

Coronary response to diadenosine triphosphate after ischemia–reperfusion in the isolated rat heart

Angel Luis García-Villalón, Nuria Fernández, Luis Monge, Miriam Granado, Gonzalo Carreño-Tarragona, Juan Carlos Figueras and Godofredo Diéguez

Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma de Madrid, Arzobispo Morcillo 4, 28029 Madrid, Spain
Corresponding author: Angel Luis García-Villalón. Email: angeluis.villalon@uam.es

Abstract

Diadenosine triphosphate (Ap3A) is a vasoactive mediator stored in platelet granules that may be released during coronary ischemia–reperfusion. To study its coronary effects in such circumstances, rat hearts were perfused in a Langendorff preparation and the coronary response to Ap3A (10^{-7} – 10^{-5} mol/L) was recorded. Both at basal coronary resting tone and after precontraction with 11-dideoxy-1a,9a-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619), Ap3A produced concentration-dependent vasodilation in the heart, which was attenuated following ischemia–reperfusion. Ap3A-induced relaxation was also attenuated in control conditions and after ischemia–reperfusion by the purinergic P2Y antagonist reactive blue 2 (2×10^{-6} mol/L), the P2Y₁ antagonist MRS 2179 (10^{-5} mol/L), the nitric oxide synthesis inhibitor *N*-omega-nitro-L-arginine methyl ester (L-NAME; 10^{-4} mol/L) and the ATP-dependent potassium channel blocker glibenclamide (10^{-5} mol/L). These results suggest that Ap3A induces coronary vasodilation, an effect attenuated by ischemia–reperfusion due to the functional impairment of purinergic P2Y receptors and K_{ATP} channels, and/or reduced nitric oxide release. This impairment of vasodilation may contribute to the coronary dysregulation that occurs during ischemia–reperfusion.

Keywords: perfused heart, purinergic P2Y receptors, coronary circulation, K_{ATP} potassium channels, nitric oxide, Langendorff preparation

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Introduction

Diadenosine polyphosphates (APnAs) are intracellular and extracellular mediators formed by two adenosine moieties that are linked by two to six phosphate groups.¹ Diadenosine triphosphate (Ap3A), diadenosine tetraphosphate (Ap4A), diadenosine pentaphosphate (Ap5A) and diadenosine hexaphosphate (Ap6A) occur naturally, and they are stored in platelets from which they can be released upon platelet activation.² In addition, these APnAs are also found in chromaffin cells³ and brain tissue.⁴ APnAs act as second messengers that mediate insulin release in pancreatic β -cells,⁵ and they can also serve as neurotransmitters⁶ and extracellular signaling molecules. These mediators have been described in human myocardial tissue^{7,8} where they exert both positive and negative inotropic effects on myocardial function.⁹ Moreover, at concentrations found in human plasma in physiological conditions (0.1 – 2×10^{-6} mol/L)¹⁰, they promote vasodilation in the coronary arteries of rats,¹¹ guinea-pigs,¹² pigs¹³ and dogs.¹⁴

Coronary vascular dysfunction frequently occurs after ischemia–reperfusion, involving reduced vasodilator and

increased vasoconstrictor responses.^{15,16} These changes may be linked to the clinical phenomenon of no-reflow,¹⁷ a persistent reduction in coronary blood flow after reperfusion. We previously reported that coronary relaxation in response to Ap5A¹⁸ and Ap4A¹⁹ is reduced after ischemia–reperfusion, suggesting that these substances may be involved in the no-reflow phenomenon. Indeed, the concentration of both Ap4A and Ap5A increases in coronary venous blood during ischemia.²⁰ Ap3A is also present in myocardial tissue^{7,8} and increases during ischemia,²⁰ suggesting that it fulfils a role in the pathophysiology of ischemia–reperfusion. Based on these observations, we analyzed the coronary effects of Ap3A in perfused rat hearts before and after ischemia–reperfusion.

