Renal interstitial fluid ATP responses to arterial pressure and tubuloglomerular feedback activation during calcium channel blockade

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Nishiyama, Akira, Keith E. Jackson, Dewan S. A. Majid, Matlubur Rahman, and L. Gabriel Navar. Renal interstitial fluid ATP responses to arterial pressure and tubuloglomerular feedback activation during calcium channel blockade. Am J Physiol Heart Circ Physiol 290: H772–H777, 2006.—A close relationship between changes in RIF ATP concentrations and renal autoregulatory or tubuloglomerular feedback (TGF)-dependent changes in renal vascular resistance (RVR) has been demonstrated, but it has not been determined whether the changes in RIF ATP are a consequence or the cause of the changes in RVR. The present study was performed in anesthetized dogs to assess the changes in RIF ATP following changes in renal arterial pressure (RAP) or stimulation of the TGF mechanism under conditions where changes in RVR were prevented by nifedipine, a calcium channel blocker. RIF ATP levels were measured by using microdialysis probes. Intra-arterial infusion of nifedipine (0.36 μg·kg⁻¹·min⁻¹) increased renal blood flow (RBF: from 4.49 ± 0.27 to 5.34 ± 0.39 ml·min⁻¹·g⁻¹) and glomerular filtration rate (GFR: from 0.84 ± 0.07 to 1.09 ± 0.11 ml·min⁻¹·g⁻¹). Under conditions of nifedipine infusion, autoregulatory adjustments in RBF, GFR, and RVR were not observed during stepwise reductions in RAP within the autoregulatory range (from 135 ± 7 to 76 ± 1 mmHg, n = 7). Furthermore, stimulation of the TGF mechanism with intra-arterial infusion of acetazolamide (100 μg·kg⁻¹·min⁻¹) did not alter RBF, GFR, and RVR (n = 7). During treatment with nifedipine, RIF ATP levels were significantly decreased in response to reductions in RAP (10.7 ± 0.7, 5.8 ± 0.7 and 2.8 ± 0.3 nmol/L at 135 ± 7, 101 ± 4, and 76 ± 1 mmHg, n = 7) and increased by acetazolamide infusion (from 8.8 ± 0.8 to 17.0 ± 1.8 nmol/L, n = 7). These results are similar to those that occurred in dogs not treated with nifedipine and thus demonstrate that the changes in RIF ATP can occur in the absence of autoregulatory or TGF-mediated changes in RVR. The data provide further support to the hypothesis that RIF ATP contributes to adjustments in RVR associated with renal autoregulation and changes in activity of the TGF mechanism.

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acetazolamide, whereas furosemide abolished this relationship (22). Collectively, these data demonstrate that RIF ATP concentrations change in association with the changes in RVR in a manner consistent with the hypothesis that RIF ATP mediates the autoregulatory and TGF-dependent changes in RVR. However, the sequence of events has not been delineated in these previous experiments. Because it has also been clearly shown that mechanical stimuli such as shear stress and stretch directly induce ATP release from various cells, including vascular smooth muscle cells (25), it is also possible that the changes in RIF ATP concentrations are the consequence of, rather than the cause of, the changes in RVR.

Accordingly, it seemed imperative to determine whether the changes in RIF ATP occur in response to the changes in RVR or can occur independently of the RVR changes. This study was performed to test the hypothesis that changes in RIF ATP concentrations following RAP changes or stimulation of the TGF mechanism occur even under conditions where the changes in RVR are prevented. To accomplish this objective, a calcium channel blocker nifedipine was used to inhibit the autoregulatory and TGF-dependent changes in RVR (18).

### MATERIALS AND METHODS

**Animal preparation.** Experiments were carried out on mongrel dogs weighing from 15 to 23 kg. 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 (16, 18, 21, 22). All experimental protocols were approved by the Tulane University and Kagawa University Medical School Animal Care and Use Committee.

