The Effect of Aprotinin (A Serine Protease Inhibitor) on Renal Function and Renin Release

Shinji Seto, M.D., Vijay Kher, M.D., A. Guillermo Scicli, Ph.D., William H. Beierwaltes, Ph.D., and Oscar A. Carretero, M.D.

SUMMARY We studied the effect of aprotinin, a reversible inhibitor of kallikrein and other serine proteases, upon urinary kallikrein and kinin excretion, renal function and hemodynamics, blood pressure, and plasma renin activity (PRA). When aprotinin was administered to anesthetized rats at 10,000 KIU/kg as a bolus, and at 1000 KIU/kg/min infusion for 60 minutes, urinary kininogenase activity and immunoreactive kallikrein, kinins, sodium, potassium, and water excretion, and PRA decreased significantly. Aprotinin also caused a 36% decrease (p < 0.001) in renal blood flow (RBF), and a 37% decrease (p < 0.001) in glomerular filtration rate (GFR), although neither blood pressure nor cardiac output changed. The effect of aprotinin on PRA was further studied in conscious rats before and after stimulation of renin release by isoproterenol or furosemide. Aprotinin (5,000 KIU/kg bolus and 1000 KIU/kg/min infusion for 60 minutes) did not alter basal or isoproterenol-stimulated PRA, but it blunted the increase in PRA as stimulated by furosemide. Aprotinin at a higher dose (20,000 KIU/kg bolus and 5000 KIU/kg/min infusion for 60 minutes) significantly lowered blood pressure and increased hematocrit and PRA. These effects may be due to inhibition of serine protease(s) or to other as yet unrecognized properties of this peptide resulting from its highly cationic nature. In conclusion, aprotinin at a low dose decreased kallikrein, kinin, sodium, and water excretion. These decreases may be due to the inhibition of kallikrein and/or other serine proteases or may be secondary to the renal hemodynamic changes. The effect of aprotinin on RBF, GFR and renin release could also be due to inhibition of a serine protease(s) that participates in the regulation of RBF, GFR and in the mechanism of renin release or renin activation. (Hypertension 5: 893-899, 1983)

KEY WORDS • kallikrein • kinin • angiotensin • renal blood flow • glomerular filtration rate • blood pressure • isoproterenol • furosemide

Aprotinin, a reversible inhibitor of kallikrein and other serine proteases, has been used as a tool to study the possible actions of the kallikrein-kinin system within the kidney under various physiological conditions. Short-term administration of aprotinin did not affect renal function in the normovolemic rats while it significantly reduced glomerular filtration rate (GFR), hippuran clearance, and electrolyte and water excretion in volume-expanded conscious rats. Aprotinin has been found to increase renal vascular resistance and reduce renal blood flow in sodium-restricted rats, but in rats with high sodium intake, aprotinin appeared to have no effect. In sodium-restricted rats, when renal perfusion was below the range of renal blood flow (RBF) autoregulation, aprotinin increased renal vascular resistance. Although these effects of aprotinin have been attributed to inhibition of the intrarenal formation of the vasodilating kinins, it is not clear whether aprotinin actually alters the intrarenal formation of kinins, since in none of these studies was kinin excretion measured.

Urinary proteases, including kallikrein, have been reported to directly stimulate renin release in vitro. This release could be blocked with aprotinin. Moreover, in humans and dogs it has been found that aprotinin suppresses renin release stimulated with a converting enzyme inhibitor. These effects were also attributed to an inhibition of renal kallikrein. However, since aprotinin inhibits dog renal kallikrein poorly or not at all, its action may be due to some other serine protease(s).

In the present study, we determined whether aprotinin would decrease the activity of the renal kallikrein-kinin system, and whether it affects renal function and hemodynamics in the anesthetized rat. The activity of
the renal kallikrein-kinin system was estimated by measuring kinins in urine collected directly from the ureters, and by measuring urinary kininogenase activity and immunoreactive kallikrein. We also studied the effect of aprotinin administration on plasma renin activity (PRA) in basal conditions and when its release was stimulated with isoproterenol or furosemide. Unanesthetized chronically instrumented rats were used in this part of the study to avoid the effect of the anesthesia and surgical manipulation on renin release.