Methods

Experimental set-up

In the present study, 36 male Sprague–Dawley rats were used (300–350 g body weight). All the experiments were conducted in accordance with the US National Institutes

of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and in compliance with all relevant laws and regulations. The use of these animals was also approved by the Institute's Animal Care and Use Committee. Each heart was used for only one experimental manipulation and five to six hearts were used for each experimental group; therefore, a large number of hearts was used. The hearts were removed from the rats under anaesthesia with intraperitoneal sodium pentobarbital (40 mg/kg) and following intravenous injection of heparin (1000 IU). Next, the ascending aorta was cannulated and the heart was subjected to retrograde perfusion with Krebs-Henseleit buffer (115 mmol/L NaCl, 4.6 mmol/L KCl, 1.2 mmol/L KH_2PO_4 , 1.2 mmol/L MgSO_4 , 2.5 mmol/L CaCl_2 , 25 mmol/L NaHCO_3 and 11 mmol/L glucose) equilibrated with 95% oxygen and 5% carbon dioxide to a pH of 7.3–7.4. Perfusion was initiated in a non-recirculating Langendorff heart perfusion apparatus at a constant flow rate of 11–15 mL/min to provide a basal perfusion pressure of approximately 70 mmHg. Both the perfusion solution and the heart were maintained at 37°C. Coronary perfusion pressure was measured through a lateral connection in the perfusion cannula and left ventricular pressure was measured using a latex balloon inflated to a diastolic pressure of 5–10 mmHg, both connected to Statham transducers (Statham Instruments, Los Angeles, CA, USA). Left ventricular developed pressure (systolic left ventricular pressure minus diastolic left ventricular pressure), left ventricular end-diastolic pressure, the first derivative of the left ventricular pressure curve (dP/dt) and heart rate were calculated from the left ventricular pressure curve. These parameters were recorded on a Macintosh computer using Chart v 3.6/s software and the MacLab/8e data acquisition system (ADInstruments, Colorado Springs, CO, USA).

Experimental procedure

After a 15-min equilibration period with constant flow perfusion, Ap3A was injected into the perfusion cannula with an infusion pump over three minutes at a constant rate to reach a final concentration of 10^{-7} , 10^{-6} or 10^{-5} mol/L. The 10^{-5} mol/L dose produced a close-to-maximal effect and the response to a 10^{-4} mol/L dose was only 10–20% higher than that of the 10^{-5} mol/L dose. Coronary arteries were maintained at a basal resting tone or precontracted with the thromboxane A_2 analogue U46619. First, 10^{-8} mol/L U46619 was added to the perfusion solution and the concentration was increased progressively until a contractile tone of ~120–140 mmHg was obtained. The

concentrations of U46619 required to achieve this effect were 1×10^{-8} to 3×10^{-8} mol/L prior to and 5×10^{-8} to 2×10^{-7} mol/L after ischemia–reperfusion. The response to Ap3A was recorded under control conditions, after which Ap3A and U46619 were washed out and the hearts were exposed to global zero-flow ischemia for 30 min. The hearts were then reperfused for 15 min at the same flow rate used before ischemia, and the response to Ap3A was again recorded at the basal coronary vascular tone or after coronary precontraction with U46619 (Figure 1). The duration of ischemia and reperfusion were chosen on the basis of previous studies demonstrating decreases in the endothelium-dependent coronary relaxation without alteration of endothelium-independent coronary relaxation.^{18,21} Time-control experiments were also performed by administering two successive injections of Ap3A (10^{-7} – 10^{-5} mol/L) separated by 45 min of perfusion without ischemia. As the experiments were performed at a constant flow rate, the coronary perfusion pressure provides a measure of the perfusion resistance and characterizes the contraction or relaxation of the coronary arteries. To assess the stability of the compound, the Ap3A-containing effluent was collected after passing through a heart and infused into another perfused heart in three experiments.

The mechanisms underlying the effects of Ap3A were analyzed by recording the response to this substance after coronary precontraction with U46619 both before and after ischemia–reperfusion, in the presence or absence of: the purinergic P_{2Y} receptor antagonist, reactive blue 2 (2×10^{-6} mol/L); the specific purinergic P_{2Y1} receptor antagonist, MRS 2179 (10^{-5} mol/L); the nitric oxide (NO) synthesis inhibitor, *N*-omega-nitro-L-arginine methyl ester (L-NAME; 10^{-4} mol/L); or the K_{ATP} channel blocker, glibenclamide (10^{-5} mol/L). These antagonists were infused five minutes before and throughout the period of Ap3A infusion, but not during ischemia or the 15-min reperfusion period.

