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Adenosine Diphosphate Ribose Dilates Bovine Coronary Small Arteries through Apyrase- and 5′-Nucleotidase-Mediated Metabolism

David X. Zhang Ai-Ping Zou Pin-Lan Li

Departments of Pharmacology and Toxicology and Physiology, Medical College of Wisconsin, Milwaukee, Wisc., USA

Key Words

Adenosine diphosphate ribose · Vasodilation · Adenosine · Apyrase · 5′-Nucleotidase · Coronary vessels · Vascular smooth muscle

Abstract

Cyclic adenosine diphosphate ribose and adenosine diphosphate ribose (ADPR) play an important role in the regulation of intracellular Ca^{2+} release and K^+ channel activity in the coronary arterial smooth muscle. The role of these signaling nucleotides in the control of vascular tone has yet to be determined. The present study was designed to determine whether ADPR produces vasodilation in coronary arteries and to explore the mechanism of action of ADPR. ADPR (10–60 μmol/l) was found to produce endothelium-independent relaxation in a concentration-dependent manner in isolated and pressurized small bovine coronary arteries. The ADPR-induced vasodilation was substantially attenuated by adenosine deaminase (0.2 U/ml), and the P_1 purinoceptor antagonist 8-(*p*-sulfophenyl)theophylline (50 μmol/l), with maximal inhibitions of 60 and 80%, respectively. When the coronary arterial homogenates were incubated with ADPR, the production of adenosine and 5′-AMP was detected. The adenosine production was blocked by the 5′-nucleotidase inhibitor, α,β-methylene adenosine 5′-diphosphate (MADP, 1 mmol/l), which was accompanied by a corresponding accumulation of 5′-AMP. This 5′-AMP accumulation was substantially inhibited by the apyrase inhibitor sodium azide (10 mmol/l). Moreover, ADPR was hydrolyzed into 5′-AMP by purified apyrase. In agreement with their inhibitory effect on the adenosine production, MADP and sodium azide significantly attenuated the vasodilator response to ADPR. The metabolism of ADPR to adenosine was only detected in cultured coronary arterial smooth muscle cells but not in endothelial cells. We concluded that ADPR produces vasodilation in small coronary arteries and that the action of ADPR is associated with the adenosine production via an apyrase- and 5′-nucleotidase-mediated metabolism.

Adenosine diphosphate ribose (ADPR) and cyclic adenosine diphosphate ribose (cADPR) are endogenous metabolites of nicotinamide adenine dinucleotide (NAD). ADPR is produced by hydrolysis of NAD by NAD glycohydrolase or hydrolysis of cADPR by cADPR hydrolase, and cADPR is formed by cyclizing NAD via ADP ribosylcyclase [1–4]. Both nucleotides have been detected in a variety of tissues such as heart, liver, spleen and brain and in red blood cells, lymphocytes, pituitary cells and renal epithelial cells, and have been demon-

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Pin-Lan Li, MD, PhD
Department of Pharmacology and Toxicology, Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee, WI 53226 (USA)
Tel. +1 414 456 8635, Fax +1 414 456 6545, E-Mail pli@mcw.edu

strated to mediate cellular signaling [5–9]. It has been reported that cADPR as a Ca^{2+}-mobilizing second messenger mediates the secretion of insulin in pancreatic β cells, the fertilization of eggs and the estrogen response in the rat uterus [10–12]. Recent studies have indicated that cADPR also importantly contributes to the effect of nitric oxide on intracellular Ca^{2+} mobilization in nonvascular and vascular tissues [13–18]. In vascular smooth muscle, the production of cADPR can be decreased by nitric oxide, thereby resulting in a decrease in intracellular Ca^{2+} concentration and vasodilation [18].

Compared with cADPR, the biological function of ADPR as a signaling molecule is less well known. ADPR is thought to participate in cell signaling primarily through the nonenzymatic ADP ribosylation, and this ADP ribosylation has been implicated in a number of biological events including DNA excision repair and translational regulation of cellular proteins [19, 20]. Other studies have demonstrated that ADPR is involved in the regulation of platelet aggregation and the gating of the fertilization channel in ascidian oocytes [21, 22]. A recent study in our laboratory has indicated that ADPR increases the activity of Ca^{2+}-activated K^+ (K_{Ca}) channels in coronary arterial smooth muscle cells (SMCs), suggesting that this nucleotide may be a vasodilator in coronary circulation [23]. Given the importance of NAD/NADH in determining the tissue redox status, ADPR as a metabolite of NAD may be involved in the regulation of tissue perfusion and redox status through its vasodilator effect. The present study was designed to determine whether ADPR produces vasodilation in isolated and pressurized small bovine coronary arteries and to explore the possible mechanisms mediating ADPR-induced vasodilation. Based on the results of arterial preparation, we went on to define the role of the apyrase- and 5′-nucleotidase-mediated metabolism in the vasodilator effect of ADPR in coronary arteries.