Materials and Methods

Study I: Kinin and Kallikrein Excretion and Renal Function During Aprotinin Administration in Anesthetized Rats

We used 34 male Sprague-Dawley rats weighing 300 to 450 g. Food was withheld for 24 hours before the study, but the animals were allowed water ad libitum. On the morning of the study, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital, 50 mg/kg body weight. A tracheotomy was performed, and the animals were ventilated with a Harvard rodent respirator (Model 680, Harvard Instruments, Harvard, Massachusetts). Supplemental doses of pentobarbital were administered as needed. The rats were placed on a thermo-regulated pad adjusted to maintain rectal temperature at 37°C ± 0.5°C throughout the experiment. Polyethylene catheters (PE-10) were placed in the abdominal aorta and inferior vena cava through the femoral artery and vein, respectively. The aortic catheter was used to sample blood and to continuously record mean arterial blood pressure; the venous catheter was used for infusion. Both ureters were dissected near their entry into the urinary bladder with a dissection microscope to avoid trauma to the vessels supplying the ureter. Polyethylene catheters (PE-10) with rounded tips were placed in each ureter. An additional tight ligature with 6-0 silk suture near the tip of the catheter facilitated collection of urine without blood (tested with Multi-Stix strips, Ames Division, Miles Laboratories, Inc., Elkhart, Indiana).

The left renal artery was dissected so that RBF could be measured with an electromagnetic flow probe connected to a square-wave electromagnetic flow meter (Model 501, Carolina Medical Electronics, Inc., King, North Carolina). Mean arterial pressure and RBF were recorded continuously throughout the experiment on a Gould Brush 440 recorder (Gould, Inc., Cleveland, Ohio).

Midway through the surgery, an infusion of 2.5% dextrose was begun at a rate of 1% body wt/hr. At the conclusion of surgery, rats were given a bolus dose of 2 μCi 14C inulin (New England Nuclear, Boston, Massachusetts) in 200 μl 2.5% dextrose followed by a continuous maintenance infusion of 0.05 μCi/min in 2.5% dextrose at a rate of 50 μl/min. Following surgery, all animals were allowed to stabilize for 1 hour before commencing three consecutive 1-hour study periods, as outlined below.

Period 1: Control Period

During the first period, the infusion of 2.5% dextrose was continued. Two 30-minute urine collections from both kidneys were obtained to measure urinary kininogenase activity, kinins, sodium, potassium, and 14C inulin. Blood was sampled for PRA and for 14C inulin concentration at the midpoint of the period, and replaced with an equal volume of blood from a 24-hour nephrectomized donor rat.

Period 2: Experimental Period

During the second period, the infusion was modified so that the rats were either maintained on the dextrose vehicle or received aprotinin (Trasylol; Bayer AG, Farben-Fabriken, Wuppertal-Elberfeld, Federal Republic of Germany, kindly supplied by Dr. G. L. Haberland) at 10,000 KIU/kg body wt as a bolus dose and at 1000 KIU/kg body wt/min as a maintenance infusion.

Period 3: Recovery Period

The infusion of aprotinin was stopped and the 2.5% dextrose vehicle resumed. All measurements done in the control period were repeated in the experimental and recovery periods. Using the protocol above, two groups of rats were studied.

Group 1 (Time Control, n = 8)

The rats received 2.5% dextrose during the three periods.

Group 2 (Aprotinin)

This group was infused with aprotinin and was subdivided into three subgroups. In Subgroup 2A (n = 11), we measured urinary kininogenase activity, kinins, sodium, potassium, urinary volume, glomerular filtration rate, and renal blood flow. In Subgroup 2B (n = 8), the only parameter measured was immunoreactive urinary kallikrein. In Subgroup 2C (n = 7), cardiac output was measured using radioactive microspheres. A catheter (PE-10) was inserted into the left ventricle via the carotid artery through which radio-labeled microspheres (°C or °Sr; New England Nuclear, Boston, Massachusetts) were injected to determine cardiac output before and during aprotinin infusion by the reference sample method.

