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Interactions of adenosine A_1 and A_{2a} receptors on renal microvascular reactivity

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Nishiyama, Akira, Edward W. Inscho, and L. Gabriel Navar. Interactions of adenosine A_1 and A_{2a} receptors on renal microvascular reactivity. *Am J Physiol Renal Physiol* 280: F406–F414, 2001.—Adenosine vasoconstricts preglomerular arterioles via adenosine A_1 receptors. Because adenosine also activates adenosine A_2 receptors, its overall renal vascular actions are complex and not fully understood. The present study was performed to determine the relative contributions of adenosine A_1 and A_{2a} receptors to the responsiveness of the renal microvasculature to adenosine. Afferent and efferent arteriolar diameters were monitored in vitro using the blood-perfused rat juxtamedullary nephron preparation. Basal afferent and efferent arteriolar diameters averaged 17.1 ± 0.5 (n = 35) and 17.8 ± 0.5 (n = 20) μm, respectively. Superfusion with 0.1 and 1 μmol/l adenosine did not significantly alter afferent and efferent arteriolar diameters; however, 10 μmol/l adenosine significantly reduced afferent and efferent arteriolar diameters (−8.2 ± 0.8 and −5.7 ± 0.6%, respectively). The afferent and efferent arteriolar vasoconstrictor responses to adenosine waned at a dose of 100 μmol/l, such that diameters returned to values not significantly different from control within 2 min. During adenosine A_1 receptor blockade with 8-noradamantan-3-yl-1,3-dipropylxanthine (KW-3902: 10 μmol/l), 10 and 100 μmol/l adenosine significantly increased afferent diameter by, respectively, 8.1 ± 1.2 and 13.7 ± 1.3% (n = 14) and efferent arteriolar diameter by 6.4 ± 1.3 and 9.3 ± 1.2% (n = 8). The afferent and efferent arteriolar vasodilatory responses to adenosine in the presence of KW-3902 were significantly attenuated by addition of the adenosine A_{2a} receptor antagonist 1,3-dipropyl-7-methyl-8-(3,4-dimethoxystyryl)xanthine (KF-17837: 15 μmol/l, n = 7 and 6, respectively). The addition of KF-17837 alone significantly enhanced afferent (n = 15) and efferent (n = 6) arteriolar vasoconstrictor responses to 1, 10, and 100 μmol/l adenosine. These results indicate the presence of adenosine A_1 and A_{2a} receptors on afferent and efferent arterioles of juxtamedullary nephrons, such that adenosine A_{2a} receptor-mediated vasodilation partially buffers adenosine-induced vasoconstriction in both pre- and postglomerular segments of the renal microvasculature.

adenosine; afferent arterioles; efferent arterioles; KW-3902; KF-17837

ADENOSINE SERVES AN important paracrine 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 exogeneous adenosine on RBF characterized by an initial transient renal vasoconstriction that wanes and becomes supplanted by a gradual vasodilation (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 A_1 and A_2 receptors to evoke renal vasoconstriction and vasodilation, respectively (20, 31, 32). Adenosine A_2 receptors are subdivided into distinct A_{2a} and A_{2b} subtypes (33), which are shown to be expressed in the kidney (14, 38). Furthermore, recent studies have demonstrated adenosine A_{2a} receptor-mediated vasodilation in isolated rabbit renal artery (29) and arcuate artery (28).

Renal microvascular responses to the adenosine A_1 or A_2 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 A_1 receptor agonist N^6-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 A_2 receptor agonist N-ethyl-carboxamide adenosine (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 A_2 receptor agonist N^6-[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 A_1 receptors on afferent arterioles. However, the presence of adenosine A_1 receptors on the efferent arteriole and the localization of adenosine A_2 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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cantly 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 A_1 and A_{2a} receptors in the control of the renal microvasculature. We hypothesized that adenosine A_{2a} 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 A_1 and A_{2a} receptor blockade on the responsiveness of afferent and efferent arteriolar responses 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-dimethoxystyryl)xanthine (KF-17837), which are new-generation adenosine A_1 and A_{2a} receptor antagonists with much higher degrees of selectivity for adenosine A_1 and A_{2a} receptors, respectively, compared with other available adenosine A_1 and A_{2a} 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. The buffy 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% O_2-5% CO_2 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 A_1 or A_{2a} blockade on afferent and efferent arteriolar responses to adenosine were determined. The selective adenosine A_1 antagonist KW-3902 (10 μmol/l; Kyowa-Hakko Kogyo, Tokyo, Japan; see Refs. 19, 25) or the selective adenosine A_{2a} antagonist KF-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 KF-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. Within each series, differences in afferent and efferent arteriolar responses to adenosine between group means were determined using the two-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 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 vasoconstrictior response in efferent arterioles that decreased efferent arteriolar diameter by

