Adenosine A2 Receptor Activation Attenuates Afferent Arteriolar Autoregulation During Adenosine Receptor Saturation in Rats

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Abstract—Adenosine is an important paracrine agent regulating renal hemodynamics via adenosine A1 and A2 receptors. To determine the interactions between adenosine A1 and A2 receptors and the possible role of adenosine as a modulator of afferent arteriolar autoregulatory responses, videomicroscopic measurements of afferent arteriolar dimensions were performed at different perfusion pressures (from 100 to 125 and 150 mm Hg) using the isolated-blood–perfused rat juxtaglomerular nephron preparation. Single afferent arterioles were visualized and superfused with low or high concentrations of adenosine, either alone or with the adenosine A1 receptor antagonist 8-noradamantan-3-yl-1,3-dipropylxanthine (10 μmol/L) or the adenosine A2 receptor antagonist dimethyl-1-propargylxanthine (10 μmol/L). Adenosine (20 μmol/L) decreased afferent arteriolar diameter by −9.0±0.9%, and this effect was enhanced by dimethyl-1-propargylxanthine (10 μmol/L) to −16.1±1.2%. However, autoregulatory capability was maintained. Adenosine-induced vasoconstriction was prevented by 8-noradamantan-3-yl-1,3-dipropylxanthine (10 μmol/L) with diameter increasing by 9.6±1.2%. Adenosine receptor saturation with a high concentration of adenosine (120 μmol/L) or blocking A1 receptors with 8-noradamantan-3-yl-1,3-dipropylxanthine in the presence of adenosine resulted in marked vasodilation and marked impairment of autoregulatory responses to increases in perfusion pressure (−1.5±1.1% and −3.5±0.9%). However, afferent arteriolar autoregulatory responses to elevations in perfusion pressure were restored after blockade of A2 receptors alone or in combination with A1 receptor blockade. During treatment with dimethyl-1-propargylxanthine in the presence of adenosine receptor saturation (120 μmol/L), afferent arteriolar autoregulatory responses were intact (−16.5±1.6% and −26.4±2.1%). These results indicate that the interactions between adenosine A1 and A2 receptors exert important modulatory influences on afferent arteriolar tone and autoregulatory capability. Activation of A2 receptors abrogates the countercoupling influences of A1 receptors leading to marked vasodilation and decreased afferent arteriolar autoregulatory efficiency. (Hypertension. 2007;50:744-749.)

Key Words: renal autoregulation ■ microcirculation ■ adenosine receptors ■ tubuloglomerular feedback ■ ATP ■ kidney

Adenosine is an endogenous adenine nucleoside that is formed by the hydrolysis of ATP. Four subtypes of adenosine receptors have been cloned: A1, A2a, A2b, and A3; all are guanine nucleotide binding proteins (G)–coupled receptors.1–4 Adenosine A2 receptors are expressed in the vasculature of most tissues and organs. In contrast, A1 receptors are not widely expressed in the vasculature. How-ever, both adenosine A1 and A2 receptors are expressed in the rat kidney.5,6 In particular, preglomerular microvessels have abundant expression of A1 and A2 receptors; A2b is the predominant A2 receptors in preglomerular arterioles.7

Adenosine modulates many physiological processes. In many tissues, adenosine elicits marked vasodilation via A2 receptors. In the renal vasculature, adenosine elicits biphasic effects with vasoconstriction via A1 receptors and vasodilation via A2 receptors. As the concentration of adenosine increases, the vasodilatory stimulus becomes predominant.8–13 Activation of adenosine A1 receptors in the renal vasculature results in vasoconstriction that reduces the glomerular filtration rate14,15 and renal blood flow16 and inhibits renin secretion.17 A2 receptor–mediated vasodilation results from stimulation of Gsα leading to increased cAMP that is partially mediated via epoxyeico-satrienoic acid release.18 Cytochrome P450 epoxygenase metabolites have been shown to alter vascular tone in afferent arterioles and modify the autoregulatory efficiency of the preglomerular microcirculation.19

