Renal Interstitial ATP Responses to Changes in Arterial Pressure During Alterations in Tubuloglomerular Feedback Activity

Akira Nishiyama, Dewan S.A. Majid, Matthew Walker III, Akira Miyatake, L. Gabriel Navar

Abstract—We recently demonstrated a direct relationship between autoregulation-related changes in renal vascular resistance (RVR) and renal interstitial ATP concentrations. To assess the possible role for extracellular ATP in the regulation of tubuloglomerular feedback (TGF)-mediated autoregulatory adjustments in RVR, renal interstitial ATP concentrations were measured with microdialysis probes in anesthetized dogs at different renal arterial pressures (RAPs) within the autoregulatory range during augmented and diminished activity of the TGF mechanism. Stepwise reductions in RAP from ambient pressure (129±3 mm Hg) to 102±2 mm Hg (step 1) and 75±1 mm Hg (step 2) resulted in significant decreases in ATP concentrations from 9.0±0.8 to 6.3±0.6 nmol/L in step 1 and to 4.2±0.5 nmol/L in step 2. Changes in RVR were highly correlated with changes in ATP concentrations (r=0.86, P<0.001, n=12). Acetazolamide (100 μg · kg⁻¹ · min⁻¹, n=6), which increases solute delivery to the macula densa, thus augmenting TGF activity, significantly decreased renal blood flow (RBF) by −16±2% and glomerular filtration rate (GFR) by −22±4% and increased ATP concentrations from 8.4±0.7 to 15.5±1.4 nmol/L. Although basal RBF and GFR levels were reduced by the acetazolamide infusion, autoregulation efficiency was maintained, and interstitial ATP concentrations were significantly decreased in response to reductions in RAP by −36±4% in step 1 and by −54±2% in step 2. The relationship between changes in RVR and interstitial ATP concentrations was preserved during acetazolamide treatment (r=0.80, P<0.01). Inhibition of the TGF mechanism by furosemide significantly increased RBF by 33±6% and GFR by 13±2% and decreased ATP concentrations from 8.9±1.4 to 5.0±0.8 nmol/L (n=6). Furosemide caused marked impairment of RBF and GFR autoregulatory efficiency (by −14±3% and −11±3% in step 1 and by −26±2% and −18±4% in step 2, respectively). In the furosemide-treated kidneys, interstitial ATP levels remained low and were not altered during reductions in RAP (4.7±0.7 nmol/L in step 1 and 4.7±0.8 nmol/L in step 2), and changes in RVR did not exhibit a correlation with changes in ATP concentrations (r=0.22, P=0.30). These data support the hypothesis that extracellular ATP contributes to autoregulatory adjustments in RVR that are mediated by changes in activity of the TGF mechanism. (Hypertension. 2001;37[part 2]:753-759.)

Key Words: adenosine ■ tubuloglomerular feedback ■ kidneys ■ acetazolamide ■ furosemide

The tubuloglomerular feedback (TGF) mechanism and the myogenic mechanism are the main mechanisms responsible for renal autoregulatory responses,1-3 which are caused by active adjustments of vascular smooth muscle tone, primarily in the afferent arterioles.1-5 The findings that the blockade of TGF activity results in significant impairment of renal autoregulation-mediated adjustments in renal blood flow (RBF)6-7 or afferent arteriolar diameter8-10,11 indicate that the normally observed high autoregulatory efficiency is dependent on the integrity of the TGF mechanism. TGF-mediated afferent arteriolar vasoconstriction occurs in response to increases in distal nephron NaCl or solute concentration at the level of the macula densa cells.1,3 The nature of the signaling mechanisms that elicit the TGF-mediated changes in afferent arteriolar tone has remained unresolved; however, the possible participation of extracellular ATP as an important mediator of this mechanism has received increased support.3,10-18 Although the macula densa cells have abundant mitochondria, they have reduced levels of Na⁺,K⁺-ATPase,12 making the macula densa cells good candidates for a source of extracellular ATP. Micropuncture and microperfusion experiments13 have demonstrated that stop-flow pressure feedback responses to increases in late proximal perfusion rate are markedly blunted during peritubular capillary infusion with saturating doses of ATP, suggesting that ATP modulates the macula densa-dependent TGF mechanism. Chan et al14 performed immunohistochemistry studies and found that the preglomerular renal vascula-
ture expresses abundant P2X receptors, whereas efferent arterioles appear to be devoid of such receptors. Studies with the blood-perfused juxtamedullary nephron preparation showed that P2-purinoceptor desensitization, receptor saturation, or blockade markedly attenuates autoregulatory adjustments in afferent arteriolar diameter after acute changes in renal perfusion pressure.15 Majid et al16 demonstrated that the ability of the renal vasculature to exhibit autoregulation-mediated changes in renal vascular resistance (RVR) in response to alterations in renal arterial pressure (RAP) is markedly attenuated during P2-purinoceptor saturation by intra-arterial infusions with high doses of ATP. Recently, Bell et al17 demonstrated that the macula densa cells have a maxi-Cl− channel that is permeable to ATP and also demonstrated that increases in macula densa NaCl concentrations result in the release of ATP from macula densa cells.

