Requisite Roles of A_{2A} Receptors, Nitric Oxide, and K_{ATP} Channels in Retinal Arteriolar Dilation in Response to Adenosine

Travis W. Hein, Zhaoxu Yuan, Robert H. Rosa, Jr, and Lih Kuo

Purpose. Adenosine is a potent vasodilator of retinal microvessels and is implicated to be a major regulator of retinal blood flow during metabolic stress. However, the receptor subtypes and the underlying signaling mechanism responsible for the dilation of retinal microvessels in response to adenosine remain unclear. In the present study, the roles of specific adenosine receptor subtypes, nitric oxide (NO), and adenosine triphosphate (ATP)–sensitive K^+ (K_{ATP}) channels in adenosine-induced dilation of retinal arterioles in vitro were examined.

Methods. Porcine second-order retinal arterioles (40–70 μm in internal diameter) were isolated, cannulated, and pressurized to 55 cmH_2O luminal pressure without flow. Diameter changes in response to agonists were recorded by using videomicroscopic techniques.

Results. All vessels exhibited basal tone and dilated dose dependently in reaction to adenosine, N^6-cyclopentyladenosine (an adenosine A_1 receptor agonist), and 2-[*p*-(2-carboxyethyl)]phenylethyl-amino-5′-*N*-ethylcarboxamidoadenosine (CGS21680; an adenosine A_{2A} receptor agonist). These responses were not altered by the selective adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine, but were significantly attenuated by the selective adenosine A_{2A} receptor antagonist 4-(2-{7-amino-2-(2-furyl)[1,2,4]-triazolo[2,3-a][1,3,5]triazin-5-ylamino}ethyl)phenol. Blockade of NO synthase, but not of cyclooxygenase or cytochrome P-450 epoxygenase, significantly attenuated the vasodilations in response to adenosine and CGS21680. The residual vasodilative reactions to both agonists was nearly abolished by the K_{ATP} channel inhibitor glibenclamide.

Conclusions. These data suggest that adenosine evokes retinal arteriolar dilation via activation of A_{2A} receptors and subsequent production of NO and opening of K_{ATP} channels. A better understanding of the fundamental signaling pathways responsible for adenosine-induced dilation of retinal arterioles may help shed light on the possible mechanisms contributing to impaired retinal blood flow regulation in patients after retinal ischemia. (*Invest Ophthalmol Vis Sci.* 2005;46:2113–2119) DOI:10.1167/iovs.04-1438

The microvascular network, especially the arteriolar vessels, plays the central role in regulating blood flow to underlying tissue for proper nutrition and function. Retinal blood flow is closely regulated to meet the metabolic demands of the retinal tissue.[1] Because the retinal circulation lacks autonomic innervation,[1] modulation of retinal vascular tone is vitally dependent on local control mechanisms such as metabolic regulation.[2] In particular, the purine metabolite adenosine has been implicated to be a major vasodilator that mediates autoregulatory adjustments in retinal blood flow.[3–6] The direct evidence for adenosine's vasodilative action has been provided in isolated large retinal arteries[7] and small retinal arterioles.[8] Although small retinal arterioles have the largest capacity for regulating retinal blood flow, the underlying mechanisms involved in the dilation of these vessels by adenosine remain unclear.

In microvascular beds that are highly regulated by local metabolism, such as those in the skeletal muscle,[9] coronary,[10,11] and cerebral circulations,[12] the adenosine A_1 and A_2 receptors have been shown to mediate the arteriolar dilation in response to adenosine. Several lines of evidence have shown that endothelial release of nitric oxide (NO) plays a role, at least in part, in the arterial dilation after adenosine receptor activation in some organ systems.[9,10,13–15] In addition, adenosine receptor-mediated activation of K^+ channels have been shown to contribute to the vasodilative response in various vascular beds,[9,10,13,16,17] including the retina. Specifically, the activation of ATP-sensitive potassium (K_{ATP}) channels appears to be involved in retinal arteriolar dilation by exposure to adenosine in vivo[5] and in vitro.[8] However, the specific adenosine receptor subtype responsible for this K_{ATP} channel–mediated response remains unknown. The possible role of NO or other endothelium-derived vasodilators, such as prostaglandins and cytochrome P-450 metabolites, in the arteriolar response has also not been investigated. Since hemodynamic changes are known to influence vascular function and to produce confounding effects on vasomotor responses to agonists in vivo, we elucidated the relative roles of receptor subtypes, endothelium-derived vasodilators, and K_{ATP} channels in the adenosine-induced dilation of porcine retinal arterioles in an isolated vessel preparation in a defined environment.

Methods

Animal Preparation

All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Scott & White Institutional Animal Care and Use Committee. Pigs (8–12 weeks old of either sex; 7–10 kg) purchased from Barfield Farms (Rogers, TX) were sedated with tiletamine plus zolazepam (4.4 mg/kg, intramuscularly) and xylazine (2.2 mg/kg, intramuscularly), anesthetized with pentobarbital sodium (30 mg/kg, intravenously), intubated, and ventilated with room air. Heparin (1000 U/kg) was administered into the marginal ear vein to prevent clotting, and the eyes were enucleated and immediately placed in a moist chamber on ice.