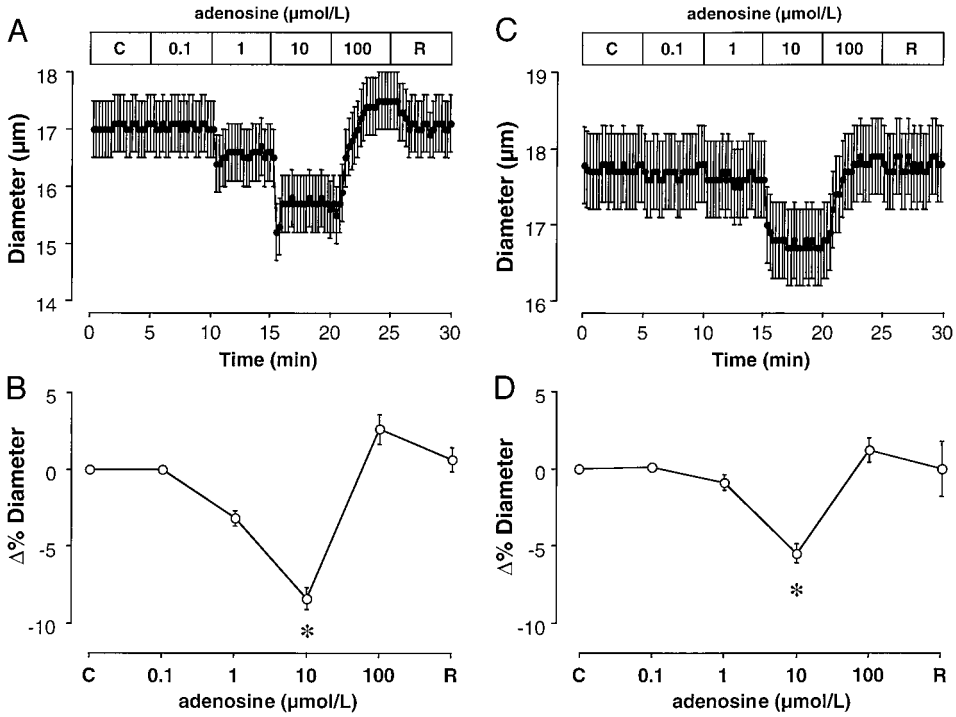

Fig. 1. Effects of adenosine on afferent (*A* and *B*, n = 35) and efferent (*C* and *D*, n = 20) arteriolar diameters. Time course of afferent (*A*) and efferent (*C*) arteriolar responses to adenosine. Data are average vessel diameters measured at 15-s intervals. Steady-state changes in afferent (*B*) 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 A_1 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 A_1 receptor blockade with KW-3902. As depicted in Fig. 2, 10 μmol/l adenosine significantly decreased afferent arteriolar diameter by 9.2 ± 1.4% and efferent arteriolar diameter by 5.5 ± 0.9%. KW-3902 treatment (10 μmol/l) did not alter basal afferent or efferent arteriolar diameters (17.3 ± 0.7 to 17.5 ± 0.7 μm and 18.4 ± 0.5 to 18.6 ± 0.7 μm). In the presence of KW-3902, 0.1 and 1 μmol/l adenosine also did not alter afferent or efferent arteriolar diameters; however, 10 and 100 μmol/l adenosine significantly increased afferent arteriolar diameter by 8.1 ± 1.2% to 19.0 ± 0.9 μm and by 13.7 ± 1.3% to 19.9 ± 0.8 μm, respectively, and efferent arteriolar diameter by 6.4 ± 1.3% to 19.8 ± 0.7 μm and by 9.3 ± 1.2% to 20.4 ± 0.8 μm, respectively (steady state). During treatment with KW-3902, 0.1–100 μmol/l adenosine did not elicit any rapid vasoconstrictor responses within the initial 45 s.