Study II: Effect of Aprotinin on Plasma Renin Activity in Conscious Rats

Male Sprague-Dawley rats weighing 250 to 300 g were anesthetized with ether, and heparin-filled (100 USP units/ml) polyethylene catheters (PE-10) were implanted into the abdominal aorta and inferior vena cava through the femoral artery and vein, respectively. The catheters were passed subcutaneously and brought through the skin at the scarapular region, as previously described. The experiments were performed 48 hours after catheter implantation. Conscious rats were kept semi-restrained in plastic restrainers, and direct mean blood pressure was recorded. All experiments were
started between 9 and 10 a.m. to minimize the effects of diurnal changes in PRA. We studied seven groups of rats using this protocol.

**Group 3 (Time Control)**

After blood pressure had stabilized, 10 rats received an initial bolus dose of 100 µl and a continuous infusion of the 5% dextrose (vehicle) at a rate of 10 µl/min for 60 minutes.

**Group 4 (Low Dose of Aprotinin)**

Nine rats received an initial dose of 5,000 KIU/kg body weight of aprotinin intravenously, followed by a continuous infusion of 1000 KIU/kg body wt/min for 60 minutes. The initial injection was given in a volume of 100 µl, followed by a 10 µl/min continuous infusion.

**Group 5 (High Dose of Aprotinin)**

Six rats received an initial dose of 20,000 KIU/kg body weight of aprotinin intravenously, followed by a continuous infusion of 5000 KIU/kg body wt/min for 60 minutes.

**Group 6 (Isoproterenol)**

Five rats were infused with 5% dextrose. Thirty minutes after the beginning of the infusion, an infusion of isoproterenol (Elkins-Sinn Inc., Cherry Hill, New Jersey) was started in a dose of 100 ng/kg body wt/min for the remaining 30 minutes. The isoproterenol was infused via an arterial catheter (PE-10) placed in the abdominal aorta with the tip of the catheter cephalad to the origin of the renal arteries in order to maximize its effect in the kidney and minimize its systemic effect. They served as control for Group 7.

**Group 7 (Isoproterenol and Aprotinin)**

Four rats received aprotinin as described for Group 4. Thirty minutes after the beginning of the aprotinin infusion, they were treated with isoproterenol as in Group 6.

**Group 8 (Furosemide)**

Fourteen rats were infused with 5% dextrose. Thirty minutes after the infusion was begun, the rats received a bolus injection of 1 mg/kg body wt of furosemide (Lasix, Hoechst-Roussel, Somerville, New Jersey). They served as a control for Group 9.

**Group 9 (Furosemide and Aprotinin)**

Fourteen rats received aprotinin as described for Group 4. Thirty minutes after the infusion was begun, they received furosemide as in Group 8.

In all groups, mean blood pressure was continuously recorded. Arterial blood samples (0.7 ml) were taken before and after the infusion period to determine hematocrit and PRA. The sample volume of the blood was immediately replaced with blood from a 48-hour nephrectomized donor rat. Rats in which the hematocrit was lower than 40% were excluded since this could indicate internal bleeding caused by surgery and could thus affect renin release. We also excluded all rats in which the body weight decreased 10% or more during the 2 days after surgery, since a decrease could indicate that the rat did not eat, affecting sodium balance and thereby the renin release.