Adenosine has been suggested as both a mediator and a modulator of renal autoregulation.13,20 Previous studies have demonstrated that autoregulatory adjustments in preglomerular resistance involve myogenic and tubuloglomerular feed-
back influences. However, the exact signaling and effector mechanisms have remained controversial. Recent studies have suggested that ATP and/or adenosine may be important mediators responsible for renal autoregulatory behavior. Both A1 and A2 receptors are present in the renal microvasculature, which makes understanding the renal vascular actions of adenosine comparatively difficult. The mechanisms underlying the occurrence of the biphasic dose responses to adenosine remain unclear, and the role of individual adenosine receptor types in autoregulation of renal afferent arterioles has not been determined. At the whole kidney level, studies with nonselective adenosine receptor blockers failed to reveal a role for adenosine receptors in autoregulation; however, this could be because of combined nonspecific blockade of adenosine receptors.

In the present experiments, we used the rat in vitro blood-perfused juxtamedullary nephron technique and selective adenosine A1and A2 receptor antagonists 8-noradamanant-3-yl-1,3-dipropylxanthine (KW3902) and 3,7-dimethyl-1-propargyloxanthine (DMPX) to determine the interactions between adenosine A1 and A2 receptors and the possible role of adenosine in the mediation of renal autoregulatory responses. To test the hypothesis that the interactions between adenosine A1 and A2 receptors play important modulatory roles in regulating afferent arteriolar tone and autoregulatory adjustments, the adenosine receptors were saturated with high concentrations of adenosine to activate the afferent arteriolar adenosine receptors, and then the effects of blocking only A1 or A2 or both A1 and A2 receptors on autoregulatory efficiency of afferent arterioles were evaluated.

Materials and Methods
The experimental protocols and procedures were approved by the Tulane University Institutional Animal Care and Use Committee. Videomicroscopic measurements of afferent arteriolar diameters were performed using the isolated blood-perfused juxtamedullary nephron preparation, as described previously. Briefly, each experiment used 1 male Sprague-Dawley rat (Charles River Laboratories, Wilmington, Mass), weighing 370 to 410 g, serving as blood and kidney donor. Rats were anesthetized with pentobarbital sodium (50 mg/kg IP), and a cannula was inserted in the left carotid artery for blood collection. Blood was collected in a heparinized (500 U) syringe via the carotid arterial cannula and centrifuged to separate the plasma and cellular fractions. Theuffy coat was removed and discarded. After sequential passage of the plasma through 5- and 0.22-μm filters (Gelman Sciences), erythrocytes were added to achieve a hematocrit of 33%. This reconstituted blood was passed through a 5-μm nylon mesh and thereafter stirred continuously in a closed reservoir that was pressurized with a 95% O2-5% CO2 gas mixture. The right kidney was perfused through a cannula inserted in the superior mesenteric artery and advanced to the right renal artery. The initial perfusate was a Tyrode’s solution (pH 7.4) containing 5.1% BSA and a mixture of L-amino acids thereafter stirred continuously in a closed reservoir that was pressurized with a 95% O2-5% CO2 gas mixture. The kidney was excised and sectioned longitudinally, retaining the papilla intact with the perfused dorsal two thirds of the organ. The papilla was reflected to expose the pelvic mucosa and tissue covering the inner cortical surface. Overlaying tissue was removed to expose the tubules, glomeruli, and related vasculature of the juxtamedullary nephrons. The arterial supply of the exposed microvasculature was isolated by ligating the larger branches of the renal artery.

After the dissection was completed, the Tyrode’s perfusate was replaced with the reconstituted blood. Perfusion pressure was monitored by a pressure catheter centered in the tip of the perfusion cannula. Renal perfusion pressure was regulated by adjusting the rate of gas inflow into the blood reservoir and set at 100 mm Hg. The inner cortical surface of the kidney was continuously superfused with a warmed (37°C) Tyrode’s solution containing 1% albumin. The tissue was transilluminated on the fixed stage of a microscope (Nikon) equipped with a water-immersion objective (×40). Video images of the microvessels were transferred by a Newvicon camera (model NC-67M, Dage-MTI) through an image enhancer (model MFJ-1452, MFJ Enterprises) to a video monitor (Conrac Display Systems). The video signal was recorded on videotape for later analysis. Afferent arteriolar inside diameters were measured at 30-second intervals using a calibrated digital image-shearing monitor (Instrumentation for Physiology and Medicine). Treatments were administered by superfusing the tissue with a Tyrode’s solution containing the agent to be tested or the vehicle solution.