Materials and Methods

Isolated Small Coronary Artery Preparation

Fresh bovine hearts were obtained from a local abattoir. The left ventricular wall was rapidly dissected and immersed in ice-cold physiological saline solution (PSS) containing the following composition (mmol/l): NaCl, 119; KCl, 4.7; $CaCl_2$, 1.6; $MgSO_4$, 1.17; NaH_2PO_4, 1.18; $NaHCO_3$, 24; EDTA, 0.026, and glucose, 5.5, pH 7.4. This myocardial section was transported immediately to the laboratory. Small intramural coronary arteries from the left anterior descending artery were carefully dissected and placed in cold PSS until cannulation (up to 4 h). Segments of small arteries (100–200 mm inner diameter) were transferred to a water-jacketed perfusion chamber and cannulated with two glass micropipettes at their in situ length, as we described previously [24]. The outflow cannula was clamped, and the arteries were pressurized to 60 mm Hg and bathed in PSS that was equilibrated with 95% O_2/5% CO_2 and maintained at pH 7.4 and 37°C. After 1 h equilibration, the internal diameter of arteries was measured with a video system composed of a stereomicroscope (Leica MZ8), a charge-coupled device camera (KP-MI AU, Hitachi), a video monitor (VM-1220U, Hitachi), a video measuring apparatus (VIA-170, Boeckeler Instrument) and a video printer (UP890 MD, Sony). The arterial images were recorded continuously with a videocassette recorder (M-674, Toshiba).

To determine the vasodilator response of ADPR, the arteries were precontracted by 50 ± 10% of their resting diameter with the thromboxane A_2 analogue U46619. Once steady-state contraction was obtained, cumulative dose response curves of ADPR (10–60 μmol/l) were determined by measuring changes in the internal diameter. Then, these arteries were either denuded to determine the dependence of ADPR action on endothelium or treated with adenosine deaminase (ADA, 0.2 U/ml), 8-(*p*-sulfophenyl)theophylline (8-PST, 50 μmol/l), α,β-methylene adenosine 5′-diphosphate (MADP, 100 μmol/l) or sodium azide (10 mmol/l) for 15 min to examine the possible mechanisms mediating ADPR-induced vasodilation. The endothelium was denuded by perfusion with air bubbles for 3–5 min [25]. The endothelial denudation was verified by determination of bradykinin-induced vasodilation after precontraction with U46619. In contrast to the vasodilator response in endothelium-intact vessels, bradykinin produced vasoconstriction in denuded arteries. All drugs were added into the bath solution. Between pharmacological interventions, the arteries were washed three times with PSS and allowed to equilibrate in drug-free PSS for 20–30 min. The vasodilator response was expressed as percent relaxation of U46619-induced precontraction based on changes in the internal diameter.

Culture of Coronary Arterial Endothelial Cells and SMCs

The bovine coronary arterial endothelial cells (ECs) and SMCs were cultured as described previously [26]. Briefly, the arteries were rinsed with medium 199 containing 5% FCS, 2% solution of antibiotics (penicillin-streptomycin-amphotericin B), 0.3% gentamycin and 0.3% nystatin, and cleaned off connective tissues. The lumen of arteries was filled with 0.25% collagenase in medium 199 and incubated at 37°C for 30 min. The arteries were then flushed with medium 199, and the detached ECs were collected and cultured in RPMI 1640 containing 25% FCS, 1% glutamine and 1% antibiotic solution. The denuded arteries were cut into small pieces, placed into dishes with the lumen side down and incubated in medium 199 containing 10% FCS, 1% glutamine, 1% antibiotic solution, 0.3% gentamycin, 0.3% nystatin and 0.1% tylosin for 3–5 days until SMCs migrated to the dishes. Once SMC growth had been established, the vessels were removed, and cells were grown in medium 199 containing 20% FCS. All cells were maintained in an incubator with 5% CO_2 in air at 37°C. The ECs were identified by morphological appearance (i.e. cobblestone array) and positive staining for von Willebrand factor antigen. The SMCs were identified by the positive staining with an anti-α-actin antibody. All studies were performed using the cells of 2–4 passages.

