Mechanism of Adenosine-Mediated Decreases in Glomerular Filtration Rate in Dogs

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SUMMARY This study evaluates the contributions of pre- and postglomerular resistances and glomerular capillary ultrafiltration coefficient (Kf) in adenosine-induced decreases in glomerular filtration rate (GFR). Experiments were performed on 19 dogs prepared for micropuncture. Whole kidney and superficial nephron functions were measured before and during adenosine infusion (0.1 μmol/min) into the renal artery. Whole kidney GFR decreased from 29.0 ± 1.7 to 23.1 ± 1.6 (SE) ml/min (P < 0.003), whereas renal plasma flow (RPF) increased slightly from 98 ± 6 to 108 ± 8 ml/min (P < 0.05), respectively. Free flow and stop flow pressures in proximal tubules decreased from 23 ± 1 to 16 ± 1 mm Hg and from 44 ± 1 to 31 ± 2 mm Hg, respectively. Kf was not significantly changed (2.55 ± 0.22 vs. 3.12 ± 0.88 nl/min per mm Hg). The hydrostatic pressures in efferent arterioles decreased from 14 ± 0.6 to 10.4 ± 1.8 mm Hg (P < 0.01). The resistance in the afferent arterioles increased more than 2-fold from 1.38 ± 0.25 to 3.04 ± 0.42 • 10^-10 dynes • sec • cm^-5 (P < 0.003), whereas efferent arteriolar resistance was only slightly increased from 1.35 ± 0.18 to 1.76 ± 0.17. We conclude that intrarenal infusion of adenosine preferentially constricts the afferent arterioles of the superficial cortex and thereby decreases superficial GFR without decreases in glomerular capillary ultrafiltration coefficient.

ADENOSINE recently has been proposed as a metabolic regulator of glomerular filtration rate. Although adenosine vasodilates various organs (brain, heart, muscle) and renal artery, it vasoconstricts when injected into the renal arteries of dogs and rats. During continuous infusion of adenosine, renal blood flow (RBF) initially decreases and then returns to preinfusion levels within 1-4 minutes. Glomerular filtration rate (GFR) decreases. In the present study we evaluated whether the depressed GFR during continuous infusion of adenosine is due to a vasoconstrictive component or can be accounted for by dilation of efferent arterioles.

Methods

Experiments were performed in mongrel dogs of either sex, weighing 16-19 kg. They were maintained on standard laboratory chow (Purina) and tap water ad libitum. Food was withheld 16 hours prior to the experiment. Dogs were anesthetized with pentobarbital (30 mg/kg, iv) supplemented with 1% chloralose during the experiment if necessary. Kidneys were exposed retroperitoneally by a flank incision. An infusion was given to establish and maintain an inulin concentration in plasma of 1 mg/ml and a p-aminohippurate (PAH) concentration in plasma of 0.02 mg/ml. The sustaining infusion was given at 1 ml/min in isotonic saline solution. A polyethylene tube (PE 100) was ad-
advanced into the renal vein from the gonadal vein. The renal artery and the kidney were dissected free of connective tissue. The kidney was placed in a holder and an electromagnetic flowmeter probe was fitted around the renal artery. Zero flow calibration was achieved by occluding the renal artery distal to the flow probe. Adenosine (0.1 μmol/min) was infused into the renal artery at a rate of 1 ml/min via a curved needle with two holes on the sides near the tip. Injection or infusion of Lissamine green resulted in a homogeneous coloration of the micropuncture area, indicating good mixture of the infused with blood. Urine was collected from a ureteral catheter (PE 150). The capsule in an area about 2 × 2 cm was removed to allow micropuncture of the surface. Isotonic saline, heated to 37°C, bathed the micropuncture area at a rate of about 0.3 ml/min. The abdominal cavity was covered with paraffin to prevent evaporation and to drain the saline bathing the surface. Tubule fluid was collected quantitatively from proximal tubules in 10 dogs as described by Windhager.7 No attempt was made to localize the site of puncture along the proximal tubule. Hydrostatic pressures were measured in the proximal tubule under free flow (Pf) and stop flow (SFP) conditions and in the efferent arteriole at the welling point where it branches into the peritubular capillaries (Pb). Pressures were measured with a servo-nulling device using pipettes (o.d. 3–6 μm) filled with 2 M NaCl solution stained with Lissamine green dye. Blood was collected from the efferent arterioles with siliconized pipettes 15 μm in outer diameter and filled with paraffin oil. Care was exercised to avoid contamination of the blood sample with the saline bathing the kidney surface. The tip of the pipette was sealed by insertion into Critoseal. Samples were centrifuged at 6000 rpm to separate plasma from red cells. Blood pressure was monitored through a catheter placed in the aorta below the renal arteries.