Experimental Protocols
A single afferent arteriole that showed robust blood flow was selected for each study. After a 10-minute equilibration period, an experimental protocol was initiated consisting of consecutive 10-minute treatment periods. Steady-state diameter determinations were calculated from the average of measurements obtained during the final 5 minutes of each treatment period at a renal arterial pressure of 100 mm Hg. The first experimental protocol was performed to determine the effects of adenosine receptor activation and saturation on afferent arterioles and the interactions between adenosine A1 and A2 receptors. Single afferent arterioles were visualized and superfused with adenosine or adenosine plus the adenosine A1 receptor antagonist KW3902 or plus the adenosine A2 receptor antagonist DMPX. Afferent arteriolar ID was measured during sequential exposure of the vessel to superfuse solutions of various compositions. In the first series, the following groups were studied: (1) adenosine (20 μmol/L) alone; (2) adenosine (120 μmol/L) alone; (3) adenosine (20 μmol/L) plus KW3902 (10 μmol/L); and (4) adenosine (20 μmol/L) plus DMPX (10 μmol/L). In the second series of experiments, we determined the effects of adenosine on afferent arteriolar autoregulatory responses. Interstitial concentrations of adenosine were increased to saturate the receptors, and autoregulatory behavior was assessed by increasing renal arterial pressure in a stepwise manner from 100 to 125 and 150 mm Hg. Renal arterial pressure was kept constant at each pressure step for ≥3 minutes before subsequent changes in pressure. After the control studies, the tissue was superfused with Tyrode’s solution containing the following: (1) adenosine (20 μmol/L) alone; (2) adenosine (20 μmol/L) plus KW3902 (10 μmol/L); (3) adenosine (20 μmol/L) plus DMPX (10 μmol/L); (4) adenosine (120 μmol/L) alone; (5) adenosine (120 μmol/L) plus DMPX (10 μmol/L); and (6) adenosine 120 μmol/L plus DMPX (10 μmol/L) and KW3902 (10 μmol/L).

Statistical Analysis
All of the data are reported as mean±SEM. Data were analyzed by 1-way ANOVA or 2-way ANOVA, followed by a Bonferroni’s multiple-comparison posthoc test. Values of P<0.05 were considered statistically significant.

Results
Effects of Adenosine Alone and With Adenosine Receptor Antagonists on Afferent Arterioles
As illustrated in Figure 1, superfusion with 20 μmol/L of adenosine caused significant constriction of afferent arterioles with diameters decreasing from 18.8±0.3 to 17.1±0.4 μm (−9.0±0.9%; n=6; P<0.01). Adding 10 μmol/L of DMPX after adenosine caused further constriction with diameters decreasing to 15.5±0.2 μm (−16.1±1.2%; n=5; P<0.01), indicating a vasodilatory
counteracting effect of A2 receptor activation. Adenosine-induced vasoconstriction was prevented by 10 μmol/L of KW3902 with diameters increasing from 18.6±0.3 to 20.4±0.4 μm (9.6±1.2%; n=5; P<0.01). Superfusion with a high concentration of adenosine alone (120 μmol/L) caused marked vasodilation increasing the afferent arteriolar diameter by 22.9±2.9% from 18.1±0.5 to 22.4±1.0 μm (n=6; P<0.01).