Preparation of Coronary Arterial and SMC Homogenates

Small bovine coronary arteries were dissected as described above. The dissected arteries were cut into very small pieces and homogenized with a glass homogenizer in ice-cold HEPES buffer containing

(mmol/l): Na-HEPES, 25; sucrose, 255; EDTA, 1; phenylmethylsulfonyl fluoride, 0.1. After centrifugation at 1,000 *g* for 10 min at 4°C, the supernatants containing the membrane protein and cytosolic components, termed homogenates, were frozen in liquid N_2 and stored at –80°C until used [24]. The SMC or EC homogenates were prepared using the same procedure. Total protein concentrations of the homogenates were measured with a Bio-Rad protein assay kit according to the procedures described by the manufacturer.

HPLC Analysis of Adenosine and 5′-AMP

To examine the conversion of ADPR to adenosine by coronary arteries, the coronary arterial, SMC or EC homogenates (100 μg protein) were incubated with 0.01–1 mmol/l ADPR and 100 μmol/l erythro-9-(2-hydroxy-3-nonyl)adenosine (EHNA), an adenosine deaminase inhibitor, at 37°C for 1 h in an assay buffer containing (mmol/l): HEPES, 10; NaCl, 148; KCl, 5; $CaCl_2$, 1.8; $MgCl_2$, 1.0, and glucose, 5.5, pH 7.4. The reaction mixture was then rapidly frozen in liquid N_2 to terminate the reaction. To address the metabolic pathway of ADPR, either the selective 5′-nucleotidase inhibitor MADP (0.1–1,000 μmol/l) or the apyrase inhibitor sodium azide (1–10 mmol/l) [27] was incubated with the homogenates 5 min before addition of ADPR. The metabolism of ADPR to 5′-AMP by purified apyrase was determined by incubating this enzyme (15 μg protein) with 0.01–10 mmol/l ADPR and 100 μmol/l EHNA at 37°C for 1 h. To determine the localization of the apyrase-mediated ADPR metabolism, the confluent ECs or SMCs (1–2 × 10^6 cells) were washed and incubated in PSS containing 1 mmol/l ADPR and 100 μmol/l EHNA for 2 h. The cells were then pelleted by a brief centrifugation, the supernatants rapidly frozen in liquid N_2. Before HPLC analysis, the reaction mixtures or supernatants were filtered with an Amicon microultrafilter to remove the proteins.

The HPLC system included a Hewlett-Packard 1090L solvent delivery system and a 1040A photodiode array detector with a 20-μl flow cell (Hewlett-Packard, Avondale, Pa., USA). Data were collected and analyzed with a Hewlett-Packard Chemstation. Nucleotides were separated on a Supelcosil LC-18T (3 μm, 4.6 × 150 mm) with a Supelcosil LC-18 guard column (5 μm, 4.6 × 20 mm; Supelco, Bellefonte, Pa., USA). A gradient system with two solvents was applied. Solvent A consisted of 5% acetonitrile in 5 mmol/l potassium dihydrogen phosphate buffer containing 5 mmol/l tetrabutylammonium hydrogen sulfate (pH 5.5). Solvent B consisted of 50% acetonitrile in the same buffer. The elution run began with 100% solvent A for 4 min, then a linear gradient was established from 100% solvent A and 0% solvent B to 0% solvent A and 100% solvent B during the next 12 min. The elution was then kept isocratic with 100% solvent B for another 8 min till completed. During the next 5 min the mobile phase was restored to 100% solvent A. A new sample was introduced after a further 10-min re-equilibration with 100% solvent A. The injection volume was 20 μl. The flow rate was 0.8 ml/min. The column eluate was monitored at 254 nm. The peak identities were confirmed by comigration with known standards and absorbance spectra compared with the known standards. Quantitative measurements were performed by comparing the peak area of the products in the samples with known concentrations of standards [23].

Statistics

Data are presented as means ± SEM. The significance of the differences in mean values between and within multiple groups was examined using an analysis of variance for repeated measures followed by Duncan's multiple range test (Sigmastat). Student's t test was used to evaluate statistical significance of the differences between two paired observations. $p < 0.05$ was considered statistically significant.