**Analytical Procedures**

Urinary samples for kinins were collected in preweighed siliconized tubes containing 80% alcohol, and urine volume was determined gravimetrically. Kinins were measured by radioimmunoassay (RIA) as previously described, and values are presented as picograms per minute (pg/min) of urine collection. Rats in which blood was detected in the urine were excluded from the study. Urinary kallikrein was measured by its kininogenase activity as previously described, and values are presented as nanograms of kinin produced per minute of incubation per minute of urine collection (ng/min/min). Immuneactive kallikrein was measured directly by RIA, and 14C inulin samples were counted with a liquid scintillation counter (Beckman Instruments, Inc., Palo Alto, California), and GFR was calculated by the clearance of inulin (\( \frac{U_i}{V - \frac{P_i}{P_o}} \)). Urinary sodium and potassium concentrations were determined using an ion electrode (Nova I sodium/potassium analyzer, Nova Biochemical, Newton, Massachusetts), and urinary values are expressed as µEq of sodium or potassium excretion/min of urine collected.

PRA was measured by RIA using a modification of the method of Haber et al., as previously described. PRA is expressed as nanogram of angiotensin I generated per milliliter of plasma per hour incubation (ng/ml/hr). The hematocrit was determined by centrifuging the blood samples at 3000 rpm for 30 minutes. All data are expressed as means ± sem. Statistical analyses were performed with Student's paired and unpaired t tests as well as Freidman's test. A p value of less than 0.05 was considered to be statistically significant.

**Results**

**Study 1: Renal Function and Renal Hemodynamics in Anesthetized Rats**

Urinary excretions of kininogenase activity, kinins, sodium, potassium, and urinary volume during the three periods in Group 1 and Subgroup 2A are shown in table 1. In the time control group (Group 1), there was no change in any of the measured parameters of excretion over the three periods. This contrasted with Subgroup 2A in which aprotinin infusion significantly decreased excretion of urinary kininogenase activity, kinins, sodium, potassium, and water.

While not statistically different, the mean basal urinary kininogenase activity (Period 1) in Subgroup 2A rats was greater than in the Group 1 time control rats. Although a mean decrease in kininogenase excretion induced by aprotinin (Subgroup 2A) occurred in all rats, it decreased to a level similar to the excretion seen in Group 1. When rats from Subgroup 2A were select-
TABLE 1. Effects of Aprotinin on Urinary Kininogenase Activity, Kinins, Sodium, Potassium, and Water Excretion in Anesthetized Rats

<table>
<thead>
<tr>
<th>Time</th>
<th>Group 1 (time controls, n = 8)</th>
<th>Subgroup 2A (experimental group, n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period 1</td>
<td>Period 1</td>
</tr>
<tr>
<td></td>
<td>(control)</td>
<td>(control)</td>
</tr>
<tr>
<td>UKA (ng/min/min collection)</td>
<td>18.88 ± 2.42</td>
<td>23.11 ± 0.34</td>
</tr>
<tr>
<td>U kinins (pg/min)</td>
<td>31.56 ± 4.95</td>
<td>36.81 ± 3.75</td>
</tr>
<tr>
<td>UNaV (µEq/min)</td>
<td>1.05 ± 0.05</td>
<td>1.04 ± 0.04</td>
</tr>
<tr>
<td>UKV (µEq/min)</td>
<td>0.67 ± 0.04</td>
<td>0.68 ± 0.05</td>
</tr>
<tr>
<td>UV (µl/min)</td>
<td>14.63 ± 1.37</td>
<td>14.43 ± 1.43</td>
</tr>
</tbody>
</table>

Aprotinin doses: 10,000 KIU/kg bolus and 1000 KIU/kg/min infusion for 60 minutes; UKA = urinary kininogenase activity excretion; UNaV = urinary sodium excretion; UKV = potassium excretion; UV = urinary volume. Values are means ± se. Significance of difference compared with Period 1: *p < 0.05; †p < 0.005; ‡p < 0.001.

