Demonstration of Kallikrein in a Rat Pancreatic Acinar Cell Carcinoma


Department of Physiology and Biochemistry, Dental Faculty, University of Oslo, 0316 Oslo 3, Norway [T. B., L. J., H. B.]; Department of Pathology, Medical School, Northwestern University, Chicago, Illinois 60611 [L. J. H., J. K. R.]; and Institute for Biochemistry, The Royal Dental College, Copenhagen, Denmark [K. P.]

ABSTRACT

Kallikrein was identified immunohistochemically and biochemically in a transplantable pancreatic acinar cell carcinoma of the rat. The concentration of immunoreactive kallikrein in tumor homogenates was the same as in the pancreas. Kallikrein in tumor cells exists as a proenzyme and is released into blood in high concentrations.

The impact of the presence of a kallikrein-producing tumor on other kallikrein-containing organs and other possibly interrelated systems was investigated. The concentration of kallikrein in the submandibular gland and pancreas of host rats was not significantly different from that of control rats. Urinary kallikrein secretion was significantly increased, although this may be a result of the high plasma glandular kallikrein concentration combined with kidney damage. The plasma concentration of kininogen, kininase, and renin was not significantly different from control rats.

Rats with tumor had significantly lower blood pressure than did control animals, and blood pressure was inversely related to the concentration of glandular kallikrein in plasma. However, it was not proven that the low blood pressure was due to the high concentration of kallikrein. Nephrectomized tumor rats gave a smaller hypotensive response to kininase inhibition than was expected from their high concentration of circulating kallikrein. This may be explained by the absence of the "free kallikrein" fraction in plasma of host rats.

INTRODUCTION

In humans, most pancreatic carcinomas are of ductal cell origin (20). However, a transplantable pancreatic acinar cell carcinoma has been developed in the rat (18). These tumor cells have been shown to function as pancreatic acinar cells in the capability to synthesize enzymes like amylase and lipase (7, 17, 19). The exocrine pancreas has been shown to contain glandular kallikrein in rats (2, 6, 25) and humans (26). It therefore seems reasonable to assume that the tumor should also contain kallikrein.

In rats with acinar cell carcinoma, the circulating levels of lipase are higher than in control rats (18), indicating that enzymes of the pancreatic tumor are released into the circulation. An elevated concentration of glandular kallikrein in plasma may therefore be expected.

The purpose of this study was to show whether the transplantable pancreatic carcinoma contains kallikrein and whether the enzyme could be detected in blood. We also wanted to study the effect of the presence of a kallikrein-producing tumor on the concentration of kallikrein in other organs, on other components of the system like kallikrein substrate and inhibitors, and kininase, as well as on a possibly interrelated system like the renin-angiotensin system. Since glandular kallikreins have been thought to play a role in blood pressure homeostasis, we measured blood pressure and the hypotensive response to kininase inhibition in tumor-carrying rats.

MATERIALS AND METHODS

A pancreatic acinar carcinoma was produced and transplanted onto weanling male F344 rats as described previously (17, 18). The tumors used in this study were of the 33rd to 35th transplant generation. The experiments were performed in 2.5- to 3.5-month-old rats carrying tumors weighing 20 to 30 g. Age- and sex-matched normal rats without tumors were used as control animals.

Identification of Kallikrein in the Tumor

Kallikrein was identified in the tumor by immunohistochemistry and biochemically in tissue homogenates. Tumor tissue was extirpated during Nembutal anesthesia (60 mg/kg body weight) and processed for immunohistochemistry or frozen (−20°) for later analysis.