Western blots

To determine the effect of Ap3A on the endothelial nitric oxide synthase (eNOS) and phosphorylated endothelial nitric oxide synthase (P-eNOS) content in the myocardium, four rat hearts were perfused for 15 min with a solution containing Ap3A (10^{-5} mol/L) injected using an infusion pump. Another four hearts were perfused for the same duration in the absence of Ap3A (control). Immediately after perfusion, the hearts were collected and frozen at -80°C . Total protein from the myocardium was isolated by homogenization of 100 mg of heart tissue in 1000 μL

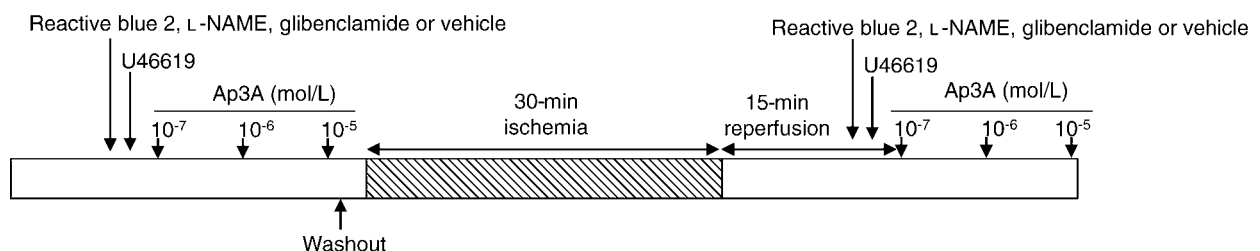


Figure 1 Diagram showing the experimental protocol and the treatment application schedule. L-NAME, *N*-omega-nitro-L-arginine methyl ester; Ap3A, diadenosine triphosphate

RIPA lysis buffer (50 mmol/L NaH_2PO_4 , 100 mmol/L $\text{Na}_2\text{H}_2\text{PO}_4$, 0.1% sodium dodecyl sulfate [SDS], 0.5% NaCl, 1% Triton X-100) with 5 mg/mL sodium deoxycholate, 1 mmol/L phenyl-methane-sulfonylfluoride and a cocktail of ethylenediaminetetraacetic acid-free protease inhibitors (Roche Diagnostics, Mannheim, Germany). The lysates were incubated overnight at -80°C and then centrifuged at $14,000 \times g$ for 20 min at 4°C . The cleared supernatant was transferred to a new tube and stored at -80°C . The total protein concentration was determined by the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA).

The total protein (100 μg) was resolved on a 10% SDS-polyacrylamide gel under denaturing conditions and electro-transferred to poly(vinylidene difluoride) membranes (Bio-Rad), determining the efficiency of transfer with Ponceau red dye. The membranes were blocked for two hours with Tris-buffered saline (TBS, 20 mmol/L) containing 5% dried non-fat milk and 0.1% Tween 20, and then incubated overnight with agitation at 4°C with the appropriate primary antibodies raised against P-eNOS Ser 1177 (1:200; BD Transduction Laboratories, Franklin Lakes, NJ, USA) and eNOS (1:1000; Abcam, Cambridge, UK). The membranes were subsequently washed and incubated with the corresponding peroxidase-conjugated secondary antibody (Pierce Biotechnology, Rockford, IL, USA), the activity of which was visualized by chemiluminescence (PerkinElmer Life Science, Boston, MA, USA), and quantified by densitometry using a Kodak Gel Logic 1500 Image Analysis system and Molecular Imaging Software, Version 4.0 (Rochester, NY, USA). All blots were reprobated for β -tubulin (Sigma-Aldrich, Madrid, Spain) to normalize each sample for gel-loading variability, and all data were normalized to the control values for each gel.

Statistical analysis

The coronary vascular response is expressed as the mean ($\pm$ SEM) change in perfusion pressure. Coronary responses before and after ischemia-reperfusion were compared using a paired Student's *t*-test. The responses in the presence and absence of antagonists were compared using one-way analysis of variance followed by Dunnett's test. A *P* value of <0.05 was considered significant.

