Adenosine signaling in outer medullary descending vasa recta

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Silldorff, Erik P., and Thomas L. Pallone. Adenosine signaling in outer medullary descending vasa recta. Am J Physiol Regulatory Integrative Comp Physiol 280: R854–R861, 2001.—We tested whether dilation of outer medullary descending vasa recta (OMDVR) is mediated by cAMP, nitric oxide (NO), and cyclooxygenase (COX). Adenosine (A; 10⁻⁶ M)-induced vasodilation of ANG II (10⁻⁸ M)-preconstricted OMDVR was mimicked by the cAMP analog 8-bromoadenosine 3',5'-cyclic monophosphate (10⁻⁶ to 10⁻⁴ M) and reversed by the adenylate cyclase inhibitor SQ-22536. Adenosine (10⁻⁴ M) stimulated OMDVR cAMP production greater than threefold. NO synthase blockade with N⁶-nitro-L-arginine methyl ester and N⁶-monomethyl-L-arginine (10⁻⁴ M) did not affect adenosine vasodilation. Adenosine induced endothelial cytoplasmic calcium transients that were small. Indomethacin (10⁻⁶ M) reversed adenosine-induced dilation of OMDVR preconstricted with ANG II, endothelin, 4-bromo-calcium ionophore A23187, or carbocyclic thromboxane A₂. In contrast, selective A₂-receptor activation dilated endothelin-preconstricted OMDVR even in the presence of indomethacin. We conclude that OMDVR vasodilation by adenosine involves cAMP and COX but not NO. COX blockade does not fully inhibit selective A₂ receptor-mediated OMDVR dilation.

Several signaling mechanisms have been proposed to mediate adenosine A₂ receptor-induced relaxation of smooth muscle cells. These include stimulation of cAMP production (8, 15), inhibition of calcium influx (11, 28), and membrane hyperpolarization (29, 30). In addition to the proposed effects on smooth muscle, there are reports that the vasodilatory effect of adenosine depends on an intact endothelium (34, 39) and the production of nitric oxide (NO) (1, 2, 9, 18, 19). Renal hemodynamic studies favor mediation of vasoconstriction via A₁ receptors (4, 22) and NO-induced vasodilation through A₂b receptors (19). Prostaglandins may play a role in the vasodilatory response to adenosine. Indomethacin does not block adenosine A₁ receptor-mediated vasoconstriction (4, 6); however, the secondary increase in renal blood flow caused by adenosine A₂-receptor stimulation is attenuated by cyclooxygenase (COX) inhibition (6).

In this study, we tested the hypothesis that adenosine vasodilates OMDVR through events that lead to stimulation of adenyl cyclase and that this process involves signaling through NO or prostaglandins. The results show the importance of cAMP and COX but fail to identify a role for NO.

METHODS

In vitro microperfusion. Details of the methods employed to perfuse OMDVR and documents of their contractility have been published (24–26, 31, 32). In brief, young female Sprague-Dawley rats (Harlan) were anesthetized by intraperitoneal injection of thiopental sodium (50 mg/kg) after which the kidneys were harvested, sliced, and placed into cold (4°C) HEPES dissection buffer (in mM: 5 HEPES, 140 NaCl, 10 Na acetate, 5 KCl, 1.2 MgCl₂, 1.71 Na₂HPO₄ 0.29 NaH₂PO₄, 1 CaCl₂, 5 alanine, 5 glucose, 0.5 g/dl albumin; pH 7.4). Details of the methods employed to perfuse OMDVR and documents of their contractility have been published (24–26, 31, 32). In brief, young female Sprague-Dawley rats (Harlan) were anesthetized by intraperitoneal injection of thiopental sodium (50 mg/kg) after which the kidneys were harvested, sliced, and placed into cold (4°C) HEPES dissection buffer (in mM: 5 HEPES, 140 NaCl, 10 Na acetate, 5 KCl, 1.2 MgCl₂, 1.71 Na₂HPO₄ 0.29 NaH₂PO₄, 1 CaCl₂, 5 alanine, 5 glucose, 0.5 g/dl albumin; pH 7.4).
Microdissected vessels were isolated from vascular bundles, transferred to the stage of an inverted microscope (Nikon diaphot), cannulated, and perfused at 37°C with the same buffer. The bath flow rate was 200 μl/min and fed by gravity through switching between separate reservoirs containing pharmacological agents. Micromanipulators and perfusion and collection apparatus were purchased from Instruments Technology and Machinery (ITM). Perfusion chambers were custom made in our laboratory. Temperature of the perfusion chamber was maintained at 37°C with a feedback system employing a CN9111A controller (Omega Engineering).

