The Effect of Hemorrhagic Hypotension on Urinary Kallikrein Excretion, Renin Activity, and Renal Cortical Blood Flow in the Pig

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SUMMARY We measured urinary kallikrein by its esterolytic and kinin-forming activity in 5-minute urine samples obtained throughout continuous bleeding experiments in pigs to correlate possible changes in urinary kallikrein excretion during hemorrhagic hypotension with renin activity and renal cortical blood flow. Renin activity was determined in venous blood samples and renal cortical blood flow was estimated by the radiolabeled microsphere technique. The rate of urinary kallikrein excretion was increased about 4-fold within an arterial pressure range of 100-70 mm Hg, whereas below 70 mm Hg, arterial pressure urinary kallikrein activity declined to undetectable values. Renin activity was increased only 2-fold in the arterial pressure range between 100 and 70 mm Hg but was increased 4-fold at pressures below 70 mm Hg. The pressure range of 100 to 70 mm Hg corresponded to the autoregulation of renal cortical blood flow and glomerular filtration rate; below that pressure range, renal cortical blood flow dropped to about 10% of the initial value. Therefore, it seems that urinary kallikrein is activated mainly during the period of autoregulation, whereas renin activity is, in the main, increased below the autoregulatory range of pressure. The vasodilatory urinary kallikrein kinin system might be involved in maintaining sufficient local blood flow during autoregulation whereas a decrease in blood pressure below the autoregulatory range leads to a major increase in renin activity, thus illustrating the attempt of the organism to reestablish sufficient blood pressure to maintain autoregulation. Circ Res 48: 386-392, 1981

Kallikreins, the enzymes that release vasoactive kinin polypeptides from kininogen substrate, are found in plasma and in organs such as pancreas, salivary glands and kidneys. Because urinary kallikrein (Urokallikrein) is functionally and structurally similar to the kallikrein found in the kidney (Nustad and Vaaje, 1975) and kidney cell suspensions (Kaizu and Margolius, 1975), it is thought that urinary kallikrein is synthesized and secreted by the kidney. Urinary kallikrein levels are abnormal in pathological conditions such as essential hypertension (Margolius et al., 1971) and primary aldosteronism (Margolius et al., 1971). The secretion of kallikrein can be altered by changes in perfusion pressure (Bevan et al., 1974; Keiser et al., 1976), sodium loading (Mills and Ward, 1975), mineralocorticoids (Nasjletti et al., 1978), or vasoactive hormones (Mills et al., 1979). These findings indicate that the renal kallikrein-kinin system can be affected by altered kidney function. During studies of experimental hemorrhagic shock, which also affects kidney function, activation of the renin-angiotensin system has been shown (Beatty et al., 1976), although no comparable studies of changes in the renal kallikrein-kinin system have been reported.

In the present study we measured urinary kallikrein excretion and plasma renin activity during the course of hemorrhagic hypotension in the pig in which renal cortical blood flow was determined using the radioactive microsphere technique. Under our experimental conditions, the rate of urinary kallikrein excretion was highest within the autoregulatory range, i.e., the range of blood pressures within the renal cortical blood flow remains constant, whereas plasma renin activity was highest at blood pressures below this range.