**Renal microdialysis technique.** For the determination of RIF ATP concentrations, we used a microdialysis method (Toyobo, Otsu, Japan) as previously reported (9, 14, 20–22). The microdialysis probes were implanted into the renal cortex and were perfused with Ringer solution (pH = 7.4) at a rate of 3 μl/min. The average in vivo equilibrium rate of ATP was 43 ± 3% (21, 22). The dialysate samples were directly collected from outflow steel tubing of two 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 by surgical exposure of the probe.

**Experimental protocols.** At least 90 min before the start of the experimental protocol, the left common carotid artery was partially constricted to elevate the basal level of RAP to above 130 mmHg. This allowed examination of the pressure-flow relationship over a wide range of arterial pressures (16, 18). The experimental protocol was started with RIF and urine collections for two consecutive 10-min periods at spontaneous RAP (n = 14). Nifedipine (Sigma Chemical, St. Louis, MO) was then infused intra-arterially for 90 min at a rate of 0.36 μg·kg⁻¹·min⁻¹. After 5 min of initiation of nifedipine infusion, three consecutive 10-min dialysate and urine samples were collected. Subsequently, RAP was reduced by using an adjustable renal arterial clamp within the renal autoregulatory range to around 100 mmHg (step 1) and 75 mmHg (step 2) while continuing the nifedipine infusion in seven dogs. At each level of RAP, 5 min was allowed for stabilization before two 10-min sampling periods were made. In seven other dogs, a continuous infusion of acetazolamide at a rate of 100 μg·kg⁻¹·min⁻¹ was added to the nifedipine infusion. The experimental protocols and sample collections performed in this study were identical to those described above. In a separate experimental series, nifedipine (0.36 μg·kg⁻¹·min⁻¹) was infused for 90 min to examine the possibility of any time-dependent changes in renal hemodynamics and functions as well as RIF ATP levels (n = 5). Ten-minute dialysate and urine samples were collected at 15, 30, 60, and 90 min after initiation of nifedipine infusion. To minimize nifedipine- and acetazolamide-induced body fluid loss, urine losses were replaced quantitatively with warm (37°C) isotonic saline containing 6 mmol/l KCl infused intravenously, the rate of which was adjusted every 2 min (22). The doses of nifedipine and acetazolamide were determined based on previous studies in dogs (16, 18, 21, 22).

**Analytical procedures.** ATP concentrations were determined by using the luciferin-luciferase assay (21, 22). Inulin and sodium concentrations in urine and plasma were measured as previously reported (16, 18).

**Statistical analysis.** The values are presented as means ± SE. Statistical comparisons of the differences were performed using the one-way or the two-way analysis of variance for repeated measures combined with Newman-Keuls post hoc test. P < 0.05 was considered statistically significant.

### RESULTS

Changes in RIF ATP concentrations in response to reductions in RAP during treatment with a calcium channel blocker. Table 1 summarizes the changes in renal hemodynamics, urine flow, and sodium excretion in response to stepwise reductions in RAP during nifedipine infusion (n = 7). Nifedipine alone slightly decreased mean arterial pressure (MAP) and significantly increased RBF and GFR. Control RVR averaged 30.0 ± 0.8 mmHg·ml⁻¹·min⁻¹ and was decreased significantly by nifedipine infusion to 24.1 ± 0.9 mmHg·ml⁻¹·min⁻¹ at 30 min (Fig. 1A). During nifedipine infusion, reductions in RAP within the autoregulatory range led to significant decreases in RBF and GFR at each pressure step (Table 1). However, there were no changes in RVR following reductions in RAP (Fig. 1A), demonstrating an absence of autoregulation. Nifedipine alone significantly increased urine volume (UV) and urinary excretion of sodium (UNaV). During treatment with nifedipine, UV and UNaV were significantly decreased in response to reductions in RAP (Table 1).