Responsiveness of afferent (n = 14) and efferent (n = 6) arterioles to increasing concentrations of adenosine (0.1–100 μmol/l) was examined before and during adenosine A_{2a} receptor blockade with KF-17837. KF-17837 treatment (15 μmol/l) did not alter basal afferent and efferent arteriolar diameters. During treatment with KF-17837, 0.1 μmol/l adenosine did not alter afferent arteriolar diameter; however, 1, 10, and 100 μmol/l adenosine significantly reduced afferent arteriolar diameters (Fig. 3*A*). As summarized in Fig. 3*B*, reductions in afferent arteriolar diameters (averaged over the last 2 min of treatment) to 1, 10, and 100 μmol/l adenosine were 6.9 ± 0.6, 13.4 ± 1.1, and 8.4 ± 1.5%, respectively. Compared with the responses observed with adenosine alone, the magnitude of the afferent arteriolar vasoconstriction to 10 μmol/l adenosine was significantly augmented during KF-17837 administration (Fig. 3*B*). However, KF-17837 did not significantly alter afferent arteriolar rapid responses (within the initial 45 s) to adenosine. During treatment with KF-17837, 0.1 μmol/l adenosine did not alter efferent arteriolar diameter; however, 1, 10, and 100 μmol/l adenosine significantly reduced efferent arteriolar diameter by −5.4 ± 0.8, −12.3 ± 1.2, and −7.5 ± 1.1%, respectively (Fig. 3*C*). The magnitude of the efferent arteriolar vasoconstriction to 10 μmol/l adenosine was significantly augmented during KF-17837 administration (Fig. 3*C*).

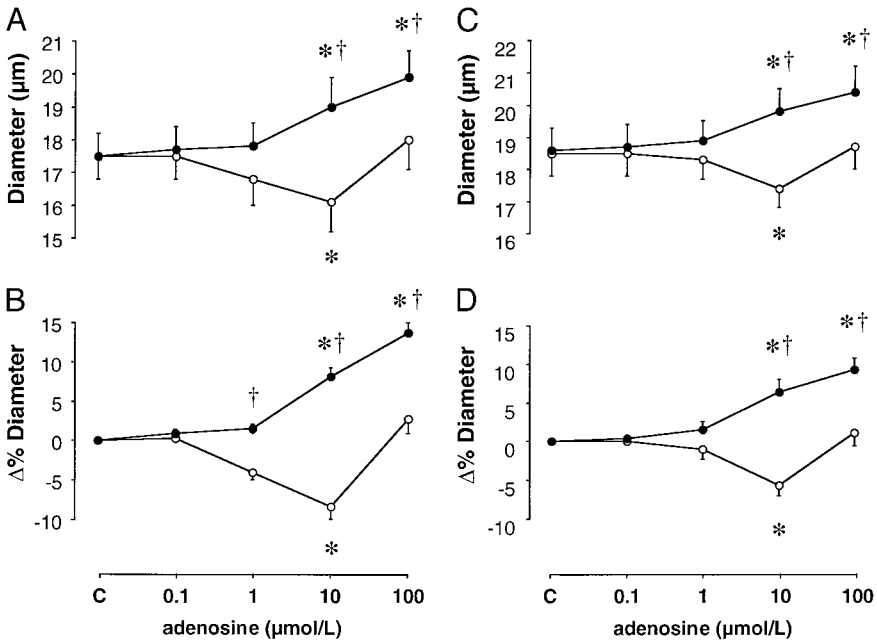

Fig. 2. Steady-state changes in afferent (*A* and *B*, n = 14) and efferent (*C* and *D*, n = 8) arteriolar diameters in response to adenosine before and during administration of adenosine A_1 receptor antagonist 8-noradamantan-3-yl-1,3-dipropylxanthine (KW-3902). ○, Adenosine alone; ●, adenosine + KW-3902. 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.