Methods

Experimental Protocol

Thirty pigs (18-30 kg), were anesthetized with sodium-thiopental (Pentothal, 30 mg/kg, iv). An endotracheal tube was inserted and the animals...
were allowed to breathe a 1% halothane-24% O₂-75% N₂O mixture spontaneously. Catheters were inserted via the left carotid artery into the left ventricle (French No. 5, pigtail), via the left jugular vein into the pulmonary artery (flow-directed thermodilution catheter, W/20 cm prox. part), into the left femoral artery and into the left femoral vein (polyethylene catheters: i.d., 1.5 mm; o.d., 2 mm). Polyethylene catheters also were inserted via a small suprapubic incision into both ureters. The surgical procedure was followed by a 60-minute stabilization period. Thereafter, hypovolemia was induced in 20 pigs by continuous bleeding from the carotid artery at a rate of 1 ml/kg per min until the death of the animal, which took about 70-120 minutes (blood loss about 60% as calculated from the body weight). Ten pigs operated in the same way and followed without bleeding for a total of 3 hours served as controls. The catheters from the pulmonary artery and the femoral artery were connected to a Statham transducer for continuous recording of systolic, diastolic, and mean arterial blood pressure (MAP), pulmonary artery pressure, and heart rate (Gould Brush polygraph). The electrocardiogram also was recorded continuously. Catheters from both ureters were connected and 5-minute urine samples were collected by means of a fraction collector. The dead space in each catheter was less than 700 µl. Care was taken to ensure free urine flow from both ureters.

Analytical Methods

In venous femoral blood samples taken three times during the first hour and four times during bleeding or during the corresponding period in the controls (interval between sampling, about 20 minutes), Na⁺, K⁺ (Eppendorf flame photometer), creatinine (Boehringer Mannheim 166 413), and renin activity (RA) were determined. In simultaneously obtained blood samples from the femoral artery, PO₂, PCO₂, and pH were measured (ABL2 Acid Base Laboratory, Radiometer Copenhagen). For each urine sample, volume and kallikrein concentration were determined. Na⁺, K⁺, and creatinine were evaluated in pooled urine samples.

Urinary kallikrein was determined as the amount of kinin released from an excess of heat-inactivated human plasma during a 5-minute incubation period and assayed on the guinea pig ileum preparation (Webster and Prado, 1970). The contractile response of the smooth muscle preparation was compared to the effect of bradykinin standards. The esterolytic activity of urinary kallikrein also was determined on the synthetic amino acid ester substrate benzoyl-arginyl-ethylster (BAE; Trautschold et al., 1970). All pigs were studied using both methods for urinary kallikrein determination and the results correlated with r = 0.81 (P < 0.001), and only substrate-dependent activities were found. RA was measured by radioimmunoassay of the angiotensin I generated after incubation of 0.1 ml of plasma samples with 0.4 ml of partially purified substrate prepared from the plasma of a nephrectomized pig (Skinner, 1967) for 1 h at 37° C. The assay was carried out according to the method of Haber et al. (1969), using a New England Nuclear assay kit. The amount of substrate added was sufficient for the generation of 1 µg angiotensin I in 1 hour at pH 6.0 by 10 µl of hog renin (Sigma Chemical Company).

Microsphere Studies

To relate urinary kallikrein excretion and RA with local blood flow in the kidneys, we evaluated renal cortical blood flow (RCBF) in four of the control animals and in 14 of the bled animals, using the radioactive microsphere technique (McNay and Abe, 1970). Microspheres (9 µm size) labeled with ¹⁴Ce, ⁵¹Cr, and ⁸⁵Sr (3M Company) were injected in a random order into the left ventricle at three times in each experiment within a 10-second period (approximately 8-10 × 10⁶ microspheres per injection). Starting 10 seconds before injection, blood was withdrawn from the femoral artery by a Harvard pump at a rate of 10 ml/min for 1 minute. At the end of the experiments, three tissue specimens were taken from both the outer and inner cortices of both kidneys. These were weighed and, together with the blood samples, were counted for their respective activities in a 3-channel Gamma-counter (Beckman 8000). Blood flow then was calculated according to McNay (McNay and Abe, 1970) in ml/g wet weight per min. The first injection was performed during the first hour and used as 100% value for the calculation. The second and third injections were done at two different blood pressures in the bled pigs and at corresponding times in the control experiments.

Statistical Methods

Results are reported as mean ± SD. Student's t-test for paired samples or analysis of variance was used to determine statistical significance (Colton, 1974).

