Urinary Excretion of Kinin in Man with Special Reference to Its Origin

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The excretion rate of urinary kinin was estimated in 71 patients under various clinical conditions. The estimated values ranged from 5.3 to 36 μg per day in 14 healthy persons. No significant change in the kinin output was recognized in 32 patients with bronchial asthma, dermatological, neurological or pancreatic diseases in which kinin is generally considered to play an etiological role.

The kinin excretion was investigated in man during the intravenous infusion of kallikrein or bradykinin, or in dogs during the bradykinin infusion into renal artery. No correlation was demonstrated between the kinin level in circulating blood and the kinin excretion in urine in six patients. It was concluded from these experiments that circulating kinin is not excreted in urine, and urinary kinin is produced and secreted by the kidney, perhaps by the tubular cells.

In 1952, Beraldo found in urine of dogs an active principle called Substance U which stimulated the contraction of the isolated guinea-pig ileum, produced the vasodilatation and was easily destroyed by the incubation with chymotrypsin. Two years later, Werle and Erdös reported a similar substance in human urine and designated it as Substance Z. Thereafter, these substances have been investigated by several researchers. According to their studies, Substance U or Z cannot be distinguished from bradykinin or kallidin in its chemical and biological properties, and the name of urinary kinin has been given to this polypeptide in urine.

Most of the studies in the past, however, have dealt with only the pharmacological properties of urinary kinin or the assay methods of this substance. In addition, studies on the kinin excretion in patients under various clinical conditions have been very few. Contrary to the original reports, however, the recovery of kinin through these methods was found very low in our laboratory. Therefore, a simple and more accurate method for the quantitative determination of urinary kinin seemed to be needed.

In order to investigate the pathophysiological significance of urinary kinin
under various diseased conditions, the origin of this substance, and the relation of
this polypeptide in urine to that in blood, following studies were carried out using
our newly established methods:

I) Urinary excretion of kinin in the patients under various clinical condi-
tions.

II) Kinin excretion in urine during the intravenous infusion of kallikrein.

III) Kinin excretion in urine during the intravenous infusion of bradykin-
in.

IV) Kinin excretion in urine during the bradykinin infusion into renal artery
in dogs.

V) Simultaneous determination of kinin in urine and blood.

These studies have been partly reported previously, and it is the main
purpose of this paper to summarize the results obtained in the experiments on
urinary kinin.10,11

METHODS

Urine collection

Twenty-four hour urine was collected in a bottle containing about 10 ml of
concentrated hydrochloric acid. The acid was applied to make the pH of urine
below 4.0, for the urinary kinin is quite stable even at room temperature in such
an acid medium.

Extraction

Twenty milliliters of acidified urine were adjusted with 2N hydrochloric acid
to pH 1.8 (indicator paper, CR), saturated with sodium chloride, and extracted
with equal volume of n-butanol under vigorous shaking for 5 minutes. After
centrifugation at 3,000 rpm for 10 minutes, the organic phase was pipetted to
another centrifuge tube. The extraction was repeated once again with half
volume of butanol. Both extracts were combined, and 15 ml of petroleum ether
were added. The mixture was re-extracted twice with 2 ml of distilled water under
shaking for 5 minutes. The combined aqueous extract was concentrated under
reduced pressure to a volume around 0.6 ml at below 40°C. The neutrality of
the extract was attained with 0.5 N sodium hydroxide, and the final volume of the
extract was adjusted to 1.5 ml with distilled water.

Bioassay

A guinea-pig (150-250 g) was killed by a blow on the head, weighed and bled
from the carotid arteries. The terminal ileum was cut out, freed from the mesen-
terium and washed thoroughly with warm Tyrode solution through the lumen.
A 2-3 cm segment of anal side was suspended in a 10 ml bath filled with Tyrode
solution containing atropine sulphate (10⁻⁶) and promethazine hydrochloride (10⁻⁷) at 34–36°C. After 2–3 hours' equilibrium with aeration, standard solution containing 0.25–0.75 μg of synthetic bradykinin in 1 ml of Tyrode solution and samples were added to the bath alternatively. Contractions of the ileum strip were recorded on a smoked kymograph with a lever.

Samples were assayed by comparing their contracting activities with that of standard solution by the four-points method.

EXPERIMENTS AND RESULTS

I) Urinary excretion of kinin in the patients with various diseases

The urinary excretion of kinin was studied on about 70 human subjects. Distribution of their values was illustrated in Fig. 1 and Table I.