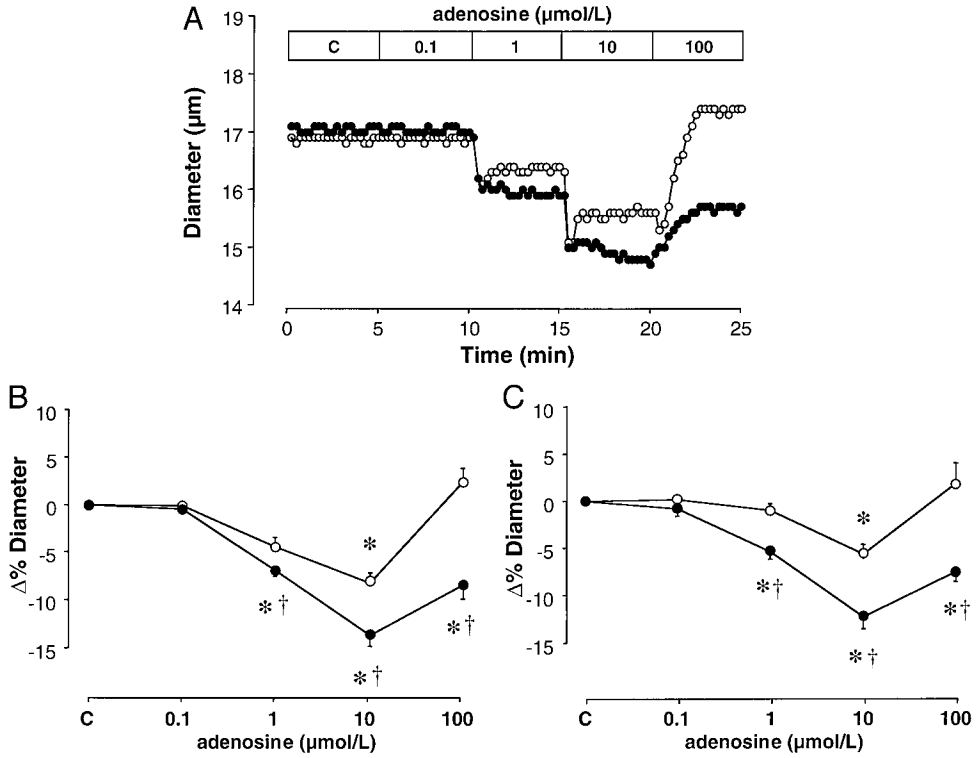

Fig. 3. Effects of adenosine A_{2a} receptor blockade with 1,3-dipropyl-7-methyl-8-(3,4-dimethoxystyryl)xanthine (KF-17837) on afferent and efferent arteriolar responses to adenosine. *A*: time course of afferent arteriolar responses to adenosine before and during administration of KF-17837 (n = 14). Data are average vessel diameters measured at 15-s intervals. SE bars have been omitted for clarity. ○, Adenosine alone; ●, adenosine + KF-17837. *B*: steady-state changes in afferent arteriolar diameter in response to adenosine before and during administration of KF-17837 (n = 14). Data are expressed as %changes of the control diameters. *C*: steady-state changes in efferent arteriolar diameter in response to adenosine before and during administration of KF-17837 (n = 6). Data are expressed as %changes of the control diameters. *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 A_1 and A_{2a} 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).

