Relation Between Renal Interstitial ATP Concentrations and Autoregulation-Mediated Changes in Renal Vascular Resistance

Akira Nishiyama, Dewan S.A. Majid, Khandaker A. Taher, Akira Miyatake, L. Gabriel Navar

Abstract—The present study was performed to examine the hypothesis that autoregulation-related changes in renal vascular resistance (RVR) are mediated by extracellular ATP. By use of a microdialysis method, renal interstitial concentrations of ATP and adenosine were measured at different renal arterial pressures (RAPs) within the autoregulatory range in anesthetized dogs (n=12). RAP was reduced in steps from the ambient pressure (131±4 mm Hg) to 105±3 mm Hg (step 1) and 80±2 mm Hg (step 2). Renal blood flow and glomerular filtration rate exhibited efficient autoregulation in response to these changes in RAP. RVR decreased by 22±2% in step 1 (P<0.01) and 38±3% in step 2 (P<0.01). The control renal interstitial concentration of ATP was 6.51±0.71 nmol/L and decreased to 4.51±0.55 nmol/L in step 1 (P<0.01) and 2.77±0.47 nmol/L in step 2 (P<0.01). In contrast, the adenosine concentrations (117±6 nmol/L) were not altered significantly. Changes in ATP levels were highly correlated with changes in RVR (r=0.88, P<0.0001). Further studies demonstrated that stimulation of the tubuloglomerular feedback (TGF) mechanism by increasing distal volume delivery elicited with acetazolamide also led to increases in renal interstitial ATP concentrations, whereas furosemide, which is known to block TGF responses, reduced renal interstitial fluid ATP concentrations. The data demonstrate a positive relation between renal interstitial fluid ATP concentrations and both autoregulation- and TGF-dependent changes in RVR and thus support the hypothesis that changes in extracellular ATP contribute to the RVR adjustments responsible for the mechanism of renal autoregulation. (Circ Res. 2000;86:656-662.)

Key Words: ATP ■ renal autoregulation ■ tubuloglomerular feedback ■ renal interstitium ■ adenosine

The purine nucleotide ATP, an intracellular energy source, is gaining recognition for its paracrine role in regulating skeletal and heart muscle contractility as well as vascular tone in several tissues. ATP has been shown to be released from endothelial cells, epithelial cells, smooth muscle cells, myocardium, and perivascular nerves. Extracellular ATP exerts a substantial influence on hemodynamic function, acting via P2 purinoceptors, on a variety of tissues and organs, including the kidney. A growing body of evidence obtained in both dogs and rats supports the hypothesis that extracellular ATP exerts a role in mediating renal autoregulatory vascular resistance responses, which are caused by active adjustments of vascular smooth muscle tone, primarily in the afferent arterioles.

Studies using the isolated blood-perfused juxtaglomerular nephron preparation demonstrated that ATP, superfused over the renal microvessels, exerts selective afferent arteriolar vasoconstriction without affecting efferent arteriolar tone, which is an important criterion for the agent mediating autoregulatory behavior. This occurrence is due to the selective localization of P2 purinoceptors, which have been clearly identified on afferent but not on efferent arteriolar smooth muscle cells. Further studies showed that P2 purinoceptor desensitization, receptor saturation, or blockade markedly attenuated autoregulatory adjustments in afferent arteriolar diameter after acute elevations in renal perfusion pressure. Recent studies performed in anesthetized dogs 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) was markedly attenuated during P2 purinoceptor saturation by intra-arterial infusions with high doses of ATP.

It has been reported that whereas the macula densa cells have abundant mitochondria, they have reduced levels of Na⁺,K⁺-ATPase, making the macula densa cells good candidates for a source of extracellular ATP. Furthermore, micropuncture and microperfusion experiments in rats have demonstrated that stop-flow pressure-feedback responses to increases in late proximal perfusion rate were markedly blunted during peritubular capillary infusion with saturating doses of ATP, suggesting that ATP modulates the macula...
densa-dependent tubuloglomerular feedback (TGF) mechanism, which contributes to renal autoregulation. On the basis of the accumulated evidence, we have hypothesized that in response to increases in RAP, ATP is secreted from the macula densa or adjoining cells into the interstitial fluid bathing the vascular pole and causes autoregulatory adjustments in the preglomerular arterial resistance. One prediction of this hypothesis is that changes in interstitial ATP concentration during changes in RAP should exhibit a relation with the autoregulation-related changes in RVR. The present experiments used an in vivo renal microdialysis method to measure renal interstitial concentrations of ATP and the changes occurring in response to changes in RAP. Experiments were also conducted to investigate the changes in renal interstitial ATP levels under conditions of enhanced activity of the TGF mechanism elicited with acetazolamide as well as during inhibition of the TGF response by furosemide. Because adenosine has also been suggested as a potential mediator of the TGF mechanism and ATP can be metabolized to adenosine, we also evaluated the changes in renal interstitial concentrations of adenosine in response to changes in RAP.

