Vascular Kallikrein in Deoxycorticosterone Acetate–Salt Hypertensive Rats

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Abstract We determined the status of vascular kallikrein in rats with severe hypertension caused by treatment with deoxycorticosterone acetate (DOCA) and drinking of 1% NaCl for 6 weeks. We assayed active and total kininogenase (kallikrein) activity in the perfusate and in arterial and venous tissues. DOCA-salt rats had higher systolic blood pressure at 6 weeks (214±5 mm Hg) than rats drinking tap water (135±4 mm Hg) or saline (145±5 mm Hg). Kininogenase in the perfusate (nanograms bradykinin per minute per kilogram body weight) increased significantly at 2 weeks, from 5.8±2.1 to 8.9±1.4 for active kallikrein and from 28.7±0.4 to 48.7±2.9 for total kallikrein. Total kallikrein returned to control values at 4 weeks, whereas it was significantly reduced at 6 weeks (20.9±0.7). Active kallikrein was significantly depressed at 4 and 6 weeks (1.08±0.1 and 0.85±0.1, respectively [P<.05]). Active kallikrein in arterial tissue (picograms bradykinin per milligram per minute) showed a small but significant increase at 2 weeks, from 156±7 to 201±10 (P<.05), finally decreasing significantly by 6 weeks to 64±3; however, total kallikrein showed a significant decrease only at 6 weeks, from 844±17 to 427±27. Both active and total kallikrein in the veins were higher than control values at 2 weeks, changing from 437±7 to 541±19 and from 1619±17 to 2062±86, respectively. Venous kallikrein remained elevated until the end of the experiment. These findings suggest that mineralocorticoids increase vascular kallikrein and that established hypertension lowers both arterial kallikrein and kallikrein released by the vasculature, whereas venous kallikrein is under a different regulatory control. The vascular kallikrein-kinin system may help counteract the elevation of vascular resistance in the earliest stages of DOCA-salt hypertension. (Hypertension. 1994;23[suppl I]:I-185-I-188.)

Key Words • kallikrein • blood pressure • hypertension, mineralocorticoid • deoxycorticosterone • vascular resistance

Kinins acting as local hormones (autocrine or paracrine) have been implicated to be among the factors that regulate blood pressure (BP).1 Kinins are released from kininogen by the proteolytic action of kallikrein, and their potent vasodilator activity is mainly due to the release of prostaglandins, nitric oxide, and other endothelium-derived vasoactive mediators. However, kinins circulate at very low concentrations and are rapidly hydrolyzed by a group of peptidases known as kininases. Thus, it has been postulated that if kinins participate in the regulation of BP, they must be released as paracrine hormones at or near the arterial wall.2 We have previously shown that kallikrein, a potent kininogenase, is present in and released from blood vessels.3 In addition, mRNA for glandular kallikrein (hereafter referred to as kallikrein) has been found in vascular tissue,4 suggesting local synthesis. We have also reported that kallikrein is released into the perfusate of isolated perfused rat hindquarters, presumably from the vasculature.5 We have previously shown that arterial kallikrein is decreased in one-kidney, one clip (1K1C) hypertensive rats, suggesting that high BP decreases arterial kallikrein. Even though the role of the kallikrein-kinin (KK) system in the development of mineralocorticoid hypertension remains unclear, a number of reports show a clear interaction between the KK system and mineralocorticoid hormones.5-8 Kallikrein has been found in the adrenal glands,9,10 and kinins stimulate release of aldosterone from adrenocortical cells.11 Hypertension is generally characterized by decreased urinary kallikrein.2 On the other hand, deoxycorticosterone acetate (DOCA)–salt hypertension or excess mineralocorticoid activity increases urinary KK excretion6-8 and circulating kinins.12 In rats deficient in low or high molecular weight kininogen, DOCA-salt hypertension developed significantly faster than in controls,13 suggesting that the KK system opposes the development of hypertension. To find out whether vascular kallikrein is affected by DOCA-salt hypertension, we examined active and total kinin-forming activity in the perfusate from isolated perfused rat hindquarters as well as in arterial and venous tissue in rats with DOCA-salt hypertension. These studies were performed during the onset of hypertension (2 weeks after DOCA-salt treatment) and during the established phase (6 weeks after treatment).