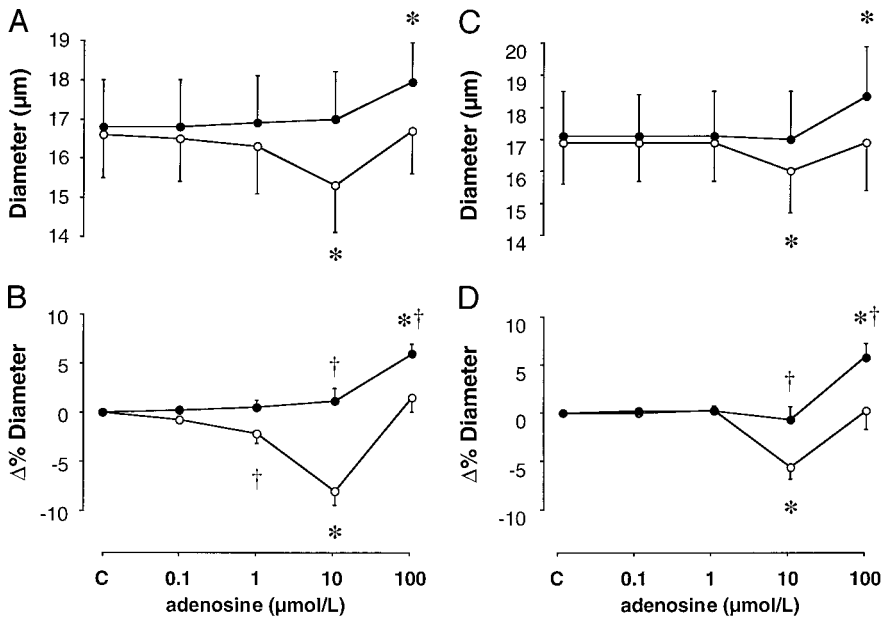

Fig. 4. Steady-state changes in afferent (*A* and *B*, n = 7) and efferent (*C* and *D*, n = 6) arteriolar diameters in response to adenosine before and during administration of KW-3902 + KF-17837. ○, Adenosine alone; ●, adenosine + KW-3902 + KF-17837. Data are expressed in μm (*A* and *C*) and as %changes of the control diameters (*B* and *D*). *P < 0.05 vs. control period. †P < 0.05 vs. adenosine alone.

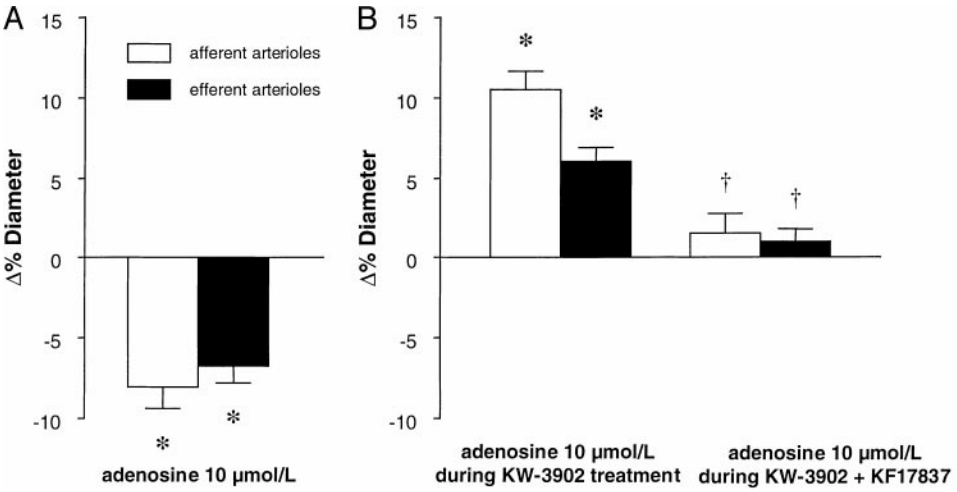

Fig. 5. Steady-state changes in afferent (n = 7) and efferent (n = 6) arteriolar diameters in response to acute application of 10 μmol/l adenosine before (A) and during (B) treatment with KW-3902 or KW-3902 + KF-17837. Open bars, afferent arterioles; filled bars, efferent arterioles. *$P < 0.05$ vs. control period. †$P < 0.05$ vs. KW-3902 alone. Data presented as %changes of the control diameters (A) and from the diameter of KW-3902 alone (B).