Materials and Methods
Experiments were carried out on mongrel dogs weighing 16 to 21 kg that had been maintained on standard laboratory chow. The animals were anesthetized with pentobarbital sodium (30 mg/kg IV) and given additional doses as required. The surgical preparation of the animals and basic experimental techniques are identical to those previously described.

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. The microdialysis probes were implanted into the renal cortex and were perfused with Ringer’s solution (pH 7.4) at a rate of 3 μL/min. The dialysates were directly collected from outflow steel tubing, and samples 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 by surgical exposure of the probe.

Characteristics of the Microdialysis Probe In Vivo
An in vitro gradient dialysis technique and mathematical model were used to estimate the in vivo efficiency of the microdialysis probe in 7 dogs. Two or 3 probes were implanted into the renal cortex and were perfused with 3 or 4 different concentrations of ATP (0 to 20 nmol/L) or adenosine (0 to 600 nmol/L) at a perfusion rate of 3 μL/min. The dialysate fluid was collected during perfusion at each concentration (25 minutes each), and the ATP or adenosine concentrations in the dialysate and perfusate were determined.

Renal Interstitial ATP and Adenosine During Stepwise Reduction in RAP
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 to 140 mm Hg. This allowed examination of the pressure-flow relation over a wider range of arterial pressure. The experimental protocol was started with urine collections for 2 consecutive 10-minute periods at spontaneous RAP. With use of an adjustable renal arterial clamp, RAP was reduced within the renal autoregulatory range to ~100 mm Hg (step 1) and 80 mm Hg (step 2). The pressure at each step was held for 25 minutes, and renal interstitial fluid samples were collected for measurements of ATP concentrations. In 6 of these dogs, separate samples from the microdialysis probes were also collected to be used for measurements of adenosine concentration. Five minutes was allowed for stabilization at each level of RAP before 2 consecutive 10-minute urine samples were collected. In the other 6 of these dogs, 2 additional consecutive 10-minute collections were performed at 30 minutes after releasing the renal arterial clamp (recovery period).

Effects of Acetazolamide and Furosemide on Renal Interstitial ATP
In this experimental series, 5-minute samples from 2 microdialysis probes were collected throughout each period. After the control dialysate sample was collected, acetazolamide (Sigma Chemical Co) was infused intra-arterially at a rate of 100 μg · kg⁻¹ · min⁻¹ for 40 minutes. After 5 minutes of acetazolamide infusion, 3 consecutive 5-minute dialysate samples were collected. A continuous infusion of furosemide at a rate of 10 μg · kg⁻¹ · min⁻¹ (Sigma) was then added to the acetazolamide infusion. After 5 minutes of furosemide infusion, 3 additional consecutive 5-minute sample collections were also performed.

Analytical Procedures
ATP concentrations were determined by using the luciferin-luciferase assay based on the Sigma ATP Bioluminescent Assay Kit. Adenosine in the dialysate was measured by using HPLC-
fluorometric analysis as previously reported.31 Inulin, sodium, and potassium concentrations in urine and plasma were measured as previously reported.18,29

### Statistical Analysis

The values are presented as mean±SE. Statistical comparisons of the differences in the responses were performed by ANOVA, followed by the Newman-Keuls test. Correlation of the responses were made by the Spearman test. A value of P<0.05 was considered statistically significant.

### Results

#### Characteristics of the Microdialysis Probe In Vivo

Figure 1 shows the linear regression analysis of the in vivo gradients for ATP and adenosine dialysis. Perfusion of the probes with ATP (0 to 20 nmol/L) or adenosine (0 to 600 nmol/L) did not change renal blood flow (RBF) and glomerular filtration rate (GFR). The relations between the net loss or gain of ATP or adenosine in the collected dialysates and the concentrations of these compounds in the perfusates were determined in each probe. The concentration at which there is no net flux of the agent across the microdialysis probe membrane represents renal interstitial concentrations, which can be derived from the x-intercept on linear regression analysis.32–34 The estimated mean equilibrium concentrations of ATP and adenosine were 4.60±0.47 nmol/L (n=12) and 103±7 nmol/L (n=7), respectively. The equilibrium rates for ATP and adenosine were calculated for each probe by dividing the concentration in the dialysate perfused with zero concentration of these agents by the estimated equilibrium concentrations. The average equilibrium rates of ATP and adenosine were 43.2±2.6% (n=12) and 40.3±2.1% (n=7), respectively.