Video microscopy and measurement of vessel diameters. To evaluate the effects of vasoactive agents on OMDVR diameters, microperfusion experiments were recorded on videotape. The inverted microscope was equipped with a 20/80% beam splitter and a side port with a video camera (Dage-MTI, CCD model 72). During experimentation, OMDVR were observed with a ×40 objective and magnified approximately ×1,300 to the video screen. OMDVR have an average internal diameter of 0.13 μm so that video projections averaged 15 mm on-screen. Experiments were recorded on a Panasonic model AG 1960 VCR with a microphone for audio recording of experimental events. During playback, diameters were measured at the point of greatest constriction using calipers. Changes in vessel diameter are expressed as percent constriction, defined in terms of the basal diameter in the absence of hormones (Do) and the experimental diameter (D) by the following expression: \% constriction = (1 – D/Do) × 100.

Measurement of endothelial intracellular calcium. OMDVR were loaded with the Ca2+-sensitive fluorescent indicator fura 2 by exposure to bath containing 2 μM fura 2-AM ester (Molecular Probes). At the time the bath was exchanged to fura 2, the feedback controller was turned on, gradually warming the vessel to 37°C over ~5 min. Total loading time was 20 min. Under these conditions, we have shown that fura 2 preferentially loads into endothelial cells with little or no fluorescent emission originating from the pericytes (25). For measurement of intracellular calcium concentration ([Ca2+]i), fura 2-loaded OMDVR were excited with the use of 350- or 380-nm dual wavelength combinations. The background-subtracted ratio of fluorescent emission (R350/380) was calculated for conversion to the equivalent [Ca2+]i, assuming a dissociation constant of 224 nM. Rmax and Rmin were measured as previously described by exposing vessels to buffer containing 5 mM CaCl2 or 0 CaCl2 and 0.5 mM EGTA, respectively, along with 10 μM 4-bromo-calcium ionophore A23187 (4-Br A23187) (25). A photon-counting photomultiplier assembly (PMT) was employed to measure fluorescent emission at 510 nm. Light for excitation of fura 2 was provided from a 75-W xenon arc lamp and directed through a computer-controlled monochromator (PTI). OMDVR were observed through a 1.3 numerical aperture, Nikon CF fluor ×40 oil-immersion objective, and the fluorescent emission from fura 2 was isolated with a 510WB40 filter (Omega Optical).

Enzyme immunoassay for cAMP. The second messenger, cAMP, was measured in microdissected OMDVR by enzyme immunoassay kit (Assay Designs). Rats were decapitated, and the left kidney was prepared for perfusion by ligating the aorta above the left renal artery. The kidney was perfused over 10 min through the aorta below the left renal artery with 10 ml ice-cold dissection solution followed by 10 ml dissection solution containing 1 mg/ml collagenase B (Boehinger Mannheim) at 37°C. The kidney was removed, decapsulated, sliced coronally, and digested for an additional 60–90 min by shaking in 1 mg/ml collagenase B at 37°C. The slices were then rinsed and maintained at 4°C during dissection. OMDVR segments were harvested until >30-mm cumulative length was obtained. These vessels were transferred in 10 μl dissection solution to a 1.5-ml centrifuge tube containing a phosphodiesterase inhibitor (Ro-20–1724, 10–4 M) in 20 μl dissection solution (total 30 μl). Blanks without OMDVR were also collected from the dissection solution. Tubes were vortexed and centrifuged at 9,000 rpm for 5 min. One-hundred microliters of supernatant were collected and stored at −20°C until assay. Samples were assayed according to the acetylated cAMP-kit protocol.