Immunohistochemistry. Immunohistochemical detection of kallikrein in the tumor cells was done after ethanol fixation and paraffin embedding as described previously (25). Tissue sections (6 μm) were stained by direct and indirect immunofluorescence technique (22, 25, 30) or by the peroxidase-antiperoxidase method (30). The anti-kallikrein antiserum applied in the first layer, was raised in rabbits against rat submandibular gland kallikrein and characterized as in a previous study (30). For direct immunofluorescence technique, a rhodamine conjugate of the IgG fraction of the anti-kallikrein antiserum (22) was used in a 0.3 g/liter dilution. For the indirect immunofluorescence technique, the anti-kallikrein IgG fraction was used at 0.3 g/liter. In addition, monospecific anti-kallikrein antiserum, preabsorbed with cross-reacting submandibular gland esterase (30), was used in the primary layer (1:10 dilution). For the peroxidase-antiperoxidase method, the IgG fraction was used at a 0.1-g/liter dilution. Immunospecificity of the staining reaction was confirmed by absorption controls adding an excess of purified kallikrein (0.4 and 0.1 g/liter for the fluorescence and peroxidase-antiperoxidase methods, respectively), or by the direct immunofluorescence technique, a rhodamine conjugate of the IgG fraction of the anti-kallikrein antiserum (22) was used in a 0.3 g/liter dilution. For the indirect immunofluorescence technique, the anti-kallikrein IgG fraction was used at 0.1 g/liter. For the indirect immunofluorescence technique, the anti-kallikrein IgG fraction was used at 0.1 g/liter. For the indirect immunofluorescence technique, the anti-kallikrein IgG fraction was used at 0.1 g/liter. For the indirect immunofluorescence technique, the anti-kallikrein IgG fraction was used at 0.1 g/liter.

Biochemical Analysis. Tumor homogenates were prepared in phosphate-buffered saline (0.01 M sodium phosphate, pH 7.4:0.15 M NaCl, 1:10, w/v) in a Potter-Elvehjem homogenizer (4", 20 strokes). After centrifugation (10,000 × g, 4°, 30 min), the supernatant was collected and frozen (−20°). The supernatant was later assayed for its concentration of glandular kallikrein as described below. For comparison, the concentrations of trypsin-like and amylase activity and protein were measured.

Glandular Kallikrein System of Other Kallikrein-containing Organs

To study the excretion of kallikrein into urine, host and age- and sex-matched control rats were placed in metabolic cages. Twenty-four-hr urine was collected using toluene to prevent bacterial growth. The submandibular glands, the pancreas, and the kidneys were extirpated under ether anesthesia, homogenized in phosphate-buffered saline (1:10,
The kidneys were also examined histologically after in vivo fixation with ethanol (23). Tissue sections were stained for kallikrein by the peroxidase-antiperoxidase method as described previously (28). Sections were also stained with hematoxylin and eosin and were examined in the microscope under regular tungsten light and for fluorescence using the same conditions as for fluorescein (24). This allowed detection of fluorescing eosinophilic structures like the basal membrane which were not easily seen under tungsten light.

**Demonstration of Prokallikrein in Tumor Tissue**

Tumor tissue homogenate was reacted with immobilized antibody overnight and assayed subsequently for amidolytic activity (9). One set of sample duplicates was tested for amidolytic activity directly, whereas another was incubated with trypsin (0.05 g/liter; 30 min, 22°), followed by SBTI (2 g/liter final concentration, 30 min, 22°). Immunoreactive kallikrein was tested in both samples by subsequent addition of radiolabeled antibody (9).

**Measurement of Kallikrein-like Amidolytic Activity**

Amidolytic activity was measured in urine, tumor, kidney, pancreas, and submandibular gland homogenates and in column fractions using the chromogenic substrate for glandular kallikrein, S-2266, as described previously (1). The samples were preincubated (30 min, 22°) with SBTI (10 g/liter final concentration) (25). SBTI and Trasylol (500 IU/ml) were added to a substrate-sample blank. One AU was equal to the amount of enzyme that split 1.0 μM of substrate per minute. Pancreatic prokallikrein was activated by leaving the homogenate at room temperature for 24 hr prior to addition of SBTI.

**Measurement of Plasma Kininogen and Kininase Concentration**

For measurements of the concentration of kininogen in plasma, a limited amount of plasma (20 μl) was incubated (30 min, 37°) with an excess of submandibular gland kallikrein (0.59 g/liter) in buffer (0.1 M sodium phosphate, pH 8.5) containing phenanthroline (108 g/liter) and EDTA (2%). Released kinin was assayed by radioimmunoassay (4). The concentration of kininogen was expressed as the amount of kinin released per ml of plasma. Kininase activity was determined by incubating 10 μl of plasma (15 min, 37°) with bradykinin (90 g/liter) as substrate using the same buffer as above but without phenanthroline or EDTA. Recovery of kinin in the assay was tested by incubation of bradykinin without the addition of plasma. The concentration of total kininase activity was expressed as the amount of bradykinin degraded per min per ml of plasma.