We recently demonstrated a direct relationship between autoregulation-related changes in RVR and changes in renal interstitial ATP levels during stepwise reductions in RAP within the autoregulatory range.11 These results support the hypothesis that autoregulation-dependent changes in RVR are mediated by corresponding changes in interstitial ATP concentrations. The aim of the present study was to examine further the relationship between renal interstitial ATP and RVR in response to changes in RAP under conditions of enhanced or attenuated TGF activity. Specially, we hypothesized that during pressure changes, autoregulation-mediated adjustments in RVR and interstitial ATP levels are closely associated to TGF activity. To test this hypothesis, renal interstitial ATP concentrations were measured using an in vivo microdialysis method11,19,20 at different RAP within the autoregulatory range under conditions of enhanced activity of the TGF mechanism elicited pharmacologically with the carbonic anhydrase inhibitor acetazolamide, which inhibits net proximal tubular reabsorption rate and thus increases NaCl and solute delivery to the macula densa cells.21–23 Further studies were also performed during inhibition of the TGF response by furosemide.4,5,7–9 Because adenosine has also been suggested as a potential mediator of the TGF mechanism24–28 and ATP can be metabolized to adenosine,3,10,18,29 we also evaluated the changes in renal interstitial concentrations of adenosine.

Methods

Renal Microdialysis Technique

For the determination of renal interstitial concentrations of ATP and adenosine, we used a microdialysis probe (Toyobo Co Ltd) as previously reported.11,19,20 The microdialysis probes were implanted into the renal cortex and were perfused with Ringers’ solution (pH 7.4) at a rate of 3 μL/min. The average in vivo equilibrium rates of ATP and adenosine were 43±3% and 40±2%, respectively.11 The dialysate samples were directly collected from outflow steel tubing of 2 microdialysis probes and were stored at −70°C before analysis. At the end of each experiment, the kidney was removed, and the location of the microdialysis membrane was confirmed through surgical exposure of the probe.

Animal Preparation

Experiments were carried out on mongrel dogs weighing from 17 to 23 kg. The animals were anesthetized with pentobarbital sodium (30 mg/kg IV) and administered additional doses as required. The surgical preparation of the animals and basic experimental techniques are identical to those previously described.11,16,30