**Effects of Lower Concentration of Adenosine on Autoregulation of Afferent Arteriolar Diameters in Response to Increased Renal Perfusion Pressure**

As shown in Figure 2, control afferent arteriolar diameter averaged 18.6±0.4 μm at 100 mm Hg and decreased significantly to 15.2±0.4 μm (−19.5±2.4%; n=6; P<0.01 versus baseline) and 12.6±0.5 μm (−32.4±2.3%; n=6; P<0.01 versus baseline) with elevations in renal perfusion pressure to 125 and 150 mm Hg, respectively. The responses to increases in renal perfusion pressure were attenuated during superfusion with 20 μmol/L of adenosine with afferent arteriolar diameter decreasing by −10.7±0.8% and −16.7±1.7% (n=5; P<0.01 versus baseline; P<0.01 versus control group) with elevations in renal perfusion pressure to 125 and 150 mm Hg. With the addition of 20 μmol/L of adenosine plus 10 μmol/L of DMPX, afferent arteriolar diameter decreased significantly from 18.5±0.2 μm to 15.5±0.2 μm at 100 mm Hg (−16.1±1.2%; n=5; P<0.01 versus baseline) and then decreased to 13.5±0.4 μm and 12.1±0.6 μm (−12.8±1.6% and −22.3±3.7%; n=5; P<0.01 versus baseline), with elevations in renal perfusion pressure to 125 and 150 mm Hg. KW3902 (10 μmol/L) in the presence of adenosine (20 μmol/L) reversed the adenosine-mediated afferent arteriolar constriction and caused marked vasodilation along with attenuation of the autoregulatory responses to elevations in renal perfusion pressure from 100 to 125 and 150 mm Hg with afferent arteriolar diameters not changing significantly (0.8±0.5% and 2.2±1.6%; n=5; P>0.05 versus baseline; P<0.01 versus control group).

**Effects of High Concentration of Adenosine on Autoregulation of Afferent Arteriolar Diameters in Response to Increased Renal Perfusion Pressure**

As illustrated in Figures 3 and 4, control afferent arteriolar diameters averaged 18.0±0.3 μm at 100 mm Hg and decreased significantly to 15.3±0.6 μm (−15.3±2.8%; n=5; P<0.01 versus baseline) and 12.9±0.6 μm (−28.7±2.2%; n=5; P<0.01 versus baseline) with elevations in renal perfusion pressure to 125 and 150 mm Hg, respectively. During superfusion with 120 μmol/L of adenosine, there was marked arteriolar dilation, and the responses to increases in renal perfusion pressure were prevented with afferent arteriolar diameters not changing significantly (−1.5±1.1% and −3.5±0.9%; n=5; P>0.05 versus baseline; P<0.01 versus control group). The addition of 10 μmol/L of DMPX significantly decreased afferent arteriolar diameter by −14.0±2.0% (from 18.6±0.3 to 16.8±0.3 μm; n=8; P<0.01). During treatment with 10 μmol/L of DMPX in the presence of 120 μmol/L of adenosine, afferent arteriolar autoregulatory responses were intact with diameter decreasing significantly by −16.5±1.6% and −26.4±2.1% (n=8; P<0.01 versus baseline; P>0.05 versus control group) with elevations in renal perfusion pressures from 100 to 125 and 150 mm Hg (Figure 4). Furthermore, when both the A2 and A1 receptors were blocked by 10 μmol/L of DMPX in combination with 10 μmol/L of KW3902, afferent arteriolar diameters returned to control levels and also exhibited autoregulation decreasing by −13.1±1.1% and −21.3±1.9% (n=5; P<0.01 versus baseline; P<0.05 versus control group) with elevations in renal perfusion pressure to 125 and 150 mm Hg, respectively.

