Adenosine Induces Vasoconstriction through Gi-Dependent Activation of Phospholipase C in Isolated Perfused Afferent Arterioles of Mice

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Abstract. Adenosine induces vasoconstriction of renal afferent arterioles through activation of A1 adenosine receptors (A1AR). A1AR are directly coupled to Gi/Go, resulting in inhibition of adenylate cyclase, but the contribution of this signaling pathway to smooth muscle cell activation is unclear. In perfused afferent arterioles from mouse kidney, adenosine and the A1 agonist N6-cyclohexyladenosine, when added to the bath, caused constriction in the concentration range of 10^{-9} to 10^{-6} M (mean diameter: control, 8.8 ± 0.3 μm; adenosine at 10^{-6} M, 2.8 ± 0.5 μm). Adenosine-induced vasoconstriction was stable for up to 30 min and was most pronounced in the most distal part of the afferent arterioles. Adenosine did not cause vasoconstriction in arterioles from A1AR−/− mice. Pretreatment with pertussis toxin (PTX) (400 ng/ml) for 2 h blocked the vasoconstricting action of adenosine or N6-cyclohexyladenosine. PTX pretreatment did not affect the constrictor response to KCl, whereas the angiotensin II dose-response relationship was shifted rightward. Reverse transcription-PCR revealed expression of Gi but not Go in kidney cortex and preglomerular vessels. The phospholipase C inhibitor U73122 (4 μM) blocked the constriction responses to both adenosine and angiotensin II. In contrast, the adenylate cyclase inhibitor SQ22536 (10 μM) and the protein kinase A antagonist KT5720 (0.1 and 1 μM) did not induce significant vasoconstriction of afferent arterioles. It is concluded that the constriction response to adenosine in afferent arterioles is mediated by A1AR coupled to a PTX-sensitive Gi protein and subsequent activation of phospholipase C, presumably through βγ subunits released from Goi.

Although the dominant effect of adenosine in most vascular beds is vasodilation, the nucleoside can cause vasoconstriction in renal resistance vessels through activation of A1 adenosine receptors (A1AR). Drury and Szent-Gyorgy (1) first observed the renal vasoconstricting effect of adenosine, at the level of the whole kidney, in 1929, and the ability of adenosine to constrict renal afferent arterioles is now well established (2–6). Adenosine released into the extracellular space produces its effects by activating the P1 class of purinergic receptors. P1 receptors belong to the family of G protein-coupled receptors with seven membrane-spanning domains (7). Four members of the P1 receptor family have been identified (designated A1AR, A2aAR, A2bAR, and A3AR), and all four receptor subtypes have been observed to be expressed in the rat kidney (8,9). In the renal vasculature, A1AR have been detected along preglomerular resistance vessels, at both the mRNA and protein levels (10–12). The precise localization and identity of A2AR in the renal vasculature are less certain. Jackson et al. (12) observed only A2bAR protein, with no expression of A2aAR, in preglomerular vessels, whereas functional studies provided evidence for the presence of A2aAR (4,5).

The intracellular signaling mechanisms linking adenosine activation of A1AR to vasoconstriction of renal afferent arterioles have not been defined. Studies in other tissues have established that A1AR can interact directly with Gi or Go proteins to inhibit adenylate cyclase (13). Therefore, it is conceivable that reductions in cAMP levels and decreases in protein kinase A (PKA) activity could be responsible for activation of the contractile apparatus. However, adenosine has also been reported to activate phospholipase C (PLC), which could cause vasoconstriction independent of decreases in cytosolic cAMP levels (14,15).

In this study, we used isolated perfused afferent arterioles from mice to answer the following specific questions. (1) Is the vasoconstriction caused by adenosine mediated by a Gi protein? (2) Does activation of PLC play a role in adenosine-elicited vasoconstriction? (3) Is inhibition of adenylate cyclase or PKA sufficient to cause vasoconstriction? (4) Is the vasoconstriction caused by adenosine transient or persistent? Our results indicate that adenosine causes persistent vasoconstriction of afferent arterioles that is mediated by Gi-coupled activation of PLC, whereas the Gi-dependent inhibition of adenylate cyclase does not seem to play a major role in the constriction response.

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Materials and Methods

Perfused Afferent Arterioles

Afferent arterioles with attached glomeruli were microdissected from A1AR+/+ and A1AR−/− mice of either gender (18 to 26 g) and perfused. The method was a modification of that used by Jensen et al. (16) and Weihprecht et al (17), and its adaptation to mouse arterioles was recently described in detail (18).