<table>
<thead>
<tr>
<th>Levels of RAP, mmHg</th>
<th>Control (Spontaneous)</th>
<th>Nifedipine</th>
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<tbody>
<tr>
<td>10 min</td>
<td>20 min</td>
<td>30 min</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>138 ± 2</td>
<td>137 ± 6</td>
</tr>
<tr>
<td>RBF, ml·min⁻¹·g⁻¹</td>
<td>4.63 ± 0.17</td>
<td>5.68 ± 0.37*</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·g⁻¹</td>
<td>0.83 ± 0.06</td>
<td>1.26 ± 0.16*</td>
</tr>
<tr>
<td>UV, μl·min⁻¹·g⁻¹</td>
<td>8.4 ± 1.4</td>
<td>24.9 ± 7.3*</td>
</tr>
<tr>
<td>UNaV, mmol·min⁻¹·g⁻¹</td>
<td>1.25 ± 0.23</td>
<td>3.58 ± 0.57*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 dogs. RAP, renal arterial pressure; MAP, mean arterial pressure; RBF, renal blood flow; GFR, glomerular filtration rate; UV, urine volume; UNaV, urinary excretion of sodium. *P < 0.05 vs. control values at respective spontaneous RAP; †P < 0.05 vs. nifedipine (30 min).
azolamide on renal hemodynamics, urine flow, and sodium excretion during nifedipine infusion (n = 7). Nifedipine alone slightly decreased MAP and significantly increased RBF, GFR, UV, and UNaV as described before. Previously, we demonstrated that stimulation of the TGF mechanism by acetazolamide infusion did not alter MAP and RAP but significantly decreased RBF and GFR, and there were no significant changes in RBF and GFR. Nifedipine infusion alone for 30 min significantly decreased RVR from 30.6 ± 2.8 to 26.7 ± 2.4 mmHg·min⁻¹·g⁻¹. During nifedipine infusion, acetazolamide did not alter RVR (26.2 ± 2.2 mmHg·min⁻¹·g⁻¹ at 30 min, Fig. 2A), indicating that the TGF-mediated RVR changes were prevented by nifedipine. Although nifedipine alone caused almost a twofold increase in UNaV, acetazolamide infusion during treatment with nifedipine elicited an additional twofold increase in UV on UNaV (Table 2).

Figure 2B illustrates the effects of acetazolamide on RIF ATP concentrations during nifedipine infusion (n = 7). During nifedipine treatment, acetazolamide infusion significantly increased RIF ATP concentrations to 17.0 ± 1.8 nmol/l. Acetazolamide-induced increases in RIF ATP concentrations during nifedipine infusion were similar to those observed without nifedipine treatment as previously reported (21, 22).

Effects of a calcium channel blocker on RIF ATP concentrations. In five other dogs, nifedipine was infused for 90 min. Nifedipine significantly increased RBF, GFR, UV, and UNaV as described before. Previously, we demonstrated that stimulation of the TGF mechanism by acetazolamide infusion did not alter MAP and RAP but significantly decreased RBF and GFR, and there were no significant changes in RBF and GFR. Nifedipine infusion alone for 30 min significantly decreased RVR from 30.6 ± 2.8 to 26.7 ± 2.4 mmHg·min⁻¹·g⁻¹. During nifedipine infusion, acetazolamide did not alter RVR (26.2 ± 2.2 mmHg·min⁻¹·g⁻¹ at 30 min, Fig. 2A), indicating that the TGF-mediated RVR changes were prevented by nifedipine. Although nifedipine alone caused almost a twofold increase in UNaV, acetazolamide infusion during treatment with nifedipine elicited an additional twofold increase in UV on UNaV (Table 2).

DISCUSSION

An important criterion for the mediator of the TGF mechanism is that there should be a direct relationship between the changes in the macula densa stimulus and the changes in the release or concentration of the TGF mediator associated with the change in RVR (23, 24). Previous studies have demonstrated that RIF ATP levels are closely associated with autoregulatory or TGF-mediated changes in RVR (21, 22). However, it has remained unclear whether changes in RIF ATP mediate RVR adjustments or the changes in RIF ATP concentrations are simply the result of the changes in RVR. To address this question directly, we determined the changes in RIF ATP following RAP changes or stimulation of the TGF mechanism with acetazolamide under conditions where the autoregulatory and TGF-dependent changes in RVR were not altered in response to reductions in RAP during nifedipine administration, indicating a loss of autoregulatory efficiency. Pressure-induced changes in RIF ATP concentrations still occurred during treatment with nifedipine. *P < 0.05 vs. control (10 min), †P < 0.05 vs. nifedipine (45 min); n = 7 dogs, respectively.