Experimental Protocol

At least 90 minutes before the start of the experimental protocol, the left common carotid artery was partially constricted to elevate the basal level of RAP to ∼130 mm Hg. This allowed examination of the pressure-flow relationship over a wider range of arterial pressure.11,16,30 The experimental protocol was started with renal interstitial fluid and urine collections for 2 consecutive 10-minute periods at spontaneous RAP (n=12). With an adjustable renal arterial clamp, RAP was reduced within the renal autoregulatory range to ∼100 mm Hg (step 1) and ∼75 mm Hg (step 2). At each level of RAP, 5 minutes were allowed for stabilization before a 10-minute sampling period. At 60 minutes after release of the renal arterial clamp, renal interstitial fluid and urine collections were performed at spontaneous RAP for 2 consecutive 10-minute periods. Next, acetazolamide (Sigma Chemical Co) was infused intra-arterially at a rate of 100 μg·kg⁻¹·min⁻¹ in 6 dogs. After a 5-minute initiation of acetazolamide infusion, 3 consecutive 5-minute dialysate and urine samples were collected. Subsequently, RAP was reduced to ∼100 mm Hg (step 1) and ∼75 mm Hg (step 2) during acetazolamide infusion. At each level of RAP, 5 minutes was allowed for stabilization before two 5-minute dialysate and urine sampling periods were made. In the other 6 dogs, furosemide (Sigma Chemical Co), instead of acetazolamide, was infused intra-arterially at a rate of 10 μg·kg⁻¹·min⁻¹. The experimental protocols and sample collections in this study were identical to those described earlier. To minimize acetazolamide- and furosemide-induced body fluid loss, urine losses were replaced quantitatively with warm (37°C) isotonic saline that contained 6 mmol/L KCl and was infused intravenously, with the rate adjusted every 2 minutes.

Analytical Procedures

ATP and adenosine concentrations were determined with the luciferin-luciferase assay and HPLC-fluorometric analysis, respectively, as previously reported.11,20 Inulin, sodium, and potassium concentrations in urine and plasma were measured as previously reported.11,16,30

Statistical Analysis

The values are presented as mean±SEM. Statistical comparisons of the differences were performed with 1- or 2-way ANOVA for repeated measures combined with Newman-Keuls post hoc test. Correlation of the responses was made with Spearman’s test. P<0.05 was considered statistically significant.

Results

Changes in Renal Interstitial Concentrations of ATP and Adenosine in Response to Reductions in RAP During Augmented Activity of the TGF Mechanism

Table 1 summarizes the changes in renal hemodynamics, urine flow, and sodium excretion during stepwise reductions in RAP before and during acetazolamide infusion (n=6). Before acetazolamide infusion, RBF and glomerular filtration rate (GFR) did not change significantly in response to reductions in RAP within this pressure range, demonstrating high autoregulatory efficiency. Urine flow, urinary excretion of sodium, and fractional excretion of sodium were significantly decreased in response to reductions in RAP, which reflect the well-established phenomenon of pressure natriuresis.11,30 Acetazolamide (100 μg·kg⁻¹·min⁻¹) infusion did not cause any significant change in mean arterial pressure (MAP) and RAP. However, acetazolamide infusion for 20 minutes significantly decreased RBF and GFR (P<0.05,
TABLE 1. Renal Responses to Alterations in RAP Before and During Treatment With Acetazolamide

<table>
<thead>
<tr>
<th>Levels of RAP, mm Hg</th>
<th>Control (Spontaneous) (128±6)</th>
<th>First Step Reduction (103±2)</th>
<th>Second Step Reduction (76±2)</th>
<th>Control (Both Spontaneous)</th>
<th>Acetazolamide</th>
<th>First Step Reduction (102±6)</th>
<th>Second Step Reduction (76±2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>136±4</td>
<td>135±4</td>
<td>134±4</td>
<td>135±4</td>
<td>134±4</td>
<td>134±4</td>
<td>135±4</td>
</tr>
<tr>
<td>RBF, mL·min⁻¹·g⁻¹</td>
<td>4.59±0.15</td>
<td>4.60±0.18</td>
<td>4.74±0.17</td>
<td>4.65±0.19</td>
<td>3.90±0.15</td>
<td>3.81±0.15</td>
<td>3.88±0.20</td>
</tr>
<tr>
<td>GFR, mL·min⁻¹·g⁻¹</td>
<td>0.86±0.06</td>
<td>0.88±0.07</td>
<td>0.85±0.04</td>
<td>0.87±0.06</td>
<td>0.67±0.03</td>
<td>0.63±0.03</td>
<td>0.67±0.06</td>
</tr>
<tr>
<td>Urine flow, μL·min⁻¹·g⁻¹</td>
<td>10.3±1.2</td>
<td>8.8±0.7*</td>
<td>5.4±0.8*</td>
<td>10.7±1.9</td>
<td>36.2±3.7</td>
<td>26.7±2.0†</td>
<td>18.3±1.7†</td>
</tr>
<tr>
<td>U_{NaV}, μmol·min⁻¹·g⁻¹</td>
<td>1.13±0.09</td>
<td>0.98±0.06*</td>
<td>0.61±0.10*</td>
<td>1.15±0.05</td>
<td>4.81±0.99</td>
<td>3.46±0.69†</td>
<td>2.56±0.55†</td>
</tr>
<tr>
<td>F_{E_{Na}}, %</td>
<td>1.08±0.10</td>
<td>0.89±0.08</td>
<td>0.62±0.07</td>
<td>1.16±0.11</td>
<td>5.98±0.98</td>
<td>4.36±0.77†</td>
<td>3.05±0.54†</td>
</tr>
</tbody>
</table>