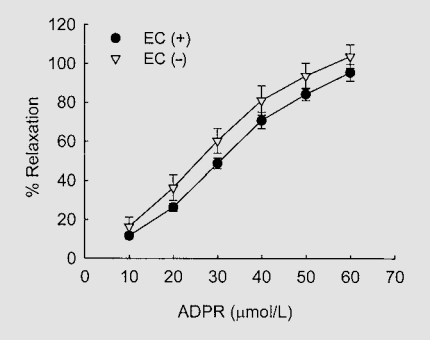

Fig. 1. ADPR-induced vasodilation in bovine coronary small arteries precontracted with the thromboxane A_2 analogue U46619. EC (+) = Endothelium-intact; EC (–) = endothelium-denuded.

Results

Effect of ADPR on Arterial Diameters in Endothelium-Intact and -Denuded Small Coronary Arteries

As shown in figure 1, ADPR (10–60 μmol/l) produced a concentration-dependent vasodilation in U46619-precontracted small bovine coronary arteries. A complete relaxation was observed at 60 μmol/l ADPR with an IC_{50} of 30 μmol/l. Removal of vascular endothelium had no effect on the vasodilator response to ADPR.

Effect of ADA and 8-PST on ADPR-Induced Vasodilation

To determine whether ADPR-induced vasodilation is associated with the production of adenosine, the arteries were pretreated with ADA (0.2 U/ml), an enzyme degrading adenosine. As shown in figure 2a, ADA markedly attenuated the vasodilator response to ADPR. In the presence of ADA, ADPR (60 μmol/l) only produced 30% relaxation, which is much less than 90% relaxation in ADA-untreated arteries. To further confirm the role of adenosine and activation of purinergic receptors in ADPR-induced vasodilation, we tested the effect of the P_1 purinoceptor antagonist 8-PST on the ADPR-induced coronary vasodilation. Pretreatment of the arteries with 8-PST (50 μmol/l) substantially blocked the vasodilator response to ADPR

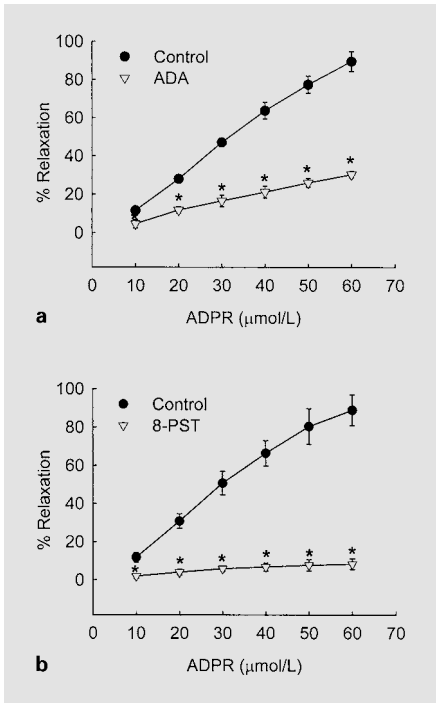

Fig. 2. Effect of ADA and the adenosine receptor antagonist 8-PST on ADPR-induced vasodilation in bovine coronary small arteries. **a** Effect of ADA (0.2 U/ml) on ADPR-induced vasodilation. **b** Effect of 8-PST (50 μmol/l) on ADPR-induced vasodilation. * $p < 0.05$ versus control.

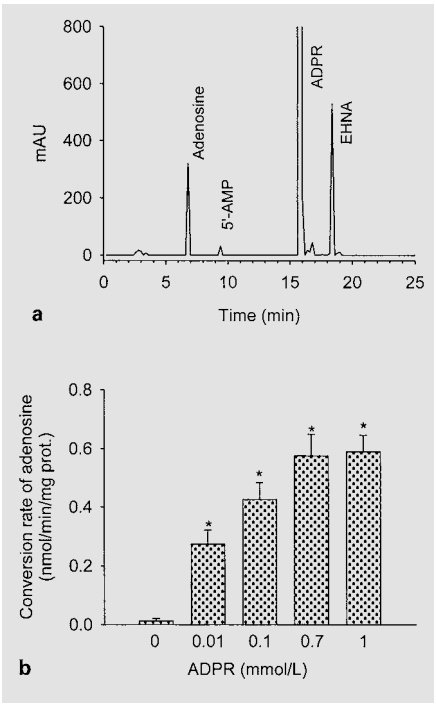

Fig. 3. Metabolism of ADPR to 5′-AMP and adenosine by bovine coronary arteries. **a** Reverse-phase HPLC depicting conversion from ADPR to 5′-AMP and adenosine by coronary arterial homogenates. **b** Conversion rate of ADPR to adenosine. * $p < 0.05$ versus control.