Signaling of adenosine in OMDVR via cAMP. We first tested the hypothesis that stimulation with adenosine or cAMP would vasodilate OMDVR. Both the cell-permeant analog 8-BrcAMP and adenosine dilated ANG II (10–9 M)-precontracted vessels in a concentration-dependent manner (Fig. 1A). Vasodilation by adenosine (10–10–10–4 M) or 8-BrcAMP was readily reversible (Fig. 1B). Furthermore, in the absence of ANG II, neither sham exchange nor 8-BrcAMP induced significant OMDVR vasoconstriction (Fig. 1C).

The results in Fig. 1, A–C support the possibility that cAMP could be a secondary mediator to induce vasodilation, but they do not establish a causal relationship between adenosine and signaling via the cAMP pathway. In view of this, we next tested the hypothesis that adenosine requires stimulation of adenyl cyclase to vasodilate OMDVR. Vessels were constricted with ANG II (10–5 M) and then vasodilated with adenosine (10–6 M). Subsequent addition of the adenyl cyclase inhibitor SQ-22536 (10–6 M) readily reversed adeno-
sine-induced vasodilation (Fig. 2). In a separate series of experiments \((n = 7)\), the lack of effect of SQ-22536 on baseline OMDVR was verified. Internal diameters averaged 11.9 ± 0.6, 11.9 ± 0.8, and 12.1 ± 0.7 μm before, during, and after 5-min application of SQ-22536 \((10^{-6} M)\).

To further establish the existence of signaling through the cAMP pathway, we directly measured cAMP generation by isolated vessels in response to adenosine. Microdissected OMDVR were exposed to vehicle, adenosine, or forskolin for 20 min. Adenosine \((10^{-4} M)\) increased cAMP generation to 326% of control values, and forskolin increased cAMP 20-fold (Fig. 3).

Fig. 1. Vasoactive effects of 8-bromoadenosine 3',5'-cyclic monophosphate (8-BrCAMP) and adenosine (ADO) on outer medullary descending vasa recta (OMDVR). A: OMDVR were preconstricted with abluminal ANG II \((10^{-9} M)\) for 10 min after which either ADO \((\bullet, n = 9)\) or 8-BrCAMP \((\bigcirc, n = 7)\) was added in log molar increments. ADO, in concentrations ≥10^{-6} M, dilated ANG II-preconstricted vessels \((^* P < 0.05 \text{ vs. sham exchange})\). Sham exchange of the bath with ANG II alone \((\bigcirc, n = 7)\) did not induce vasodilation. B: the vasoconstrictive effects of ADO \((10^{-6} M, n = 12)\) and 8-BrCAMP \((n = 11)\) were reversible \((^* P < 0.05 \text{ vs. first ANG II application}; ^# P < 0.05 \text{ recovery period vs. ANG II + agonist})\). C: neither 8-BrCAMP \((\bigcirc, n = 6)\) nor vehicle \((\bullet, n = 8)\) induced significant vasoconstriction.

Fig. 2. Reversal of ADO-induced vasodilation by adenylate cyclase inhibition. OMDVR were preconstricted with abluminal ANG II \((10^{-9} M)\) for 10 min after which ADO \((10^{-6} M)\) was added to the bath for 5 min. The adenylate cyclase inhibitor SQ-22536 \((\bigcirc, 10^{-4} M)\) was then added for 5 min, reversing the ADO-induced vasodilation \((n = 7)\). Finally, the effects of SQ-22536 were reversible after its removal \((^* P < 0.05 \text{ vs. ADO}, ^# P < 0.05 \text{ vs. ADO + SQ-22536})\). Sham exchange of the bath with ANG II alone was without effect \((n = 7)\).

Fig. 3. Enzyme-linked immunoassay for cAMP in microdissected OMDVR. OMDVR were microdissected from collagenase-digested kidneys (collagenase B, 1 mg/ml for 60 min at 37°C) and incubated with vehicle \((n = 16)\), ADO \((n = 12)\), or forskolin \((n = 15)\) at 10^{-4} M for 15 min at 37°C. ADO and forskolin significantly increased cAMP production compared with vehicle-treated vessels or respective blank tubes without vessels \((^* P < 0.05 \text{ vs. vehicle-treated OMDVR})\).
NO as a possible mediator of adenosine-induced vasodilation. Having established that cAMP is generated in response to adenosine and is required for vasodilation, we next tested the hypothesis that adenosine-induced vasodilation is mediated through NO generation. Vessels were constricted with ANG II (10^{-9} M) and then dilated with adenosine in either the presence or absence of NO synthase (NOS) inhibitors. Neither L-NMMA nor L-NAME (10^{-4} M) blocked the effect of adenosine (10^{-6} M) to dilate ANG II-preconstricted OMDVR, implying that NO generation is unlikely to contribute to adenosine-induced vasodilation (Fig. 4).