Figure 1B illustrates the changes in RIF ATP concentrations in response to reductions in RAP during nifedipine infusion (n = 7). Control RIF ATP concentrations averaged 10.4 ± 1.2 nmol/l and were not different during nifedipine infusion (9.9 ± 0.8 nmol/l at 30 min). ATP levels were significantly decreased during stepwise reductions in RAP by 42 ± 8% to 5.8 ± 0.7 nmol/l in step 1 and by 71 ± 2% to 2.8 ± 0.3 nmol/l in step 2 (P < 0.05, respectively). The pressure-induced reductions in RIF ATP concentrations during nifedipine infusion were similar to those observed without nifedipine treatment as previously reported (21, 22).

Changes in RIF ATP concentrations in response to the activation of the TGF mechanism during treatment with a calcium channel blocker. Table 2 summarizes effects of acetazolamide on renal hemodynamics, urine flow, and sodium excretion during nifedipine infusion (n = 7). Nifedipine alone slightly decreased MAP and significantly increased RBF, GFR, UV, and UNaV as described before. Previously, we demonstrated that stimulation of the TGF mechanism by acetazolamide infusion did not alter MAP and RAP but significantly decreased RBF and GFR, and there were no significant changes in RBF and GFR. Nifedipine infusion alone for 30 min significantly decreased RVR from 30.6 ± 2.8 to 26.7 ± 2.4 mmHg·min⁻¹·g⁻¹. During nifedipine infusion, acetazolamide did not alter RVR (26.2 ± 2.2 mmHg·min⁻¹·g⁻¹ at 30 min, Fig. 2A), indicating that the TGF-mediated RVR changes were prevented by nifedipine. Although nifedipine alone caused almost a twofold increase in UNaV, acetazolamide infusion during treatment with nifedipine elicited an additional twofold increase in UV on UNaV (Table 2).

**Table 2. Renal responses to acetazolamide during treatment with nifedipine**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Nifedipine</th>
<th>Acetazolamide</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
<td>20 min</td>
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<tr>
<td>MAP, mmHg</td>
<td>134 ± 7</td>
<td>137 ± 7</td>
<td>132 ± 7</td>
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<tr>
<td>RAP, mmHg</td>
<td>128 ± 6</td>
<td>128 ± 6</td>
<td>126 ± 7</td>
</tr>
<tr>
<td>RBF, ml·min⁻¹·g⁻¹</td>
<td>4.35 ± 0.37</td>
<td>5.02 ± 0.37*</td>
<td>5.01 ± 0.39*</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·g⁻¹</td>
<td>0.85 ± 0.07</td>
<td>0.99 ± 0.07*</td>
<td>0.99 ± 0.08*</td>
</tr>
<tr>
<td>UV, μl·min⁻¹·g⁻¹</td>
<td>8.2 ± 1.6</td>
<td>26.1 ± 4.1*</td>
<td>29.6 ± 5.8*</td>
</tr>
<tr>
<td>UNaV, μmol·min⁻¹·g⁻¹</td>
<td>1.35 ± 0.19</td>
<td>3.64 ± 0.54*</td>
<td>3.99 ± 0.66*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 dogs. *P < 0.05 vs. control; †P < 0.05 vs. nifedipine (30 min).
Early studies showed that macula densa cells have abundant mitochondria but little transport activity as demonstrated by evidence for diminished Na\(^{+}\)-K\(^{+}\)-ATPase activity (28), indicating the possibility that the mitochondria present along the basolateral aspects of macula densa cells subserve the role of synthesizing ATP for export as an extracellular signaling molecule. Recently, Bell et al. (2) utilized a biosensor technique and the isolated perfused thick ascending limb of the loop of Henle-macula densa preparation, and they demonstrated that increases in luminal NaCl concentrations sufficient to elicit TGF responses result in the release of ATP from the basolateral surface of macula densa cells. Further studies showed that ATP release from the macula densa cells in response to increases in NaCl concentration was enhanced by dietary salt restriction (15). In the present study, we utilized a microdialysis method and demonstrated that ATP is released into the RIF in response to increases in the activity of the TGF mechanisms caused by treatment with acetazolamide. The results of the present experiments obtained in intact animal experiments do not allow us to address the exact sources of ATP in the dialysate. Nevertheless, these data support the hypothesis based on the previous studies (2, 5, 11–13, 15–17, 19, 21–24, 35), indicating that the changes in RIF ATP concentrations are closely associated with alterations in TGF and renal autoregulatory activities.