The effects of adenosine were determined by using a step-up protocol in which adenosine in the concentration range of 10⁻⁹ to 10⁻⁶ M was added to the bath for 3 min, via changing of the solution in the perfusion chamber. The effect of prolonged exposure to adenosine was examined by applying adenosine at 10⁻⁷ to 10⁻³ M to the bath for 30 min and determining the luminal diameter at 5-min intervals. To assess the role of Go/Gi proteins, we determined dose-response relationships for adenosine, the A1AR agonist N⁶-cyclohexyladenosine (CHA), and angiotensin II before and after pertussis toxin (PTX) (400 ng/ml; Sigma Chemical Co., St. Louis, MO) pretreatment for 2 h.

Time control experiments were performed by generating two dose-response relationships for adenosine and CHA, with an interval of 2 h (the time used for PTX treatment).

The effect of adenylate cyclase inhibition was examined with the adenylate cyclase inhibitor SQ22536 (RBI, Natick, MA). With vessels known to contract in response to 10⁻⁷ M adenosine, SQ22536 (10 or 20 μM) was added to the bath for 30 min; the luminal diameter was then determined. In another set of experiments, the constriction responses to adenosine (10⁻¹⁰ to 10⁻⁷ M) before and after treatment with 10 μM SQ22536 (30 min) were established. The involvement of PKA in the constriction mechanism of adenosine was investigated by adding the specific PKA antagonist KT5720 (10⁻⁷ or 10⁻⁶ M; Sigma) for 45 min. The role of PLC was determined by assessing the responses to adenosine before and after a 30-min exposure to the PLC antagonist U73122 (2 or 4 μM; Cayman Chemical, Ann Arbor, MI). The effects of U73122 on angiotensin II (10⁻⁹ M)-induced and high-potassium solution-induced responses were tested in control experiments. The viability of the specimens was examined at the end of all experiments, with the addition of high-potassium solution.

Reverse Transcription-PCR

To identify the inhibitory G protein coupled to A1AR, we determined the mRNA expression of Go and the three Gi isoforms in the kidney cortex and in preglomerular vessels. Preglomerular vessels, including afferent arterioles and interlobular arteries, were microdissected from slices of mouse kidney in RNAlater solution (Ambion, Austin, TX) at 4°C, and the total length of all branches was determined. RNA was isolated by using Trizol reagent and was reverse-transcribed by using Superscript (Invitrogen, San Diego, CA) and oligo(dT) (Pharmacia, Piscataway, NJ). PCR amplification of identical amounts of cDNA was performed for 35 cycles. The preglomerular vessel cDNA used for PCR analysis corresponded to 1.7 mm of vessel, and the PCR was performed for 38 cycles. Negative control samples contained water instead of cDNA for the PCR, with the addition of RNA instead of cDNA. mRNA obtained from brain tissue was used in positive control experiments.

Statistical Analyses

All data are expressed as means ± SEM. Statistical analyses were performed with ANOVA followed by the Newman-Keuls test or t test for paired data. P < 0.05 was considered significant.

Results

The average baseline diameter for a total of 28 afferent arterioles isolated from A1AR+/+ mice was 8.8 ± 0.3 μm. Successive addition of adenosine at increasing concentrations reduced the arteriolar diameter to 7.8 ± 0.4 μm at 10⁻⁹ M, 6.1 ± 0.5 μm at 10⁻⁸ M, 3.9 ± 0.5 μm at 10⁻⁷ M, and 2.8 ± 0.5 μm at 10⁻⁶ M (Figure 1). The EC⁵₀, as calculated in nonlinear regression analyses with GraphPad Prism software (GraphPad Software, San Diego, CA), averaged 1.7 × 10⁻⁹ M. With all concentrations of adenosine, the effects were most pronounced in the glomerular entrance segment of the arterioles. Baseline diameters of six arterioles from A1AR−/− mice averaged 8.7 ± 0.6 μm, which was not significantly different from the value for A1AR+/+ mice. Adenosine was unable to induce significant constriction in the arterioles from A1AR−/− mice.

During 30-min exposure of afferent arterioles to 10⁻⁷ M adenosine, arteriolar diameters promptly decreased from 7.3 ± 0.36 μm to 4.9 ± 0.8 μm (n = 5, P < 0.05, ANOVA), with no waning of the effect in the subsequent time period (Figure 2). In fact, the constriction effect tended to increase, although the difference between the 1-min and 30-min values was not
With 30 min of washout, the luminal diameter returned to $7.4 \pm 0.3$ $\mu$m (NS, compared with control values).