To further establish that calcium-sensitive isoforms of NOS are unlikely to be activated by adenosine, we next compared the endothelial [Ca^{2+}]_i responses of adenosine with that induced by bradykinin. Interestingly, responses were consistently observed with adenosine at either 10^{-9} or 10^{-5} M, but these were much smaller than that observed with the true endothelium-dependent vasodilator bradykinin (Fig. 5).

Prostaglandins as mediators of adenosine-induced vasodilation. To test the hypothesis that prostaglandins mediate the response of OMDVR to adenosine, we examined the effect of COX inhibition with indomethacin on vasodilation. The effects were complicated by the observation that ANG II preconstriction is itself substantially inhibited by indomethacin (10^{-6} M) (Fig. 6). This implies that vasoconstrictor prostaglandins such as thromboxanes are likely to be responsible for ANG II-induced OMDVR vasoconstriction. Nonetheless, consistent with the notion that adenosine-induced vasodilation requires COX product(s) (e.g., Figs. 1, 2, and 4), adenosine acted as a vasoconstrictor rather than as a vasodilator in the presence of indomethacin (Fig. 6). The potentiation of adenosine-induced vasoconstriction by COX inhibition has been substantiated by several reports (27, 33).

We next verified that adenosine-induced vasodilation of OMDVR is prostaglandin dependent when vasoconstriction is induced by other agents. Specifically, we tested vessels preconstricted with the thromboxane analog cTxA_2 (10^{-5} M), 4-Br A23187 (10^{-5} M), or ET-1 (10^{-10} M). In all cases, constriction stabilized within 15 min (Fig. 7A). In a first set of experiments, indomethacin (10^{-6} M) was subsequently added to the bath. As in the case of ANG II (Fig. 6), a tendency for indomethacin to vasodilate the preconstricted vessels was observed (Fig. 7B). This vasodilatory effect was less pronounced than that associated with ANG II (Fig. 6), and it achieved significance only with the most potent vasoconstrictor ET-1.

In a separate series of experiments, the ability of indomethacin to reverse adenosine-induced vasodilation was examined (Fig. 8, A–C). After 15-min applica-
ADENOSINE SIGNALING IN OMDVR

rather than vasodilation as observed in
accompanied by significant and reversible vasoconstriction (\(\text{ANG II}\)).

indomethacin from the bath (Fig. 8) to verify the reversibility of this effect on removal of
the respective vasoconstrictor, adenosine was
added to the bath for 5 min. Significant vasodilation was observed in all cases. Subsequently, indomethacin
was introduced along with adenosine for another 5-min
period. As in the case of ANG II (Fig. 6), indomethacin
was uniformly reversed adenosine-induced vasodilation. In
the case of ET-1, an additional period was included to
verify the reversibility of this effect on removal of
indomethacin from the bath (Fig. 8C).

\(\text{A}_2\) activation during COX inhibition. We previously
showed that adenosine constricts OMDVR at low concentration and dilates OMDVR at high concentration
and that these actions are mediated by \(\text{A}_1\) and \(\text{A}_2\)
receptors, respectively (31). To test the hypothesis that
exaggerated vasoconstriction of OMDVR by adenosine
during COX inhibition in preconstricted vessels (Fig. 8)
is accounted for by blockade of \(\text{A}_2\)-receptor signaling,
we performed two series of experiments. First, we
tested the hypothesis that during COX inhibition,
adenosine would act as a vasoconstrictor at low con-
centration, where it typically does so, and at high
concentration where \(\text{A}_2\)-receptor activation favors va-
sodilation. At 5-min intervals, in the presence of indo-
methacin (\(10^{-6}\) M), OMDVR were sequentially exposed
to adenosine at 10 M and then 10 M. For maximum
sensitivity, vessels that did not exhibit at least 25% constriction in response to 10 M adenosine were
eliminated. As shown in Fig. 9, in the presence of indomethacin, the concentration-dependent, biphasic effect of adenosine was observed. OMDVR constricted
by 10 M adenosine (baseline internal diameter 12.3 \pm 1.6 \(\mu\)m) dilated in response to 10 M adeno-
sine, a concentration at which \(\text{A}_2\) receptors should be activated. This finding shows that \(\text{A}_2\) receptor-mediated signaling remains during COX inhibition.