**Measurement of Trypsin, Amylase, and Renin Activity**

Trypsin activity of tumor and pancreatic homogenates was assayed by caseinolytic activity (11). One caseinolytic unit (CU) was defined as the amount of enzyme that increased the OD280 by 1 unit in 1 min. In addition, trypsin activity was assayed by the chromogenic substrate for glandular kallikrein as described above but without the preincubation with SBTI. Trypsinogen of tumor and pancreatic homogenates were autolytically inactivated by leaving the homogenate at room temperature for 24 hr. Amylase activity of tumor and pancreatic homogenates was assayed by the Phadebas Amylase Test. Amylase converting-enzyme activity of kidney homogenates was measured by the method of Cushman and Cheng (5). Protein was measured by the method of Lowry et al. (15) using bovine serum albumin as standard. Plasma renin activity was measured by the antibody trapping method (14).
RESULTS

Identification of Kallikrein in the Pancreatic Acinar Carcinoma. Immunohistochemistry revealed the presence of kallikrein in the cytoplasm of the pancreatic acinar carcinoma cells (Fig. 1). The immunospecificity of this staining reaction was confirmed by the absorption controls; the staining was abolished by addition of purified kallikrein to the primary antibody but not by addition of the cross-reacting submandibular gland antigens. The presence of kallikrein in the pancreatic tumor cells was confirmed by measurements of tissue homogenates by the immunoradiometric assay with a dose-response curve that paralleled the standard curve (Chart 1). The immunoreactive kallikrein of tumor and pancreatic tissue both eluted from the column in the same position as kallikrein-like amidolytic activity and as purified submandibular gland kallikrein (Chart 2). The specific activity of tumor immunoreactive kallikrein after gel filtration was 297 AU/mg, only 37% of that of submandibular gland kallikrein. Kallikrein absorbed directly from tumor tissue homogenates to immobilized antibody did not show enzymatic activity unless activated by trypsin. The activated enzyme had a specific activity of 685 ± 60 (S.E.) AU/mg. These results indicate that kallikrein in tumor tissue was present as a proenzyme.

The concentration of kallikrein and other pancreatic enzymes (amylase and trypsin-like activity) in tumor and pancreatic homogenates is shown in Table 1. The concentration of kallikrein per mg protein measured in tumor tissue was not significantly different (p > 0.05) from that of the pancreas of host or control rats. The concentration of trypsin (caseinolytic) and amylase activity per mg protein was, on the other hand, significantly lower in tumor tissue than in the pancreas of host and control rats.

Effect of the Tumor on Other Kallikrein-containing Organs. In host rats, the concentration of immunoreactive kallikrein in the pancreas or the submandibular gland was not significantly different from that of the control group (p > 0.05; Table 1). In the submandibular gland, kallikrein-like and trypsin-like amidolytic activity was, however, significantly lower in the host than in the control group both per mg protein and per gland (Table 1). If the amount of kallikrein was calculated using the kallikrein-like amidolytic activity and the specific activity of submandibular gland kallikrein (694 AU/mg), the concentration was 29.0 and 49.4 mg/mg protein for the host and control group, much higher than that measured immunologically (Table 1). Since the recovery of the immunological measurements in the submandibular gland was close to 100%, these results show that, in the submandibular gland, the amidolytic assay was not specific for kallikrein only, even after addition of SBTI. Measurements of kallikrein immunoreactivity was positively correlated to measurements of kallikrein-like amidolytic activity (PR, 0.64; p < 0.05).