Fig. 1. Urinary excretion of kinin in the patients under various clinical conditions.

Normal control

Fourteen healthy persons were studied as normal control. Daily excretion rates of urinary kinin ranged from 5.3 to 36 μg (mean 19.6±9.1 μg), and were in good accordance with Horton's report.¹²

Dermatological diseases

The kinin excretion in urine was examined on 13 patients with various dermatological diseases in whom 2 cases of systemic lupus erythematosus, 2 of systemic panniculitis (Weber-Christian's syndrome) and 3 of urticaria were included. Normal amounts of kinin were excreted in 11 cases. But in a patient with Weber-Christian's syndrome, the estimated values were very high as 98, 140
TABLE I. The Excretion Rates of Urinary Kinin in the Patients with Various Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of subjects</th>
<th>Urinary kinin (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Healthy persons</td>
<td>14</td>
<td>5.3~36</td>
</tr>
<tr>
<td>Dermatological diseases*</td>
<td>12</td>
<td>3.7~29</td>
</tr>
<tr>
<td>Neurological diseases</td>
<td>14</td>
<td>7.5~37</td>
</tr>
<tr>
<td>Hypertension</td>
<td>10</td>
<td>5.3~34</td>
</tr>
<tr>
<td>Diseases of the alimentary tract</td>
<td>6</td>
<td>6.0~26</td>
</tr>
<tr>
<td>Renal diseases</td>
<td>7</td>
<td>3.1~35</td>
</tr>
<tr>
<td>Bronchial asthma</td>
<td>3</td>
<td>5.6~15</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>4</td>
<td>6.0~10</td>
</tr>
</tbody>
</table>

* The patient with Weber-Christian's syndrome who excreted a large quantity of urinary kinin was excluded.

and 190 µg per day on three occasions. In a patient with erythema annulare centrifugum, the excretion rate was low.

**Neurological diseases**

Fourteen neurological patients, 13 central nervous and one peripheral nervous disease, were studied on their kinin excretions. In 2 cases of migraine, the severe attacks of the headache were complained of. No change from the normal values was found in the kinin output in all cases.

**Hypertension**

Estimation of urinary kinin was made in 10 hypertensive patients; 5 cases of essential hypertension, 3 of primary aldosteronism, one of coarctation of the aorta and one of renovascular hypertension. Abnormally high or low excretions were found in none.

**Diseases of the alimentary tract**

Four patients with gastric ulcer and two patients with pancreatitis were examined. A case in the former was accompanied with intense hematemesis and was in a state of shock, and was treated with blood transfusions. Urinary kinin was studied in such a condition. In the latter, both cases showed slight elevation of amylase in urine, but severe signs were not observed. The estimated values of urinary kinin were 6.0~26 µg per day in six cases.

**Renal diseases**

Measurement of urinary kinin was performed in 7 patients, 3 with nephrotic syndrome and 4 with glomerulonephritis. The diagnosis was made clinically or histologically. In two cases of severe renal failure in whom glomerular filtration rate was lower than 10 ml per minute and non-protein nitrogen in the blood elevated over 100 mg per dl., the kinin excretions were very low. The values were 3.1 µg in one and 3.8 µg in the other. But the remaining 5 patients
maintaining normal renal functions excreted normal amounts of kinin.

**Bronchial asthma**

Three patients were studied on their kinin output. The values were 5.6 and 15 µg per day in 2 cases under the state of asthmatic attack with wheezing, cough and expiratory dyspnea and 14 µg in the other case having no-attack at the time of examination.

**Hyperthyroidism**

In hyperthyroidism, lower normal values were obtained in all of the 4 patients examined.

II) **Excretion of kinin during the intravenous infusion of kallikrein**

The kinin excretion was studied during the intravenous infusion of kinin-forming enzyme.

Kallikrein (Padutin, Bayer) was diluted with 5% glucose and infused intravenously in a patient in a total dose of 90 units for 30 minutes (Fig. 2). The fall of blood pressure was observed following infusion and the patient felt itching on the face and chest, but serious side-effects were not recognized during the infusion. The urines collected separately before, during and after the infusion were extracted and assayed for their kinin content. The excretions of kinin were 5.3, 11 and 5.2 ng per minute, respectively. This degree of increase is quite insignificant, because normal urinary kinin excretion ranged from 3.7 to 25 ng per minute.

![Fig. 2. Changes of blood pressure, pulse rate and urinary kinin excretion during intravenous infusion of kallikrein.](image)
Fig. 3. Changes of blood pressure, pulse rate and urinary kinin excretion during intravenous infusion of synthetic bradykinin.