#### Changes in Renal Interstitial Concentrations of ATP and Adenosine During Changes in RAP Within the Autoregulatory Range

The Table summarizes the changes in renal hemodynamics and function during stepwise reductions in RAP (n=12). RBF and GFR did not change significantly within this pressure range, demonstrating high autoregulatory efficiency. Urine flow, urinary excretion of sodium, fractional excretion of sodium, and urinary excretion of potassium were significantly decreased during reductions in RAP, which are consistent with the well-established phenomenon of pressure natriuresis.29

The basal average dialysate ATP concentration, which was measured at 90 minutes after the implantation of the microdialysis probes, was 2.81±0.37 nmol/L. Reduction in RAP significantly decreased ATP concentrations to 1.95±0.25 nmol/L in step 1 (P<0.01) and 1.20±0.22 nmol/L in step 2 (P<0.01) (Figure 2). When a probe equilibrium rate of 43.2% was taken into account, renal interstitial concentrations of ATP were estimated to be 6.51±0.71 nmol/L in control conditions and to be decreased to 4.51±0.55 nmol/L in step 1 and 2.77±0.47 nmol/L in step 2 (n=12). Control RVR averaged 33.1±2.6 mm Hg·mL⁻¹·min⁻¹·g⁻¹. RVR decreased significantly by 22±2% in step 1 (P<0.01) and 38±3% in step 2 (P<0.01) (Figure 2). When individual responses were considered, each experiment showed the same pattern between RAP and the dialysate ATP concentration and also between the ATP concentration and RVR. Figure 3 illustrates the relations between the percent changes in RAP and ATP (Figure 3A) and between ATP and RVR (Figure 3B) (n=12). The percent changes in ATP concentrations were highly correlated with the percent changes in RAP (r=0.80, P=0.0005) and in RVR (r=0.88, P=0.0001). After allowing RAP to return to ambient conditions, ATP concentrations returned to their respective control levels, with an average of 2.44±0.51 nmol/L (n=6), which was not significantly different from the average control value from these 6 experiments.

The control average dialysate adenosine concentration was 47±2 nmol/L (n=6, Figure 2). When a probe equilibrium rate of 40.3% (see above) was taken into account, renal interstitial concentrations of adenosine were estimated to be 117±6 nmol/L in control conditions. However, interstitial adenosine concentrations were not altered in response to changes in RAP within this autoregulatory pressure range. In addition, the percent changes in adenosine concentrations did not exhibit any correlation with RAP (r=0.07, P>0.999) or RVR (r=0.10, P=0.72) (Figure 4).
Time-control experiments were performed to determine the stability of the renal interstitial concentrations of ATP and adenosine (n=3). Dialysate sampling (30-minute duration) was started 90 minutes after the implantation of the microdialysis probes and was continued for 180 minutes. GFR, urinary excretion of sodium and potassium, and fractional excretion of sodium were also measured. At 180 minutes after the initiation of sampling, ATP and adenosine concentrations in the dialysate were 3.25±0.81 and 53±9 nmol/L, which were not significantly different from basal ATP and adenosine concentrations (3.12±0.84 and 49±11 nmol/L, respectively). During this period, RAP was not significantly changed (from 124±7 mm Hg in control to 123±6 mm Hg at 180 minutes). GFR, urinary excretion of sodium and potassium, and fractional excretion of sodium also did not change during this period (data not shown).

