DURING ADENOSINE A1 RECEPTOR BLOCKADE WITH 8-NORADAMANTAN-
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METERS, SUCH THAT DIAMETERS RETURNED TO VALUES NOT SIGNIFICANTLY DIFFERENT FROM CONTROL WITHIN 2 MIN.
DURING ADENOSINE A1 RECEPTOR BLOCKADE WITH 8-NORADAMANTAN-3-YL-1,3-DIPROPYLXANTHINE (KW-3902: 10 
MOl/L), 10 AND 100 
MOl/L ADENOSINE SIGNIFICANTLY INCREASED EFFERENT AND EFFERENT ARTERIOLE DIAMETERS (−8.2 ± 0.8 AND −5.7 ± 0.6%, RESPECTIVELY). THE AFFERENT AND EFFERENT ARTERIOLE VASOCONSTRICTOR RESPONSES TO ADENOSINE WANED AT A DOSE OF 100 
MOl/L, SUCH THAT DIAMETERS RETURNED TO VALUES NOT SIGNIFICANTLY DIFFERENT FROM CONTROL WITHIN 2 MIN.

Adenosine; afferent arterioles; efferent arterioles; KW-3902; KP-17837

ADENOSINE SERVES AN IMPORTANT PARACINRE FUNCTION IN REGULATING RENAL HEMODYNAMICS (1, 15, 20, 31, 32, 36). MEASUREMENTS OF TOTAL RENAL BLOOD FLOW (RBF) IN THE whole kidney have demonstrated an effect of exogenous adenosine on RBF characterized by an initial transient renal vasoconstriction that wanes and becomes supplanted by a gradual vasodilatation (1, 20, 31, 32). The mechanism for this biphasic response remains poorly understood, although it is presumed to involve the differential binding of adenosine to A1 and A2 receptors to evoke renal vasoconstriction and vasodilatation, respectively (20, 31, 32). Adenosine A2 receptors are subdivided into distinct A2a and A2b subtypes (33), which are shown to be expressed in the kidney (14, 38). Furthermore, recent studies have demonstrated adenosine A2a receptor-mediated vasodilation in isolated rabbit renal artery (29) and arcuate artery (28).

Renal microvascular responses to the adenosine A1 or A2 receptor agonists have been evaluated using several different systems. Holtz and Steinhausen (6) performed studies using the rat hydronephrotic kidney model and observed that topical application of the selective adenosine A1 receptor agonist N6-cyclohexyladenosine (CHA) reduced afferent arteriolar diameter without affecting efferent arteriolar diameter. In contrast, Dietrich and Steinhausen (5) used the same method and demonstrated CHA-induced efferent arteriolar vasoconstriction. The authors also reported that application of the selective adenosine A2 receptor agonist N-ethyl-carboxamidoadenosine (NECA) dilated both afferent and efferent arterioles. Studies performed using isolated perfused rabbit afferent arterioles also demonstrated a vasoconstrictor response to CHA; however, the selective adenosine A2 receptor agonist N6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine (DPMA) had no effect on afferent arteriolar diameter (35). Collectively, these observations support the presence of adenosine A1 receptors on afferent arterioles. However, the presence of adenosine A1 receptors on the efferent arteriole and the localization of adenosine A2 receptors on the renal microvasculature remain unclear.

The effects of adenosine or nonselective adenosine analogs on renal microvascular reactivity have also been evaluated (4, 6, 8, 9, 34, 35). The nonselective adenosine agonist, 2-chloroadenosine (2-CA), signifi-

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significantly reduced afferent arteriolar diameter but increased efferent arteriolar diameter in the hydronephrotic kidney (6). Using the juxtamedullary nephron preparation, Inscho et al. (8) demonstrated that superfusion of 2-CA over juxtamedullary afferent arterioles elicits vasoconstriction but that, at the higher concentrations, the vasoconstriction changes to a vasodilation. Further studies showed that adenosine reduces both afferent and efferent arteriolar diameters (4, 9). In contrast to these observations, adenosine and 2-CA caused monophasic diameter reductions of afferent arterioles isolated from rabbits (35). Thus the fact that adenosine can act as either a vasoconstrictor or a vasodilator of the renal microvasculature has led to uncertainty in the characterization of adenosine-mediated renal microvascular reactivity.