Figure 1. Effects of aprotinin infusion on glomerular filtration rate (GFR), renal blood flow (RBF), urinary kininogenase activity, and kinin excretion presented as a percentage change from control values. Asterisks represent significant differences compared with control period at the levels of: *p < 0.05; **p < 0.005; ***p < 0.001.
ed so that the basal kininogenase excretion matched the range of the control rats (Group 1), the effect of aprotinin was still evident. Kininogenase excretion in these rats (n = 7) dropped from 20.52 ± 2.4 to 12.75 ± 1.65 ng/min/kg body wt (p < 0.005), and remained depressed during the recovery period (17.06 ± 2.90 ng/min/kg body wt, p < 0.02).

Figure 1 shows the changes in GFR, RBF, urinary kininogenase activity, and kinin excretion as a percentage of the control period (Period 1). The GFR was significantly depressed by 37%, from 6.73 ± 0.51 to 4.21 ± 0.39 ml/min/kg body wt (p < 0.001) during aprotinin infusion. It remained depressed some 17% (5.04 ± 0.52 ml/min/kg body wt, p < 0.005) during the recovery period. The RBF decreased by 36% from 12.41 ± 0.64 to 8.01 ± 0.66 ml/min/kg body wt (p < 0.001) during aprotinin, and remained depressed by 22% (10.15 ± 0.68 ml/min/kg body wt, p < 0.05) during the recovery period. Excretions of kininogenase activity and kinins were also significantly reduced during the infusion of aprotinin (43% and 35%, respectively) and remained depressed during the recovery period (see table 1 and fig. 1).

During the infusion of aprotinin, PRA decreased 21% from 23.7 ± 3.3 to 18.7 ± 2.8 ng/ml/hr (p < 0.05) and increased to 28.2 ± 4.3 ng/ml/hr during recovery. In the time control group, PRA increased progressively from 18.2 ± 4.4 in period 1 to 35.9 ± 13.1 ng/ml/hr in Period 2 (p < 0.05), and to 45.3 ± 19.5 ng/ml/hr in Period 3 (p < 0.05).

Blood pressure was not changed in the aprotinin-treated group (Period 1 = 113 ± 5; Period 2 = 105 ± 5; Period 3 = 106 ± 5 mm Hg). However, in the time control group, a small decrease in blood pressure (p < 0.05) was observed in Period 3 (Period 1 = 124 ± 7; Period 2 = 114 ± 6; Period 3 = 107 ± 7 mm Hg).

In Subgroup 2B, urinary excretion of immunoreactive kallikrein during aprotinin infusion decreased from 131 ± 26 to 49 ± 11 ng/min (p < 0.025), and remained significantly lower at 50 ± 7 ng/min (p < 0.025) during Period 3.

In Subgroup 2C, cardiac output was not altered when measured before and at the end of aprotinin infusion (before, 14.7 ± 1.3; after, 14.2 ± 2.4 ml/min/100 g body wt).

Study II: Renin Release Studies in Conscious Rats

Group 3 (Time Controls)
Continuous infusion with the dextrose vehicle over 60 minutes prompted no change in PRA, hematocrit, or blood pressure (table 2).

Group 4 (Low Dose of Aprotinin)
In these rats PRA did not change during aprotinin infusion. Neither hematocrit nor mean blood pressure changed throughout these experiments (table 2).

Group 5 (High Dose of Aprotinin)
Administration of the high dose of aprotinin for 60 minutes caused a 5.6-fold increase in PRA (p < 0.005, table 2), but this was accompanied by increased hematocrit (p < 0.05, table 2) and a 25% decrease in blood pressure (p < 0.005).

Groups 6 and 7 (Isoproterenol, and Isoproterenol with Aprotinin)
Infusion of isoproterenol significantly increased PRA, and this increase was not affected by aprotinin. PRA increased approximately threefold (table 3) in both groups. Intraarterial infusion of isoproterenol did not alter the blood pressure or hematocrit in either aprotinin-treated or control rats (table 3).