**Discussion**

The present study demonstrates the biphasic responses to adenosine mediated via A1 and A2 receptors. These findings are consistent with previous studies showing that exogenous adenosine elicits biphasic effects on total renal blood flow in the whole kidney, which are characterized by an initial transient renal vasoconstriction that wanes and becomes supplanted by a gradual vasodilation.8–11 We found that A2
receptor blockade with DMPX (10 μmol/L) significantly decreased afferent arteriolar diameter, suggesting a regulatory influence of endogenous adenosine via both A1 and A2 receptors, which is supported by a recent study showing that A1 and A2 receptors are well expressed in preglomerular microvessels.7 Superfusion with a low concentration of adenosine (20 μmol/L) caused slight constriction of afferent arterioles, but adenosine receptor saturation by high concentrations of adenosine (120 μmol/L) resulted in marked vasodilation. Adenosine-induced vasoconstriction was enhanced by the A2 receptor antagonist (DMPX) but was prevented by the A1 receptor antagonist (KW3902). These data indicate that interactions between adenosine A1 and A2 receptors play important modulatory roles in regulating afferent arteriolar tone. Thus, during superfusion with adenosine, both A1 and A2 receptors were activated to produce opposing effects; A1 receptor blockade causes marked vasodilation, whereas A2 receptor blockade restored vascular tone and autoregulatory responses. A1 receptors evoke vasoconstriction by inhibiting adenylate cyclase activity, thereby reducing cAMP generation in vascular smooth muscle. A1 receptor activation also seems to induce vasoconstriction through inhibitory G protein-dependant activation of phospholipase C.32–34 A2 receptors produce vasodilation by stimulating adenylate cyclase activity to increase cAMP generation by coupling to stimulatory G proteins and also by activating vasodilation products of arachidonic acid.18,35 Our results demonstrate that A1 receptors are more sensitive to adenosine than A2 receptors. At the low concentrations, adenosine predominantly activates A1 receptors leading to vasoconstriction, but at the high concentration, adenosine mainly activates the A2 receptors, resulting in marked vasodilation. Interestingly, once the A2 receptors are fully activated, there seems to be no counteracting influences of A1 receptors, suggesting an abrogation of A1 receptor activity. Thus, activation of A2 receptors nullifies the counteracting influence of A1 receptors leading to vasodilation. These data

Figure 2. Effects of low concentration of adenosine on autoregulation of afferent arteriolar diameters in response to increased renal perfusion pressure. Control afferent arteriolar diameter in the presence of 20 μmol/L of adenosine plus 10 μmol/L of DMPX decreased significantly with elevations in renal perfusion pressure from 100 to 125 and 150 mm Hg (n=6; P<0.01). The responses to increases in renal perfusion pressure were attenuated by 20 μmol/L of adenosine alone (Ado; n=5; P<0.01 vs baseline; P<0.01 vs control group) and were prevented by treatment with 10 μmol/L of KW3902 (KW; n=5; P<0.05 vs baseline; P<0.01 vs control group) in the presence of 20 μmol/L of adenosine. Values are mean±SE. **P<0.01 vs baseline. #P<0.05, ##P<0.01 vs control group.

Figure 3. Effects of a high concentration of adenosine on autoregulation of afferent arteriolar diameters in response to increases in renal perfusion pressure. Afferent arteriolar diameter decreased significantly with elevations in renal perfusion pressure from 100 to 125 and 150 mm Hg (n=5; P<0.01). The responses to increases in renal perfusion pressure were prevented by treatment with 120 μmol/L of adenosine alone (Ado; n=5; P<0.05 vs baseline; P<0.05 vs control group). During treatment with DMPX (10 μmol/L) in the presence of adenosine (120 μmol/L) or combined DMPX plus KW3902 (KW), afferent arteriolar autoregulatory responses were maintained with diameter decreasing significantly (n=8; P<0.01 vs baseline; P<0.05 vs control group; and n=5; P<0.01 vs baseline; P<0.01 vs control group) with elevations in renal perfusion pressures from 100 to 125 and 150 mm Hg. Values are mean±SE. **P<0.01 vs baseline. #P<0.05, ##P<0.01 vs control group.
help to explain the biphasic dose responses often induced by adenosine and the marked loss of autoregulatory response and tubuloglomerular feedback responses when adenosine A1 receptors are blocked or missing.\(^{13,20,36}\)

The present study demonstrates that renal adenosine A1 and A2 receptor saturation by high concentrations of adenosine (120 \(\mu\)mol/L) and blocking A1 receptors with KW3902 (10 \(\mu\)mol/L) in the presence of adenosine (20 \(\mu\)mol/L) results in marked impairment of autoregulatory efficiency in renal afferent arterioles. However, afferent arteriolar autoregulatory responses to elevations in renal perfusion pressure from 100 to 125 and 150 mm Hg were restored during treatment with DMPX (10 \(\mu\)mol/L) even in the presence of a saturating concentration of adenosine (120 \(\mu\)mol/L). The above results indicate that activation of A2 receptors in the absence of the counteracting influence of A1 receptors diminishes afferent arteriolar autoregulatory responses.