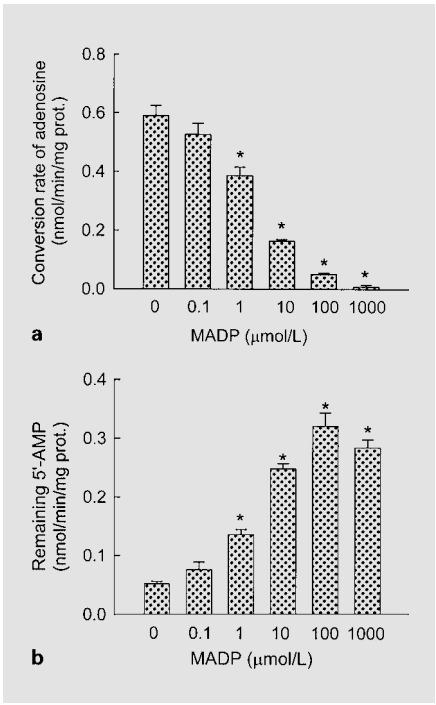

Fig. 4. Effect of MADP, a 5′-nucleotidase inhibitor, on the metabolism of ADPR (1 mmol/l) to 5′-AMP and adenosine in bovine coronary arteries. **a** Effect of MADP on the production of adenosine. **b** Effect of MADP on the production of 5′-AMP. * $p < 0.05$ versus control.

(fig. 2b), at a dose that blocked the adenosine-induced vasodilation. ADA and 8-PST had no effect on the basal vascular diameter and the vasodilation induced by bradykinin, an endothelium-dependent vasodilator.

Production of Adenosine from ADPR in Coronary Arteries

The coronary arterial homogenates were incubated with 1 mmol/l ADPR as a substrate and EHNA as an inhibitor of adenosine degradation. A typical HPLC chromatogram depicting the production of adenosine is presented in figure 3a. A peak with a retention time of 6.6 min in the reaction filtrates was detected and coeluted with synthetic adenosine. A small peak with a retention time of 9.4 min was confirmed as 5′-AMP. The peak at 15.8 min coeluted with ADPR, indicating presence of unreacted ADPR. The peak at 18.3 min coeluted with EHNA. Figure 3b summarizes the data showing the conversion rate of ADPR to adenosine by coronary arterial homogenates. There was a concentration-dependent production of adenosine from ADPR, with a maximal conversion rate of 0.59 ± 0.06 nmol/min/mg protein. 5′-AMP was also detected in the reaction mixture with a rate of 0.052 ± 0.004 nmol/min/mg protein, which represented a remaining amount of 5′-AMP under the actions of endogenous 5′-nucleotidase. In the absence of EHNA, the production of adenosine was also detected when the arterial homogenates were incubated with ADPR (data not shown).

Effect of MADP on the Metabolism of ADPR in Coronary Arteries

To determine the enzymatic activity responsible for the conversion of ADPR to adenosine, we examined the effect of 5′-nucleotidase inhibition on the adenosine production from ADPR. The coronary arterial homogenates were incubated with the 5′-nucleotidase inhibitor MADP (0.1–1,000 μmol/l), and the production of adenosine and 5′-AMP from ADPR was measured. A high concentration of ADPR (1 mmol/l) was used to maximize the activity of

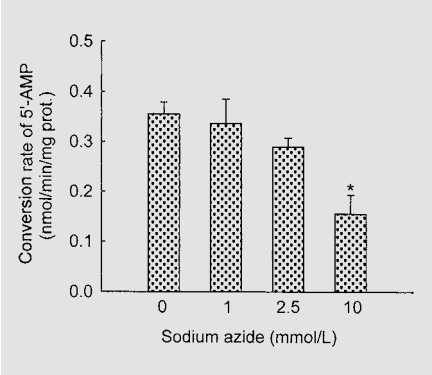

Fig. 5. Effect of sodium azide, an apyrase (ecto-ATP diphosphohydrolase) inhibitor, on the metabolism of ADPR (1 mmol/l) to 5′-AMP in bovine coronary arteries. * $p < 0.05$ versus control.