Isolation and Cannulation of Microvessels

The lens and vitreous body were removed carefully under a dissection microscope. The eye cup was placed in a cooled dissection chamber

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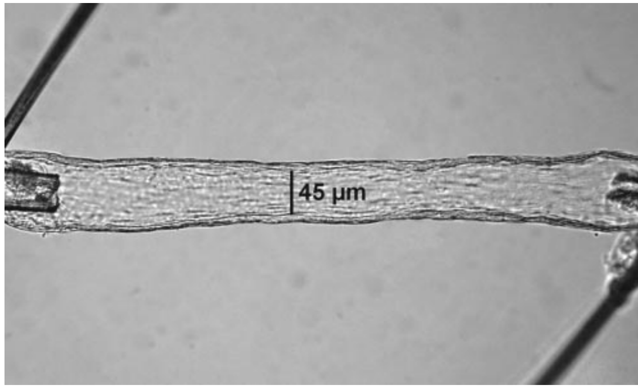

FIGURE 1. An isolated retinal arteriole cannulated with glass micropipettes and secured with ophthalmic sutures. The vessel was transferred to the stage of an inverted microscope and was allowed to develop resting basal tone (45-μm internal diameter) at 55 cmH_2O intraluminal pressure. The image was taken through the video port of an inverted microscope. Magnification, ×20.

(~8°C) containing a physiological salt solution (PSS; in mM: NaCl 145.0, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.17, NaH_2PO_4 1.2, glucose 5.0, pyruvate 2.0, EDTA 0.02, and MOPS 3.0 [3-(*N*-morpholino)propanesulfonic acid]) with 1% albumin (USB, Cleveland, OH). Single second-order retinal arterioles (in the range of 40–70 μm in internal diameter, 0.6–1.0 mm in length) were carefully dissected with a pair of Dumont microdissection forceps (Fine Science Tools, Foster City, CA) with the aid of a stereomicroscope (model SZX12; Olympus, Melville, NY). After careful removal of any remaining neural/connective tissues, the arteriole was then transferred for cannulation to a Lucite vessel chamber containing PSS-albumin solution equilibrated with room air at ambient temperature. One end of the arteriole was cannulated with a glass micropipette (tip outer diameter of 30–40 μm) filled with PSS-albumin solution, and the outside of the arteriole was securely tied to the pipette with an 11-0 ophthalmic suture (Alcon, Fort Worth, TX). The other end of the vessel was cannulated with a second micropipette and also secured with a suture. After cannulation, the vessel and pipettes were transferred to the stage of an inverted microscope (model CKX41; Olympus) coupled to a video camera (Sony DXC-190; Labtek, Campbell, CA) and video micrometer (Cardiovascular Research Institute, Texas A&M University System Health Science Center, College Station, TX) for continuous measurement of the internal diameter throughout the experiment (Fig. 1). The micropipettes were connected to independent pressure reservoirs. Adjusting the height of the reservoirs pressurized the vessel to 55 cmH_2O intraluminal pressure without flow. This level of pressure was used based on pressure ranges that have been documented in retinal arterioles in vivo[18] and in the isolated, perfused retinal microcirculation.[19] Preparations with visible side branches and leaks were excluded from further study.

Experimental Protocols

Cannulated arterioles were bathed in PSS-albumin at 36°C to 37°C, to allow development of basal tone. After vessels developed a stable basal tone (~30–40 minutes), the dose-dependent vasodilations after addition of the natural ligand adenosine (0.1 nM to 100 μM), the A_1 receptor agonist N^6-cyclopentyladenosine (CPA; 0.1 nM to 10 μM), and the A_{2A} receptor agonist 2-[p-(2-carboxyethyl)]phenylethyl-amino-5′-*N*-ethylcarboxamidoadenosine (CGS21680; 0.1 nM to 10 μM; Tocris Cookson, Ellisville, MO) were independently recorded. The vessels were exposed to each concentration of agonist for 3 to 5 minutes until a stabile diameter was established. After the control responses were recorded, the vasodilation elicited by adenosine and by the adenosine receptor agonists was reexamined after a 30-minute incubation of vessels with the selective A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX; 0.1 μM)[10,11,20] or the selective A_{2A} receptor antagonist 4-(2-{7-amino-2-(2-furyl)[1,2,4]-triazolo[2,3-a][1,3,5]triazin-5-ylamino}ethyl)phenol (ZM241385, 1 μM; Tocris Cookson).[10,11,21] In these experiments, usually one or two agonist and antagonists were studied in each vessel, and the sequence of administration of the agonists was alternated in each experiment. After completing the dose-diameter curve, the vessel was washed at least three times, and the other run of the drug study was performed after equilibration of the vessel with PSS-albumin for at least 30 minutes. The reproducibility of the response was confirmed in our pilot studies.