Groups 8 and 9 (Furosemide, and Furosemide with Aprotinin)
A bolus dose of furosemide significantly increased PRA by 157% in control rats (p < 0.01), while in the rats pretreated with aprotinin, this increase in PRA was only 37%. The increment of PRA caused by furosemide administration in the rats pretreated with aprotinin was significantly lower than this increment in groups 8 and 9.
The aprotinin-kallikrein complex did not alter kininogenase activity of renal kallikrein. However, the decrease in kinin excretion may be secondary to the inhibition of serine proteases other than kallikrein within the kidney. We and others have observed that the stress of pentobarbital anesthesia and surgery causes a constant increase in PRA over time. However, despite this time-dependent increase, and the decrease in RBF which should stimulate renin, aprotinin eliminated the increase in renin release. This phenomenon was reversed when aprotinin was withdrawn. These findings suggest that the stimulus for increased PRA under these conditions was directly or indirectly due to serine protease(s). The suppressive effect of aprotinin on PRA in anesthetized rats could be due to its inhibition of serine proteases which might convert prorenin or inactive renin to active renin. Recently, Barrett et al.21 demonstrated the presence of prorenin or inactive renin in rat plasma. However, the results of Osmond et al.24 as well as our own observations (unpublished results) did not show prorenin or inactive renin in rat plasma.

We performed additional studies in conscious rats with minimal surgical intervention to further elucidate the influence of aprotinin upon renin release. With rats maintained under conditions in which neither blood pressure nor PRA was changed, a low dose of aprotinin had no effect on basal renin release. This might suggest that the effect of aprotinin becomes manifest only when renin has been stimulated beyond basal conditions. Increasing the dose of aprotinin resulted in a substantial decrease in blood pressure, which stimulated PRA. Olsen25 has also reported that a high dose of aprotinin infusion significantly decreased blood pressure in anesthetized rats, although the cause of this hypotension was unclear. However, aprotinin has been reported to inhibit myocardial contractility in vitro.26 Also, aprotinin is a highly cationic peptide27 which at a high dose may exert charge-related effects such as an increase in capillary permeability. This effect might also explain the observed increase in hematocrit in the presence of lowered blood pressure.

To test the possibility that low doses of aprotinin inhibit stimulated renin release (as opposed to basal release), two known stimuli of renin were used in the conscious rat. 3-Adrenergic stimulation of renin release with isoproterenol was unaltered by infusion of aprotinin. There is also evidence in vivo28 and in vitro29 that isoproterenol stimulation of renin release is independent of prostaglandin mediation. Thus, the combined results would suggest that 3-adrenergic stimulation of renin is neither mediated by serine proteases nor prostaglandins.

The loop diuretic furosemide stimulates renin, but this response is blunted by aprotinin in conscious rats. Shimoda et al.30 have reported similar results in rabbits. Overlack et al.31 demonstrated that the combination of indomethacin and aprotinin had a greater suppressive effect on furosemide-stimulated PRA than indomethacin alone, although aprotinin alone did not

| Table 4. Effect of Aprotinin in Furosemide-Stimulated Plasma Renin Activity (PRA) |
|-----------------|-----------------|-----------------|
| Control (n = 14) | Aprotinin low dose (n = 14) |
| Minutes of infusion | PRA (ng/ml/hr) | delta |
| 0 | 4.5 ± 0.7 | 3.5 ± 0.9 |
| (p < 0.01) | 11.7 ± 2.3 | 11.2 ± 2.3 |
| 60 | 7.2 ± 2.4 | 6.7 ± 1.3* |
| Hct (%) | 44.7 ± 0.6 | 43.5 ± 0.8 |
| (p < 0.005) | (p < 0.005) |
| BP (mm Hg) | 116.7 ± 2.1 | 113.0 ± 3.2 |
| Low Dose = 5000 KIU/kg bolus and 1000/kg/min infusion for 60 minutes; delta indicates the difference between PRA at 60 and 0 minutes of infusion. *p < 0.05 when compared to control group; values in parentheses represent significance between 0 and 60 minutes within the same group. |  |  |

control rats (p < 0.05, table 4). Hematocrit increased approximately 3% (p < 0.005), while blood pressure remained unchanged (table 4).