ADP-ribosylation of the Goi protein with PTX (400 ng/ml) pretreatment for 2 h abolished adenosine-induced vasoconstriction ($n = 6$) (Figure 3A). PTX also completely prevented the constriction response to the A1AR agonist CHA in the concentration range of $10^{-9}$ to $10^{-7}$ M ($n = 5$) (Figure 4A). Time control experiments demonstrated that the initial vasoconstriction produced by adenosine ($10^{-7}$ M, $n = 4$) or CHA ($10^{-7}$ M, $n = 5$) could be repeated after a 2-h waiting period, indicating that the elapsed time itself did not affect the adenosine dose-response relationship (Figures 3B and 4B). These results indicate that the adenosine- and CHA-induced constriction of afferent arterioles is initiated by activation of Goi/Go$\alpha$ proteins.

PTX pretreatment caused a significant rightward shift of the angiotensin II dose-response relationship, with an increase in the EC$_{50}$ from $1.6 \times 10^{-10}$ M under control conditions to $1.2 \times 10^{-9}$ M after PTX treatment ($n = 6$) (Figure 5A). Representative photographs of an arteriole under resting conditions and during exposure to angiotensin II ($10^{-9}$ M), before and after PTX treatment, are presented in Figure 5C. Time control experiments demonstrated no sign of tachyphylaxis ($n = 5$) (Figure 5C).

To identify the G protein activated by A1AR, we determined the presence of mRNA encoding the three Goi isoforms and Go$\alpha$ in the renal cortex and in dissected preglomerular microvessels. In renal cortical tissue, PCR products of the expected size were obtained for Goi1 (135 bp), Goi2 (201 bp), and Goi3 (218 bp) but not Go$\alpha$, although a band of the predicted size (183 bp) was detected in cDNA from brain tissue, which was used as a positive control sample. In micro-dissected preglomerular vessels, bands of the expected size were obtained for Goi1 and Goi2 but not Goi3 or Go$\alpha$ (Figure 6B).

To determine the direct vascular effect of non-ligand-induced reductions in adenylate cyclase activity. For vessels that responded to adenosine ($10^{-7}$ M) with vasoconstriction (from $8.2 \pm 0.5$ $\mu$m to $3.5 \pm 0.7$ $\mu$m, $n = 6$), bath administration of 10 $\mu$M SQ22536 did not significantly change the luminal vessel diameters (from $8.3 \pm 0.5$ $\mu$m to $7.6 \pm 0.5$ $\mu$m) (Figure 7A). A higher concentration of SQ22536 (20 $\mu$M) was also unable to induce vasoconstriction ($n = 3$). After washout of SQ22536, the vessels constricted in response to KCl, indicating intact contractile mechanisms. To assess whether the effect of adenylate cyclase inhibition would be manifest during binding of a ligand, we determined the dose-response relationship for

*Figure 2.* Effect of exposure of afferent arterioles to adenosine ($10^{-7}$ M) for 30 min. Luminal diameters (mean + SEM) before (Basal) and during adenosine addition to the bath are shown; measurements were made every 5 min. After 30 min of washout, the diameter returned to the basal level.

*Figure 3.* (A) Dose-response relationship between the bath adenosine concentration ($10^{-8}$ to $10^{-6}$ M) and the mean luminal diameter of six perfused afferent arterioles before (□) and after (▲) 2-h pretreatment with pertussis toxin (PTX) (400 ng/ml). Values are means + SEM. (B) Time control experiments, showing the luminal diameter response to adenosine ($10^{-8}$ to $10^{-6}$ M) before (□) and after (▲) a 2-h waiting period with no PTX added.


The leftward shift in the dose-response relationship, but the EC$_{50}$ of adenosine (10$^{-9}$ to 10$^{-6}$ M) reduced the diameters by 71.8 ± 5.6%, whereas the diameters were decreased by only 35 ± 10.8% in the presence of 2 μM U73122. Although this dose of U73122 was associated with smaller percentage reductions at all adenosine concentrations, significance was achieved only with 10$^{-7}$ M adenosine (Figure 8A). In contrast, 4 μM U73122 completely eliminated the vasoconstricting effect of adenosine at all concentrations tested (Figure 8B). As demonstrated previously, U73122 also prevented the vasoconstriction caused by angiotensin II at 10$^{-9}$ M (−11.3 ± 7.8% and −1.8 ± 2.3% with 2 and 4 μM U73122, respectively). In contrast, the constriction effect of the high-potassium solution was not affected by U73122.