To elucidate the possible signaling mechanisms involved in the retinal arteriolar dilation induced by adenosine and its receptor agonists, the following series of experiments were performed. The contribution of K_{ATP} channels to the adenosine-receptor–mediated vasodilation was examined before and after incubation of isolated arterioles with the specific inhibitor glibenclamide (5 μM). The involvement of prostaglandins, NO, and cytochrome P-450 metabolites in mediating the vascular responses was assessed before and after incubation of vessels with known effective concentrations of the specific inhibitors indomethacin (10 μM),[22,23] N^G-nitro-L-arginine methyl ester, (L-NAME, 10 μM),[10,13] and sulfaphenazole (1 μM),[24] respectively. In a separate series of experiments, we studied the effect of glibenclamide (5 μM), in the presence of L-NAME (10 μM), on adenosine- and CGS21680-induced vasodilations. To confirm the efficacy of glibenclamide and L-NAME, vasodilations induced by the K_{ATP} channel opener pinacidil[10,13] and NO-mediated agonist bradykinin[8] were examined. At the end of each study, a complete dose-dependent vasodilative response to sodium nitroprusside was examined to ensure that the vasodilative function (or vessel preparation) had not deteriorated. Because >90% of glibenclamide binds albumin,[25] the series of experiments using this drug were performed in PSS without albumin. All drugs were administered extraluminally, and each antagonist was incubated for at least 30 minutes.

Chemicals

Drugs were obtained from Sigma-Aldrich (St. Louis, MO) except when specifically stated otherwise. Adenosine, bradykinin, sodium nitroprusside, and L-NAME were dissolved in PSS. CPA, CPX, indomethacin, pinacidil, and sulfaphenazole were dissolved in ethanol, and CGS21680, glibenclamide, and ZM241385 were dissolved in dimethylsulfoxide (DMSO) as stock solutions (10 mM). Subsequent concentrations of these drugs were diluted in PSS. The final concentration of ethanol or DMSO in the vessel bath was 0.1%. Vehicle control studies indicated that this final concentration of solvent had no effect on the arteriolar function.

Data Analysis

At the end of each experiment, the vessel was relaxed with 100 μM sodium nitroprusside in EDTA (1 mM) and calcium-free PSS, to obtain its maximal diameter at 55 cmH_2O intraluminal pressure. All diameter changes in response to agonists were normalized to this maximal vasodilation and expressed as a percentage of maximal dilation.[26] Data are reported as the mean ± SEM and *n* values represent the number of vessels studied. In each set of interventions, the vessels have their own control, with each vessel being from a different eye. The control vessels were pooled for comparison of a series of experiments examining the effect of antagonists on vasodilation. Statistical comparisons of vasomotor responses to the same agonist under various treatments were performed using two-way analysis of variance, with or without repeated measures when appropriate, followed by a Bonferroni multiple-range test. Comparisons of basal tone and vasodilations to pinacidil and bradykinin were performed using the paired Student's *t*-test. $P < 0.05$ was considered significant.

RESULTS

Vasodilation of Retinal Arterioles to Adenosine and Adenosine Receptor Agonists

In this study, all vessels ($n = 47$) showed a similar level of basal tone (constricted to 58% ± 2% of their maximal diameter) at

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36°C to 37°C bath temperature with 55 cmH_2O intraluminal pressure. The average resting and maximal diameters of the vessels were 60 ± 3 and 101 ± 3 μm, respectively. Adenosine, the A_1 agonist CPA, and the A_{2A} agonist CGS21680 produced dose-dependent dilation of isolated retinal arterioles (Fig. 2). The relative order of potency of these agonists was CGS21680 = adenosine > CPA. The higher concentrations of CPA and CGS21680 (i.e., >10 μM) could not be evaluated because of the toxic effect of solvent (>0.1% ethanol and DMSO, respectively), which caused nonspecific dilation of arterioles and loss of basal tone.

Role of Adenosine A_1 and A_{2A} Receptors in Retinal Arteriolar Dilation Induced by Adenosine and Adenosine Receptor Agonists

To evaluate the contribution of A_1 and A_{2A} adenosine receptors to retinal arteriolar dilation in response to adenosine, dose-dependent responses to adenosine analogues were examined in the absence and presence of competitive adenosine receptor antagonists. Blockade of A_1 receptors by CPX had no effect on vasodilation in response to adenosine (Fig. 2A). In contrast, the A_{2A} receptor antagonist ZM241385 abolished vasodilation induced by the lower concentrations of adenosine (≤10 μM) and reduced the response to the highest concentration of adenosine (0.1 mM) from 81% (control) to 25% (Fig. 2A). The dilation of arterioles after addition of CPA was not altered by CPX, but was abolished by ZM241385 (Fig. 2B). Retinal arteriolar dilation in response to CGS21680 also was not altered by CPX, but was almost completely inhibited by ZM241385 (Fig. 2C). It should be noted that ZM241385 did not significantly alter resting basal tone (control: 60% ± 3% versus ZM241385: 58% ± 4%).