III) Excretion of kinin during the intravenous infusion of synthetic bradykinin

Synthetic bradykinin was dissolved in 5% glucose and infused into an antecubital vein of another patient in a total dose of 450 µg for 27 minutes (Fig. 3). Tachycardia and flushing on the face and chest were immediately appeared after the infusion and the blood pressure dropped as illustrated in Fig. 3, but no other side-effects were observed.

The urinary excretion of kinin was studied prior to and during the intravenous infusion of synthetic bradykinin. The values were 9.8 ng per minute prior to and 19 ng per minute during the infusion. The increase of excreted kinin during the infusion was 9.2 ng per minute and the ratio of increased kinin to the total infused dose was only 0.054%. Therefore, any significant change was not recognized during the intravenous infusion of synthetic bradykinin.

IV) Excretion of kinin during the infusion of synthetic bradykinin into renal artery in dogs

Synthetic bradykinin was infused into the renal arteries in three dogs and their kinin excretions in urine were examined during the infusion.

The dogs were anesthetized with sodium pentobarbital, and thin polyethylene tube was inserted into one renal artery via the femoral artery. Urine was obtained from ipsilateral ureter which was also cannulated by polyethylene tube through a low midline abdominal incision. Three different doses of synthetic
bradykinin, 0.75, 1.5 and 3.0 μg per kg, were dissolved separately in 15 ml of 5% glucose and each solution was infused into the renal artery for 15 minutes. Control urine was obtained by the infusion of glucose only.

Remarkable diuresis was observed during the bradykinin infusions, but the systemic arterial pressure measured in the carotid artery was not altered. Table II showed the result on a dog weighed 13 kg. The kinin excretion in urine was not significantly increased during the infusion of bradykinin into the renal artery. In other two dogs, similar results were obtained.

V) Simultaneous determination of kinin in urine and blood

A method for the quantitative determination of kinin in blood has been studied for several years in this laboratory, and a practical method has been recently established. The author intends to publish the details of this method in the near future.

Using this new method, the content of kinin in blood was estimated simultaneously with the determination of urinary kinin in six patients (Table III). A patient with dermatomyositis in whom erythema and edema were conspicuous on the face and four extremities showed a high level of kinin in blood of 40 ng per ml (normal value 0–2 ng per ml) whereas his excretion of urinary kinin was within normal range. On the other hand, in a case of Weber-Christian’s syndrome who excreted a large quantity of urinary kinin, the estimated value of kinin in blood was normal. No correlation was found between the blood kinin level and the urinary kinin excretion in the remaining 4 patients.

| Table II. Changes in Kinin Output during Bradykinin Infusion into Renal Artery in a Dog |
|-----------------------------------------------|---|---|---|---|
| Bradykinin infusion rate | μg/kg/min | 0 | 0.05 | 0.1 | 0.2 |
| Total dose infused | μg | 0 | 9.7 | 18.4 | 37.5 |
| Bradykinin excretion | μg/15 min | 0.24 | 0.19 | 0.40 | 0.39 |
| Increase of excretion during infusion | μg/15 min | -0.05 | +0.16 | +0.15 |
| Ratio of increase | % | -0.27 | +0.86 | +0.40 |

<p>| Table III. Simultaneous Determination of Kinin in Blood and Urine |
|-------------------|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Disease</th>
<th>Age</th>
<th>Sex</th>
<th>Kinin in blood ng/ml</th>
<th>Kinin in urine μg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weber-Christian’s syndrome</td>
<td>14</td>
<td>M</td>
<td>1.4</td>
<td>140</td>
</tr>
<tr>
<td>Weber-Christian’s syndrome</td>
<td>49</td>
<td>F</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Dermatomyositis</td>
<td>55</td>
<td>F</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td>Essential hypertension</td>
<td>56</td>
<td>M</td>
<td>0</td>
<td>6.2</td>
</tr>
<tr>
<td>Migraine</td>
<td>29</td>
<td>M</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Leukemia</td>
<td>16</td>
<td>M</td>
<td>1.6</td>
<td>8.0</td>
</tr>
</tbody>
</table>
DISCUSSION

It has been confirmed that kinin plays an important role in various diseases such as angioneurotic edema\textsuperscript{13} acute pancreatitis\textsuperscript{14} or migraine.\textsuperscript{15} In the present experiments, the kinin excretion in urine was investigated in the patients with various diseases which have been assumed to have something to do with this polypeptide.