Effects of Acetazolamide and Furosemide on Renal Interstitial ATP

Acetazolamide infusion (100 μg · kg⁻¹ · min⁻¹) for 20 minutes did not cause any significant change in RAP (from 121±4 mm Hg in control and 122±4 mm Hg at 20 minutes). As expected, the sodium excretion rate increased from 1.14±0.06 to 6.39±0.71 μmol · L⁻¹ · min⁻¹ · g⁻¹ (P<0.01, n=5). RBF and GFR were significantly decreased from 3.63±0.15 and 0.89±0.06 mL · min⁻¹ · g⁻¹ to 3.12±0.21 and 0.77±0.03 mL · min⁻¹ · g⁻¹, respectively (P<0.01). Accordingly, RAP was significantly increased from 33.5±2.0 to 38.3±2.7 mm Hg · mL⁻¹ · min · g (P<0.01). Figure 5 illustrates the changes in ATP concentrations in dialysate. Acetazolamide significantly increased dialysate ATP levels from 3.16±0.42 nmol/L to a peak concentration of 8.00±1.78 nmol/L during the first sampling period (P<0.05). ATP concentrations waned slightly during the next sampling periods, as shown in Figure 5. After addition of furosemide (10 μg · kg⁻¹ · min⁻¹) to the intrarenal arterial infusion line, RBF and GFR were significantly increased to 3.52±0.32 and 1.17±0.12 mL · min⁻¹ · g⁻¹, respectively (P<0.05). Dialysate ATP concentrations were significantly decreased to 2.46±0.46 nmol/L during the first sampling period and slightly lower during the subsequent periods (P<0.05, Figure 5). Interstitial ATP concentrations during furosemide were significantly lower than those measured during the control period (P<0.05, Figure 5). In the other 3 dogs, acetazolamide (100 μg · kg⁻¹ · min⁻¹) was infused alone for 40 minutes to examine the possibility of any time-dependent changes in renal interstitial ATP levels. Acetazolamide significantly increased dialysate ATP levels from 2.87±0.52 to 6.21±1.89 nmol/L (5- to 10-minute sampling period, P<0.05), and these concentrations remained elevated for the duration of sampling up to 40 minutes (6.04±1.73 nmol/L).

Discussion

The present study has demonstrated that dialysate from the cortical renal interstitium contains detectable amounts of
ATP and that renal interstitial ATP concentrations are closely correlated with autoregulation-mediated changes in RVR. The renal interstitial concentration of ATP in dogs was estimated to be 6.51 ± 0.71 nmol/L. Although microdialysis studies have measured interstitial concentrations of ATP in the brain of the freshwater turtle35 and in rat heart, 36 to our knowledge, no previous investigation has reported quantitative assessments of renal interstitial ATP concentrations. We assessed renal interstitial ATP and adenosine concentrations to determine whether they were associated with autoregulation-dependent changes in RVR. Interestingly, we found that renal interstitial concentrations of ATP were decreased consistently in response to reductions in RAP, whereas the adenosine levels were not altered significantly over this pressure range. These changes in ATP concentration were positively correlated with the changes in RAP as well as the autoregulation-associated alterations in RVR. The association between the autoregulatory adjustments in RVR and renal interstitial ATP concentrations provides further support for the hypothesis that extracellular ATP contributes to the changes in RVR that occur during renal autoregulation.14–18

At present, 2 mechanisms are considered to be responsible for renal autoregulation: the TGF mechanism and the myogenic mechanism.14,15 Both mechanisms involve signals that impinge on afferent arteriolar resistance.14,15 The nature of the signaling mechanisms that mediate the afferent arteriolar vasoconstriction in response to increases in arterial pressure has remained an unresolved issue; however, the possible participation of extracellular ATP in this mechanism has received increased attention recently.14–18 Chan et al21 performed immunohistochemistry studies and found that the preglomerular renal vasculature expresses abundant P2X receptors, whereas efferent arterioles appear to be devoid of such receptors. It has also been demonstrated that P2 purinoceptors located in afferent arteriolar vascular smooth muscle cells act as Ca2+-permeable ion channels and thus contribute to activation of Ca2+ influx.37 Studies using the blood-perfused juxtamedullary nephron preparation have shown that selective afferent arteriolar responses to ATP occur with a response time that is compatible with normal autoregulatory responses,14,15,19,20 In addition, normal autoregulatory responses are significantly attenuated by P2 purinoceptor saturation16,18 or receptor desensitization16 and are impaired by blockade of P2X purinoceptors.16 These observations are consistent with the hypothesis that ATP mediates autoregulatory adjustments in RVR. The present results provide further support for this hypothesis by demonstrating that ATP concentrations in the interstitial fluid are closely associated with the autoregulatory adjustments in RVR that occur in response to changes in RAP.

Although the exact mechanism by which extracellular ATP regulates RVR remains to be identified, it is possible that signals originating as a consequence of alterations in RAP initiate a sequence of events that alters the rate of ATP production by the cells surrounding the vascular pole of the glomerulus.14,15 It has been reported that renal epithelial cells7 as well as vascular smooth muscle cells8 and endothelial cells6 release ATP into the surrounding pericellular fluid and that flow-induced shear stress on vessel walls stimulates ATP release from endothelial cells.8,39 Furthermore, macula densa cells are also proposed as a potential source of ATP during changes in RAP.14,15 The present experiments demonstrate that ATP is released into the interstitial fluid in response to increases in the activity of the TGF mechanism caused by either increases in RAP or treatment with acetazolamide.23,24

Figure 4. A, Relation between percent changes in RAP and percent changes in renal interstitial concentrations of adenosine (n=6). B, Relation between percent changes in renal interstitial concentrations of adenosine and percent changes in RVR (n=6). Data are expressed as percent change of the control values at spontaneous RAP.