F_{E_{Na}} indicates fractional excretion of sodium; U_{NaV}, urinary excretion of sodium. Values are mean±SEM, n=6.  
*P<0.05 vs control values at respective spontaneous RAP.  
†P<0.05 vs acetazolamide.

At these lower levels for RBF and GFR elicited by acetazolamide, equally high efficiency autoregulatory capability was observed in response to reductions in RAP. As expected, urine flow, urinary excretion of sodium, and fractional excretion of sodium were significantly increased by acetazolamide infusion. These parameters were significantly decreased in step 1 and step 2 (P<0.05, respectively) during acetazolamide infusion, demonstrating the maintenance of pressure natriuresis.

Figure 1A illustrates the changes in RVR in response to reductions in RAP before and during acetazolamide infusion (n=6). Control RVR averaged 28.4±1.9 mm Hg·mL⁻¹·min⁻¹ and decreased significantly by −20±3% in step 1 and −41±2% in step 2 (P<0.05, respectively) before acetazolamide infusion. Acetazolamide infusion for 20 minutes significantly increased RVR from 28.0±2.1 to 33.1±2.5 mm Hg·mL⁻¹·min⁻¹·g⁻¹ (P<0.05). During acetazolamide infusion, RVR decreased significantly in response to reductions in RAP by −19±3% to 26.4±1.6 mm Hg·mL⁻¹·min⁻¹·g⁻¹ in step 1 and by −40±2% to 19.9±1.2 mm Hg·mL⁻¹·min⁻¹·g⁻¹ in step 2 (P<0.05, respectively; Figure 1A). The pressure-induced reductions in RVR in the presence of acetazolamide infusion were not significantly different from the changes in RVR observed before acetazolamide infusion (by −20±3% in step 1 and by −41±2% in step 2, respectively). As reported previously, reductions in RAP significantly decreased renal interstitial concentrations of ATP from 9.0±1.0 to 6.2±0.8 nmol/L in step 1 and to 4.2±0.8 nmol/L in step 2 (P<0.05, respectively) under control conditions (Figure 1B). Within this pressure range, the percent changes in ATP concentrations were highly correlated with the percent changes in RAP (r=0.77, P<0.05) and RVR (r=0.80, P<0.01) (Figure 1C).

Acetazolamide significantly increased renal interstitial concentrations of ATP from 8.4±0.7 to 15.5±1.4 nmol/L. During acetazolamide infusion, ATP levels were significantly decreased during stepwise reductions in RAP by −36±4% to 9.5±0.8 nmol/L in step 1 and by −54±2% to 7.2±0.7 nmol/L in step 2 (P<0.05, respectively). The pressure-induced reductions in renal interstitial ATP concentrations during acetazolamide infusion were similar to those observed before acetazolamide administration (by −31±2% in step 1 and by −55±4% in step 2). Furthermore, the percent changes in ATP concentrations were highly correlated with the percent changes in RVR during acetazolamide infusion (r=0.71, P<0.01). As shown in Figure 1C, the slopes of the regression equations for both conditions were very similar (0.69 versus
Changes in Renal Interstitial Concentrations of ATP and Adenosine in Response to Reductions in RAP During Diminished Activity of the TGF Mechanism