In a second series of experiments, OMDVR were
preconstricted by ET-1 (10^{-10} M) in the presence of
indomethacin (\(10^{-6}\) M) and allowed to stabilize for 15
min. Subsequently, to test the ability of \(\text{A}_2\) stimulation
to induce vasodilation, either adenosine (10^{-5} M, mixed \(\text{A}_1\), \(\text{A}_2\) agonist), adenosine (10^{-5} M) + \(\text{A}_1\)-receptor
antagonist DPCPX (3 \times 10^{-8} M), or the \(\text{A}_2\) agonist
CGS-21680 (10^{-7} M) was added to and then removed
from the bath at 5-min intervals. In the presence of
indomethacin, ET-1 constricted each group from base-
line internal diameters of 12.1 \pm 1.6, 11.3 \pm 1.0, and
11.3 \pm 0.7 \(\mu\)m, respectively, to ~50% of the original
value. When \(\text{A}_1\)-receptor stimulation was blocked
(DPCPX + adenosine) or absent (CGS-21680), dilation
of preconstricted vessels occurred (Fig. 10). In contrast,
as previously observed (Fig. 8C), mixed \(\text{A}_1\) and \(\text{A}_2\)
stimulation with adenosine resulted in exaggeration of

![Fig. 6. Effect of cyclooxygenase (COX) inhibition on ADO-induced vasodilation. A: vessels were preconstricted with ANG II (10^{-9} M, 10 min, \(n = 9\)) and then sham exchanged (no significant effect). Subsequently, ADO was added to the bath, inducing significant vasodilation (*\(P < 0.05\) vs. ANG II preconstriction). B: comparison of sham-exchanged time controls (\(A\)), ANG II preconstriction (10^{-9} M, 10 min, \(n = 6\)) was significantly reversed by indomethacin (Indo; 10^{-6} M, *\(P < 0.05\) vs. ANG II + Indo). Subsequent addition of ADO during COX blockade was accompanied by significant and reversible vasoconstriction (#\(P < 0.05\)) rather than vasodilation as observed in \(A\).](image)

![Fig. 7. Effect of COX inhibition on vasoconstriction by carbocyclic thromboxane \(\text{A}_2\) (cTxA_2), 4-bromo-calcium ionophore A23187 (4-Br A23187), and endothelin-1 (ET-1). A: baseline diameters of OMDVR were recorded for 1 min after which cTxA_2 (10^{-5} M), 4-Br A23187 (10^{-5} M), or ET-1 (10^{-10} M) was introduced into the bath (\(n = 6\) each). By 15 min, vasoconstriction stabilized in all cases. B: after 15 min of application of 4-Br A23187 (10^{-6} M, \(n = 8\), 4-Br A23187 (10^{-6} M, \(n = 7\), or ET-1 (10^{-10} M, \(n = 8\)), Indo (10^{-6} M) was added to and then removed from the bath at 5-min intervals. In each case, slight reversal of vasoconstriction was observed, but this achieved significance only in the case of ET-1 (\(*P < 0.05\) Indo vs. ET-1 alone). Some vessels are the same as those in \(A\), but the diameters were recorded only at the end of each period.](image)
vasoconstriction. Thus, in ET-1-constricted vessels, vasodilation through A2-receptor activation continues during COX inhibition.

DISCUSSION

In most vascular beds, adenosine is generated during periods of increased oxygen demand or decreased supply to enhance blood flow by inducing local vasodilation. The regional effects of adenosine in the kidney include cortical vasoconstriction, an increase in medullary blood flow, and a reduction of glomerular filtration rate (3, 20, 21). Presumably, these actions serve to enhance medullary oxygen delivery and to reduce the vasoconstriction. Thus, in ET-1-constricted vessels, vasodilation through A2-receptor activation continues during COX inhibition.