Methods All procedures involving animals were in accordance with institutional guidelines for animal experimentation. Male Wistar rats weighing 180 to 200 g were anesthetized with ether, and the left kidney was removed via a left flank incision. Three days later, the animals were randomly divided into three treatment groups, and the right kidney was removed with rats under ether anesthesia. The animals in group 1 (n=15) served as controls and were given free access to tap water. Group 2 (n=15) was given 1% (wt/vol) NaCl. Group 3 (n=70) drank 1% NaCl and received injections of DOCA (Sigma Chemical Co, St Louis, Mo; 25 mg/kg body wt IM) suspended in sesame seed oil twice a week. Animals were kept in a temperature-
controlled room (20°C) illuminated between 8 AM and 8 PM. They were fed standard rat chow ad libitum, and systolic BP was determined by tail sphygmomanometry. Kallikrein activity in vascular tissues and in the perfusate of the isolated perfused hindquarters was measured 0, 2, 4, and 6 weeks after the beginning of the experiment.

Preparation of Tissue Homogenates

Male Wistar rats weighing 250 to 300 g were anesthetized with ether and decapitated. The thoracic and abdominal aorta, tail artery, thoracic segment of the inferior vena cava, and tail vein were removed and rinsed several times with ice-cold 0.01 mol/L Tris-HCl buffer (pH 7.4). O.25 mol/L sucrose, and 3 mol/mL EDTA. After being cleaned of connective tissue and fat, the vessels were weighed, minced, and homogenized with 0.1 mol/L Tris-HCl buffer (pH 7.4).

The homogenate was centrifuged at 1000g for 10 minutes, the supernatant separated out, and the pellet washed and centrifuged again at 2000g for 20 minutes. Both supernatants were pooled (final concentration, 100 mg wet tissue per milliliter) and kept at −20°C until needed for measurement of active and total kininogenase activity.1,4

Release of Kallikrein From the Isolated Perfused Rat Hindquarters

Rats were anesthetized with sodium pentobarbital (Nemb- utal, Abbott Laboratories) and given 1000 U heparin intrave-
nously. After 3 minutes, the animal was decapitated to facil-
tate blood drainage. An abdominal incision was made, and the aorta and vena cava were carefully dissected from the renal vessels to the bifurcation. The rectum was cut between double ligatures and the distal sigmoid displaced to the upper abdo-
men. All major and minor tributaries of the descending aorta and abdominal vena cava were ligated except for the femoral and tail arteries. A 20-gauge catheter was placed in the aorta just below the renal arteries. The vena cava was also catheter-
ized, and warmed (37°C) Krebs-Henseleit solution containing 3.5% Ficoll 70 and gassed with 95% O2-5% CO2 was perfused through the aorta. The buffer was passed through a 0.45-μm filter before use. In the perfusion system, the medium was oxygenated by passage through the blood compartment of a C-DAK hollow-fiber artificial kidney (Cordis-Dow, Miami, Fla) and gassed with the O2-CO2 mixture. The isolated hind-
quarters were perfused in a single-pass (non-recirculating) system using a peristaltic pump while perfusion pressure was constantly recorded. Details of the procedure were similar to those reported previously.15 During the initial perfusion pe-
riod, the hindquarters preparation was thoroughly rinsed with the buffer for 30 minutes until the effluent was sufficiently clear. The flow rate was adjusted to obtain the desired perfusion pressure, normally 60 to 80 mm Hg.

Kininogenase Activity

We measured active and total kininogenase activity by incubating 400 μL of the homogenate supernatant (80 mg wet tissue), 1000 μL of the medium in which the slices were bathed, or 1000 μL of the hindquarters perfusate (previously concentrated five times) with 200 μL of partially purified dog kininogen (2000 ng kinin-releasing capability) for 5 hours at 37°C in the presence of 1000 μL fresh 0.1-mol/L Tris-HCl buffer (pH 8.5) containing EDTA (15 mg/mL), 1-10 phenan-
threnol (1 mg/mL), 8-OH-quinoline (1 mg/mL), and soybean trypsin inhibitor (SBTI) (100 μg/mL). Vascular kininogenase is inhibited by aprotinin and phenylmethylsulfonyl fluoride but is resistant to SBTI. SBTI was included in the incubation buffer to inhibit plasma kallikrein and trypsin-like enzymes that could contaminate the homogenates. Total kininogenase ac-
tivity was measured by incubating 500 μL homogenate with 20 μg SBTI for 30 minutes at 37°C. The reaction was stopped by adding 100 μg SBTI, after which the homogenate was incu-