Table 2 summarizes the changes in renal hemodynamics, urine flow, and sodium excretion during stepwise reductions in RAP before and during furosemide infusion (n=6). Before furosemide infusion, RBF and GFR did not change significantly in response to reductions in RAP within this pressure range, and urine flow, urinary excretion of sodium, and fractional excretion of sodium were significantly decreased during reductions in RAP, as described previously. Furosemide (10 μg·kg⁻¹·min⁻¹) did not cause any significant change in MAP and RAP but significantly increased RBF and GFR (P<0.05, respectively). During furosemide infusion, there was marked impairment of autoregulatory efficiency. RBF and GFR significantly decreased in response to reductions in RAP (by −14±3% and −11±3% in step 1 and by −26±2% and −18±4% in step 2, P<0.05, respectively). Urine flow, urinary excretion of sodium, and fractional excretion of sodium were significantly increased by furosemide infusion (P<0.05, respectively). During furosemide infusion, urine flow, urinary excretion of sodium, and fractional excretion of sodium did not change in step 1, but these values were significantly decreased in step 2 (P<0.05, respectively).

<table>
<thead>
<tr>
<th>Table 2: Renal Responses to Alterations in RAP Before and During Treatment With Furosemide</th>
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<tr>
<td>Levels of RAP, mm Hg</td>
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<td>----------------------</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
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<tr>
<td>RBF, mL·min⁻¹·g⁻¹</td>
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<tr>
<td>GFR, mL·min⁻¹·g⁻¹</td>
</tr>
<tr>
<td>Urine flow, μL·min⁻¹·g⁻¹</td>
</tr>
<tr>
<td>UNaV, μmol·min⁻¹·g⁻¹</td>
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<tr>
<td>FEna,%</td>
</tr>
</tbody>
</table>

FEna indicates fractional excretion of sodium; UNaV, urinary excretion of sodium. Values are mean±SEM, n=6.

*P<0.05 vs control values at respective spontaneous RAP.
†P<0.05 vs furosemide.

0.66). Control average dialysate adenosine concentration was 114±14 nmol/L, which was not altered significantly in response to changes in RAP (113±14 nmol/L in step 1 and 109±14 nmol/L in step 2). In addition, the percent changes in adenosine concentrations did not exhibit any correlation with the percent changes in RAP (r=0.19, P=0.54) or RVR (r=0.03, P=0.76). Acetazolamide did not alter renal interstitial concentrations of adenosine (from 99±10 to 100±12 nmol/L). Furthermore, adenosine levels were not altered in response to reductions in RAP during acetazolamide infusion (93±12 nmol/L in step 1 and 94±12 nmol/L in step 2).
In agreement with previous data, we observed that renal interstitial concentrations of ATP decreased consistently in response to reductions in RAP before and during furosemide administration. Acetazolamide- or furosemide-induced changes in urine flow, urinary excretion of sodium and potassium, and fractional excretion of sodium also remained stable during the observation period. Furthermore, acetazolamide or furosemide infusion for 50 minutes did not significantly alter MAP, RAP, plasma concentrations of sodium and potassium, and renal interstitial concentrations of adenosine.

**Discussion**

In agreement with previous data, we observed that renal interstitial concentrations of ATP decreased consistently in response to reductions in RAP. Furthermore, the changes in ATP concentrations are positively correlated with the autoregulation-associated alterations in RVR. The association between the autoregulatory adjustments in RVR and renal interstitial ATP concentrations are consistent with the hypothesis that autoregulation-dependent changes in RVR are closely associated with alterations in interstitial ATP concentration. To examine further the possible role for extracellular ATP in the regulation of TGF-mediated autoregulatory adjustments in RVR during alterations in RAP, pressure-induced changes in renal interstitial ATP concentrations were assessed under conditions of enhanced activity of the TGF mechanism elicited pharmacologically by acetazolamide and during inhibition of the TGF response by furosemide. We recently investigated the oscillatory patterns in RBF of anesthetized dogs and confirmed that the TGF signal operates at 0.028 to 0.033 Hz, is significantly enhanced by intra-arterial infusion of acetazolamide, and is completely eliminated by the administration of furosemide.