The kidneys of the host rats had undergone hypertrophic changes with an increase in kidney weight but with no significant change in the concentration of protein per g, wet weight (Table 2). Histochemistry of the kidney of the tumor rats revealed interstitial glomerulonephritis with atrophic changes, particularly in the proximal tubules, and with a thickening of the basal membrane (Fig. 2). The concentration of kallikrein in the kidney was significantly increased (p < 0.005) in the tumor group, both per kidney and per mg protein (Table 2). However, immunohistochemistry revealed no increase in kallikrein in tubular cells of the host kidney but seemed to be rather less than in the control group. The renal concentration of amidolytic activity both without or with SBTI was significantly higher in the host than in the control group (Table 2).

The concentration and 24-hr secretion of immunoreactive kallikrein into urine was significantly higher in the host group than in the control group (Table 3). The specific amidolytic activity of immunoreactive kallikrein in urine was 726 and 652 AU/mg for the host and the control group, respectively. An increase was also observed in kallikrein-like amidolytic activity (Table 3). The immunoreactive kallikrein in urine of both groups eluted similarly as submandibular gland, pancreatic, and tumor kallikrein on the ACA22 column (Charts 2 and 3). Unlike the pancreatic and tumor kallikrein, the specific activity of the urinary kallikrein-peak was 1228 and 768 AU/mg, for the host and the control group, respectively. Since purified submandibular gland kallikrein has a specific activity of 694 AU/mg, these results indicate that the kallikrein extracted from urine was fully active. The peak of kallikrein in urine of host rats was 10 times higher than in the control urine.

Amidolytic activity in urine was significantly higher (p < 0.005) without the presence of SBTI than with, and the difference...
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Chart 2. Gel filtration on Ultrogel ACA22 of tumor (a) and pancreatic (b) tissue homogenates. *, absorbance at 280 nm; □, amidolytic kallikrein-like activity; △, immunoreactive kallikrein; ○, enzymatic activity of the immunoreactive kallikrein. The kallikrein in tumor tissue eluted in the same position as that of pancreatic tissue and as purified submandibular gland kallikrein (arrow).

Chart 3.

Fraction number

between the host and the control group was more pronounced for trypsin-like than for kallikrein-like amidolytic urinary concentration and 24-hr secretion (Table 3).

Glandular Kallikrein in Plasma. The concentration of immunoreactive kallikrein and enzyme activity and the degree of enzyme inhibition of glandular kallikrein in plasma of host and normal rats is given in Table 4. The concentration of immunoreactive kallikrein and kallikrein enzyme activity was, respectively, 147 and 159 times higher in host than in control rats. The specific amidolytic activity or degree of inhibition of kallikrein in plasma from host rats was not significantly different from that of control rats (p > 0.05). These observations were confirmed by gel filtration of host and normal plasma (Chart 4). The molecular weight range of kallikrein-inhibitor complexes was comparable for the 2 plasmas, the peaks being largely elevated in the tumor plasma compared to that of control animals. However, some differences were observed in the pattern of enzymatic activity; the peak which may represent free glandular kallikrein in normal plasma (Chart 4b) was not observed in host plasma but, in the latter, one small additional peak of residual enzymatic activity was observed (Chart 4a). Recovery of immunoreactive kallikrein from the column was 76 and 97% for host and normal plasma, respectively and, for enzymatic activity, it was 59 and 68%.

The concentration of glandular kallikrein in plasma was positively correlated to tumor wet weight (PR, 0.70; p < 0.05).

Effect of the Tumor on Other Enzymes, Possibly Related to Blood Pressure Regulation. Table 5 shows the concentration of renin, total kininase activity, and kininogen in plasma and the concentration of angiotensin converting enzyme in the kidney. Plasma renin and kininase activity and kininogen concentration was not significantly different in the 2 groups (p > 0.05). However, the concentration of angiotensin-converting enzyme was significantly elevated in the kidney of the host group compared to the control group (p < 0.025).