Results

Fig 1 (top) shows changes in systolic BP in DOCA-salt rats (n=32). At the beginning of the experiment (week 0), BP was 135±2.1 mm Hg; at 2, 4, and 6 weeks it was 143±1.1, 172±1.2, and 214±3.9 mm Hg, respectiv
e. These changes were statistically significant (P<.05). Active and total kininogenase released into the perfusate are shown in the bottom panel of Fig 1. At 2 weeks, active KK increased significantly from 5.8±2.1 to 8.9±1.4 and total KK from 28.7±0.4 to 48.7±2.9 ng bradykinin/min per kilogram body weight (P<.05). Total KK returned to control values at 4 weeks, whereas it was significantly reduced at 6 weeks (20.9±0.7). Active KK was significantly depressed at both 4 and 6 weeks (1.0±0.1 and 0.85±0.1, respectively [P<.05]). Total kallikrein released into the perfusate of normotensive controls that were drinking water (time control) did not change across time; KK values were 31.2±1.7, 32.5±1.7, and 33.0±1.3 at 0, 2, 4, and 6 weeks, respectively (P=NS). There were no differences between controls that were drinking water and rats drinking saline. When active and total KK in the perfusate of DOCA-salt rats were compared with time controls, significant differences were seen at 2 weeks (higher in DOCA-salt rats) and 6 weeks (lower in DOCA-salt rats) (P<.05).

Fig 2 represents the second group of DOCA-salt rats (n=32). The top panel shows changes in systolic BP. Values were quite similar to those shown in Fig 1. Active kininogenase in the arteries was significantly higher than initial values at 2 weeks. At 4 weeks, kininogenase did not differ from baseline (P=NS), whereas at 6 weeks it was significantly lower. Only at 6 weeks was total kininogenase (active plus inactive) significantly lower than initial values.
Fig 1. Top: Line graph shows blood pressure in rats treated with deoxycorticosterone acetate-salt; bottom: bar graph shows kininogenase activity in perfusate from isolated perfused hindquarters. Significant differences (P<.05, Dunnett's multiple comparison): blood pressure was higher at 6, 4, and 2 weeks than at time 0; active kininogenase, higher than controls at 2 weeks, lower than controls at 4 and 6 weeks; and total kallikrein, higher than controls at 2 weeks, lower than controls at 6 weeks. BK indicates bradykinin; BW, body weight.

Fig 2. Top: Line graph shows blood pressure in rats treated with deoxycorticosterone acetate-salt; bottom: bar graph shows kininogenase activity in arterial tissue. Significant differences (P<.05, Dunnett's multiple comparison): blood pressure was higher at 6, 4, and 2 weeks than at time 0; active kininogenase, higher than controls at 2 weeks, lower than controls at 6 weeks; and total kininogenase, lower at 6 weeks than at time 0. BK indicates bradykinin.

Fig 3. Bar graph shows kininogenase activity in veins of deoxycorticosterone acetate-salt hypertensive rats. Tissue was obtained from the same group of rats shown in Fig 2. Significant differences (P<.05, Dunnett's multiple comparison): active and total kininogenase were higher at 2, 4, and 6 weeks than at time 0. BK indicates bradykinin.