We observed that during acetazolamide infusion, RBF and GFR were decreased but autoregulated at the lower plateau in response to reductions in RAP; thus, the autoregulatory adjustments in RVR were fully preserved. These results are consistent with recent studies by Ichihara and Navar demonstrating that the TGF-mediated autoregulatory efficiency of afferent arteriolar diameter responses to alterations in renal perfusion pressure is maintained in acetazolamide-treated kidneys. As previously reported, acetazolamide increased renal interstitial concentrations of ATP at control arterial pressures. During acetazolamide infusion, the RAP-induced changes in renal interstitial concentrations of ATP were maintained, although at a different level, and were positively correlated with the changes in RVR. In addition, the slopes of the correlations between changes in ATP levels and RVR were similar before and during acetazolamide treatment. These results indicate that increased activity of the TGF mechanism does not alter the pattern of the relationship between the autoregulation-induced adjustments in RVR and the renal interstitial ATP concentrations. In contrast, inhibition of the TGF response with furosemide resulted in significant decreases in renal interstitial concentrations of ATP. Furthermore, furosemide elicited a marked impairment of RBF and GFR autoregulatory efficiency as well as the autoregulation-associated alterations in RVR, as previously reported by other investigators. The key finding is that pressure-induced changes in renal interstitial ATP levels were not observed in furosemide-treated kidneys. In addition, the association between the autoregulatory adjustments in RVR and renal interstitial ATP concentrations was not observed during furosemide infusion. Thus, these results support a prediction of the hypothesis that during changes in RAP, autoregulation-mediated adjustments in RVR due to changes in activity of the TGF mechanism are elicited by the corresponding changes in interstitial ATP concentrations.

Although the present results demonstrate that renal interstitial concentrations of ATP change in response to alterations in TGF activity, the exact sources of renal interstitial fluid ATP have remained uncertain. It has been shown that renal epithelial cells, vascular smooth muscle cells, and endothelial cells release ATP into the surrounding pericellular fluid. Furthermore, flow-induced shear stress on vessel walls can stimulate ATP release from mesenteric and aortic endothelial cells. The present experiment demonstrates that interstitial fluid ATP levels are highly responsive to alterations in the activity of the TGF mechanism caused by...
changes in RAP as well as by treatment with acetazolamide or furosemide. In addition, preliminary reports by Bell et al. indicate that ATP is released from macula densa cells into the pericellular fluid. These observations suggest the possibility that at least part of the released extracellular ATP comes from macula densa cells. It should be recognized, however, that changes in interstitial concentrations of ATP are the result of release, removal, and metabolism and are not solely reflected by the changes in ATP release. The activity of ATPases on cell membranes has been reported to be quite high, and extracellular ATP could be constantly being degraded, thus indicating that the interstitial ATP concentrations represent residual levels of those present at their direct effector sites.

Several micropuncture studies demonstrating that local administration of high doses of adenosine receptor antagonists reduce the magnitude of TGF-mediated reductions in RAP within the autoregulatory range. In the present study, we observed that renal interstitial adenosine levels were not altered in response to reductions in RAP within the autoregulatory range. In the present study, we observed that renal interstitial adenosine levels were not altered in response to reductions in RAP within the autoregulatory range. In the present study, we observed that renal interstitial adenosine levels were not altered in response to reductions in RAP within the autoregulatory range. In the present study, we observed that renal interstitial adenosine levels were not altered in response to reductions in RAP within the autoregulatory range. In the present study, we observed that renal interstitial adenosine levels were not altered in response to reductions in RAP within the autoregulatory range.

In conclusion, the present study demonstrates a positive correlation between the autoregulation-induced adjustments in RVR and renal interstitial ATP concentrations during control conditions and when intensity of the TGF-dependent signals is augmented. Furthermore, blockade of TGF-dependent actions on RVR resulted in loss of this relationship. These data are consistent with the hypothesis that autoregulation-dependent adjustments in RVR that are elicited by alterations in activity of the TGF mechanism are mediated by the corresponding changes in interstitial ATP concentrations.

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References


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