Results

Results are summarized in Table 1 as means and standard deviations for period I, II, and III, as described in Methods for bled animals and controls. The mean arterial blood pressure (MAP), pulmonary artery pressure (PAP), and heart rate throughout a whole experiment are shown for one representative pig in Figure 1 (upper panel). During the first hour, MAP, PAP, and heart rate remained at a constant, normal level. Whereas these values did not change in the control animals, about 15-20 minutes after the initiation of bleeding, both MAP and PAP started to decline simultaneously with an increase in the heart rate. These changes in circulatory parameters continued until the pig died. Uri-
TABLE 1  Pattern of Hemodynamics, Kidney Function, Renin Activity, and Urinary Kallikrein Excretion in Control and Bled Animals*

<table>
<thead>
<tr>
<th></th>
<th>Control experiments (n = 10)</th>
<th>Hemorrhagic hypotension (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr</td>
<td>2 hr</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>116 ± 10</td>
<td>108 ± 14</td>
</tr>
<tr>
<td>PAP (mm Hg)</td>
<td>22 ± 3</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>HR/min</td>
<td>151 ± 6</td>
<td>145 ± 10</td>
</tr>
<tr>
<td>V_u (ml/min)</td>
<td>0.31 ± 0.17</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>RA (ng/ml per hr)</td>
<td>67.5 ± 12.9</td>
<td>82 ± 18.5</td>
</tr>
<tr>
<td>UKall (μg kinin/ml)</td>
<td>1.5 ± 0.6</td>
<td>1.3 ± 0.7</td>
</tr>
<tr>
<td>UKall (μg kinin/min)</td>
<td>0.46 ± 0.1</td>
<td>0.39 ± 0.07</td>
</tr>
<tr>
<td>UKall (U/ml)</td>
<td>0.16 ± 0.07</td>
<td>0.15 ± 0.07</td>
</tr>
<tr>
<td>UKall (U/min)</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.007</td>
</tr>
<tr>
<td>(Na⁺) (mmol/l)</td>
<td>28.7 ± 9.5</td>
<td>18.2 ± 3.5</td>
</tr>
<tr>
<td>% Na⁺ reabs.</td>
<td>99.8 ± 0.05</td>
<td>99.87 ± 0.06</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>26.7 ± 4.2</td>
<td>23.9 ± 5.6</td>
</tr>
</tbody>
</table>

* Mean arterial blood pressure (MAP), pulmonary artery pressure (PAP), heart rate (HR), urine volume (V_u), renin activity (RA), urinary kallikrein (UKall) as kinin-generating activity in μg kinin and as esterolytic activity on BAEe in units, sodium concentration in urine samples [(Na⁺)]_u, percent sodium reabsorption (% Na⁺ reabs.), and glomerular filtration rate (GFR). Data are presented as means and standard deviations for the periods indicated (period I = first hour; period II = between initiation of bleeding and a MAP of 70 mm Hg; period III = below a MAP of 70 mm Hg).

† Statistical significance (P < 0.005) was calculated by analysis of variance.