The present study was performed to delineate the interaction between adenosine A1 and A2a receptors in the control of the renal microvasculature. We hypothesized that adenosine A2a receptor-mediated vasodilation buffers adenosine-induced vasoconstriction in both afferent and efferent arterioles. To test this hypothesis, we used the in vitro blood-perfused juxtamedullary nephron technique (4, 8, 9) and determined the effects of selective adenosine A1 and A2a receptor blockers on the responsiveness of afferent and efferent arterioles to adenosine. In this study, we used 8-noradamantan-3-yl-1,3-dipropylxanthine (KW-3902) and 1,3-dipropyl-7-methyl-8-(3,4-dimethoxy-styryl)xanthine (KF-17837), which are new-generation adenosine A1 and A2a receptor antagonists with much higher degrees of selectivity for adenosine A1 and A2a receptors, respectively, compared with other available adenosine A1 and A2a receptor antagonists (10, 19, 25, 26).

MATERIAL AND METHODS

Assessment of afferent and efferent arteriolar reactivity.

The experiments were performed in accordance with the guidelines and practices established by the Tulane University Animal Care and Use Committee.

Afferent and efferent arteriolar diameters were assessed in vitro using the blood-perfused juxtamedullary nephron technique combined with videomicroscopy, as previously described (4, 8, 9). Each experiment used two male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 325–450 g, with one rat serving as the blood donor and the second rat as the kidney donor. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and a cannula was inserted in the left carotid artery of the blood donor. Donor blood was collected in a heparinized (500 units) 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, Ann Arbor, MI), 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 of the kidney donor was perfused through a cannula inserted in the superior mesenteric artery and advanced into the right renal artery. The perfusate was a Tyrode solution (pH 7.4) containing 5.1% BSA and a mixture of L-amino acids as previously described (4, 8, 9). 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. Overlying 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 perfusate was replaced with the reconstituted blood. Perfusion pressure was monitored by a pressure cannula 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 setting at 100 mmHg. The inner cortical surface of the kidney was continuously superfused with a warmed (37°C) Tyrode solution containing 1% BSA. The tissue was transilluminated on the fixed stage of a Leitz Laborlux-12 microscope equipped with a water-immersion objective (40). Video images of the microvessels were transferred by a Newvicon camera (model NC-67M; Dage-MTI, Michigan City, IN) through an image enhancer (model MFJ-1452; MFJ Enterprises, Starkville, MS) to a video monitor (Conrac Display Systems, Covina, CA). The video signal was recorded on videotape for later analysis (Super VHS videocassette recorder; Panasonic, Secaucus, NJ). Afferent and efferent arteriolar inside diameters were measured at 15-s intervals using a calibrated digital image-shearing monitor (Instrumentation for Physiology and Medicine, San Diego, CA).

Afferent and efferent arteriolar diameters were measured at a location as close to the glomerulus as possible (within 100 μm of the glomerulus). Efferent arterioles were studied between the glomerulus and the first bifurcation. Treatments were administered by superfusing the tissue with a solution containing the agent to be tested or vehicle (0.2% DMSO). Superfusion directly over the adventitial side of afferent and efferent arterioles permits investigation of the actions of adenosine from the interstitial side as would occur if adenosine is serving as a paracrine agent.

Experimental protocols. After a 15-min equilibration period, an experimental protocol was initiated consisting of consecutive 5-min treatment periods. Steady-state diameter determinations were calculated from the average of measurements obtained during the final 2 min of each 5-min treatment period and were used for statistical analysis. We also evaluated the rapid responses to adenosine occurring during the first 45 s.

