A Kallikrein-like Enzyme in Blood Vessels of One-Kidney, One Clip Hypertensive Rats

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Active and inactive kallikrein or a kallikrein-like enzyme are found in the aorta, vena cava, and tail artery and veins of the rat. We studied the concentration of vascular kininogenase in rats with one-kidney, one clip renovascular hypertension and in unilaterally nephrectomized normotensive rats. Six weeks after surgery, active and total vascular kininogenase activity (active plus trypsin-activated) was measured. Blood pressure was 212±4 mm Hg in the hypertensive rats (n=33) and 120±1 mm Hg in the normotensive rats (n=32) (p<0.001). Active kininogenase was lower in the hypertensive rats; although the difference was not significant in the thoracic aorta (56±8 versus 77±15), it was highly significant in the abdominal aorta (63±13 versus 167±17, p<0.001) and tail artery (48±8 versus 197±31, p<0.003). Total vascular kininogenase activity (active plus trypsin-activated) was lower in the hypertensive rats in all arteries examined: thoracic aorta (185±36 versus 380±38, p<0.003), abdominal aorta (565±61 versus 1,093±74, p<0.001), and tail artery (532±112 versus 1,243±135, p<0.003). Active kininogenase in the vena cava was higher in the hypertensive rats (213±56 versus 131±31); however, this difference was not statistically significant, whereas in the tail veins it was highly significant (1,803±221 versus 771±79, p<0.001). Total venous kininogenase activity was significantly higher in the hypertensive rats (vena cava, 1,850±171 versus 998±149, p<0.01; tail vein, 4,261±261 versus 2,521±212, p<0.001). In conclusion, we found that in rats with one-kidney, one clip renovascular hypertension both active and total vascular kininogenase are decreased in the arteries and increased in the veins. These changes may contribute to the pathogenesis of hypertension. (Hypertension 1990;16:436–440)

The kallikrein-kinin system has been implicated in the regulation of renal function and blood pressure and in the pathogenesis of various forms of hypertension.1–3 We found that kallikrein or a kallikrein-like enzyme is present in the tail arteries and veins of the rat in both active and inactive (trypsin-activated) forms. Both its molecular weight and inhibition profile were similar to glandular kallikrein. It was inhibited by aprotinin and by polyclonal and monoclonal antibodies against glandular kallikrein and was resistant to soybean trypsin inhibitor (SBTI).4 Although the function of this vascular kininogenase is not known, it could participate in circulatory homeostasis through local generation of kinins.

The present study was designed to determine whether one-kidney, one clip renovascular hypertension alters the concentration of vascular kininogenase. We measured active and total kininogenase activity in the thoracic and abdominal aorta, vena cava, and tail artery and veins of rats with one-kidney, one clip renovascular hypertension and in unilaterally nephrectomized normotensive rats.

Methods

Male Sprague-Dawley rats weighing 200–250 g were housed in a room under conditions of constant temperature and a 12-hour light/dark cycle; they had free access to tap water and rat chow. All surgical procedures were performed with the rats under ether anesthesia, using disappearance of the corneal reflex to adjust the depth of anesthesia. Animals were divided into two groups. In one-kidney, one clip hypertension (n=33), the right kidney was removed and a silver clip (0.22 mm gap) was placed around the left renal artery via a flank incision. In unilateral nephrectomy (n=32), the right kidney was removed and the left renal artery dissected but not clipped.
Blood pressure was monitored weekly by the tail-cuff method. Six weeks after surgery, the rats were decapitated and the thoracic and abdominal aorta, vena cava, and tail artery and veins were removed. Comparable vessels of three rats were pooled, and each pool was rinsed several times with 5 ml ice-cold 0.01 M Tris-HCl, 0.25 M sucrose solution (pH 7.4). The vessels were cleaned of adipose and connective tissue, blotted, weighed, and homogenized in a glass homogenizer containing 0.1 M Tris-HCl buffer (pH 7.4) with 0.25 M sucrose and 3 mg/ml sodium EDTA. The homogenate was centrifuged at 1,000g for 10 minutes; the supernatant was separated out and the pellet washed and centrifuged at 2,000g for 20 minutes. Both supernatants were pooled (final concentration, 200 mg wet tissue/ml) and kept at -20°C until needed for measurement of active and total kininogenase activity and protein concentration.