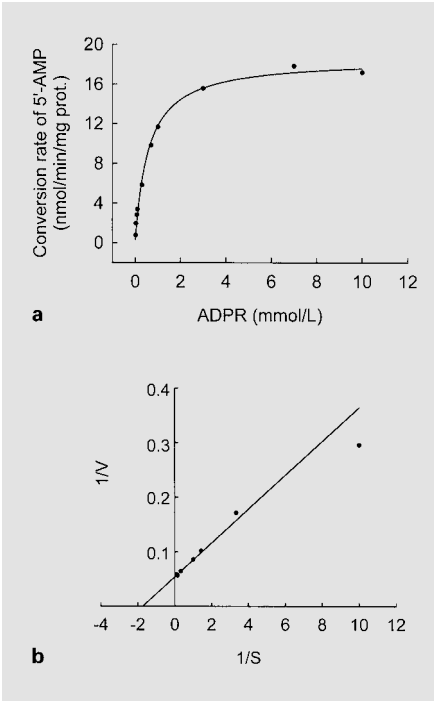

Fig. 6. Kinetic study of conversion of ADPR to 5′-AMP by purified apyrase. **a** Concentration-dependent conversion of ADPR to 5′-AMP. **b** Lineweaver-Burk plot of the data from **a**. 1/V = 1/conversion rate of 5′-AMP; 1/S = 1/[ADPR].

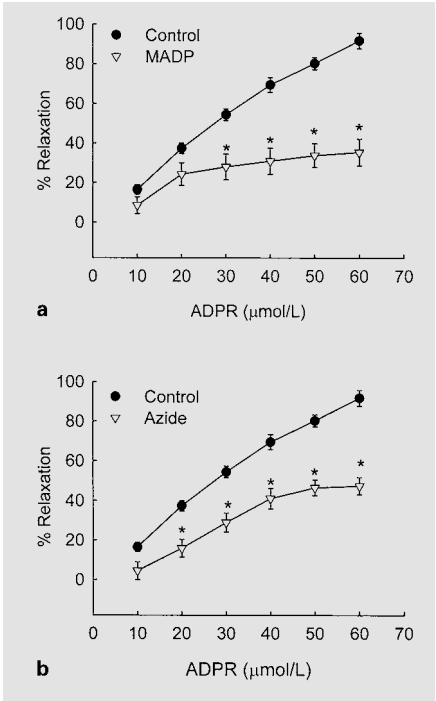

Fig. 7. Effect of MADP and sodium azide on ADPR-induced vasodilation in bovine coronary small arteries. **a** Effect of MADP (100 μmol/l) on ADPR-induced vasodilation. **b** Effect of sodium azide (10 mmol/l) on ADPR-induced vasodilation. * $p < 0.05$ versus control.

enzymes responsible for the production of adenosine. MADP concentration-dependently inhibited the production of adenosine from ADPR, which was accompanied by a concentration-dependent increase in 5′-AMP, an intermediate metabolite of ADPR in the reaction mixture (fig. 4). Similarly, concanavalin A, another inhibitor of 5′-nucleotidase, decreased the production of adenosine and increased the 5′-AMP production from ADPR in a concentration-dependent manner (data not shown).

Effect of Sodium Azide on the Metabolism of ADPR in Coronary Arteries

To further explore the mechanism by which ADPR is converted to 5′-AMP, then to adenosine in the coronary arteries, we examined the effect of the apyrase inhibitor sodium azide on the production of 5′-AMP when coronary arterial homogenates were incubated with ADPR. As shown in figure 5, sodium azide (1–10 mmol/l) concentration-dependently inhibited the production of 5′-AMP from ADPR in coronary arterial homogenates, with 60% inhibition at the highest concentration of sodium azide studied.

Kinetic Study on the Production of 5′-AMP from ADPR by Purified Apyrase

To confirm the activity of apyrase in converting ADPR to 5′-AMP, the purified apyrase (15 μg protein) was incubated with ADPR and the production of 5′-AMP was detected. Apyrase was found to catalyze the conversion of ADPR to 5′-AMP. The K_m and V_{max} of this enzyme using ADPR as a substrate were 0.58 mmol/l and 18.6 nmol/min/mg protein, respectively (fig. 6).

Effect of MADP and Sodium Azide on ADPR-Induced Vasodilation

To confirm that ADPR-induced vasodilation was mediated by the production of adenosine via 5′-nucleotidase and apyrase, the arteries were pretreated with MADP (100 μmol/l) or sodium azide (10 mmol/l). As shown in figure 7, MADP and sodium azide significantly attenuat-

ed the vasodilator response to ADPR. The inhibition of ADPR-induced vasodilation by MADP and sodium azide correlated well with their inhibitory action on the production of adenosine or 5′-AMP from ADPR.