Afferent and efferent arteriolar responsiveness to increasing concentrations (0.1, 1, 10, and 100 μmol/l) of adenosine was assessed. After the control dose-response relationship to adenosine was obtained, the kidneys were superfused with the control buffer solution for 20 min to allow restoration of control diameters. Next, the effects of adenosine A1 or A2a blockade on afferent and efferent arteriolar responses to adenosine were determined. The selective adenosine A1 antagonist KW-3902 (10 μmol/l; Kyowa-Hakko Kogyo, Tokyo, Japan; see Refs. 19, 25) or the selective adenosine A2a antagonist KY-17837 (15 μmol/l; Kyowa-Hakko Kogyo; see Refs. 10, 19, and 26) was added to the superfusate for 5 min, and the afferent and efferent responses to adenosine (0.1–100 μmol/l) were reassessed in the presence of these adenosine antagonists. In a separate experimental series, the afferent and efferent arteriolar responses to adenosine (0.1–100 μmol/l) were also assessed before and during superfusion with KW-3902 (10 μmol/l) plus KY-17837 (15 μmol/l).
The assessment of afferent arteriolar responsiveness to adenosine (0.1–100 μmol/l) was repeated to determine the afferent arteriolar responses to repeated administration of adenosine. After the first dose-response relationship to adenosine was obtained, the kidney was superfused for 20 min with the control buffer solution. Next, the second afferent arteriolar dose-response relationship to adenosine was determined. To avoid the possibility that prolonged exposure to extracellular adenosine results in desensitization of adenosine receptors, the response of afferent arterioles to a single application of 10 μmol/l adenosine was repeated in a separate group of vessels. This adenosine concentration was chosen because it evoked vasoconstriction from each of the afferent arterioles previously studied (4, 9). After the first response to 10 μmol/l adenosine was obtained, the kidney was superfused for 20 min with the control buffer solution. Subsequently, the second afferent arteriolar response to 10 μmol/l adenosine was evaluated.

In a separate experimental series, the responses of afferent and efferent arterioles to 10 μmol/l adenosine were assessed before and during treatment with KW-3902 (10 μmol/l) and KW-3902 (10 μmol/l) plus KF-17837 (15 μmol/l). After afferent and efferent arteriolar responses to a single application of 10 μmol/l adenosine were assessed, the kidney was superfused for 20 min with the control buffer solution. Next, KW-3902 was added to the superfusate for 5 min, and the afferent and efferent arteriolar responses to 10 μmol/l adenosine were reassessed in the presence of KW-3902. Subsequently, KF-17837 was added to the superfusate containing KW-3902 and 10 μmol/l adenosine.

Drugs. KW-3902 and KF-17837 were supplied by Kyowa Hakko Kogyo. Adenosine was obtained from Sigma Chemical (St. Louis, MO).

Statistical analysis. Analysis of changes in afferent and efferent arteriolar responses to adenosine were performed using the one-way ANOVA for repeated measures combined with the Newman-Keuls post hoc test. Differences between groups from different experimental series were determined using Student’s t-test for unpaired data. P < 0.05 was considered statistically significant. Data are presented as means ± SE.

RESULTS

Effects of extracellular adenosine on afferent and efferent arteriolar diameters. Figure 1 shows the afferent and efferent arteriolar responses to increasing concentrations of adenosine (0.1, 1, 10, and 100 μmol/l). Basal afferent and efferent arteriolar diameters were similar in both arterioles and averaged 17.1 ± 0.5 and 17.8 ± 0.5 μm (n = 35 and 20, respectively). Superfusion with 0.1 μmol/l adenosine did not alter afferent and efferent arteriolar diameters (17.1 ± 0.5 and 17.7 ± 0.5 μm, respectively). Increases in superfusate adenosine concentration to 1 μmol/l caused a transient reduction in arteriolar diameter by 4.9 ± 1.1% within the initial 45 s (P < 0.05). With continued application of 1 μmol/l adenosine, this effect waned, and afferent arteriolar diameter returned to values that were only 3.2 ± 0.5% below control values (not significant). Afferent arteriolar diameter was significantly reduced with 10 μmol/l adenosine by 11.3 ± 1.2% to 15.2 ± 0.5 μm within the initial 45 s and remained significantly smaller than control. Afferent arteriolar diameter averaged 15.8 ± 0.5 μm (91.7 ± 0.7% of the control diameter) over the last 2 min of adenosine treatment. In contrast, 1 μmol/l adenosine did not alter efferent arteriolar diameter significantly during the treatment period. Furthermore, 10 μmol/l adenosine elicited a slowly developing vasoconstrictor response in efferent arterioles that decreased efferent arteriolar diameter by