Fig. 8. Effect of COX inhibition on vasodilation by ADO. A: OMDVR were preconstricted with cTxA2 (10^{-5} M, 15 min, n = 8) after which ADO (10^{-5} M, 5 min) was added to the bath, inducing significant dilation (*P < 0.05 vs. cTxA2 alone). Subsequently, Indo (10^{-6} M, 5 min) was also added, resulting in reversal of the ADO effect (#P < 0.05 vs. cTxA2 + ADO). B: same protocol as in A, except that 4-Br A23187 (10^{-5} M, n = 7) was used to induce vasoconstriction. C: same protocol as in A and B, except that ET-1 (10^{-10} M, n = 8) was used to induce vasoconstriction. Recovery periods were also included to demonstrate reversibility of the effects of ADO and Indo. ADO induced vasodilation (*P < 0.05 vs. ET-1 alone) that was reversed by Indo (#P < 0.05 vs. ET-1 + ADO).

Fig. 9. ADO-induced constriction during COX inhibition. Vasoconstriction by ADO was measured sequentially at 5-min intervals at 10^{-8} and 10^{-5} M while Indo (10^{-6} M) was continuously present in the bath (n = 8). Significant vasoconstriction by ADO at 10^{-8} M reversed at 10^{-5} M (#P < 0.05, %constriction at 8, 9, and 11 min vs. that at 5 min).

Fig. 10. A2-mediated dilation of ET-1-constricted OMDVR during COX inhibition. OMDVR preconstricted for 15 min with ET-1 (10^{-10} M) in the presence of Indo (10^{-6} M) were exposed to ADO (10^{-5} M), ADO + 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (3 × 10^{-8} M), or 2-p-[2-carboxyethyl]phenethyl-amino-5'-N-ethylcarboxamido-adenosine (CGS-21680) (10^{-8} M) from 15 to 20 min (n = 7, 9, and 8, respectively). By repeated-measures ANOVA, compared with constriction at 15 min, ADO significantly constricted vessels at 20 min, whereas the other treatments consistently and significantly dilated the vessels.

Fig. 11. Effect of A2-receptor agonists on vasodilation by ADO. OMDVR preconstricted with cTxA2 (10^{-5} M, 15 min, n = 8) were exposed to ADO (10^{-5} M) and the indicated A2-receptor agonists for 5 min, except for DPCPX, which was added with ADO and remained for 5 min. Significant dilation by ADO was reversed by DPCPX (#P < 0.05 vs. ADO alone).
oxygen demand that arises from sodium transport in the thick ascending limb. Consistent with this notion, adenosine infused into the renal interstitium increases both medullary oxygen tension and blood flow (3, 10). On the basis of these observations, it is reasonable to hypothesize that adenosine produced by the medullary thick ascending limb (mTAL) (5) diffuses to and dilates OMDVR on the periphery of vascular bundles. Because OMDVR on the bundle periphery supply the adjacent interbundle region where the mTAL resides, a feedback system would serve to enhance oxygen delivery to the inner stripe of the outer medulla. The possibility that OMDVR participate in such a process is supported by the finding that the mTAL has the capacity to produce adenosine (5) and that A1 and A2 receptor mRNA is expressed in OMDVR (14, 31). We previously demonstrated that A1 and A2 adenosine-receptor stimulation on OMDVR favor vasoconstriction and vasodilation, respectively. The A1-receptor agonist cyclohexyladenosine constricts OMDVR, and the A2-receptor agonist CGS-21680 dilates OMDVR preconstricted by ANG II. Consistent with these observations, adenosine itself constricted OMDVR at low concentrations (10^-12 to 10^-8 M) where high-affinity A1-receptor effects are expected to dominate but not at high concentrations (10^-7 to 10^-5 M) where A2 receptor-mediated effects should occur (31).