Active and total kininogenase activity were measured by incubating 400 µl homogenate supernatant (80 mg wet tissue) for 4 hours at 37°C with 200 µl partially purified dog kininogen (2,000 ng kinin-releasing capability) in the presence of 1,000 µl 0.1 M Tris-HCl fresh buffer (pH 8.5) containing EDTA (15 mg/ml), 1,10-phenanthroline (1 mg/ml), 8-OH-quinoline (1 mg/ml), and SBTI (100 µg/ml). The vascular kininogenase is inhibited by aprotinin and phenylmethylsulfonyl fluoride (PMSF) but is resistant to SBTI,4 which is characteristic of glandular kallikrein.2 SBTI was included in the incubation buffer to inhibit plasma kallikrein and trypsin-like enzymes that could contaminate the homogenates. Kinins generated during incubation were measured by radioimmunoassay.6 Total kininogenase activity was measured by incubating 500 µl of the homogenates with 20 µg trypsin for 30 minutes at 37°C. The reaction was stopped by adding 100 µg SBTI, after which the homogenates were incubated with kininogen as described above for active kininogenase.

Under these conditions, the kininogen itself released small amounts of kinins (kininogen blank). In every assay, duplicate tubes containing the reagents but no homogenates were run to assess for kininogen blank. Vascular kininogenase activity was calculated as kininogenase activity in the sample minus kininogen blank.

Because glandular kallikrein-like activity has been found in plasma,7,8 kallikrein activity in vessel walls could be due to plasma trapped in the tissues. If so, it would be removed by extensive washing. To assess the effects of extensive washing on vascular kininogenase activity, a separate experiment was carried out. Male rats (270–310 g) were decapitated, and the thoracic and abdominal aorta, tail artery, and tail veins were removed. Comparable vessels were pooled separately (pools, n=5; each pool contained vascular tissue from three rats). Each pool was suspended in 50 ml ice-cold 0.01 M Tris-HCl (pH 7.4), the beaker containing the vessels placed on an automatic shaker and lightly shaken for 10 minutes. The fluid was renewed three times, after which the vessels were cleaned of adipose and connective tissue, blotted, weighed, and homogenized in a glass homogenizer containing 0.01 M Tris-HCl buffer, 0.25 M sucrose, and 3 mg/ml sodium EDTA (pH 7.4). The homogenate was centrifuged at 1,000g for 10 minutes. The supernatant was separated out, the pellet resuspended in 0.5 ml buffer, centrifuged, and the supernatants pooled. Protein concentration was measured and then adjusted to 200 mg/ml. The homogenates were kept at -20°C until used.

To strengthen the evidence that vascular kininogenase is glandular kallikrein, the effects of preincubation with kallikrein antibodies were studied. Kallikrein antibodies (KKAb) were obtained as described previously.9 Serum from nonimmunized rabbits (normal rabbit serum [NRS]) was used as a control. The globulin fraction was precipitated with 40% ammonium sulfate and dialyzed against 0.02 M potassium phosphate (pH 8.0), then further purified by passage through a DEAE-afil-gel blue column (Bio-Rad, Richmond, Calif.) equilibrated with the same buffer. Immunoglobulin G (IgG) in the voided volume was concentrated by ultrafiltration (Amicon, Danvers, Mass.) and then dialyzed against 0.9% NaCl. To determine the effects of KKAb on vascular kininogenase, 0.4 ml supernatant from vascular homogenates was preincubated for 30 minutes at 37°C with 0.2 mg globulins from either NRS or KKAb. Kallikrein dissolved in a buffer containing peptidase inhibitors and SBTI was added, and kininogenase was measured as described before.