Production of Adenosine from ADPR by the Coronary Arterial SMCs and ECs

To further localize the enzyme activity catalyzing the conversion of ADPR to adenosine, cultured coronary arterial SMCs and ECs or homogenates were incubated with ADPR, and the adenosine level in the incubation media or reaction mixture was measured. Adenosine was found to accumulate in the incubation media of ADPR-treated SMCs, and the conversion rate was 0.29 ± 0.04 nmol/min/10^5 cells. However, there was no detectable adenosine or 5′-AMP in the incubation media of ADPR-treated ECs. For SMC homogenates, the conversion rate of adenosine was 0.28 ± 0.01 nmol/min/mg protein.

Discussion

In the present study, we demonstrated that ADPR produced a concentration-dependent vasodilation in small resistant coronary arteries. Removal of the endothelium had no effect on the vasodilator response to ADPR in this coronary arterial preparation. These results provide the direct evidence that ADPR is a potent vasodilator in small coronary arteries and this ADPR-induced vasodilation is endothelium independent. To our knowledge, little is known regarding the effect of ADPR on coronary arterial tone. However, previous studies have shown that the precursor of ADPR, NAD, lowers blood pressure, relaxes smooth muscle from isolated guinea pig taenia coli and dilates dog coronary and rat mesenteric arteries [28–31], but it is unknown whether these effects of NAD are associated with the formation of ADPR. Recently, we have reported that nicotinamide, a co-product of ADPR from NAD hydrolysis, produced a significant coronary vasodilation, but nicotinamide-induced vasodilation does not appear to be related to the effect of ADPR, rather than to the blockade of cADPR-induced intracellular Ca^{2+} mobilization [32]. The findings in the present study indicate that ADPR dilates coronary arteries, which may contribute to the vasodilator effect of NAD in coronary circulation. Since ADPR has been reported to produce vasodilation in rat mesenteric arteries, it is possible that this nucleotide may serve as a common vasodilator in systemic circulation and different organs to regulate a circulatory adaptive response to tissue metabolic activity.

In previous studies, the vasodilator effects of NAD were found to be associated with the production of adenosine and activation of P_1 purinoceptors in vascular SMCs [31]. Since ADPR is a metabolite of NAD and they are structurally similar, we hypothesized that adenosine production and activation of P_1 purinoceptors may contribute to ADPR-induced coronary vasodilation. The present study provides three lines of evidence to support this hypothesis. First, addition of adenosine deaminase into the arterial bath substantially blocked ADPR-induced coronary vasodilation. Second, blockade of P_1 purinoceptor by 8-PST completely abolished the vasodilator response to ADPR. It is clear that activation of P_1 purinoceptors is importantly involved in ADPR-induced coronary vasodilation. However, a previous study demonstrated that blockade of P_{2Y} purinoceptors but not P_1 purinoceptors attenuated the ADPR-induced decrease in perfusion pressure in the isolated perfused mesenteric bed, suggesting that P_{2Y} purinoceptors are activated by ADPR in mesenteric vessels [31]. The reason for this discrepancy is unknown. It is possible that the different vascular beds express different purinoceptors that are responsible for the vasodilator response to ADPR. Moreover, we used the isolated arterial preparation to study the effect of ADPR, which avoided the possible indirect effects of ADPR via other tissues surrounding the vessels in the perfused mesenteric bed used in that previous study [31]. Finally, using HPLC analysis, we detected the conversion of ADPR into adenosine by coronary arterial homogenates and cultured coronary arterial SMCs. This provides direct evidence that coronary arterial smooth muscle can convert ADPR into adenosine. It is well known that adenosine dilates coronary arteries through activation of P_1 purinoceptors including A_2 receptor [33, 34].

Another important aspect of the present study is the demonstration of a metabolic pathway responsible for the metabolism of ADPR to adenosine in the coronary arteries. First, we examined the role of 5′-nucleotidase in the conversion of ADPR into adenosine since 5′-nucleotidase represents an important enzymatic pathway responsible for adenosine production. It has been reported that 5′-nucleotidase, especially ecto-5′-nucleotidase, is expressed in a variety of vascular and nonvascular tissues, and it dephosphorylates 5′-AMP to produce adenosine [35–40]. In the present study, we found that incubation of coronary arterial homogenates with ADPR resulted in the production of adenosine, and the adenosine production was blocked by the 5′-nucleotidase inhibitor MADP, suggesting that 5′-nucleotidase is involved in the conversion of