Drugs and chemicals used

The following substances were all obtained from Sigma (Madrid, Spain): P1,P3-di(adenosine-5') triphosphate ammonium salt (Ap3A), 1-amino-4[[4-[4-chloro-6-[[3-sulfophenyl]amino]-1,3,5-triazin-2-yl]amino]-3-sulfophenyl]-amino]-9,10-dihydro-9,10-dioxo-2-anthracenesulfonic acid (reactive blue 2), 2'-deoxy-N6-methyladenosine 3',5'-diphosphate diammonium salt (MRS 2179), 9,11-dideoxy-1 α ,9 α -epoxymethano-prostaglandin $\text{F}_{2\alpha}$ (U46619), N-omega-nitro-L-arginine methyl ester hydrochloride (L-NAME), 5-chloro-N-[4-(cyclohexylureidosulfonyl)phenethyl]-2-methoxybenzamide and N-p-[2(5-chloro-2-methoxy-benzamido)ethyl] benzenesulfonyl-nN'-cyclohexylurea (glibenclamide, glyburide).

Results

Effects of ischemia in perfused hearts

When the hemodynamic parameters in hearts perfused at basal coronary resting tone were assessed ($n = 5$, Table 1), coronary perfusion pressure remained unaltered after 30 min of ischemia and 15 min of reperfusion, and no significant change in heart rate was observed (239 ± 10 versus 179 ± 49 beats/min). However, we found significant decreases in the left ventricular developed pressure ($P < 0.01$) and maximal dP/dt ($P < 0.01$), and significant increases in the left ventricular end-diastolic pressure (15 ± 5 versus 44 ± 2 mmHg; $P < 0.01$). Treatment with U46619 increased the coronary perfusion pressure ($n = 5$, Table 1; $P < 0.01$), but had no effect on the left ventricular developed pressure, left ventricular end-diastolic pressure, maximal dP/dt or heart rate, parameters that remained similar to those of untreated hearts before ischemia-reperfusion. Ischemia-reperfusion in precontracted hearts had no effect on coronary perfusion pressure, maximal dP/dt or heart rate, although the left ventricular developed pressure was reduced ($P < 0.01$) and the left ventricular end-diastolic pressure increased. In time-control experiments, hearts perfused at basal coronary resting tone exhibited no changes in coronary perfusion pressure, left ventricular developed pressure, left ventricular end-diastolic pressure, maximal dP/dt or heart rate between the beginning and end of the experiment ($n = 4$, Table 1).

Ap3A response in hearts perfused at basal coronary resting tone

At basal coronary tone, injection of Ap3A (10^{-7} – 10^{-5} mol/L, $n = 5$) reduced perfusion pressure and this decrease was stronger before ischemia-reperfusion than after (Figure 2). Ap3A induced small fluctuations in left ventricular developed pressure, left ventricular end-diastolic pressure, maximal dP/dt and heart rate which were not statistically significant, both before and after ischemia-reperfusion (Table 2).

In time-control experiments with hearts perfused at coronary basal tone ($n = 4$), a second injection of Ap3A 45 min after the first injection had similar effects on perfusion pressure (Figure 2), left ventricular developed pressure, left ventricular end-diastolic pressure, maximal dP/dt and heart rate. Moreover, the Ap3A-containing effluent collected after passing once through a heart produced similar effects to freshly prepared Ap3A when injected into another heart, suggesting that the compound was relatively stable under our experimental conditions.

Ap3A response after coronary precontraction with U46619

When compared with hearts at basal tone, precontraction of the coronary vasculature with U46619 enhanced the Ap3A-mediated decrease in perfusion pressure both before and after ischemia-reperfusion ($n = 5$). In precontracted hearts, the decrease in perfusion pressure induced by Ap3A was significantly attenuated after ischemia-reperfusion. The

Table 1 Coronary perfusion pressure (mmHg), left ventricular developed pressure (mmHg) and maximal dP/dt (mmHg/s) in perfused rat hearts: before and after 45 min of perfusion without ischemia (time-control); before and after 30 min of ischemia plus 15 min of reperfusion in hearts at basal coronary tone; or after coronary precontraction with U46619 in the presence or absence of reactive blue (2×10^{-6} mol/L), MRS2179 (10^{-4} mol/L), L-NAME (10^{-4} mol/L) or glibenclamide (10^{-5} mol/L)