The studies regarding the identification of the chemical mediator of autoregulatory adjustments in preglomerular resistance have yielded conflicting results. Insoho et al\(^{22}\) reported that mice lacking ATP-sensitive P2X\(_1\) receptors exhibit impaired autoregulatory responses but retain responsiveness to adenosine, indicating that P2X\(_1\) receptor activation plays a critical role in mediating autoregulatory adjustments in resistance and implicates endogenously released ATP as the chemical mediator responsible for autoregulatory behavior and perhaps tubuloglomerular feedback-mediated adjustments in preglomerular resistance. In contrast, other studies have shown that renal blood flow and glomerular filtration rate autoregulatory responses were significantly impaired in A1AR\(^{-/-}\) compared with A1AR\(^{+/+}\) mice, indicating reduced regulatory responsiveness in the knockout mice.\(^{20}\) It was suggested that A1 receptor activation plays a role in mediating autoregulatory behavior. However, the role of unopposed activation of A2 receptors was not considered. Macula densa cells generate and release ATP, which is broken down to form AMP in the extracellular space. AMP, in turn, is degraded by ecto-5'-nucleotidases to adenosine.\(^{13,37}\)

Several studies have suggested that adenosine mediates tubuloglomerular feedback response, inhibits renin release, and may contribute to autoregulatory response.\(^{17,36,38,39}\) Based on the current studies, the possible role of unopposed activation of A2 receptors should be considered. Our data showing moderate alterations in efficiency of autoregulation suggest that adenosine contributes an important modulating influence with A1 receptor activation enhancing autoregulatory responses, whereas A2 receptor activation markedly diminishes autoregulatory responses. Maintained autoregulation during combined blockade of both A1 and A2 receptors indicates the existence of multiple mechanisms, suggesting a direct mediating role for ATP through P2 receptors.\(^{22}\)

In summary, A2 receptor blockade with DMPX significantly decreased afferent arteriolar diameter. Low concentrations of adenosine decreased afferent arteriolar diameter, and this effect was enhanced by DMPX. Adenosine-induced vasoconstriction was prevented by the A1 receptor antagonist KW3902, and vasodilation resulted. High concentrations of adenosine markedly increased afferent arteriolar diameter. Blocking A1 receptors with KW3902 in the presence of adenosine increased afferent arteriolar diameter and prevented afferent arteriolar constriction in response to elevations in renal perfusion pressure, indicating a powerful vasodilation effect of unopposed A2 receptor activation. A high concentration of adenosine alone also caused loss of ability to respond to increases in perfusion pressure. However, afferent arteriolar autoregulatory responses to elevations in renal perfusion pressure were restored by treatment with DMPX alone or combined with KW3902 in the presence of adenosine. These results indicate that interactions between adenosine A1 and A2 receptors exert important modulatory roles in regulating afferent arteriolar tone and autoregulatory adjustments. Full activation of A2 receptors abrogates the counteracting influence of A1 receptors leading to marked vasodilation and diminished afferent arteriolar autoregulatory efficiency. Thus, unopposed activation of A2 receptors may be primarily responsible for the loss of autoregulatory and tubuloglomerular feedback responses. Blockade of A2 receptors restored afferent arteriolar autoregulatory capability.

**Perspectives**

Adenosine has been suggested as an important paracrine agent regulating renal hemodynamics. In this study we used
the in vitro blood-perfused juxtamedullary nephron technique to directly measure diameters of afferent arterioles near the macula densa, the key site of renal autoregulation. We demonstrated the biphasic responses to adenosine mediated through activation of A1 and A2 receptors. The interactions between adenosine A1 and A2 receptors are important in autoregulatory adjustments in afferent arterioles of juxtamedullary nephrons. Our data suggest that the counteracting effect of adenosine A1 and A2 receptors should be considered in interpreting renal hemodynamic responses during blockade or deficiency of A1 receptors.

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Reference
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