Role of K_{ATP} Channels in Adenosine-Induced Retinal Arteriolar Dilation

To determine the relative contribution of K_{ATP} channels to adenosine-mediated vasodilation, dose-dependent responses to adenosine were examined in the absence and presence of the K_{ATP} channel inhibitor glibenclamide. As shown in Figure 3A, glibenclamide abolished vasodilation induced by the lower concentrations of adenosine (≤1 μM) and reduced the response to the highest concentration (0.1 mM) from 87% (control) to 42%. Glibenclamide did not significantly alter resting basal tone (control: 59% ± 5% vs. glibenclamide: 58% ± 5%). The concentration of glibenclamide used in this study appears to be effective, because this antagonist abolished vasodilation in response to the K_{ATP} channel opener pinacidil (Fig. 3B).

Role of Endothelium-Derived Factors in Retinal Arteriolar Dilations in Response to Adenosine and CGS21680

The relative contribution of prostaglandins, cytochrome P-450 metabolites, and NO to adenosine- and CGS21680-induced vasodilations was examined and compared in the absence and presence of their respective inhibitors. Neither indomethacin nor sulfaphenazole inhibited the vasodilation elicited by adenosine (Fig. 4A). However, L-NAME significantly shifted the vasodilative response curves of adenosine (Fig. 4A) and CGS21680 (Fig. 4B) to the right. The L-NAME concentration was effective for blocking NO synthase because it significantly reduced the retinal arteriolar dilation by the NO-mediated agonist bradykinin (10 nM; control: 79% ± 4% of maximal dilation versus L-NAME: 30% ± 5% of maximal dilation; $n = 5$). The resting basal tone was slightly increased by L-NAME, but not significantly (control: 61% ± 4% versus L-NAME: 58% ± 4%; $P = 0.07$). The residual vasodilative responses to adenosine (Fig. 4A) and CGS21680 (Fig. 4B) in the presence of L-NAME