Effect of the Tumor on Arterial Blood Pressure. Arterial blood pressure was significantly lower in the host than in the control group, both for anesthetized (p < 0.025) and awake (p < 0.005) animals (Table 6). The BP of host rats was inversely related to the concentration of glandular kallikrein in plasma (PR, 0.87 and 0.87; p < 0.05 for anesthetized and awake rats). In 24-hr nephrectomized male rats, captopril induced a hypotensive response in host rats, whereas the response was practically
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Table 1
Concentration of kallikrein, trypsin, and amylase activity in homogenates of tumor, pancreas, and submandibular gland tissue in host and control animals

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Kallikrein concentration</th>
<th>Trypsin activity</th>
<th>Amylase concentration</th>
<th>Amylase activity without SBTI (AU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IRMA (μU/mg)</td>
<td>Amidolytic with SBTI (AU/mg)</td>
<td>Casenolytic (CU/mg)</td>
<td>Amidolytic without SBTI (AU/mg)</td>
</tr>
<tr>
<td>Tumor</td>
<td>0.29 ± 0.04</td>
<td>0.11 ± 0.02</td>
<td>1.3 ± 0.3</td>
<td>0.21 ± 0.19</td>
</tr>
<tr>
<td>Host pancreas</td>
<td>0.29 ± 0.04</td>
<td>0.09 ± 0.03</td>
<td>6.1 ± 1.4</td>
<td>1.31 ± 0.59</td>
</tr>
<tr>
<td>Control pancreas</td>
<td>0.22 ± 0.03</td>
<td>0.07 ± 0.02</td>
<td>6.5 ± 2.0</td>
<td>0.73 ± 0.42</td>
</tr>
<tr>
<td>Host submandibular gland</td>
<td>17.7 ± 2.0</td>
<td></td>
<td>Not done</td>
<td>29.8 ± 2.2</td>
</tr>
<tr>
<td>Control submandibular gland</td>
<td>23.9 ± 1.4</td>
<td></td>
<td>Not done</td>
<td>44.6 ± 2.5</td>
</tr>
</tbody>
</table>

Mean ± S.E. p < 0.005 for host rats versus control rats in all parameters.

Table 2
Wet weight, concentration of protein, immunoreactive kallikrein, and kallikrein-like and trypsin-like amidolytic activity in the kidney of host (n = 6) and control (n = 6) rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Host rats</th>
<th>Control rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>g, wet wt/kidney</td>
<td>1.58 ± 0.19</td>
<td>0.92 ± 0.04</td>
</tr>
<tr>
<td>mg protein/g, wet wt</td>
<td>55.5 ± 7.1</td>
<td>61.7 ± 1.2</td>
</tr>
<tr>
<td>Kallikrein (ng/mg protein)</td>
<td>74.0 ± 8.9</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>S-2266 (AU/mg protein × 10^-8)</td>
<td>77 ± 17</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Kallikrein-like trypsin-like</td>
<td>140 ± 30</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Tumor (ml/24 hr)</td>
<td>31.6 ± 5.8</td>
<td>8.6 ± 1.5</td>
</tr>
<tr>
<td>Kallikrein concentration (μg/ml)</td>
<td>7.3 ± 0.8</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Kallikrein excretion (μg/24 hr)</td>
<td>212.0 ± 25.3</td>
<td>12.0 ± 2.0</td>
</tr>
<tr>
<td>Amidolytic activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With SBTI (AU/24 hr)</td>
<td>108.5 ± 31.1</td>
<td>12.0 ± 2.0</td>
</tr>
<tr>
<td>Without SBTI (AU/24 hr)</td>
<td>499.7 ± 286.5</td>
<td>19.3 ± 2.4</td>
</tr>
<tr>
<td>Protein (mg/ml)</td>
<td>28.5 ± 5.2</td>
<td>7.5 ± 1.6</td>
</tr>
</tbody>
</table>

Mean ± S.E. p < 0.005 for host rats versus control rats in all parameters.

Discussion

Kallikrein was demonstrated in a pancreatic acinar cell carcinoma, biochemically in tissue homogenates, and immunohistochemically in the cytoplasm of the tumor cells. As in the normal rat pancreas (15, 25), kallikrein in tumor cells exists as a proenzyme. The low specific activity of kallikrein in tumor tissue after autoactivation compared to that of trypsin-activated tumor kallikrein extracted by immobilized antibody and that of purified submandibular gland kallikrein was probably due to incomplete activation by the present procedure. The concentration of kallikrein in tumor tissue was not significantly different from that of both host and normal pancreas, whereas the concentration of trypsin-like and amylase activity was significantly lower. The high concentration of kallikrein in plasma of host rats indicated that kallikrein was released from tumor cells into the vascular compartment. An endocrine release of kallikrein is probably taking place also in the pancreas, since a 1.5-times higher concentration of glandular kallikrein was observed in plasma sampled from the vena porta compared to that in arterial blood (3). The present results show that a transplantable pancreatic acinar cell carcinoma resembles normal pancreatic acinar cells in that it contains and secretes glandular kallikrein, as it also contains and secretes other enzymes of the exocrine pancreas (18, 19).