FIGURE 1  Time course of experimental hypovolemia in the pig. The upper panel depicts the changes in intravascular pressure and urinary kallikrein levels at 5-minute intervals during a single experiment on a bled animal. Bleeding was initiated (arrows) after a 50-minute equilibration period. Mean arterial blood pressure (MAP), pulmonary artery pressure (PAP), and heart rate were recorded continuously. Urinary kallikrein concentration is given as kinin-generating activity (KGA; μg kinin/ml of urine). In the lower panel are shown for the same experiment urine flow, ml/5 min; rate of urinary kallikrein excretion, KGA, μg kinin/5 min; and renin activity, RA, μg angiotensin I/ml per hour of incubation.
Urinary kallikrein excretion, shown as kinin-generating activity in 5-minute urine fractions, increased initially from an average value of 0.8 ± 0.3 μg kinin per ml of urine to 3.2 μg kinin per ml, declined thereafter, and remained at a value of 1.5 μg kinin per ml until the MAP had dropped below 70 mm Hg. At this point the kallikrein level began to decline and was undetectable by the end of the experiment. For all 20 experimental animals, the average value of kinin-generating activity increased significantly from 1.2 ± 0.9 μg kinin per ml in the stabilization period to 4.8 ± 1.8 μg kinin per ml as the MAP fell from 100 to 70 mm Hg, but then gradually became undetectable with further decreases in MAP; this also was significant (Table 1). An initial overshoot beyond the mean increase in urinary kallikrein excretion like that shown in Figure 1 was seen in 14 of the 20 experimental animals as the MAP started to decrease. In the control animals, urinary kallikrein excretion remained almost constant to a MAP of about 70 mm Hg, the mean RA was highest in period III in the pig shown in Figure 1, lower panel). Despite a decline in RA towards the end of all experiments at blood pressures lower than 70 mm Hg (Fig. 1, lower panel), the mean RA was highest in period III in the bled animals (Table 1). Although urine flow was almost constant to a MAP of about 70 mm Hg, the rate of urinary kallikrein excretion (Table 1: Fig. 1, lower panel) showed a 3- to 4-fold increase after initiation of bleeding. At blood pressures below 70 mm Hg, both the rate of urinary kallikrein excretion and urine flow declined to nondetectable values (Table 1: Fig. 1, lower panel).

In both control and bled animals, arterial P0₂, Pco₂, and pH as well as plasma concentration of Na⁺ and K⁺, remained unchanged and at normal values throughout the experiments. Urinary sodium excretion, calculated percent sodium reabsorption, and glomerular filtration rate (GFR) calculated from the creatinine values also are shown in Table 1.

The relationship of renal cortical blood flow, urinary kallikrein excretion, and renin activity to the mean arterial pressure are shown in Figure 2 for 14 bled animals in which microsphere studies were performed. After about 25% blood loss (100 mm Hg MAP), the renal cortical blood flow was decreased to about 65-70% of the value observed during the stabilization period. Thereafter, renal cortical blood flow remained constant despite continuation of bleeding until a MAP of about 70 mm Hg was reached (45% blood loss). Further lowering of the mean arterial blood pressure led to a decline in the renal cortical blood flow to 10% of control value. The increase in urinary kallikrein excretion described above was seen during the period of constant RCBF. In contrast, although renin activity doubled during the period of constant RCBF, the greatest increase in renin activity was observed at the time RCBF was further diminished. In the control animals, no change in RCBF occurred. The absolute values for RCBF in ml/g wet weight per min for controls, as well as for bled animals, are shown in Table 2.

**Discussion**

We have studied the response of the renal kallikrein-kinin system and the renin-angiotensin system to hemorrhagic hypotension in the anesthetized pig, determining urinary kallikrein excretion and renin activity. Bleeding was followed by an increase in renin activity, a finding which has been reported previously (Beatty et al., 1976). This effect probably is mediated by lowering the perfusion pressure (Schmid, 1972) and/or by stimulation of the renal sympathetic nerves (Lagrange et al., 1973). There was an additional sharp increase in renin activity when the MAP dropped below 70 mm Hg. This sharp increase correlates well with a study by Schmid (1972), who altered the perfusion pressure by constriction of the renal artery in dogs. A significant increase in renin production occurred only when the decrease in perfusion pressure exceeded the autoregulatory ability of the kidney to maintain both flow and filtration or when a dissociation occurred between autoregulation of blood flow and filtration rate. On the other hand, externally decreased perfusion pressure for 5 minutes led to an increased renin secretion in uninephrectomized ewes (Blaine and Zimmerman, 1979) within the autoregulatory pressure range. Although renin release increased further below the autoregulatory range, this increase was less pronounced. Therefore, two different mechanisms to control renin release, one within the autoregulatory pressure range and one below it, have been suggested.