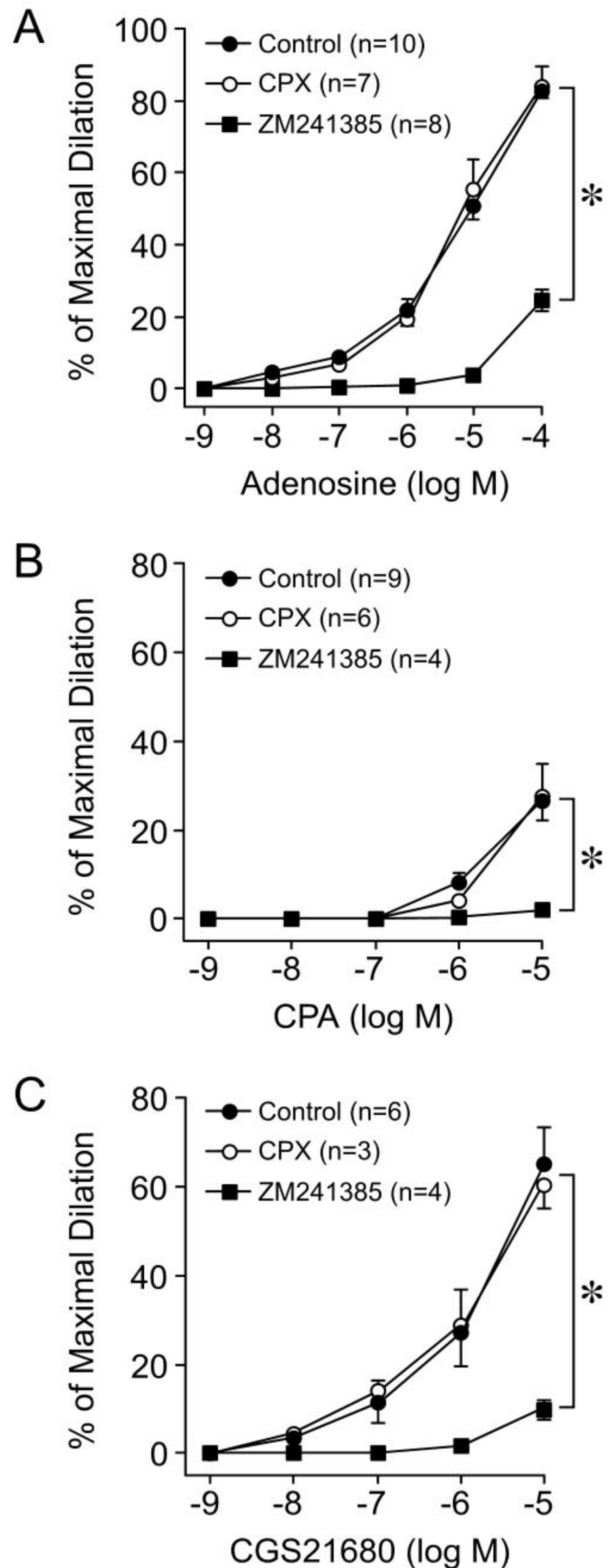

FIGURE 2. Effect of A_1 and A_{2A} receptor blockade on dilation of isolated retinal arterioles to (**A**) adenosine, (**B**) the A_1 agonist CPA, or (**C**) the A_{2A} agonist CGS21680. Dose-dependent vasodilation was examined before (control) and after incubation with the A_1 antagonist CPX (0.1 μM) or the A_{2A} antagonist ZM241385 (1 μM). n, number of vessels. $^*P < 0.05$ versus control.

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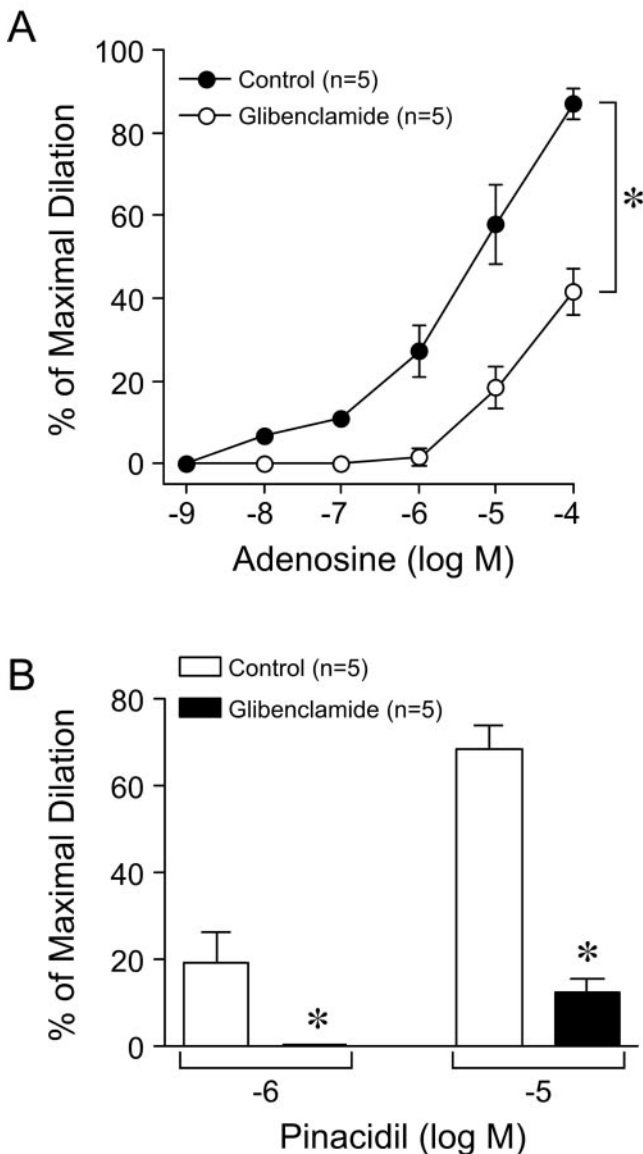

FIGURE 3. Role of K_{ATP} channels in adenosine-induced dilation of isolated retinal arterioles. (**A**) The dose-dependent vasodilative response to adenosine was examined before and after incubation with the K_{ATP} channel inhibitor glibenclamide (5 μM). (**B**) Vasodilation induced by pinacidil was examined before (control) and after incubation with glibenclamide (5 μM). *n*, number of vessels. $^*P < 0.05$ versus control.

were further reduced by subsequent treatment with glibenclamide. It does not appear that L-NAME+glibenclamide or ZM241385 influenced retinal arteriolar function through a nonspecific effect, because vasodilation by sodium nitroprusside was not altered by these agents (Fig. 5).

DISCUSSION

Adenosine, a breakdown product of cellular adenosine triphosphate, is a potent vasodilator in most vascular beds, including the retinal circulation.[5–8] This nucleoside has been proposed to play a significant role in the metabolic regulation of retinal blood flow.[3,4,6,27] The putative regulatory role of adenosine is based on the observations that intravitreal administration of adenosine[5,6,27,28] and adenosine reuptake inhibitors[4,27] evokes dilation of retinal microvessels in vivo. In addition, direct evidence of adenosine's vasodilative action has been provided in isolated large retinal arteries[7] and small retinal arterioles.[8] Our present results demonstrate that the dilation of isolated porcine small retinal arterioles induced by adenosine is mediated by the activation of A_{2A} receptors and subsequent production of NO and opening of K_{ATP} channels.