Fig. 1. Effects of adenosine on afferent (A and B, n = 35) and efferent (C and D, n = 20) arteriolar diameters. Time course of afferent (A) and efferent (C) arteriolar responses to adenosine. Data are average vessel diameters measured at 15-s intervals. Steady-state changes in afferent (B) and efferent (D) arteriolar diameters in response to adenosine. Steady-state diameter determinations were calculated from the average of measurements obtained during the final 2 min of each 5-min treatment period. Data are expressed as % changes of the control diameters. C, control; R, recovery. *P < 0.05 vs. control period.
5.7 ± 0.6% to 16.7 ± 0.5 μm. The afferent and efferent arteriolar vasoconstrictor responses to 10 μmol/l adenosine waned at a dose of 100 μmol/l, such that diameters returned to values not significantly different from control within 2 min (17.5 ± 0.5 and 17.9 ± 0.5 μm).

The time control experiments (n = 4) demonstrated no differences in afferent arteriolar responses to repeat administration of adenosine (0.1–100 μmol/l; data not shown). The afferent arteriolar response to a single application of 10 μmol/l adenosine was also determined (n = 7). Administration of 10 μmol/l adenosine elicited a rapid vasoconstrictor response, with afferent diameter declining by −11.8 ± 2.5% from 18.0 ± 1.0 to 15.9 ± 1.0 μm within the initial 45 s. With continued application of 10 μmol/l adenosine, afferent diameter increased slightly but remained significantly smaller than control. Afferent diameter averaged 16.5 ± 0.9 μm (91.5 ± 0.8% of the control diameter) over the last 2 min of adenosine treatment. Removal of adenosine from the superfusate bathing solution allowed recovery of afferent diameter to 17.9 ± 0.9 μm. No differences in the repeat afferent arteriolar vasoconstrictor response to 10 μmol/l adenosine were observed (data not shown).

Effects of adenosine A₁ receptor blockade on afferent and efferent arteriolar responses to adenosine. Responsiveness of afferent (n = 14) and efferent (n = 8) arterioles to increasing concentrations of adenosine (0.1–100 μmol/l) were examined before and during adenosine A₂a receptor blockade with KW-3902. As depicted in Fig. 2, 10 μmol/l adenosine elicited a rapid vasoconstrictor response, with afferent diameter declining by −11.8 ± 2.5% from 18.0 ± 1.0 to 15.9 ± 1.0 μm within the initial 45 s. With continued application of 10 μmol/l adenosine, afferent diameter increased slightly but remained significantly smaller than control. Afferent diameter averaged 16.5 ± 0.9 μm (91.5 ± 0.8% of the control diameter) over the last 2 min of adenosine treatment. Removal of adenosine from the superfusate bathing solution allowed recovery of afferent diameter to 17.9 ± 0.9 μm. No differences in the repeat afferent arteriolar vasoconstrictor response to 10 μmol/l adenosine were observed (data not shown).

Fig. 2. Steady-state changes in afferent (A and B, n = 14) and efferent (C and D, n = 8) arteriolar diameters in response to adenosine before and during administration of adenosine A₁ receptor antagonist 8-noradaman-tan-3-yl-1,3-dipropylxanthine (KW-3902). ○ Adenosine alone; ● adenosine + KW-3902. Data are expressed in μm (A and C) and as % changes of the control diameters (B and D). *P < 0.05 vs. control period. †P < 0.05 vs. adenosine alone.
Afferent (n = 7) and efferent (n = 6) arteriolar responses to 0.1, 1, 10, and 100 μmol/l adenosine were also assessed during both adenosine A₁ and A₂a receptor blockade, as shown in Fig. 4. Basal afferent and efferent arteriolar diameters were similar in both arterioles and averaged 16.6 ± 1.1 and 16.9 ± 1.2 μm, respectively. Treatment with KW-3902 (10 μmol/l) plus KF-17837 (15 μmol/l) did not alter basal afferent and efferent arteriolar diameters (16.8 ± 1.2 and 17.1 ± 1.4 μm, respectively). During treatment with KW-3902 plus KF-17837, 0.1, 1, and 10 μmol/l adenosine did not alter afferent and efferent arteriolar diameters. However, 100 μmol/l adenosine significantly increased afferent and efferent arteriolar diameters by 5.9 ± 1.0% to 17.8 ± 1.1 μm and by 5.8 ± 0.9% to 18.1 ± 1.5 μm, respectively (steady state). On the basis of group comparisons, the afferent and efferent arteriolar responses to 100 μmol/l adenosine in kidneys treated with KW-3902 plus KF-17837 were significantly smaller than those observed in kidneys treated with KW-3902 alone, as shown in Figs. 2 and 4 (P < 0.05).