The experimental protocol consisted of three observation periods: First, a control period of two 10-minute collections of urine, including withdrawal of blood samples from the iliac artery and renal vein at the midpoint of urine collections; second, two 10-minute collection periods during infusion of adenosine into the renal artery. The collections were started when the RBF had reached a steady state about 5–7 minutes after onset of the adenosine infusion. The third observation period consisted of two 10-minute recovery periods, which were started 10 minutes after cessation of the adenosine infusion.

The volume of tubule fluid was measured with a micropipette that had been calibrated with a radioactive tracer. Inulin concentration in tubule fluid samples was determined in duplicate by the microfluorometric method.8 Plasma and urine inulin concentrations were measured by the anthrone method.9 PAH was measured in plasma from arterial and venous blood and urine.10

Calculations

The resistances along the afferent (Rf) and efferent (Re) glomerular arterioles were calculated as follows:

\[
R_f = \frac{R_{AP} - P_{GC}}{GBF_f} \quad \text{and} \quad R_e = \frac{P_{GC} - P_{E}}{GBF_e}
\]

The mean hydrostatic pressure in the aorta below the renal arteries was taken as the renal artery pressure (RAP). The glomerular capillary pressure (Pf) was calculated from the stop flow pressure (SFP) plus the colloid oncotic pressure of systemic arterial blood (Pf). The albumino-globulin ratio in the dogs' blood was 0.65 ± 0.04 (n = 7), and the correlation between protein concentration (c) and colloid oncotic pressure (π) was described by the equation \( π = 1.4c + 0.22c^2 + 0.005c^3 \). The glomerular blood flow through the afferent arteriole (GBFA) equals QA/(1 − Hct). QA, the initial glomerular plasma flow, is given by the single nephron filtration rate (SNFF) and single nephron filtration fraction (SNFF):

\[
QA = \frac{SNFF}{SNFR};
\]

\[
SNFF = \frac{\dot{V}}{P} \ln \text{In} \quad \text{and} \quad SNFR = 1 - \frac{C_A}{C_E}
\]

where \( \dot{V} \) is the tubule flow rate in nl/min, and \( TF/P_{in} \) is the tubular fluid-to-plasma inulin concentration ratio. \( C_A \) and \( C_E \) denote the protein concentrations in the systemic arterial blood (A) and in the efferent arterioles (E). The blood flow at the efferent end of the glomerulus (GBF_e) = GBFA − SNFR. One resistance unit [(mm Hg)/(nl/min)] was converted into one cgs unit (dyne·sec·cm⁻³·10⁻⁵) by multiplying with the factor 7.962. Protein concentration in systemic plasma and in efferent arteriole plasma was determined in duplicate with an ultramicrocolorimeter.12 The glomerular ultrafiltration coefficient, \( K_f \), was determined from the single nephron filtration rate and the effective filtration pressure, \( \Delta P \), as \( K_f = SNFF/ΔP \). Data are expressed as mean ± SE. Student's t-test for paired and unpaired comparisons was used for statistical analysis.

Results

The effect of adenosine infusion on renal blood flow (RBF) and arterial blood pressure is shown in Figure 1. Adenosine infusion causes an initial decrease in RBF but, within 5–10 minutes, RBF increases slightly above the preinfusion level. After cessation of adenosine infusion, there is a transient increase in RBF lasting for about 5–10 minutes. Table 1 summarizes the data from 14 dogs. The mean arterial blood pressure was not affected by adenosine infusion. GFR decreased 29.0 ± 1.7 to 23.1 ± 1.6 ml/min during adenosine infusion (n = 14; P < 0.003) and recovered to 28.0 ± 1.0 ml/min.
Adenosine O.1 μmoles/min into the renal artery

Start

Stop

Dog # 7
16.5 Kg B.W.