**Discussion**

Previous pharmacologic evidence indicated that adenosine-induced vasoconstriction is mediated by the A1AR subtype. A1AR are directly coupled to Go/Gi proteins to inhibit adenylate cyclase, but PLC activation is another effect of adenosine binding to A1AR. Therefore, it is not clear whether adenosine-induced vasoconstriction is the result of decreased cAMP levels or increased cytosolic calcium concentrations and whether adenylate cyclase inhibition and PLC activation are interdependent. Our data indicate that adenosine-induced vasoconstriction is initiated by activation of A1AR coupled to a PTX-sensitive Gi protein but that crosstalk between activated Gi and PLC, rather than inhibition of adenylate cyclase, is responsible for activation of the contractile apparatus.

Vasoconstriction of afferent arterioles by adenosine has been demonstrated in a number of different preparations. With the use of micropuncture techniques, afferent arteriolar resistance was calculated to double during intrarenal infusion of adenosine in dogs (19). In the blood-perfused juxtamedullary nephron preparation, adenosine caused significant steady-state reductions of afferent arteriolar vessel diameters, with conversion to vasodilation at higher adenosine concentrations (2,4). Finally, adenosine caused vasoconstriction of isolated perfused afferent arterioles from rabbit kidneys, with maximal constriction of 30% at a concentration of 10$^{-6}$ M (6). It was consistently observed in those experiments that adenosine was a more powerful constrictor in the most distal part of the afferent arterioles, near the glomerular entrance, where the maximal diameter reduction was 45% at a concentration of 10$^{-4}$ M (6). Our studies in a similar arteriolar preparation from mouse kidneys confirmed the constriction potential of adenosine and the longitudinal heterogeneity along afferent arterioles. Compared with rabbits, maximal adenosine-induced constriction in the terminal afferent arterioles of mice was greater (68% versus 45%) and EC$_{50}$ values were approximately 10-fold lower (17 nM versus 220 nM). It is unclear whether these differences are attributable to higher levels of expression of A1AR or lower levels of expression of dilatory A2AR in mice, compared with rabbits. The failure of adenosine to cause vasoconstriction in A1AR-knockout mice directly indicates that adenosine-induced vasoconstriction is the result of A1AR activation. It is noteworthy that the monotonic dose-dependence observed in our experiments did not indicate attenuation of A1AR-medi-

**Figure 4.** (A) Dose-response relationship between the bath concentration of N$^\omega$-cyclohexyladenosine (CHA) (10$^{-9}$ to 10$^{-7}$ M) and the mean luminal diameter of five perfused afferent arterioles before (□) and after (▲) 2-h pretreatment with PTX (400 ng/ml). Values are means ± SEM. (B) Time control experiments, showing the luminal diameter response to CHA (10$^{-9}$ to 10$^{-7}$ M) before (□) and after (▼) a 2-h waiting period with no PTX added.
aded vasoconstriction by simultaneous dilation effects mediated by A2AR, which are known to be expressed in preglomerular arterioles (12). This finding was most likely attributable to the fact that a clear dilation response is usually observed at concentrations higher than $10^{-8}$ M (4), a concentration range not examined in our studies.

In an attempt to address the temporal characteristics of adenosine-induced vasoconstriction, we documented the persistence of A1AR-mediated constriction in afferent arterioles for as long as 30 min. Within this time period, we did not detect any response waning; instead, the degree of constriction tended to increase with exposure time. Previous observations in other preparations also demonstrated constriction maintained for periods longer than 2 min (2,4,6). The absence of rapid desensitization of A1AR in afferent arterioles is in agreement with...
earlier studies that demonstrated that native A1AR were desensitized with a time frame of hours to days (13). Furthermore, human recombinant A1AR expressed in CHO cells were not desensitized with 30 min of exposure to an A1AR agonist, in experiments in which desensitization was assessed as the inhibition of forskolin-stimulated adenylate cyclase activity (20). Nevertheless, in clear contrast to the ability of adenosine