Our data from the microsphere studies clearly show that the sharp increase in renin activity is concomitant with the sharp decline in RCBF and GFR, as will be discussed. Since hemorrhage leads to a continuous slow decline of the perfusion pressure, different patterns of renin activities might be explained by different time courses in the decrease of the perfusion pressure.

Severe hemorrhagic hypotension causes a reduction in arterial pressure and in total renal blood flow (Grandchamp et al., 1971; Rector et al., 1972). The radioactive microsphere technique (McNay and Abe, 1970) was used to determine renal cortical blood flow distribution and to calculate the actual RCBF for each 10 mm Hg step reduction in MAP. By plotting these data as the percentage of the 100% control obtained during the stabilization period vs. the MAP, we could demonstrate a pressure range between 100 and 70 mm Hg within which RCBF (Table 2) and GFR (Table 1) remained constant. This effect is known as autoregulation,
although the mechanisms that contribute to this effect are not clearly understood. In addition, hemorrhagic hypotension produces a typical redistribution of renal blood flow to inner cortical nephrons with marked decreases in perfusion of the outer cortical nephrons (Rector et al., 1972). Since, in these studies, the cortex had been divided into four zones (Grandchamp et al., 1971; Rector et al., 1972), whereas, in our experiments, RCBF had been measured only in the outer and the inner half of the renal cortex, we found insignificant differences between the two zones. However, there was a tendency in every experiment for the inner cortex to be better perfused (Fig. 2). The main common factor for redistribution of RCBF could be identified as decreased renal resistance (Stein et al., 1973) which is caused by vasodilation (Vatner, 1974; Rector et al., 1972; Data et al., 1976) and most likely mediated by a local intrarenal mechanism (Stein et al., 1973). Decreased afferent arteriolar resistance is also considered to contribute to autoregulation of RCBF (Hall, 1977).

Since the activity of kallikrein found in urine is thought to resemble the activity of kidney kallikrein, our results show for the first time increased activity of the vasodilatory renal kallikrein-kinin system (Nasjletti et al., 1978; Mills et al., 1979). Bevan et al. (1974) reported that mechanical changes in renal artery pressure by a clamp produced corresponding changes in excretion of kallikrein. However, there are no data given concerning the degree of renal artery pressure lowering that occurred in this study. Reducing the blood flow to one kidney by more than 75% using chronic renal artery stenosis in the dog, Keiser et al. (1976) found a significantly reduced kallikrein excretion from this kidney which correlated with renal blood flow and glomerular filtration rate. Although these results are based on total renal blood flow, they are consistent with our experiments, which show a correlation between MAP, RCBF, GFR and urinary kallikrein excretion below the autoregulatory range.

By correlation of the observed changes in renin activity, urinary kallikrein excretion and RCBF during hemorrhagic hypotension neither a direct nor an inverse relationship between these parameters could be found. As can be seen from Table 1 and Fig. 2 urinary kallikrein excretion per time and per ml of urine was significantly increased within that blood pressure range in which RCBF remained constant, i.e. within the autoregulatory range. Despite a two-fold increase during this period renin showed its highest activity concomitant with the final decline of RCBF that occurs below the autoregulatory range, a period when urinary kallikrein excretion dropped below control values. Therefore, it appears that different mechanisms may be responsible for excretion of urinary kallikrein and secretion of renin within the autoregulatory pressure range and below it. Among the mechanisms known to influence renin activity and urinary kallikrein excretion, RCBF might be the determining factor for renin and kallikrein activation below the autoregulatory range. Determining factors for renin activity and kallikrein excretion within the autoregulatory pressure range also might be responsible for autoregulation itself. Since inhibition of prostaglandin synthesis abolishes the autoregulatory ability of the kidney, vasodilatory prostaglandins might be involved in autoregulation of renal cortical blood flow (Herbaczynska-Cedro and Vane, 1973; Data et al., 1976). Prostaglandin production increases when