Fig. 4. Steady-state changes in afferent (A and B, n = 7) and efferent (C and D, n = 6) arteriolar diameters in response to adenosine before and during administration of KW-3902 + KP-17837. ○, Adenosine alone; ●, adenosine + KW-3902 + KP-17837. Data are expressed in μm (A and C) and as %changes of the control diameters (B and D). *P < 0.05 vs. control period. †P < 0.05 vs. adenosine alone.
Afferent \((n = 7)\) and efferent \((n = 6)\) arteriolar responses to acute application of 10 \(\mu\)mol/l adenosine were assessed before (Fig. 5A) and during KW-3902 treatment alone and in combination with KF-17837 treatment (Fig. 5B). Basal afferent and efferent arteriolar diameters were similar in both arterioles and averaged 17.1 ± 0.9 and 17.6 ± 1.5 \(\mu\)m, respectively. Adenosine alone (10 \(\mu\)mol/l) significantly reduced afferent arteriolar diameter by 8.1 ± 1.3% to 15.7 ± 0.8 \(\mu\)m and efferent arteriolar diameter by 6.7 ± 1.1% to 16.5 ± 1.5 \(\mu\)m (Fig. 5A). Removal of adenosine from the superfusate solution allowed recovery of afferent diameter to 17.0 ± 1.1 \(\mu\)m and efferent diameter to 17.0 ± 1.9 \(\mu\)m. Similar to the studies described above, KW-3902 (10 \(\mu\)mol/l) treatment did not alter afferent and efferent arteriolar diameters (17.1 ± 1.1 and 17.0 ± 1.7 \(\mu\)m, respectively). During treatment with KW-3902, 10 \(\mu\)mol/l adenosine significantly increased afferent arteriolar diameter by 10.5 ± 1.1% to 18.9 ± 1.2 \(\mu\)m and efferent arteriolar diameter by 5.8 ± 1.0% to 18.1 ± 1.9 \(\mu\)m. The adenosine-induced afferent and efferent arteriolar vasodilation was recovered by the addition of 15 \(\mu\)mol/l KF-17837 (afferent arterioles: to 17.6 ± 1.2 \(\mu\)m, efferent arterioles: to 19.5 ± 1.8 \(\mu\)m), as shown in Fig. 5B.

**DISCUSSION**

Recently, the mRNAs for A1 and A2a receptors were detected in renal arterial tissue (29); however, the localization and functional role of these receptors in the renal microvasculature remain unclear. The present experiments using the blood-perfused juxtamedullary nephron preparation establish that superfusion with 10 \(\mu\)mol/l adenosine evokes sustained afferent and efferent arteriolar vasoconstriction, whereas with higher concentrations of adenosine the vasoconstrictor effect is attenuated. To evaluate the possible interaction between adenosine A1 receptor-mediated vasoconstriction and A2a receptor-mediated vasodilation, we examined the afferent and efferent arteriolar responses to adenosine during selective adenosine A1 and A2a receptor blockade.