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Figure 7. (A) Effect of exposure of afferent arterioles to SQ22536 for 30 min. Luminal diameters of the same vessels were determined under basal conditions (time 0), after addition of $10^{-7}$ M adenosine (Ado) (time, 30 min), after washout of adenosine (Basal) (time, 55 min), after 30 min of exposure to SQ22536, and after washout of SQ22536 and addition of 100 mM KCl ($K^+$) (time, 100 min). Data shown are means ± SEM for six vessels. * $P < 0.05$, significantly different from basal values. (B) Dose-response relationship between the adenosine concentration ($10^{-9}$ to $10^{-6}$ M) and the luminal diameter of five afferent arterioles before (○) and after (▲) SQ22536 treatment. The basal diameter of the arterioles before exposure to adenosine is also shown. Values are means ± SEM. (C) Effect of the protein kinase A inhibitor KT5720 on afferent arterioles. Luminal diameters of the same vessels were determined under basal conditions (time 0), after addition of $10^{-7}$ M adenosine (Ado) (time, 30 min), after washout of adenosine (Basal) (time, 55 min), after 45 min of exposure to KT5720, and after washout of KT5720 and addition of 100 mM KCl ($K^+$) (time, 115 min). (Left) Treatment with 0.1 mM KT5720 ($n = 6$). (Right) Treatment with 1 mM KT5720 ($n = 3$). Data are means ± SEM.

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Figure 8. (A) Average diameter decrease (percentage of basal diameter) of afferent arterioles in response to bath addition of adenosine ($10^{-9}$ to $10^{-6}$ M) before and during exposure to the phospholipase C (PLC) inhibitor U73122 at 2 mM. Values are means ± SEM for four vessels. Numbers under the bars indicate $P$ values for the effect of U73122 at each adenosine concentration (paired $t$ test). (B) Average diameter decrease (percentage of basal diameter) of afferent arterioles in response to bath addition of adenosine ($10^{-9}$ to $10^{-6}$ M) before and during exposure to the PLC inhibitor U73122 at 4 mM. Values are means ± SEM for five vessels. Numbers under the bars indicate $P$ values for the effect of U73122 at each adenosine concentration (paired $t$ test).
to cause persistent A1AR activation and vasoconstriction, the increase in vascular resistance elicited by adenosine at the whole-kidney level is a transient phenomenon (21). Furthermore, the vasoconstriction response of afferent arterioles in the hydronephrotic kidney preparation has been observed to wane within approximately 1 to 2 min (3,5). Because A1AR activation does not seem to be intrinsically transient, alternative explanations for the short-lasting constricting effect of adenosine in some preparations are required. It is noteworthy that, in most studies in which vasoconstriction was observed to be transient, adenosine was administered from the luminal side of the vessel (5,22). In contrast, in our experiments and in other studies that reported longer-lasting constriction effects, adenosine was applied abluminally. Therefore, luminally applied adenosine may cause the release of endothelial dilating agents, blunting its constricting actions and enhancing its dilating actions (23–25), whereas abluminally applied adenosine may interact directly with AR on smooth muscle cells. In regions in which A1AR predominate, this would cause persistent vasoconstriction. Nevertheless, in one study with the hydronephrotic kidney preparation, adenosine caused only transient constriction of afferent arterioles despite abluminal administration (3). The reasons for this unexpected outcome must be further explored.

Coupling to a Gi protein, with subsequent inhibition of adenylate cyclase, has been one of the defining characteristics of the A1AR class of receptors (26). In addition, there is convincing evidence that adenosine can activate PLC, but it is not clear whether this activation of PLC is an event downstream of Gi coupling or Gi proteins are bypassed. Our experiments demonstrated that the vasoconstriction of afferent arterioles induced by adenosine or by the A1AR agonist CHA was completely blocked by PTX, indicating that coupling of A1AR to a Gi/Go protein in preglomerular smooth muscle cells initiates the vasomotor response. Our studies confirmed an earlier report that demonstrated that the CHA-induced vasoconstriction of isolated perfused rat kidneys could be blocked by PTX (27). Gi/Go-mediated initiation of afferent arteriolar smooth muscle cell activation by adenosine is in agreement with conclusions from studies with smooth muscle cells from cat de-trusor, cat esophagus, and rabbit airways, in which contraction or inositol 1,4,5-trisphosphate accumulation were observed to be PTX-sensitive (15,28,29). PTX sensitivity was also demonstrated for the vasoconstriction of afferent arterioles caused by high concentrations of PGE2, an effect initiated by another Gi-coupled receptor, namely, the EP3 receptor (30). In contrast to the complete blockade of A1AR-induced vasoconstriction, PTX caused a rightward shift in the dose-response curve for the arteriolar constriction caused by angiotensin II, without blocking the full constriction response at a higher concentration range. It was previously demonstrated that angiotensin II receptors are coupled to Gi proteins and that some of the effects of angiotensin II are sensitive to PTX (31,32). However, because angiotensin II uses multiple PTX-insensitive transduction pathways, with the most important for vasoconstriction of afferent arterioles being coupling to Gq, PTX does not entirely eliminate vasomotor responses (33). This is consistent with the previous demonstration that angiotensin II-induced constriction of afferent arterioles was not inhibited by the Gi/Go antagonist N-ethylmaleimide (34).