ADPR into adenosine. Since inhibition of 5′-nucleotidase is accompanied by the accumulation of 5′-AMP, we next determined the enzyme activity catalyzing the conversion of ADPR to 5′-AMP. Apyrase, which is also known as ecto-ATP-diphosphohydrolase, is a membrane-bound enzyme present in a large variety of tissues including brain, heart, nonvascular and vascular smooth muscle [41–46]. It catalyzes the hydrolysis of ATP and ADP to 5′-AMP. We hypothesized that this apyrase may also be responsible for the conversion of ADPR into 5′-AMP in coronary arteries, as ADPR and ADP share the similar purine nucleotide structure. To test this hypothesis, we examined the effect of apyrase inhibition by sodium azide on the accumulation of 5′-AMP in the reaction mixture of coronary arterial homogenates with ADPR in the presence of 5′-nucleotidase inhibitor. As expected, inhibition of apyrase significantly blocked the accumulation of 5′-AMP in the reaction mixture. This supports the view that apyrase accounts for the conversion of ADPR into 5′-AMP, which is then metabolized into adenosine by 5′-nucleotidase. Using purified apyrase, we demonstrated that this enzyme hydrolyzed ADPR into 5′-AMP with a K_m of 0.58 mmol/l, which is generally in agreement with the K_m for ADP [36, 37]. Taken together, these results indicate that ADPR is metabolized into 5′-AMP by apyrase and 5′-AMP is further metabolized into adenosine by 5′-nucleotidase in coronary arteries. This represents a novel enzymatic pathway for adenosine production in coronary circulation.

To further explore the role of this enzymatic pathway in the adenosine production and ADPR-induced vasodilation, we determined the effect of 5′-nucleotidase and apyrase inhibition on the vasodilator response to ADPR in coronary arteries. We found that the inhibition of adenosine production by MADP or sodium azide significantly attenuated the ADPR-induced vasodilation in coronary arteries. These functional data further confirm the contributing role of 5′-nucleotidase and apyrase in the adenosine production from ADPR in coronary arteries.

Since ADPR-induced vasodilation is endothelium independent, it appears that the conversion of ADPR into adenosine is primarily localized in the vascular smooth muscle. To test this view, we examined adenosine production from ADPR in cultured coronary vascular SMCs and ECs. We found that the conversion of ADPR into adenosine occurred primarily in the vascular SMCs but not the ECs. This is consistent with the small arterial studies indicating the endothelium-independent vasodilator effect of ADPR. In addition, detection of adenosine in the incubation medium when cells were incubated with ADPR suggests that adenosine is produced extracellularly, which is further supported by our finding that ADPR-induced vasodilation was blocked by the adenosine receptor antagonist 8-PST and ADA (which is not cell permeable). Numerous studies have indicated that tissue interstitial adenosine exerts its physiological regulatory role on vascular tone [47, 48]. Taken together, these results suggest that vascular SMCs may contribute importantly to the conversion of ADPR into adenosine, and adenosine then serves as an autocrine to relax these cells, thereby producing vasodilation.

The present study did not attempt to determine the physiological significance of this novel pathway for adenosine production. However, it has been shown that adenosine as an important protective factor participates in the adaptation to ischemia and reperfusion in different tissues, especially the myocardium [49–52]. Adenosine produced in the coronary vascular cells including SMCs has been indicated to contribute to the regulation of coronary blood flow and the protection of the ischemia/reperfusion injury of the heart [33, 34, 53, 54].

Since tissue NAD could be increased during ischemia and reperfusion by changing the NAD transport mechanism or tissue redox status [55, 56], ADPR may be increasingly produced in the tissues under these circumstances. By the action of apyrase and 5′-nucleotidase, ADPR can be converted into adenosine. Adenosine then dilates arteries and increases blood flow to the tissue, thereby protecting the tissues or cells from hypoxic and ischemic injury [33, 34]. Interestingly, this adenosine production occurs primarily in vascular SMCs. It may represent an autoregulatory mechanism in vascular smooth muscle in response to tissue ischemia/reperfusion or oxidant stress.

In summary, the present study demonstrated that ADPR produces endothelium-independent vasodilation in small coronary arteries and the vasodilator action of ADPR is largely dependent on the adenosine production by vascular smooth muscle via the apyrase and 5′-nucleotidase metabolism. The conversion of ADPR into adenosine represents another novel metabolic pathway for adenosine production in coronary circulation, which may importantly contribute to the myocardial protection during ischemia and reperfusion through improving tissue perfusion and oxygenation.

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