RBF
ml/min

BP
mm Hg

Control periods
K-GFR 33.4 ml/min
SNFGR 51  nl/min

Adenosine periods
27.5 ml/min
14  nl/min

Recovery periods
32.5 ml/min
68  nl/min

FIGURE 1  Original tracing of renal blood flow (RBF) and arterial blood pressure (BP) in dog #7 during control, adenosine, and recovery periods. Kidney and superficial nephron GFRs during the experimental periods are given at the bottom of the figure.

(n = 12). Renal plasma flow calculated from the flowmeter reading (n = 12) or from the PAH extraction and clearance (n = 2) increased from 98 ± 6 to 108 ± 8 ml/min (P < 0.05). Filtration fraction decreased from 0.30 ± 0.02 to 0.22 ± 0.02. Urine flow rate and sodium excretion decreased in parallel with GFR, whereas potassium excretion was only slightly decreased.

The GFR of single superficial nephrons (SNFGR) decreased from 68.8 ± 5.3 to 39.9 ± 6.4 nl/min

TABLE 1  Summary of the Data Obtained in 14 Dogs before (Control), during (Adenosine), and after (Recovery) Infusion of Adenosine (0.1 μmol/min) into the Renal Artery

<table>
<thead>
<tr>
<th></th>
<th>Period</th>
<th></th>
<th>Control</th>
<th>Adenosine</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td></td>
<td></td>
<td>133 ± 2.8</td>
<td>132 ± 2.7</td>
<td>138 ± 2.2</td>
</tr>
<tr>
<td>RPF (ml/min)</td>
<td></td>
<td>(14)</td>
<td>98.0 ± 5.7</td>
<td>108 ± 7.9</td>
<td>100 ± 7.1</td>
</tr>
<tr>
<td>V (ml/min)</td>
<td></td>
<td>(14)</td>
<td>0.4 ± 0.1</td>
<td>0.27 ± 0.06</td>
<td>0.55 ± 0.1</td>
</tr>
<tr>
<td>K-GFR (ml/min)</td>
<td></td>
<td>(14)</td>
<td>29.0 ± 1.7</td>
<td>23.1 ± 1.6</td>
<td>28.0 ± 2.0</td>
</tr>
<tr>
<td>FF</td>
<td></td>
<td>(14)</td>
<td>0.30 ± 0.02</td>
<td>0.22 ± 0.02</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>FEK (%)</td>
<td></td>
<td>(8)</td>
<td>1.33 ± 0.33</td>
<td>0.70 ± 0.18</td>
<td>1.31 ± 0.22</td>
</tr>
<tr>
<td>E_{PAH} (%)</td>
<td></td>
<td>(8)</td>
<td>27.2 ± 2.9</td>
<td>25.0 ± 3.4</td>
<td>38.5 ± 3.9</td>
</tr>
<tr>
<td>SNGFR (nl/min)</td>
<td></td>
<td>(10)</td>
<td>76.6 ± 4.1</td>
<td>67.4 ± 2.1</td>
<td>79.6 ± 2.3</td>
</tr>
<tr>
<td>K-GFR</td>
<td></td>
<td>(10)</td>
<td>68.6 ± 5.3</td>
<td>39.9 ± 4.9</td>
<td>68.3 ± 2.1</td>
</tr>
<tr>
<td>P_{T} (mm Hg)</td>
<td></td>
<td>(7)</td>
<td>2.42 ± 0.23</td>
<td>1.70 ± 0.28</td>
<td>2.63 ± 0.21</td>
</tr>
<tr>
<td>P_{E} (nl/ml)</td>
<td></td>
<td>(7)</td>
<td>16.3 ± 1.0</td>
<td>16.3 ± 1.0</td>
<td>26.0 ± 0.7</td>
</tr>
<tr>
<td>SFP (mm Hg)</td>
<td></td>
<td>(7)</td>
<td>23.0 ± 0.7</td>
<td>10.4 ± 0.9</td>
<td>15.0 ± 0.7</td>
</tr>
<tr>
<td>SNGFR (nl/min)</td>
<td></td>
<td>(7)</td>
<td>43.6 ± 1.2</td>
<td>31.1 ± 1.8</td>
<td>47.0 ± 2.1</td>
</tr>
</tbody>
</table>