To assess whether changes in vascular kininogenase are specific or whether a similar pattern can be observed with a generalized cellular enzyme, lactic dehydrogenase activity (LDH) was measured following standard protocols. Male rats (100–150 g) were anesthetized with ether and the right kidney removed. In one group (n=9), one-kidney, one clip hypertension was induced as described before; in another group (n=9), the left kidney was dissected but not clipped. Blood pressure was measured by the tail-cuff method. After 7 weeks, the rats were decapitated and vascular tissues were obtained as described previously. Comparable tissues from three rats were pooled; the tissues were homogenized, and proteins, LDH, and kininogenase activity were measured. Proteins were determined by Bradford’s method, using albumin as a standard.10

Statistics

Two-sided, two-sample t tests were used to examine kininogenase activity in arteries and veins between normotensive and hypertensive rats. The inhibition of kininogenase activity in extensively washed arteries and veins from normotensive rats was examined with two-sided paired t tests. The Bonferroni adjustment for multiple comparisons was used. Unless otherwise noted, all results are expressed as mean±SEM.
Blood pressure at the time of decapitation was 120±0.8 mm Hg in the controls and 212±4 mm Hg in the hypertensive rats (p<0.001). Active and total kininogenase activity in arteries from hypertensive and normotensive rats are shown in Figure 1. Active kininogenase was lower in the hypertensive rats; although the difference was not significant in the thoracic aorta (56±8 versus 77±15), it was highly significant in the abdominal aorta (63±13 versus 167±17, p<0.001) and tail artery (48±8 versus 197±31, p<0.003). Total vascular kininogenase activity (active plus trypsin-activated) was lower in the hypertensive rats in all arteries examined: thoracic aorta (183±16 versus 380±38, p<0.003), abdominal aorta (565±61 versus 1,093±74, p<0.001), and tail artery (532±112 versus 1,243±135, p<0.003).

Figure 2 shows active and total kininogenase activity in veins from hypertensive and normotensive rats. Active kininogenase in the vena cava was higher in the hypertensive rats (213±56 versus 131±31); however, this difference was not statistically significant, whereas in the tail veins it was highly significant (1,803±221 versus 771±79, p<0.003). Total venous kininogenase activity was significantly higher in the hypertensive rats (vena cava, 1,850±171 versus 998±149, p<0.01; tail vein, 4,261±261 versus 2,521±212, p<0.001).

Neither kininogenase activity nor the pattern of distribution was altered by extensive washing. Preincubation with antibodies against rat urinary kallikrein resulted in 80–90% inhibition of both active and total kininogenase activity in all tissues analyzed (p<0.001) (Figure 3).
vascular rats, no differences in LDH concentrations were observed (Table 1).

**Discussion**

We found both active and inactive kininogenase activity in the blood vessels. In one-kidney, one clip renovascular hypertension, vascular kininogenase was decreased in the arteries and increased in the veins. Although decreased arterial kininogenase activity may contribute to the pathogenesis of hypertension, we cannot overlook the possibility that it is decreased because of the higher blood pressure. Lowered kininogenase concentrations in the arterial wall may be due in part to hypertrophy secondary to hypertension, which may not be accompanied by a proportional increase in kininogenase activity. Because kininogenase activity was increased in veins from hypertensive rats and because changes in arterial blood pressure are not accompanied by changes in venous pressure, we speculate that the changes in the arterial wall are secondary to the increase in blood pressure, and venous changes are due to neuroendocrine factors. These hypothetical neuroendocrine factors may also act in the arterial wall, but the effects could have been overridden by the hemodynamic changes. At present, we do not know whether these alterations occur only in the one-kidney, one clip model, which is known to be volume dependent, or whether it occurs in other hypertensive models as well.

Measurement of LDH, a general cell marker, showed little or no difference between normotensive and hypertensive rats. Although the number of samples was small, normalization of kininogenase by LDH maintained the differences observed in the previous group: hypertensive arteries had less kininogenase activity and hypertensive veins more. Thus, the changes in kallikrein-like activity (kininogenase) observed in hypertension appear to be specific.

Both the present data and those published previously suggest that vascular tissue contains a kallikrein-like enzyme capable of generating kinins from kinogen. Because plasma contains glandular kallikrein, vascular kininogenase activity could be due to trapped plasma; however, this is unlikely, as extensive washing did not remove the kininogenase from the vessel wall. We found messenger RNA (mRNA) for glandular kallikrein in rat vascular tissue, suggesting that the kininogenase activity was due to locally synthesized glandular kallikrein. We used SBTI to inhibit the possible kinin-generating activity of plasma kallikrein or trypsin-like enzymes. Although SBTI resistance is characteristic of glandular kallikrein, this is not sufficient to identify the vascular kininogenase as glandular kallikrein, which is why we call the kininogenase activity “kallikrein-like” rather than kallikrein. However, we may be overly cautious, as retention of activity despite extensive washing, resistance to inhibition by SBTI, blockade of kininogenase activity by both polyclonal and monoclonal antibodies against rat glandular kallikrein, enzymatic activity at alkaline pH (the present study and Reference 4), and the presence of mRNA for glandular kallikrein in the vascular wall are strong evidence of an endogenous vascular kallikrein-kinin system.