Discussion

We examined the status of vascular kininogenase (considered equivalent to vascular kallikrein) in rats during the development of DOCA-salt hypertension, using kininogenase activity in the effluent of isolated perfused hindquarters as an index of kallikrein released from vascular tissue. After treatment with DOCA-salt, we found that vascular kallikrein released into the perfusate initially increased but later returned to control values and was significantly reduced at the end of the experimental period. Total kallikrein showed only minor changes at the outset (P=NS) but subsequently decreased and was lowest at 6 weeks. In contrast, kallikrein in the veins showed a persistent increase. Thus, during the 6 weeks of DOCA-salt treatment used in this protocol, kallikrein in the perfusate showed a biphasic pattern. The decrease in arterial kallikrein during established DOCA-salt hypertension is in agreement with our previous findings in 1K1C hypertension. In the present work we extended these findings and demonstrated that release is also lessened during hypertension. Therefore, because arterial kallikrein was found to decrease in two different models of experimental hypertension, the decrease in arterial content and vascular release of kallikrein is most likely secondary to high BP. On the other hand, the stimulated kallikrein release at 2 weeks of DOCA-salt treatment was unexpected. BP was only slightly elevated at this time. Kallikrein in the perfusate showed a profile similar to that of the arteries and distinct from that of the veins. Although the isolated perfused hindquarters technique does not allow us to determine the relative contribution of arteries and veins as sources of the
kallikrein seen in the perfusate, the data suggest that it may reflect arterial release. Although release of kallikrein into the perfusate was clearly increased, the changes in arterial kallikrein content showed a similar tendency only for active kallikrein. Increased release with little change in content suggests that arterial kallikrein synthesis has increased as well. One possible explanation is that vascular kallikrein synthesis and release are heightened by mineralocorticoids, similar to renal kallikrein.\textsuperscript{2,6-8,12,16} The fact that venous kallikrein also increased during DOCA-salt treatment suggests that vascular kallikrein may respond to excess mineralocorticoids with increased synthesis.

The reasons for the decreased arterial kallikrein and vascular kallikrein release during hypertension are not clear. Decreased kallikrein concentration in the arterial wall may be partially due to hypertrophy secondary to hypertension, unaccompanied by a proportional increase in kallikrein activity; however, if that were the case, kallikrein release would have been constant. The fact that kallikrein released into the perfusate was also decreased suggests that the changes are not entirely due to dilution attributable to higher vascular mass. In addition, we do not know whether the vascular cells that release kallikrein into the perfusate are the same type we are measuring in vascular homogenates. We found mRNA coding for kallikrein in vascular smooth muscle cells in culture,\textsuperscript{4} and Oza et al\textsuperscript{17} found kallikrein activity in cultured vascular smooth muscle cells. This suggests that the kallikrein in the perfusate originated in the smooth muscle. Furthermore, although the existence of an endothelial KK system has been suggested, no firm evidence has been shown.\textsuperscript{18} As we found previously in 1K1C hypertension, venous kallikrein is increased in DOCA-salt hypertension. It is not clear how venous kallikrein is regulated during hypertension. A possible link is that 1K1C and DOCA-salt models are both volume-expanded.

The role of the vascular KK system in regulating vascular resistance remains speculative. Majima et al\textsuperscript{13} reported that hypertension developed faster in DOCA-salt–treated Brown Norway Katholiek rats with kininogen deficiency and in normal DOCA-salt–treated rats receiving aprotinin, a nonspecific but effective blocker of kallikrein activity. However, final BP levels were no different in DOCA controls or kininogen-deficient rats. Thus, during DOCA-salt treatment, absence or blockade of endogenous kinin production removes an antihypertensive mechanism. In control animals having an intact KK system, the antihypertensive effect delays development but is not important in setting final BP levels. The reason for this loss of antihypertensive activity is not clear. Although Majima suggested that increased activity of renal kallikrein and kinin may be responsible for the antihypertensive effects, the present results suggest that the vascular KK system could also play a role. It is possible that increased vascular kallikrein in the early stages resulted in increased local vasodilator activity, thus counteracting the rise in BP. As hypertension developed, kallikrein release decreased, possibly explaining the lack of a more sustained antihypertensive effect. The present data are consistent with the hypothesis that BP control is the result of a balance between vasopressor and vasodilator influences. The vascular KK system may act as a vasodepressor.\textsuperscript{1,2,19}

In summary, the present study indicates that in the early stages of DOCA-salt hypertension, kallikrein released by vascular tissue increases, suggesting that the vascular KK system may counteract the elevation of vascular resistance induced by DOCA-salt treatment. Arterial kallikrein and vascular kallikrein release are depressed in the established phase of DOCA-salt hypertension. A secondary effect of hypertension may be a lessened release of kallikrein from arteries and therefore decreased antihypertensive activity.

References

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_Hypertension_. 1994;23:I185
doi: 10.1161/01.HYP.23.1_Suppl.I185

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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