Majid et al. (16) examined RBF autoregulatory efficiency in anesthetized dogs during intrarenal infusion of ATP. These authors showed that P2 receptor saturation by infusion of high doses of ATP resulted in decreases in basal RBF and marked impairment of whole kidney autoregulatory efficiency. Interestingly, administration of norepinephrine caused similar reductions in RBF but did not cause any impairment in autoregulatory efficiency of RBF, indicating that the responses to ATP were not simply due to the associated renal vasoconstriction (16). We previously demonstrated that RVR and RIF ATP concentrations decreased consistently in responses to reductions in RAP (21, 22). In the present study, blockade of autoregulatory adjustments in RVR by nifedipine increased basal RBF but did not alter RIF ATP concentrations. However, the pressure-induced reductions in RIF ATP concentrations still occurred during nifedipine infusion, indicating that the changes in RIF ATP concentrations are not the result of changes in RVR.

High renal autoregulatory efficiency is dependent on the integrity of the TGF mechanism as evidenced by the fact that inhibition of the TGF response results in an impairment of

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**Table 3. Effects of nifedipine on renal hemodynamics, functions, and renal interstitial fluid ATP**

<table>
<thead>
<tr>
<th></th>
<th>Control, min</th>
<th>10 min</th>
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<th>60 min</th>
<th>90 min</th>
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<tr>
<td>MAP, mmHg</td>
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<td>131±3</td>
<td>130±3*</td>
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<td>129±3*</td>
</tr>
<tr>
<td>RAP, mmHg</td>
<td>123±2</td>
<td>120±2*</td>
<td>119±2*</td>
<td>119±2*</td>
<td>119±2*</td>
</tr>
<tr>
<td>RBF, ml/min⁻¹·g⁻¹</td>
<td>4.04±0.22</td>
<td>4.83±0.25*</td>
<td>4.86±0.26*</td>
<td>4.79±0.30*</td>
<td>4.78±0.29*</td>
</tr>
<tr>
<td>GFR, ml/min⁻¹·g⁻¹</td>
<td>0.76±0.06</td>
<td>1.01±0.10*</td>
<td>1.02±0.08*</td>
<td>1.06±0.09*</td>
<td>1.08±0.07*</td>
</tr>
<tr>
<td>RVR, mmHg/ml⁻¹·min⁻¹·g⁻¹</td>
<td>30.7±1.6</td>
<td>25.1±1.1*</td>
<td>24.8±1.2*</td>
<td>25.1±1.6*</td>
<td>25.2±1.5*</td>
</tr>
<tr>
<td>RIF ATP, nmol/l</td>
<td>10.9±1.4</td>
<td>10.9±1.4</td>
<td>10.8±1.3</td>
<td>10.7±1.5</td>
<td>10.6±1.5</td>
</tr>
<tr>
<td>UV, µmol/min⁻¹·g⁻¹</td>
<td>8.7±1.6</td>
<td>23.2±4.1*</td>
<td>22.4±4.1*</td>
<td>23.8±4.5*</td>
<td>25.0±5.0*</td>
</tr>
<tr>
<td>UoV, µmol/min⁻¹·g⁻¹</td>
<td>1.11±0.22</td>
<td>2.99±0.55*</td>
<td>3.01±0.53*</td>
<td>2.99±0.51*</td>
<td>2.96±0.54*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 dogs. RIF, renal interstitial fluid. *P < 0.05 vs. control values.
autoregulation-associated alterations in RVR (19, 31, 32). In the present study, whole kidney stimulation of the TGF mechanism was elicited by the administration of acetazolamide, a carbonic anhydrase inhibitor, to inhibit proximal reabsorption rate and increase distal solute delivery (26). We previously demonstrated that acetazolamide infusion resulted in decreases in both RBF and GFR, indicating a predominant preglobular vasoconstriction (21, 22). We also observed that acetazolamide consistently increased RVR and RIF ATP concentrations. In addition, inhibition of the TGF response with furosemide prevented acetazolamide-induced increases in RVR and RIF ATP concentrations (21, 22). The results of the present study show that acetazolamide-induced increases in RIF ATP concentrations still occurred during treatment with nifedipine, which prevented the RVR changes. Thus these data indicate that the changes in RIF ATP concentrations are not the consequence of changes in RVR induced by stimulation of the TGF activity and further support the hypothesis that RIF ATP contributes to TGF-mediated changes in RVR.