	Time-control (n = 4)	Basal tone (n = 5)	Precontracted untreated (n = 5)	Precontracted + reactive blue 2 (n = 5)	Precontracted + MRS2179 (n = 5)	Precontracted + L-NAME (n = 6)	Precontracted + glibenclamide (n = 6)
Before time-control or before ischemia-reperfusion							
Coronary perfusion pressure	85 ± 5	83 ± 3	139 ± 7 ^{###††}	128 ± 6 ^{##††}	148 ± 9 ^{###††}	148 ± 6 ^{##††}	148 ± 11 ^{###††}
Left ventricular developed pressure	93 ± 16	83 ± 16	111 ± 12	149 ± 12	90 ± 19	86 ± 8	119 ± 8
Maximal dP/dt	1382 ± 234	1774 ± 388	2423 ± 521	2165 ± 172	1801 ± 459	1305 ± 146	2335 ± 219
After 45 min of perfusion (time control) or after ischemia-reperfusion							
Coronary perfusion pressure	81 ± 7	82 ± 4	146 ± 8 ^{##††}	134 ± 8 ^{##††}	118 ± 3 ^{###††}	161 ± 5 ^{##††}	136 ± 6 ^{##††}
Left ventricular developed pressure	106 ± 31	23 ± 11 ^{###}	57 ± 20 ^{##}	34 ± 5 ^{##}	52 ± 24 ^{##}	29 ± 8 ^{##}	51 ± 7 ^{##}
Maximal dP/dt	1229 ± 230	512 ± 246 ^{###}	1705 ± 667 ^{††}	566 ± 119 ^{##}	1090 ± 342 ^{##}	459 ± 144 ^{##}	924 ± 135 ^{##}

L-NAME, N-omega-nitro-L-arginine methyl ester

n = number of experiments

Significant difference as compared with pre-ischemia-reperfusion values (** $P < 0.01$)

Significant difference with time-control ([#] $P < 0.05$; ^{##} $P < 0.01$)

Significant difference with respect to hearts at basal tone (^{††} $P < 0.01$)

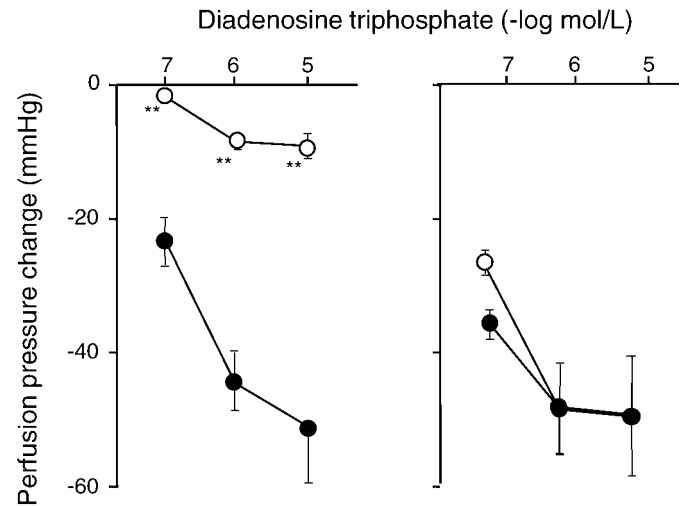


Figure 2 Coronary dilation induced by diadenosine triphosphate (Ap3A; 10^{-7} – 10^{-5} mol/L) in perfused rat hearts. Measurements were taken before (control) and after 30 min total ischemia followed by 15 min of reperfusion (left panel), or repeatedly before (first test) and after (second test) 45 minutes of perfusion in time-control experiments (right panel). Left panel: control (•); ischemia-reperfusion (○). Right panel: first test (•); second test (○). Values represent the mean ($\pm$ SEM) of five experiments. **Significant difference with respect to controls ($P < 0.01$). Ap3A, diadenosine triphosphate

effects of Ap3A on left ventricular developed pressure, left ventricular end-diastolic pressure, maximal dP/dt and heart rate were similar in precontracted hearts and those at basal tone, both before and after ischemia-reperfusion (Table 2). We also investigated whether increases in myocardial function and metabolism after U46619 treatment might increase Ap3A-induced vasodilation in this condition. However, increases in left ventricular developed pressure and maximal dP/dt in response to the beta-adrenergic agonist isoproterenol (10^{-7} mol/L) attenuated the vasodilatory response to Ap3A (data not shown).