In agreement with the previous studies (4, 9), we observed that adenosine constricts both afferent and efferent arterioles of juxtamedullary nephrons. Although adenosine A1 receptor-mediated afferent arteriolar vasoconstriction has been shown in several experimental models (4, 5, 6, 8, 9, 11, 34, 35), the efferent arteriolar response to adenosine A1 receptor activation has been less consistent (4–6, 11). Some studies reported efferent arteriolar vasoconstriction in response to adenosine or the adenosine A1 agonist CHA (4, 5), whereas others observed no change with these compounds (6, 11). Dietrich and Steinhausen (5) found that CHA constricts proximal efferent arterioles (within 50 \(\mu\)m of the glomerulus), whereas no effect was observed on distal efferent arterioles (near the welling point). In the present study, the measurement sites along the efferent arterioles were selected at a location as close to the glomerulus as possible. Therefore, one possible explanation for the discrepancy between the current report and others (6, 11) may be due to regional heterogeneity of the adenosine A1 receptor population or to the sensitivity of the efferent arterioles studied. It is also possible that there is a heterogeneity in the distribution of vascular adenosine A1 receptors between superficial and juxtamedullary nephrons, as suggested by other investigators (27, 35).

We found that adenosine dilates both afferent and efferent arterioles during adenosine A1 receptor blockade. These results are consistent with the recent observations that adenosine dilates isolated rabbit afferent arterioles in the presence of the adenosine A1 receptor antagonist 6-oxo-3-[2-phenylpyrazole(1,5-a)pyridine-3-yl]-l(6H)-pyridazinebutyric (37). We also observed that afferent and efferent arteriolar vasodilatory responses to adenosine during adenosine A1 receptor blockade were significantly attenuated by adenosine A2a receptor inhibition. Thus these data support the hypothesis that adenosine A1 and A2a receptors are expressed on both afferent and efferent arterioles and support the postulate that adenosine A2a receptor-mediated vasodilation may buffer adenosine A1 receptor-mediated vasoconstriction. These counter-
acting actions may help explain why some studies have failed to show significant effects of nonselective adenosine agonists or antagonists in various studies.

As reported previously (9, 34), we observed a biphasic afferent arteriolar response to adenosine characterized by a transient vasoconstriction that wanes within a minute. One possible explanation for the waning vasoconstrictor response is the delayed activation of a secondary vasodilatory response. During adenosine A2a receptor blockade, the rapid afferent arteriolar vasoconstrictor responses to adenosine (within the initial 45 s) were not altered; however, the constrictor responses to adenosine averaged over the last 2 min of treatment were significantly augmented. These results indicate that adenosine A2a receptors participate in the apparent waning of the initial afferent arteriolar vasoconstrictor response to adenosine. In contrast, adenosine did not elicit a rapid vasoconstrictor response in efferent arterioles. The reason for the discrepancy between afferent and efferent arteriolar responses to adenosine is unclear; however, the fact that adenosine A2a receptor blockade augmented adenosine-induced efferent arteriolar vasoconstriction suggests that a similar vasodilatory effect of adenosine A2a receptors also exists on efferent arterioles.

The presence of adenosine A2 receptors on afferent and efferent arterioles is supported by studies performed in vivo (1, 13) and in vitro (5, 6, 16, 17). Miura et al. (13) demonstrated that renal arterial administration of the selective adenosine A2a agonist 2-[p-(2-carboxethyl)phenethylamino]-5′-N-ethylcarboxamido adenosine significantly increased RBF without affecting glomerular filtration rate in anesthetized dogs. Thus these results indicate that adenosine A2a receptors mediate afferent and efferent arteriolar vasodilation. Similar results were reported by Murray and Churchill (16, 17) using the nonselective adenosine A2 receptor agonist NECA in the isolated perfused rat kidney. It was also demonstrated that NECA significantly increased both afferent and efferent arteriolar diameters in the hydromephrotic rat kidney (5, 6). In contrast to these observations, Weihprecht et al. (35) showed that NECA constricts afferent arterioles and the more selective A2 analog DPMA had no effect on the isolated afferent arteriolar diameter. The reason for the discrepancy between these results is unclear; however, it is possible that such varied responses may be due to the relatively low selectivity of NECA and DPMA for adenosine A2 receptors and may reflect some interaction between the A2 agonists and adenosine A1 receptors. Recently, Yaoita et al. (37) showed that, during adenosine A1 receptor blockade, adenosine dilates isolated afferent arterioles preconstricted with norepinephrine but does not affect the diameter of afferent arterioles not treated with norepinephrine. Therefore, another possibility exists that adenosine A2 receptor-mediated afferent arteriolar reactivity could be influenced by the basal vascular tone. Indeed, Weihprecht et al. (35) performed studies using nonpreconstricted isolated afferent arterioles and assessed the response to an adenosine A2 agonist.