The findings that GFR and U_{NaV} were significantly increased by nifedipine infusion suggest that nifedipine infusion alone should have also increased basal RIF ATP levels. However, intra-arterial infusion of nifedipine did not significantly increase RIF ATP levels, indicating that the stimulus to the TGF mechanism by nifedipine was not sufficient to increase RIF ATP levels or that other counteracting mechanisms associated with decreases in RAP or increased RBF prevented the anticipated changes in RIF ATP. Micropuncture and clearance studies have shown that the primary tubular action sites of dihydropyridine calcium antagonists are at distal convoluted tubules and collecting ducts (7). It has also been suggested that the diuretic action of dihydropyridine calcium antagonists is partially dependent on the increases in medullary blood flow (1). Therefore, it is possible that although nifedipine increases GFR, the amount of nifedipine-induced increases in solute delivery at the macula densa cells may be lower compared with acetazolamide infusion, which inhibits proximal reabsorption rate. Another possibility is that our methods using microdialysis technique may fail to detect small increases in RIF ATP concentrations in response to nifedipine infusion. It is also possible that the increase in RBF caused by nifedipine led to increased washout of RIF ATP, which counteracted the effects of increased ATP release to increase RIF ATP levels. Nevertheless, the failure to demonstrate an increase in ATP levels directly in response to the administration of nifedipine introduces a note of caution in our interpretation.

Several micropuncture studies (8, 29) have demonstrated that local administration of high doses of adenosine receptor antagonists decreases the magnitude of TGF-mediated reductions in stop-flow pressure and single nephron filtration rate in response to increases in the distal nephron perfusion rate. Furthermore, normal TGF responses are not present in adenosine A1 receptor-deficient mice (3, 30). These data suggest a role for adenosine in the transmission of the TGF signals. However, this hypothesis has remained controversial because adenosine antagonists do not block RBF and GFR autoregulation (10, 27) or TGF responses (8, 17). Furthermore, recent studies have shown that RIF adenosine levels were not altered in response to reductions in RAP within the autoregulatory range (21, 22). In addition, RIF adenosine levels were not altered significantly during augmented TGF activity by acetazolamide or inhibition of the TGF response by furosemide, indicating that there is no relationship between the change in the macula densa stimulus and RIF concentrations of adenosine (21, 22). It should also be noted that, although ATP can be metabolized to ADP, AMP, and adenosine (19), complete and immediate hydrolysis of all available ATP would still not yield sufficiently high levels of these substances to cause comparable vasoconstriction, as described previously (21–24).

In summary, the present study demonstrates that the changes in RIF ATP concentrations can occur in the absence of autoregulatory or TGF-related changes in RVR. The results are consistent with the hypothesis that autoregulatory and TGF-dependent adjustments in RVR are mediated by the corresponding changes in RIF ATP concentrations (2, 5, 11–13, 15–17, 19, 21–24, 35). It has been recognized that impairment of renal autoregulation and the TGF mechanism closely contribute to the development of hypertension and renal injury (19, 24, 34). In addition, potential pathological roles of renal interstitial ATP have also been suggested by several recent studies (4, 6, 24). It is possible that TGF- and autoregulatory-mediated increases in RIF ATP concentrations, if sustained for a prolonged period, could contribute to hypertension-related renal injury. Accordingly, future studies will be needed to determine the dynamics of RIF ATP and its roles in the development of hypertension and renal injury.

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