Afferent (n = 7) and efferent (n = 6) arteriolar responses to acute application of 10 μmol/l adenosine were assessed before (Fig. 5*A*) and during KW-3902 treatment alone and in combination with KF-17837 treatment (Fig. 5*B*). Basal afferent and efferent arteriolar diameters were similar in both arterioles and averaged 17.1 ± 0.9 and 17.6 ± 1.5 μm, respectively. Adenosine alone (10 μmol/l) significantly reduced afferent arteriolar diameter by 8.1 ± 1.3% to 15.7 ± 0.8 μm and efferent arteriolar diameter by 6.7 ± 1.1% to 16.5 ± 1.5 μm (Fig. 5*A*). Removal of adenosine from the superfusate solution allowed recovery of afferent diameter to 17.0 ± 1.1 μm and efferent diameter to 17.0 ± 1.9 μm. Similar to the studies described above, KW-3902 (10 μmol/l) treatment did not alter afferent and efferent arteriolar diameters (17.1 ± 1.1 and 17.0 ± 1.7 μm, respectively). During treatment with KW-3902, 10 μmol/l adenosine significantly increased afferent arteriolar diameter by 10.5 ± 1.1% to 18.9 ± 1.2 μm and efferent arteriolar diameter by 5.8 ± 1.0% to 18.1 ± 1.9 μm. The adenosine-induced afferent and efferent arteriolar vasodilation was recovered by the addition of 15 μmol/l KF-17837 (afferent arterioles: to 17.6 ± 1.2 μm, efferent arterioles: to 19.5 ± 1.8 μm), as shown in Fig. 5*B*.

DISCUSSION

Recently, the mRNAs for A_1 and A_{2a} 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 μmol/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 A_1 receptor-mediated vasoconstriction and A_{2a} receptor-mediated vasodilation, we examined the afferent and efferent arteriolar responses to adenosine during selective adenosine A_1 and A_{2a} 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 A_1 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 A_1 receptor activation has been less consistent (4–6, 11). Some studies reported efferent arteriolar vasoconstriction in response to adenosine or the adenosine A_1 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 μm 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 A_1 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 A_1 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 A_1 receptor blockade. These results are consistent with the recent observations that adenosine dilates isolated rabbit afferent arterioles in the presence of the adenosine A_1 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 A_1 receptor blockade were significantly attenuated by adenosine A_{2a} receptor inhibition. Thus these data support the hypothesis that adenosine A_1 and A_{2a} receptors are expressed on both afferent and efferent arterioles and support the postulate that adenosine A_{2a} receptor-mediated vasodilation may buffer adenosine A_1 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 A_{2a} 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 A_{2a} 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 A_{2a} receptor blockade augmented adenosine-induced efferent arteriolar vasoconstriction suggests that a similar vasodilatory effect of adenosine A_{2a} receptors also exists on efferent arterioles.

The presence of adenosine A_2 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 A_{2a} agonist 2-[p-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamido adenosine significantly increased RBF without affecting glomerular filtration rate in anesthetized dogs. Thus these results indicate that adenosine A_{2a} receptors mediate afferent and efferent arteriolar vasodilation. Similar results were reported by Murray and Churchill (16, 17) using the nonselective adenosine A_2 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 hydronephrotic rat kidney (5, 6). In contrast to these observations, Weihprecht et al. (35) showed that NECA constricts afferent arterioles and the more selective A_2 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 A_2 receptors and may reflect some interaction between the A_2 agonists and adenosine A_1 receptors. Recently, Yaoita et al. (37) showed that, during adenosine A_1 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 A_2 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 A_2 agonist.

Because adenosine A_{2a} 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 A_{2a} receptor antagonist. However, the magnitude of the afferent and efferent arteriolar response to 100 μmol/l adenosine during adenosine A_{2a} 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 A_1 and A_{2a} receptor blockers (Fig. 4). These data suggest the possibility that the dose of the adenosine A_{2a} 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 A_{2a} receptors compared with other adenosine A_{2a} 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 A_{2b} 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 hydronephrotic rat kidney: one response corresponds to activation of low-affinity adenosine A_{2b} receptors and the second response is mediated by high-affinity adenosine A_{2a} receptors. The authors suggested that adenosine concentrations >10 μmol/l are required for adenosine A_{2b} receptor-mediated afferent arteriolar vasodilation in in vitro preparations (34). Although recent studies indicate that adenosine A_{2a}-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 A_{2b} 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 endogeneous 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 endogeneous 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 A_1 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 A_{2a} receptor blockade. During adenosine A_{2a} receptor blockade, the adenosine-induced vasoconstrictor responses are enhanced. These data indicate the presence of adenosine A_1 and A_{2a} receptors on juxtamedullary afferent and efferent arterioles and are consistent with the hypothesis that adenosine A_{2a} receptor-mediated vasodilation buffers adenosine A_1 receptor-mediated vasoconstriction in the renal microvasculature.

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