Four adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3) have been cloned from various cell types, tissues, and species.[29] In microvascular beds that are highly regulated by local metabolism, the A_1 and A_2 receptors have been shown to

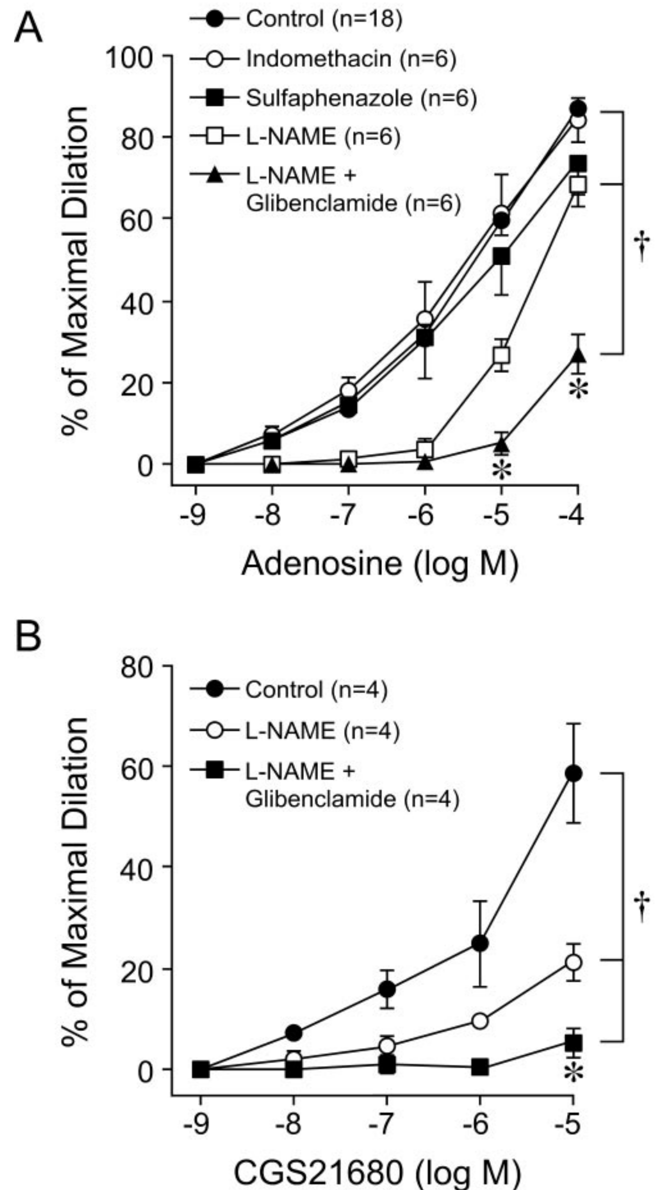

FIGURE 4. Roles of endothelium-derived factors and K_{ATP} channels in isolated retinal arteriolar dilation in response to adenosine and CGS21680. (**A**) Dose-dependent vasodilation after exposure to adenosine was examined before (control) and after incubation with the cyclooxygenase inhibitor indomethacin (10 μM), the cytochrome P-450 inhibitor sulfaphenazole (10 μM), or the NO synthase inhibitor L-NAME (10 μM). Residual vasodilation in the presence of L-NAME was examined after coincubation with the K_{ATP} channel inhibitor glibenclamide (5 μM). (**B**) Dose-dependent vasodilation induced by CGS21680 was examined before (control) and after incubation with L-NAME (10 μM). Residual vasodilation in the presence of L-NAME was examined after coincubation with glibenclamide (5 μM). *n*, number of vessels. $^*P < 0.05$ L-NAME+glibenclamide versus L-NAME; $^†P < 0.05$ versus control.

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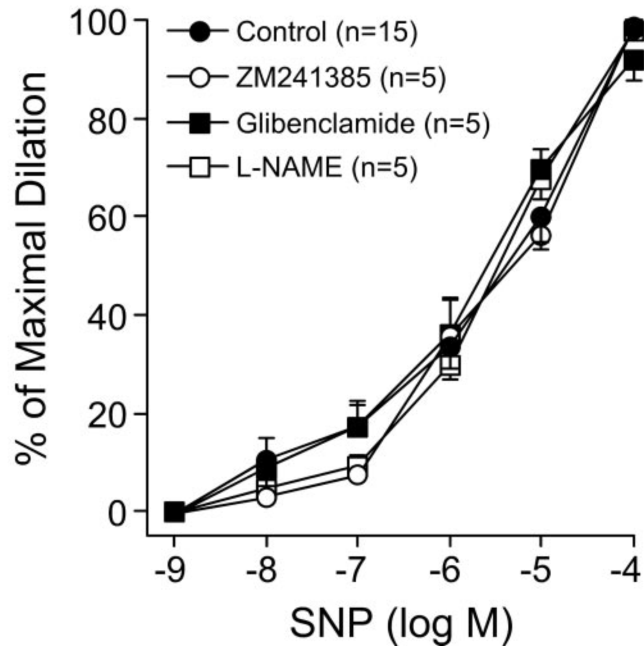

