aldosterone system, suggesting that urinary PGE excretion is more dependent on urinary Na excretion than that on kallikrein and that urinary kallikrein excretion is more dependent on the renin-angiotensin-aldosterone system than on urinary PGE excretion.

In conclusion, renal depressor substances, kallikrein and PGE generated in the renal tubular compartment are considered to be involved in the regulation of blood pressure through the interaction with the renin-angiotensin-aldosterone system and through influencing renal sodium handling. The present data showed that basal level of urinary excretion of PGE and kallikrein was lower in essential hypertension than in normal subjects and that the release of renal kallikrein and PGE after furosemide administration was also suppressed in essential hypertension compared with that in normal subjects, suggesting that there is an impairment in the defense mechanism against the renin-angiotensin-aldosterone system and sodium retention.

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