The micropuncture data in each period represent the mean of one to four observations in each dog. Numbers in parentheses indicate the number of dogs. Abbreviations: MAP = mean arterial blood pressure; RPF = renal plasma flow from flowmeter reading and hematocrit; V = urine volume; K-GFR = kidney glomerular filtration rate; FF = filtration fraction; FE = fractional excretion; E_{PAH} = percent extraction of p-aminophenol; SNGFR = single nephron filtration rate; P_{T} and P_{E} = hydrostatic pressures in the proximal tubule and efferent arterioles, SFP = stop flow pressure.
during adenosine infusion or by 45 ± 7% (P < 0.001; n = 10). The corresponding GFR decreased 16 ± 4% (n = 10). The ratio of SNGFR to GFR decreased from 2.4 ± 0.23 to 1.7 ± 0.28, indicating that adenosine preferentially constricts vessels in the superficial cortex (Fig. 2). PAH extraction decreased from 76.6 ± 1.4% to 67.4 ± 2.1% (n = 6; P < 0.05).

Free flow and stop flow pressures measured in proximal tubules were reduced from 23.0 ± 0.7 to 16.3 ± 1.0 mm Hg and from 43.6 ± 1.1 to 31.1 ± 1.8 mm Hg, respectively.

The hydrostatic pressure in the efferent arterioles decreased from 13.9 ± 0.6 to 10.4 ± 0.9 mm Hg (n = 7, P < 0.01). During the recovery periods, the pressures in proximal tubules and efferent arterioles were slightly increased as compared with the control periods (see Table 1). As shown in Figure 3 and Table 2, the resistance of the afferent arterioles was markedly increased from 1.4 to 3.0 units (P < 0.003), whereas the efferent resistances were 1.4 and 1.8 units before and during adenosine, respectively. There was no significant change in Kf following adenosine infusion (Table 2).

Discussion

These experiments indicate that the decrease in GFR during adenosine infusion is not due to a decrease in Kf but, rather, is due to a decrease in glomerular capillary pressure. Tagawa and Vander concluded from clearance experiments in dogs that adenosine constricts afferent arterioles and dilates efferent arterioles. Our results support the effect of adenosine to constrict afferent arterioles, at least in the superficial cortex. If the decrease in glomerular capillary pressure, as indicated by the stop flow pressure, were also the result of a decrease in efferent arteriole resistance, an increase in hydrostatic pressures at the end of the efferent arterioles would be expected. However, the efferent arteriolar pressure decreased from 13.9 to 10.4 mm Hg during adenosine infusion. Calculation of the segmental resistances, as shown in Table 2, indicates that...
superficial efferent arterioles slightly constricted rather than dilated during adenosine infusion.

Since the glomerular plasma flow of superficial nephrons decreased by 38% and the total renal plasma flow was not changed, the plasma flow to the deep cortex must have been increased following adenosine infusion. Further, the decreases in the SNGFR and the PAH extraction ratios also indicate a redistribution of blood flow from the superficial to the deep cortex. Thus, our results are consistent with the thesis that the unchanged whole kidney resistance is a consequence of superficial vasoconstriction and deep cortical vasodilation. This conclusion is supported by a study reported in a preliminary communication during which microspheres were used to show a redistribution of blood flow to the deep cortex in the dog kidney following adenosine infusion.\(^4\)

Since the renal vasoconstrictor response to adenosine is associated with renal renin concentrations,\(^6\),\(^10\),\(^16\) the higher sensitivity of the outer cortex to adenosine may be related to the higher renin content of superficial glomeruli compared to that of juxtamedullary glomeruli.\(^17\)-\(^19\) Recent evidence indicates that prostaglandins mediate the increased renal blood flow in the deep cortex during adenosine infusion. Pretreatment of cats with meclofenamate or indomethacin enhanced the adenosine-induced vasoconstriction, and the characteristic return of RBF to control levels during continuous infusion of adenosine was abolished.\(^20\)

The physiological significance of the intrarenal action of adenosine may relate to the metabolic regulation of renal blood flow. Since adenosine is present in the normal as well as in the hypoxic kidney,\(^21\) it has the potential to play a role in the regulation of blood flow and GFR. Increased ATP consumption associated with increased tubular reabsorption of electrolytes could lead to an increased formation of adenosine. The subsequent role of adenosine as a transmitter in the tubuloglomerular feedback is discussed in detail elsewhere.\(^22\) Briefly, changes in adenosine concentration in the tissue adjacent to the afferent vessels (i.e., macula densa) could mediate changes in the resistance of the afferent arterioles.

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