FIGURE 5. Effect of pharmacological inhibitors on isolated retinal arteriolar dilation induced by sodium nitroprusside (SNP). Dose-dependent SNP-induced vasodilation was examined before (control) and after incubation with ZM241385 (1 μM), glibenclamide (5 μM), or L-NAME (10 μM). *n*, number of vessels.

mediate adenosine-induced dilation. Specifically, coronary arterioles dilate in response to A_{2A} and A_{2B} receptor activation,[10,11] cerebral arterioles dilate after A_{2A} receptor activation,[12] and skeletal muscle arterioles dilate after A_1 receptor activation.[9] The receptor subtypes involved in adenosine-induced dilation of retinal arterioles have not been clearly identified. For example, in vivo studies in pigs have shown that intravitreal administration of a nonselective adenosine A_2 antagonist inhibited the dilation of retinal arterioles to adenosine reuptake inhibitors.[27,28] Although the investigators also found that the relative order of potency of adenosine receptor agonists for producing arteriolar dilation was consistent with an A_2 receptor profile, they cautioned that the retinal blood flow and vessel diameter changes after intravitreal administration of adenosine and adenosine receptor agonists may reflect, in part, their indirect effect on the adenosine receptors located on neuronal and glial cells.[30] It is important to note that unequivocal determination of the direct role of adenosine receptors in vascular regulation from the intact organ/tissue[27,28] or in vivo[27,28] study is difficult because blood flow regulation in this situation is mingled with humoral, hemodynamic, and local vessel control mechanisms (i.e., myogenic and flow-induced responses). In our study, we obviated these potential confounding influences by characterizing the adenosine receptor subtypes in a defined environment in an isolated vessel preparation.

Previous interpretations of adenosine receptor involvement in adenosine-induced vasodilation have relied on the reported binding affinities of the adenosine receptor agonists. Two of the most selective agonists that are commercially available for A_1 and A_{2A} receptors are CPA and CGS21680, respectively. CPA has been shown to be 15-fold selective for A_1 compared with A_2 receptors.[20] CGS21680 is 170-fold more selective for A_{2A} than for the other adenosine receptors.[20] In the present study, retinal arterioles dilated in response to adenosine, CPA, and CGS21680. The relative order of potency was CGS21680 = adenosine > CPA, suggesting a prominent role for A_{2A} receptors in retinal arteriolar dilation to adenosine.

The essential role of A_{2A} receptors for retinal arteriolar dilation is further evident in the results showing that A_{2A} antagonist ZM241385, but not A_1 antagonist CPX, significantly reduced adenosine-induced dilation. Precise interpretation of these pharmacological results relies on the selectivity and specificity of the A_{2A} antagonist ZM241385. This recently developed nonxanthine compound is currently the most potent and selective A_{2A} antagonist available.[21] Ligand-binding studies have demonstrated that ZM241385 has 6700-fold selectivity for A_{2A}- over A_1-binding sites.[21] In the present retinal microvascular preparation, ZM241385 specifically abolished the vasodilation induced by both CGS21680 and adenosine, but did not affect vasodilation by sodium nitroprusside. These results not only indicate that ZM241385 is a specific and selective antagonist for the A_{2A} receptor but also suggest that A_{2A} receptor activation is responsible for the adenosine-induced retinal arteriolar dilation. In an unexpected finding, the retinal arteriolar dilation induced by A_1 agonist CPA was not affected by its antagonist CPX, but was effectively inhibited by ZM241385. Although CPA is the most selective A_1 agonist that is commercially available, it has also been reported to activate A_{2A} receptors at higher concentrations.[20] Therefore, CPA-induced dilation is expected to be blocked by ZM241385 if A_{2A} receptors are activated. These results suggest that the dilation elicited by CPA is mediated by the activation of A_{2A} receptors and further imply the lack of functional A_1 receptors in retinal arterioles.

One of the underlying signaling mechanisms implicated in contributing to adenosine receptor-induced dilation in a number of vascular beds is the opening of smooth muscle K^+ channels.[9,10,13,16,17] The smooth muscle K_{ATP} channels are important in the dilation of coronary[10,13] and skeletal muscle arterioles.[9] These earlier studies, as well as numerous others, have investigated the possible functional role of K_{ATP} channels using the pharmacological blocker glibenclamide.[31] An in vivo study in pigs has shown that intravitreal administration of glibenclamide attenuates retinal blood flow in response to exogenous adenosine,[5] indicating that activation of these K^+ channels may be involved in retinal vasodilation caused by adenosine. However, these results are difficult to interpret because the in vivo administration of glibenclamide may affect the activity of retinal pigment epithelium[32] and retinal neurons.[33] The evidence for a possible role of K_{ATP} channels was recently suggested by an in vitro study showing that glibenclamide abolished the dilation of isolated porcine retinal arterioles induced by adenosine.[8] However, a pharmacological preconstrictor was used in that study for vascular tone development, which could have altered the signaling pathways and confounded vasomotor function, as demonstrated in various isolated vessel preparations.[34–40] Our present results showing that glibenclamide attenuated, but did not abolish, the adenosine-induced dilation of porcine retinal arterioles that developed spontaneous myogenic tone supports this idea. Thus, it appears that activation of K_{ATP} channels may not be the sole signaling pathway in the vasodilative response of retinal arterioles to adenosine.