<table>
<thead>
<tr>
<th>MAP (mm Hg)</th>
<th>Outer cortex</th>
<th>Inner cortex</th>
<th>Min</th>
<th>Outer cortex</th>
<th>Inner cortex</th>
</tr>
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<tbody>
<tr>
<td>≥110</td>
<td>4.73 ± 0.5</td>
<td>4.0 ± 0.5</td>
<td>30</td>
<td>4.7 ± 0.55</td>
<td>4.2 ± 0.55</td>
</tr>
<tr>
<td>100</td>
<td>3.07 ± 0.1</td>
<td>2.8 ± 0.2</td>
<td>60</td>
<td>4.65 ± 0.48</td>
<td>4.2 ± 0.42</td>
</tr>
<tr>
<td>90</td>
<td>3.1 ± 0.28</td>
<td>2.92 ± 0.25</td>
<td>90</td>
<td>4.75 ± 0.45</td>
<td>4.31 ± 0.45</td>
</tr>
<tr>
<td>80</td>
<td>3.0 ± 0.15</td>
<td>2.7 ± 0.22</td>
<td>120</td>
<td>4.74 ± 0.5</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>70</td>
<td>3.2 ± 0.09</td>
<td>2.71 ± 0.2</td>
<td>150</td>
<td>4.6 ± 0.5</td>
<td>4.25 ± 0.38</td>
</tr>
<tr>
<td>60</td>
<td>1.64 ± 0.05</td>
<td>1.4 ± 0.07</td>
<td>180</td>
<td>4.68 ± 0.53</td>
<td>4.19 ± 0.45</td>
</tr>
<tr>
<td>40</td>
<td>0.46 ± 0.05</td>
<td>0.4 ± 0.03</td>
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</table>

* Absolute values for renal cortical blood flow in ml/g wet weight per min for outer and inner half of renal cortex. Means and standard deviations for different time periods (min) in the control experiments and for different mean arterial blood pressure levels (MAP) in the bleeding experiments.
perfusion pressure is decreased (Herbacynska-Cedro and Vane, 1973), or during renal nerve stimulation (Dunham and Zimmerman, 1970), both of which can occur during hemorrhagic hypotension. However, it is not known whether these mechanisms can release prostaglandins directly or if they act via mediators. One system that can participate in the release of prostaglandins is the renal kallikrein-kinin system (Vargaftig and Hai, 1972; Wong et al., 1977). Autoregulation also might be mediated by reduced activity of an intrarenal renin-angiotensin system (Thurau, 1964), although this mechanism is not generally accepted (Murray and Marvin, 1979). There is evidence also for a relationship between the renin-angiotensin system and the renal kallikrein-kinin system. In addition to the fact that angiotensin I converting enzyme is identical with kininase II (Yang et al., 1971), recently it could be shown that in vitro kallikrein is an activator of the inactive precursor of renin, prorenin (Sealey et al., 1978a). Based on that finding Sealey et al. (1978b) suggested that both vasoactive peptides could act concurrently at different sites to regulate systemic arterial pressure without at the same time compromising local kidney perfusion.

By determination of renin activity, urinary kallikrein excretion and renal cortical blood flow during hemorrhagic hypotension we have shown that both renal hormones are activated and are neither directly nor inversely correlated to each other. The increase in urinary kallikrein excretion was related strongly only to the autoregulation of renal cortical blood flow and glomerular filtration rate. Our data cannot be explained sufficiently by one of the proposed mechanisms for urinary kallikrein excretion or renin release. Therefore, the following hypothesis might be suggested: the vasodilatory urinary kallikrein-kinin system activated by mechanisms not completely clarified appears to be involved with maintaining sufficient local blood flow—either directly or as mediator—within the autoregulatory pressure range. A fall of the blood pressure below the autoregulatory range leads to a major increase in renin activity reflecting the attempt of the organism to reestablish sufficient blood pressure to maintain autoregulation.

**Acknowledgments**

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