Figure 5. Effects of acetazolamide and furosemide on renal interstitial ATP levels expressed as percent change (Δ) from control value (n=5). *P<0.05 vs basal values; #P<0.05 vs acetazolamide alone (period 3).
Furthermore, the ATP levels were decreased after infusion of furosemide, which is known to block the TGF response.\textsuperscript{25,26} Thus, the results of the present experiments support the hypothesis based on the results of previous studies\textsuperscript{14,15,17,22} indicating that ATP released from the macula densa cells, at least in part, caused the changes in interstitial concentrations of ATP during changes in RAP.

It should be recognized that the concentrations noted in the renal interstitial fluid in the present study may not reflect the actual effective concentrations of ATP at the vascular smooth muscle cells. Because extracellular ATP is rapidly hydrolyzed by membrane-bound ectoenzymes,\textsuperscript{5,14,15,40} the interstitial ATP concentrations would be expected to be lower than the actual concentration in the vascular site. It should also be noted that the dialysis membrane is located in the mid cortex and superficial cortex. Therefore, ATP levels in the collected dialysate sample reflect the mid and superficial cortical ATP concentrations. It is possible that there are differential responses of renal regional interstitial ATP to RAP. Further experiments are needed to assess the regional differences of ATP levels between superficial cortex, mid cortex, and medulla.

Studies using the isolated perfused guinea pig heart showed that flow-induced shear stress released ATP in the perfusate, which then caused coronary vasodilation, possibly via P2Y purinoceptors.\textsuperscript{3} In renal vessels, P2Y purinoceptors are located on endothelial cells and mediate large arcuate artery vasodilation through nitric oxide and prostacyclin release from vascular endothelium.\textsuperscript{5,11,14,15} Several studies have concluded that P2Y purinoceptors on endothelial cells are activated from the luminal side of the vasculature to cause vasodilation, whereas P2X-mediated constriction occurs predominantly from direct exposure of ATP from the adventitial side.\textsuperscript{5,14–20} Because ATP released from adjoining epithelial cells would approach the vascular smooth cells from the interstitium, their consequences would be consistent with renal vasoconstriction.\textsuperscript{5,14,15}

Adenosine has also been postulated as a candidate for mediating renal autoregulation and the TGF mechanism;\textsuperscript{27} however, this hypothesis remains controversial because of the conflicting reports of the ability of various adenosine receptor agonists and antagonists to influence renal autoregulatory responses.\textsuperscript{14,15,17,21,22} The present estimated concentration of renal interstitial adenosine in the dog cortex is \(\approx 100 \text{ nmol/L}\), which is similar to that observed in previous renal microdialysis studies of the rat \textsuperscript{22} and rabbit\textsuperscript{11} cortex. In the present experiments, it has been shown that interstitial adenosine levels are not altered in response to decreases in RAP within the autoregulatory range, whereas ATP concentrations are significantly decreased. It has also been shown that the renal microvasculature is essentially unresponsive to \(10^{-8} \text{ mol/L}\) of ADP\textsuperscript{20} and AMP,\textsuperscript{19} Although ATP can be metabolized to ADP, AMP, and adenosine,\textsuperscript{5,14,15,28,40} complete and immediate hydrolysis of ATP would still not yield sufficiently high levels of these substances to cause comparable vasoconstriction.

Although it is possible that the changes in renal interstitial ATP concentrations were simply the result of metabolic activity of nephrons, this seems unlikely because the changes in ATP concentrations did not exhibit a significant correlation with changes in sodium excretion rate \((r=0.35, P=0.236)\). In addition, we have found that furosemide, which inhibits sodium transport and markedly increases urinary sodium excretion, actually decreases ATP levels in the interstitium. These results indicate that renal interstitial concentrations of ATP may not reflect the metabolic activity of nephrons.

In conclusion, the present study demonstrated a positive correlation between the autoregulatory and TGF-mediated adjustments in RVR and renal interstitial ATP concentrations and supports the hypothesis that autoregulation-dependent changes in RVR are mediated by corresponding changes in interstitial ATP concentrations.

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