The presence of an elevated concentration of glandular kallikrein enzyme activity in plasma of host rats was also confirmed by the hypotensive response to captopril. However, the hypotensive response was not so great as could be expected from the measured concentration of kallikrein in plasma. In a previous study (27, 31), sympathetic stimulation of the submandibular gland induced a rise in the concentration of glandular kallikrein in plasma which was about one-tenth of that observed in the host rats. Still, the hypotensive effect of kallikrein inhibition in nephrectomized tumor rats was only 18.4 ± 4.2 mm Hg whereas, after sympathetic nerve stimulation, a fall of 43 ± 8.3 mm Hg was observed. This difference may be due to an altered binding pattern to inhibitors, since plasma from host rats, unlike that after submandibular gland stimulation, did not show kallikrein

*a T. Berg and L. Johansen, unpublished data.*

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Table 4

Determination of glandular kallikrein and its enzymatic activity in arterial plasma in host and control rats

The concentration of kallikrein was also measured in 24 hr binephrectomized rats, 30 min after administration of captopril. Specific enzyme activity, expressed in AU, represents residual amidolytic activity per mg kallikrein adsorbed from plasma to the solid-phase antibody. Percentage of inhibition was calculated by comparing this specific activity with that of purified kallikrein standard in buffer adsorbed to the solid phase.

<table>
<thead>
<tr>
<th></th>
<th>Plasma kallikrein concentration (ng/ml)</th>
<th>Kallikrein enzymatic activity (mAU/ml)</th>
<th>Specific enzyme activity (AU/mg)</th>
<th>Inhibition (%)</th>
<th>Rats (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>Not nephrectomized</td>
<td>3063.4 ± 1089.9* a</td>
<td>380.5 ± 137.6 b</td>
<td>0.13 ± 0.02 b</td>
<td>NS</td>
<td>84.1 ± 1.9 b 86.3 ± 2.3 NS</td>
</tr>
<tr>
<td>Host rats</td>
<td>20.9 ± 2.4</td>
<td>2.4 ± 0.6</td>
<td>0.11 ± 0.02</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Control rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hr nephrectomized</td>
<td>5667.6 ± 1010.4 a</td>
<td>890.2 ± 167.8 a</td>
<td>0.17 ± 0.03 a</td>
<td>77.3 ± 4.6</td>
<td>5</td>
</tr>
<tr>
<td>Host rats</td>
<td>58.3 ± 6.6</td>
<td>2.5 ± 0.4</td>
<td>0.04 ± 0.01</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Control rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± S.E.

b \( p < 0.005 \)

NS, not significant \( p > 0.05 \).
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Chart 4. Gel filtration on Ultragel ACA22 of host (a) and normal (b) rat plasma. \( A \), absorbance at 280 nm; \( \bullet \), immunoreactive kallikrein; \( O \), enzymatic activity of the immunoreactive kallikrein. The percentage of total inhibition of enzymatic activity was not significantly different between the 2 plasmas but the peak of enzymatic activity in normal plasma which may represent free kallikrein (asterisk, b) was not detected in host plasma. On the other hand, in host rat plasma, one additional peak of enzymatic activity was observed in a higher-molecular-weight region (asterisk, a).