Discussion

Infusion of the serine protease inhibitor, aprotinin, significantly lowered the excretion of kallikrein, kinins, sodium, potassium, and water, and also resulted in lower RBF and GFR. Since these changes occurred in the absence of significant changes in either blood pressure or cardiac output, these results suggest that aprotinin exerted a selective effect upon the kidney.

The observed changes in excretion of kinins after aprotinin administration could be due to its inhibitory effect upon the kininogenase activity of renal kallikrein. However, the decrease in kinin excretion may be secondary to a decrease in kallikrein since aprotinin also induced decreases in immunoreactive kallikrein. The aprotinin-kallikrein complex did not alter kallikrein immunoreactivity in our radioimmunoassay.19 thus, the decrease in immunoreactive kallikrein excretion indicated a decrease in kallikrein secretion by the nephron. This decrease in kallikrein secretion may be secondary to the inhibition of a serine protease(s) involved in the mechanism of kallikrein release or it may be secondary to hemodynamic changes.

The decrease in intrarenal formation of kinins may modify RBF, GFR, and salt and water excretion. However, the changes in salt and water excretion may be secondary to the renal hemodynamic alterations.

Kramer et al.2 also found that aprotinin decreased RBF, GFR, and sodium, potassium, and water excretion in volume-expanded rats, and they attributed the effects of aprotinin to inhibition of renal kallikrein. However, in their study, unlike ours, they did not measure kallikrein or kinin excretion. Kinins have been shown to stimulate the PGE2 secretion rate in the dog30 and PGE2 formation in papillary collecting tubules.21 Aprotinin has been associated with decreased prostaglandin excretion,2,3 thus, some of the effect of aprotinin may be due to a decrease in prostaglandins secondary to the decrease in kinins. The effects we observed with aprotinin may also be due to the inhibition of serine proteases other than kallikrein within the kidney.

We and others have observed that the stress of pentobarbital anesthesia and surgery causes a constant increase in PRA over time. However, despite this time-dependent increase, and the decrease in RBF which should stimulate renin, aprotinin eliminated the increase in renin release. This phenomenon was reversed when aprotinin was withdrawn. These findings suggest that the stimulus for increased PRA under these conditions was directly or indirectly due to serine protease(s). The suppressive effect of aprotinin on PRA in anesthetized rats could be due to its inhibition of serine proteases which might convert prorenin or inactive renin to active renin.21 Recently, Barrett et al.21 demonstrated the presence of prorenin or inactive renin in rat plasma. However, the results of Osmond et al.24 as well as our own observations (unpublished results) did not show prorenin or inactive renin in rat plasma.

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alter this response in normotensive humans. Thus, furosemide stimulation of renin may be mediated by a serine protease and/or prostaglandins.\textsuperscript{23, 32}

The mechanism or site of the aprotinin-renin interaction cannot be determined by our study. When in the bloodstream, aprotinin can be filtered and has been shown to accumulate within the renal tissue,\textsuperscript{23} so that it could act at almost any level, intra- or extrarenal. It has been speculated that aprotinin might inhibit serine proteases that convert inactive to active renin.\textsuperscript{33} Osmond et al.\textsuperscript{34} as well as our own unpublished observations have failed to show inactive renin in rat plasma, suggesting that the effect of aprotinin on conversion must occur at an intrarenal site.\textsuperscript{34} However, other results that support the theory that rat plasma has inactive renin\textsuperscript{33} make this conclusion controversial.

In conclusion, aprotinin at high doses had a systemic effect that caused a decrease in blood pressure and an increase in renin release and hematocrit. Aprotinin at a low dose caused a decrease in renin release, RBF, and GFR without systemic hemodynamic changes. Furthermore, low doses of aprotinin caused a decrease in urinary excretion of kinins, kininogenase activity, immunoreactive kallikrein, electrolytes, and water excretion. These changes may be due to inhibition of the kallikrein-kinin system, other serine proteases, or other unrecognized properties of aprotinin due to its highly cationic nature.

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