This is the first study of the signaling pathways responsible for OMDVR dilation by adenosine. Before the development of adenosine analogs and antagonists, the observation that adenosine caused an increase in cAMP in some tissues and a decrease in others led to the conclusion that two receptor populations exist (36). Designated A1 and A2, they were found to interact with adenyylate cyclase through inhibitory and stimulatory G proteins, respectively (35). Stimulation of the high-affinity A1 receptor results in diminished cAMP levels and vasoconstriction, whereas stimulation of the lower-affinity A2 receptors increases cAMP levels and causes vasodilation. Some studies have favored other effects of A2 receptor-subtype stimulation through specific subtypes (A2a and A2b). Cushing et al. (8) found that although adenosine vasodilates coronary arteries via cAMP, the A2 agonist 5’-(N-ethylcarboxamido)adenosine (NECA) and CGS-21680 failed to increase cAMP production. In addition, Martin and Potts (19) demonstrated that NECA and the A2 agonist N6-cyclopentyladenosine mediated vasodilation of isolated renal arteries and required an intact endothelium. On the basis of the notion that CGS-21680 is more A2a receptor selective (7), it has been hypothesized that adenosine receptor-signaling mechanisms may be complex, involving agonist interaction with more than one adenylyl cyclase-coupled A2a-receptor subtype. In our hands, both pharmacological manipulations (Figs. 1, 2, and 4) and direct microassay of cAMP production (Fig. 3) indicate that cAMP is involved in adenosine-induced vasodilation of OMDVR.

With regard to the identity of the diffusible mediators that are generated on adenosine stimulation, we investigated two pathways, NO and prostaglandins. The NO pathway is known to be very important in the modulation of OMDVR vasomotor tone and renal medullary blood flow (21, 26, 37). It has been shown that acetylcholine and bradykinin vasodilate OMDVR in an NO-dependent fashion and that NOS blockade can be reversed with excess L-arginine. Abluminal application of L-arginine also produces a concentration-dependent dilation of preconstricted OMDVR, favoring a role for constitutive expression of NOS in these vessels (25, 37). In isolated OMDVR of this study, however, NOS inhibition had no effect on adenosine-induced vasodilation of ANG II-preconstricted vessels (Fig. 4). Interestingly, however, adenosine did elicit an [Ca^2+]_i response in fura 2-loaded OMDVR at both 10^-5 and 10^-9 M. This response was small compared with that generated by the endothelium-dependent vasodilator bradykinin (Fig. 5). On the basis of this comparison, it seems unlikely that Ca^2+ plays a role in adenosine-induced vasodilation. The small responses in Fig. 5 might represent localized effects within the cytoplasm sufficient to stimulate phospholipases or COX. A less likely explanation is that it is due to sizable responses in a small fraction of the endothelial cells that comprise the OMDVR wall.

In addition to NO, vascular endothelial cells can generate vasodilatory prostaglandins (e.g., prostacyclin), and a role for prostaglandins to modulate regional blood flow within the kidney has been established (26). Prostaglandin synthesis leads to redistribution of cortical blood flow toward the juxtamedullary region (13, 16), and blockade of prostaglandin synthesis reduces inner medullary blood flow (17). Interestingly, in this study, indomethacin reversed constriction of OMDVR, implying a role for vasoconstrictor prostaglandins to mediate constriction by the peptide hormones ANG II and ET-1 (Figs. 5 and 7). Such a role for thromboxanes in ANG II-induced vasoconstriction within the kidney has been described by others (21).

Despite the tendency of indomethacin to dilate preconstricted OMDVR, its effect in the presence of adenosine was to promote additional vasoconstriction (Figs. 5, 8, and 10). This observation supports the conclusion that generation of vasodilatory prostaglandin(s) mediates the dilation of OMDVR by adenosine. The results shown in Figs. 9 and 10 imply that this cannot be explained solely by blockade of A2-receptor signaling. An alternate hypothesis is that the apparent effect of vasoconstrictors is enhanced during COX inhibition because arachidonic acid liberated by phospholipase A2 is metabolized to form other vasoconstrictors by the lipoxygenase or 5,450 pathways.

In conclusion, micromolar concentrations of adenosine vasodilate OMDVR in a cAMP-dependent manner. The adenosine response is independent of NOS and does not involve large changes in endothelial cytoplasmic calcium concentration. Nonspecific blockade of COX in OMDVR abrogates vasoconstriction by ANG II and ET-1, implying a role for the generation of prostaglandins in their signaling pathways. In contrast, in
the presence of adenosine, COX blockade in preconstricted OMDVR led to vasoconstriction rather than vasodilation, supporting the hypothesis that prostaglandins are involved in vasodilation by adenosine.

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