We found evidence that mRNA for glandular kallikrein is present in an established rat vascular smooth muscle cell line; in addition, Oza et al recently reported that vascular smooth muscle cells in culture release kallikrein. In preliminary work, we found that vascular kininogenase activity is still present after removal of the endothelium by collagenase treatment. Thus, at least part of the vascular kininogenase activity is likely present in the smooth muscle cells. Up to now, attempts to locate kallikrein in the vascular wall by immunohistochemistry have not been successful (T. Berg, personal communication). This may be because the concentration of kallikrein is below the sensitivity of this technique or because the epitopes recognized by the antibodies we used are not exposed.

Whether kallikrein or a kallikrein-like enzyme acts as a paracrine hormone, releasing kinins within the vasculature, is not known. Our preliminary studies indicate that blood vessels release kininogenase into the circulation, where it could form kinins (unpublished results). Indirect evidence is consistent with the hypothesis that kinins acting as paracrine hormones may be involved in circulatory homeostasis. For example, high doses of a kinin antagonist produce a transient increase in blood pressure. Furthermore, angiotensin converting enzyme inhibitors can lower blood pressure in hypertensive states.

### Table 1. Lactic Dehydrogenase and Kininogenase Activity In Vascular Tissue From Normotensive and Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>Tail veins</th>
<th></th>
<th>Tail arteries</th>
<th></th>
<th>Thoracic aorta</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Normotensive</td>
<td>Hypertensive</td>
<td>Normotensive</td>
<td>Hypertensive</td>
<td>Normotensive</td>
<td>Hypertensive</td>
</tr>
<tr>
<td>LDH</td>
<td>8.3±0.5</td>
<td>9.5±0.4</td>
<td>15.6±1.6</td>
<td>13.6±0.1</td>
<td>5.4±0.6</td>
<td>5.7±0.8</td>
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<tr>
<td>Active Kgase</td>
<td>620±81</td>
<td>1025±41</td>
<td>156±29</td>
<td>78±8</td>
<td>104±22</td>
<td>28±12</td>
</tr>
<tr>
<td>Total Kgase</td>
<td>2,134±300</td>
<td>3,880±122</td>
<td>1,334±400</td>
<td>541±65</td>
<td>416±35</td>
<td>93±21</td>
</tr>
</tbody>
</table>

Values are mean±SD. Lactic dehydrogenase (LDH) (units/ml) was measured in three pools; each pool contained tissue from three rats. Kininogenase (Kgase) (pg bradykinin/mg protein/min) was also measured (n=2; third pool was lost).
where plasma renin is low or normal. This effect may be due to blockade of angiotensin II formation not in the circulation but in the arterial wall, where all components of the renin-angiotensin system are present. Alternatively, because angiotensin converting enzyme is a potent kininase (kininase II), part of the antihypertensive effect of angiotensin converting enzyme inhibitors may be due to accumulation of kinins in tissue. A kinin antagonist blunted the hypotensive effect of enalaprilat, indicating that kinins contribute to the hypotensive effect of angiotensin converting enzyme inhibitors; however, kinins in arterial blood were not increased by the angiotensin converting enzyme inhibitor. Therefore, the antagonist appears to block the effect of accumulated kinins in the arterial wall rather than in the blood.

In summary, the present study shows that in the one-kidney, one clip model of renovascular hypertension, kininogenase activity is decreased in the arteries and increased in the veins. A decrease in the local concentration of arterial kinins may contribute to increased peripheral resistance. Increased formation of kinins in the veins may result in their constriction and decreased compliance.

References


KEY WORDS • kinins • kallikrein • Goldblatt hypertension • slow-reacting substances • blood pressure • vascular tissue
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