Another signaling pathway that may be involved in retinal arteriolar dilation in response to adenosine is through an endothelium-dependent mechanism. The endothelium has been shown to produce three major vasodilators: NO,[41] prostaglandins,[42] and cytochrome P-450 metabolites.[43] These factors are worth investigating, because the endothelial release of the potent vasodilator NO has been shown to play a role in the dilation of coronary[10,13] and skeletal muscle[9] arterioles in response to adenosine. Because it is difficult to distinguish between possible neuronal- or vascular-mediated mechanisms of action of NO in the retina in in vivo preparations, the isolated vessel preparation provides the most appropriate approach for unambiguous identification of this signaling pathway. In the present study, it does not appear that prostaglandins or cyto-

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chrome P-450 metabolites are involved in retinal arteriolar dilation in response to adenosine because blockade of cyclooxygenase or cytochrome P-450 epoxygenase did not alter the response. In contrast, the blockade of NO synthase with L-NAME reduced the adenosine-induced vasodilation, suggesting that NO contributes in part to the response. Likewise, L-NAME attenuated vasodilation after application of CGS21680, indicating that the adenosine-induced NO production is mediated by A_{2A} receptors. In contrast to in vivo findings in the retinal circulation,[44,45] it is important to note that L-NAME (10 μM) did not significantly increase basal tone of isolated retinal arterioles. This may be due to the absence of luminal flow in our in vitro study, since it has been shown that endothelial cells respond to increased flow (or shear stress) by releasing NO.[23,46] In this regard, it is expected that the NO component would be more pronounced in vivo (i.e., with luminal flow) compared with that in vitro (i.e., without luminal flow) in resting conditions. Therefore, the effect of L-NAME on basal vascular tone would be less apparent in our in vitro study. The present results are consistent with our previous findings in isolated porcine coronary arterioles.[10,13] In the presence of L-NAME, we found that the residual dilation of retinal arterioles to both adenosine and CGS21680 was further reduced by glibenclamide. These data support the idea that both endothelium-derived NO and smooth muscle K_{ATP} channels contribute to the adenosine receptor-activated dilation of retinal arterioles.

Our current findings may provide further insight into the possible adenosine receptor signaling mechanisms associated with retinal ischemia–reperfusion injury. One of the manifestations of ischemia–reperfusion injury in the retinal vasculature is the reduction of retinal blood flow.[47,48] Increased blood flow during early reperfusion is a compensatory reactive hyperemic response to ischemia; however, it may also promote edema, which can directly compress the blood vessels and contribute to hypoperfusion.[49] In addition, postischemic hypoperfusion may result from altered vascular reactivity during prolonged periods of reperfusion.[47] Although retinal microvessels are exposed to an elevated level of adenosine during experimental ischemia[50] and reperfusion,[51] the role of specific vascular adenosine receptors in ischemia–reperfusion injury remain unclear. Previous studies in cats have shown that the nonselective adenosine receptor antagonist 8-sulfophenyltheophylline attenuates postischemic retinal hyperemia, suggesting the involvement of adenosine.[52,53] Based on our results, it is reasonable to speculate that the activation of arteriolar adenosine A_{2A} receptors could contribute to the hyperemic response. An in vivo study in rats has shown that A_{2A} receptor blockade protects retinal function (i.e., recovery of electroretinogram a- and b-waves) and structure after ischemia–reperfusion.[54] Although retinal blood flow was not measured, a possible explanation for the postischemic damage could be hyperemia-induced retinal edema. It is also plausible that the activation of A_{2A} receptors on retinal neuronal cells caused the deleterious effect. However, the direct role of neuronal or vascular A_{2A} receptors in the ischemia–reperfusion injury was not determined in this earlier study. Future studies examining the function of small retinal arterioles after ischemia and reperfusion are necessary to identify the precise role of vascular adenosine A_{2A} receptor activation, as well as NO synthase/K_{ATP} channel signaling, in retinal ischemia–reperfusion injury.

In summary, the results of this study provide the first evidence that retinal arteriolar dilation in response to adenosine is mediated predominantly by A_{2A} receptors. The stimulation of NO synthase and the opening of K_{ATP} channels appear to be two independent mechanisms responsible for the dilation of retinal arterioles in response to the activation of adenosine A_{2A} receptors. Because modulation of retinal vascular tone is fundamentally dependent on local control mechanisms such as metabolic regulation, these findings provide the framework for investigating potential vascular signaling pathways involved in impaired retinal blood flow after retinal ischemia.

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