Table 5

<table>
<thead>
<tr>
<th>Concentration of plasma renin and kininase activity, plasma kallikrein, and renal angiotensin-converting enzyme in the host and the control groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host rats</td>
</tr>
<tr>
<td>Plasma renin concentration (GU ( \times 10^{-3} )/ml)</td>
</tr>
<tr>
<td>Plasma kallikrein (mg bradykinin/ml)</td>
</tr>
<tr>
<td>Plasma kallikrein activity (ng bradykinin/ml/min)</td>
</tr>
<tr>
<td>Renal angiotensin-converting enzyme (U/mg protein)</td>
</tr>
</tbody>
</table>

\( ^a \) Mean ± S.E.
\( ^b \) Numbers in parentheses, number of rats.
\( ^c \) NS, not significant (\( p > 0.05 \)).

The highly increased concentration of circulating glandular kallikrein may also be due to the fact that binding to inhibitors is a progressive process. When kallikrein is released from the tumor, it is likely that this happens more slowly than that after submandibular gland stimulation, thus allowing the time for more efficient inhibition. It is also possible that the constantly elevated concentration of kallikrein in host rats may induce an increased production of inhibitors to kallikrein. Alterations at the level of kinin receptors is another possibility. Further studies are needed to elucidate this problem.

Table 6

Effect of the presence of the kallikrein-producing tumor on arterial blood pressure

<table>
<thead>
<tr>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake rats</td>
</tr>
<tr>
<td>Host rats</td>
</tr>
<tr>
<td>107.8 ± 5.2 ( ^a ) (6)</td>
</tr>
<tr>
<td>77.4 ± 5.0 (12)</td>
</tr>
</tbody>
</table>

\( ^a \) Mean ± S.E.
\( ^b \) Numbers in parentheses, number of animals.
\( ^p < 0.025 \).
\( ^d \) \( p < 0.005 \).
kallikrein in host rats did not influence the concentration of kallikrein in kallikrein-containing organs like the submandibular gland and pancreas. This indicates that there is no direct feedback mechanism between local kallikrein synthesis in these organs and the concentration of kallikrein in plasma. On the other hand, renal kallikrein concentration and urinary kallikrein secretion was 19 and 18 times higher than in the control rats, respectively. Since there was no indication of an increase of kallikrein in kidney tissue sections, this elevated concentration of kallikrein in tissue homogenates was probably due to a high concentration of kallikrein in the preurine, filtered from the kallikrein-rich plasma. The filtration of kallikrein may be unspecific and a result of the proteinuria due to the interstitial glomerular nephritis in the host rats. However, the increase in the concentration of circulating kallikrein following nephrectomy in normal rats, as also observed by others (12, 16), may indicate that filtration of circulating glandular kallikrein is a normal physiological mechanism.

The high concentration of kallikrein and kallikrein enzyme activity in plasma of host rats may induce a continuous formation of kinins. This kinin formation may be responsible for the low blood pressure observed in these animals, even though this was not proven by the present experiments. BP was also inversely related to the concentration of kallikrein in plasma. The higher concentration of angiotensin-converting enzyme/kininase II in the kidney of the host rats compared to normal rats may be a protective response against an elevated plasma or renal kinin formation. The concentration of plasma renin was, however, not altered, suggesting that no direct feedback mechanisms exist between the concentration of renin and glandular kallikrein in plasma.

In conclusion, the present paper describes the demonstration of kallikrein in a transplantable pancreatic acinar cell carcinoma of the rat. The presence of a kallikrein-producing tumor did not alter the concentration of kallikrein in other kallikrein-containing organs or the concentration of other components of the system like kininogen and kininase activity. Of components of possibly interrelated systems, the plasma renin concentration was not altered, whereas a minor elevation of renal angiotensin-converting enzyme was observed. The high concentration of glandular kallikrein released into the circulation may be responsible for the hypotension in the host rats.

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Fig. 1. Demonstration of glandular kallikrein in cells of a pancreatic acinar cell carcinoma by immunofluorescence. × 956.

Fig. 2. Kidney from host rat (A) and from control rat (B) fixed in ethanol and stained with hematoxylin and eosin. The kidney of host rats showed interstitial glomerulonephritis with atrophic changes, particularly in the proximal tubules, with hematuria, interstitial fibrosis, and a thickening of the basal membrane (arrow). This basal membrane was easily visualized by its eosinophilic characteristics under fluorescent microscopic conditions. × 485.
Demonstration of Kallikrein in a Rat Pancreatic Acinar Cell Carcinoma


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