The identity of the inhibitory G proteins coupled to A1AR in afferent arterioles or other parts of the kidney is still unknown. Gi1, Gi2, Gi3, and Go proteins have all been observed to couple to A1AR (35,36). Our results documented the presence of mRNA for all three Go isoforms (Go1, Go2, and Go3) in renal cortical tissue, whereas Goiso mRNA was undetectable. Expression of Gi1 and Gi2 mRNA was also observed in microdissected preglomerular vessels (interlobular arteries and afferent arterioles), whereas Gi3 and Go were undetectable. The presence of Gi1, Gi2, and Gi3 protein in isolated glomeruli and preglomerular arterioles was previously demonstrated with immunoblotting assays (37,38). Immunohistochemical analyses demonstrated a staining pattern consistent with expression of Gi1 protein in glomerular endothelial cells (39). Therefore, the available evidence is insufficient to establish which type of Gi protein is coupled to A1AR in renal microvessels, but we conclude that the vasoconstriction response mediated by A1AR is probably dependent on Gi, rather than Go.

The relationship between the decreases in intracellular cAMP levels and PKA activation resulting from Gi activation and the vascular vasoconstriction response is not entirely clear. This is somewhat in contrast to the evidence supporting the converse, namely, vasodilation caused by Gs activation. For example, the dilation response initiated by A2AR in mesenteric arteries or descending vasa recta is likely to be related to increases in cytosolic cAMP concentrations and PKA activation (40,41). Activation of the cAMP/PKA pathway may cause vasodilation by enhancing the activity of ATP-sensitive potassium channels, by inhibiting voltage-activated calcium channels, or by reducing myosin light chain phosphorylation (40,42,43). However, cAMP may not be directly involved in the signaling pathway leading to constriction of afferent arterioles, because inhibition of adenylate cyclase by SQ22536 or of PKA by KT5720 was not sufficient to induce vasoconstriction in afferent arterioles. Furthermore, the dose-dependence of vasoconstriction caused by adenosine was not significantly altered by adenylate cyclase inhibition. If progressive inhibition of adenylate cyclase were an important factor contributing to the vasoconstriction response resulting from Goi activation, then marked enhancement of the constricting effect of low doses of adenosine under conditions of continuous adenylate cyclase inhibition might be expected.

Crosstalk between ligand-activated Gi and PLC was strongly suggested by our observation that the adenosine-induced vasoconstriction was prevented by a PLC inhibitor. Activation of PLC by adenosine is most likely mediated by βγ subunits released from Goi. There is strong evidence that some isoforms of PLC-β, specifically PLC-β2, can be directly activated by βγ subunits in reconstituted membranes (44,45). Our findings are in agreement with observations in nonvascular smooth muscle cells, in which adenosine-induced contraction was prevented by inhibitors of both Gi proteins and PLC (15,28,29,45). Activation of PLC through coupling to Gi/Go has also been demonstrated for M2 muscarinic, somatostatin,
and opioid receptors (46–48). We conclude that activation of Gi proteins and subsequent activation of PLC are required for A1AR-mediated vasoconstriction of renal afferent arterioles. Activation of PLC is expected to stimulate inositol 1,4,5-trisphosphate-mediated release of calcium from the endoplasmic reticulum. In fact, adenosine and A1AR agonists have been observed to increase intracellular calcium concentrations in rabbit afferent arterioles and intestinal smooth muscle cells (49, 50). We speculate that the calcium influx is maintained by activation of voltage-dependent calcium channels after depolarization, which is most likely produced through calcium-activated chloride channels.

In summary, adenosine mediated a sustained vasoconstriction effect in the renal afferent arterioles of wild-type mice but not A1AR−/− mice, indicating that adenosine acts through A1AR to cause constriction. Adenosine-induced vasoconstriction was fully blocked by PTX pretreatment and by the PLC inhibitor U73122 but was not affected by the adenylate cyclase inhibitor SQ22536 or the PKA inhibitor KT5720. These data suggest that A1AR-dependent activation of Gi is coupled to PLC, causing the release of calcium from intracellular stores and the initiation of vasoconstriction.

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