Because adenosine A2a receptor blockade significantly augmented the vasoconstrictor response to 10 μmol/l adenosine, we anticipated that 100 μmol/l adenosine would further reduce afferent and efferent arteriolar diameters in the presence of the adenosine A2a receptor antagonist. However, the magnitude of the afferent and efferent arteriolar response to 100 μmol/l adenosine during adenosine A2a receptor blockade was actually less than the response to 10 μmol/l adenosine (Fig. 3, A-C). We also observed that 100 μmol/l adenosine slightly but significantly dilated both afferent and efferent arterioles during combined administration of adenosine A1 and A2a receptor blockers (Fig. 4). These data suggest the possibility that the dose of the adenosine A2a receptor antagonist KF-17837 (15 μmol/l) is insufficient to prevent the vasodilatory responses to 100 μmol/l adenosine. KF-17837 has great selectivity for the adenosine A2a receptors compared with other adenosine A2a receptor antagonists (10, 19, 26); however, Scatchard analysis demonstrated that KF-17837 functions as a competitive inhibitor (26). Therefore, we could not use a higher concentration of KF-17837 in the present experimental setting because high concentrations of KF-17837 may affect other adenosine receptors. Another possibility is that the low-affinity adenosine A2a receptors may be activated at high adenosine concentrations and elicit a vasodilatory response. Tang et al. (34) reported that there are two different types of adenosine-induced afferent arteriolar vasodilatory responses in the in vitro perfused hydromecephrotic kidney: one response corresponds to activation of low-affinity adenosine A2a receptors and the second response is mediated by high-affinity adenosine A2a receptors. The authors suggested that adenosine concentrations >10 μmol/l are required for adenosine A2b receptor-mediated afferent arteriolar vasodilation in vitro preparations (34). Although recent studies indicate that adenosine A2a-mediated afferent (34) and arcuate arteriolar (28) vasodilation are linked to the activation of ATP-sensitive potassium channels, the higher concentrations of adenosine may also activate potassium channels via adenosine A2b receptors, leading to vasodilation, as shown for other vessels (12, 18).

The microdialysis experiments performed in anesthetized rats (3, 30, 38), dogs (21–23), and rabbits (24) indicate that resting renal interstitial adenosine concentrations normally remain in the 0.1–1 μmol/l range. Therefore, the fact that a significant vasoconstriction to adenosine occurs at 10 μmol/l suggests the possibility that endogenous adenosine concentrations are lower than appears necessary for significant adenosine-mediated renal vasoconstriction in resting conditions. In the present study, we observed that KW-3902 treatment did not alter afferent or efferent arteriolar diameters under resting conditions. These results are consistent with previous observations that intrarenal administration of KW-3902, which prevents the vasoconstrictor influence of exogeneous adenosine, did not alter basal RBF or glomerular filtration rate (2), thus indicating that the basal influence of endogenous adenosine is relatively low. Recent studies have shown,
however, that renal interstitial adenosine concentrations are increased by chronic salt loading (30, 38) and during acute renal failure (21, 23, 24). Therefore, it is possible that endogenous adenosine contributes to renal hemodynamic changes in some pathological circumstances.

In summary, the availability of more selective adenosine antagonists has allowed us to demonstrate that extracellular adenosine can evoke vasoconstrictor and vasodilator actions on both afferent and efferent arterioles of juxtamedullary nephrons. During adenosine A1 receptor blockade, adenosine elicits afferent and efferent arteriolar vasodilation with a slightly greater effect on afferent arterioles. These vasodilator effects are markedly attenuated by adenosine A2A receptor blockade. During adenosine A2A receptor blockade, the adenosine-induced vasoconstrictor responses are enhanced. These data indicate the presence of adenosine A1 and A2A receptors on juxtamedullary afferent and efferent arterioles and are consistent with the hypothesis that adenosine A2A receptor-mediated vasodilatation buffers adenosine A1 receptor-mediated vasoconstriction in the renal microvasculature.

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