It has been postulated that kinin plays an important role in hyperemia or increased vascular permeability in the inflammation or allergy. But, as described above, in various dermatological patients, the kinin excretion was not increased. As an exception, abnormally high excretion of kinin was found in a patient with systemic panniculitis. As marked proteinuria and polyuria were observed in this case, the kidney must be involved in this disease. Why was found such a high excretion of kinin in this patient? Is it caused by non-suppurative inflammation in adipose tissue or renal lesion? In the present investigation, it is not possible to answer the question.

In 1958, Chapman and his colleagues\textsuperscript{16} observed that the cerebrospinal fluid collected from the patients with various central nervous diseases contained kinin-like substance or kinin-forming enzyme. Furthermore, according to their recent studies,\textsuperscript{17} kinin is released as a result of neural activity evoked by noxious stimulation, and pain-threshold is lowered by released kinin. However, in all patients with various neurological diseases, the kinin excretion was found to be within normal range.

Recently, it has been confirmed that the local edema, pain and hypotension of acute pancreatitis in human is caused by kinin produced by activated kallikrein or trypsin in pancreas tissue.\textsuperscript{14} Although the author have had no opportunity to study patients with typical acute pancreatitis, the kinin output in urine was normal in two cases of mild pancreatitis.

Any increase in the kinin excretion rate was not recognized in bronchial asthma in which kinin seemed to be a mediator in the attack of asthma.\textsuperscript{18} As the activity of the kinin-destructive enzyme, kininase, is very high in blood of patients with hyperthyroidism,\textsuperscript{19} it might be assumed that its activity in the kidney tissue is elevated as well, and supposedly it must lead to the low level of urinary kinin in all cases of this disorder studied.

In two patients with severe renal failure, the kinin excretion was very low.

Then, the great interest was aroused in the problem of whether urinary kinin originates from the circulating blood through the glomeruli or is produced and secreted by tubular cells. The former possibility seemed to take a place from the fact that the kinin excretion in urine was markedly reduced in the patient with severe renal failure. On the other hand, however, the fact that the kinin output was within normal range in the patients with dermatological diseases, mi-
graine, pancreatitis or bronchial asthma in whom kinin is generally considered to play an etiological role in the diseases may lead to the tubular origin of kinin.

To investigate the origin of urinary kinin, several experiments were performed.

The kinin-forming enzyme kallikrein, or synthetic bradykinin was infused intravenously. As flushing or itching on the face and chest, hypotension, and tachycardia appeared during the infusion, it was no doubt that kinin increased in peripheral circulating blood. But no significant change on the kinin excretion was recognized in urine during the infusion periods.

From this result, urinary kinin seemed not to originate from the circulating blood. However, the destructive enzyme of kinin, kininase, is so active in human blood or in various tissues that added bradykinin is too rapidly inactivated to be recovered after few minutes. According to Saameli and Eskes, the half-life of bradykinin was shorter than one minute in circulating blood. Therefore, if bradykinin would have been infused into antecubital vein, kininase in blood would act and destroy the most part of infused kinin before this polypeptide reaches to glomeruli. In order to take away the effect of the kinin-destructive enzyme, synthetic bradykinin was directly infused into the renal artery in dogs. Even under such conditions, however, any notable increase in kinin output was not observed in urine.

From these experiments, it was evident that circulating kinin is not excreted in urine in any significant amount. Since the molecular weight of bradykinin is around 1,000, it may easily be filtered through glomeruli. But it does not appear in urine. This fact seems to suggest that bradykinin is re-absorbed by tubular cells perhaps in the proximal part of the nephrons or completely destroyed in the glomeruli by kininase in the kidney.

If urinary kinin does not originate from circulating blood, where does it come from? As mentioned previously, it seems very probable that kinin is produced and secreted by renal tubular cells perhaps in the distal part of the nephrons.

If the source of urinary kinin would be renal tubular cells, the correlation could not be demonstrated between the kinin content in circulating blood and the kinin excretion in urine. In order to prove this presumption, the simultaneous determination of this polypeptide was carried out in blood and urine on six patients. The values of blood kinin ranged from 0 to 2 ng per ml in the healthy persons. In a patient with dermatomyositis, the content of blood kinin was elevated up to 40 ng per ml, but the kinin output in urine was not increased. On the contrary, the level of blood kinin was within normal range in a patient with systemic panniculitis, in spite of the fact that a large quantity of kinin was excreted in urine. In the other 4 cases, no correlation was recognized between both values, too.
Acknowledgment

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