Ap3A response in hearts treated with reactive blue 2

Treatment of precontracted hearts with reactive blue 2 (2×10^{-6} mol/L; $n = 5$) before ischemia-reperfusion attenuated the vasodilatory response to the highest concentration of Ap3A (10^{-5} mol/L), when compared with that observed in precontracted hearts not exposed to reactive blue 2. By contrast, reactive blue 2 treatment after ischemia-reperfusion attenuated the vasodilatory response to all concentrations of Ap3A used. Following reactive blue 2 pretreatment, Ap3A reduced left ventricular developed pressure and maximal dP/dt without significantly modifying the left ventricular end-diastolic pressure or heart rate, both before and after ischemia-reperfusion (Table 2). Coronary perfusion pressure, left ventricular developed pressure, left ventricular end-diastolic pressure, maximal dP/dt and heart rate were not affected by exposure to reactive blue 2, both before and after ischemia-reperfusion (Table 1).

Ap3A response in hearts treated with MRS2179

In precontracted hearts, MRS2179 treatment (10^{-5} mol/L, $n = 5$) before ischemia-reperfusion attenuated the

Table 2 Percentage increase (positive values) or decrease (negative values) in left ventricular developed pressure and maximal dP/dt produced by injection of diadenosine triphosphate (Ap3A, 10^{-7} – 10^{-5} mol/L) before (control) and after ischemia–reperfusion in untreated hearts (control) at basal tone and in hearts precontracted with U46619 in the presence or absence of reactive blue 2 (2×10^{-6} mol/L), MRS2179 (10^{-5} mol/L), L-NAME (10^{-4} mol/L) or glibenclamide (10^{-5} mol/L)

	Control			Ischemia–reperfusion		
	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}
Control at basal tone ($n = 5$)						
Left ventricular developed pressure	-4 ± 2	-2 ± 8	-6 ± 13	-29 ± 20	21 ± 29	23 ± 28
Maximal dP/dt	-4 ± 2	-3 ± 7	15 ± 15	1 ± 1	19 ± 21	22 ± 30
Control precontracted ($n = 7$)						
Left ventricular developed pressure	-9 ± 3	1 ± 5	13 ± 9	-1 ± 2	-8 ± 6	10 ± 12
Maximal dP/dt	-7 ± 2	1 ± 5	16 ± 9	-6 ± 7	7 ± 10	27 ± 9
Reactive blue 2 ($n = 5$)						
Left ventricular developed pressure	-21 ± 3	-20 ± 6	-11 ± 2	5 ± 9	-13 ± 14	-21 ± 6
Maximal dP/dt	-24 ± 5	-20 ± 5	-15 ± 3	1 ± 2	-18 ± 10	-11 ± 14
MRS2179 ($n = 5$)						
Left ventricular developed pressure	-10 ± 4	-17 ± 6	-11 ± 4	4 ± 2	2 ± 5	-7 ± 9
Maximal dP/dt	-15 ± 5	-20 ± 6	-13 ± 5	-1 ± 5	-12 ± 5	-8 ± 7
L-NAME ($n = 6$)						
Left ventricular developed pressure	2 ± 1	3 ± 12	14 ± 9	-16 ± 18	13 ± 4	20 ± 17
Maximal dP/dt	1 ± 1	1 ± 14	19 ± 10	12 ± 8	26 ± 14	26 ± 26
Glibenclamide ($n = 6$)						
Left ventricular developed pressure	2 ± 1	10 ± 3	27 ± 6	-5 ± 8	8 ± 2	2 ± 17
Maximal dP/dt	2 ± 1	7 ± 3	29 ± 5	1 ± 4	14 ± 2	22 ± 12

L-NAME, *N*-omega-nitro-L-arginine methyl ester

n = number of experiments

Values represent the mean $\pm$ SEM

vasodilatory response induced by the highest concentration of Ap3A (10^{-5} mol/L), when compared with precontracted hearts not exposed to MRS2179. After ischemia–reperfusion, the same dose of MRS2179 attenuated the vasodilatory response to the two highest concentrations of Ap3A used (10^{-6} and 10^{-5} mol/L). Moreover, after MRS2179 treatment, Ap3A reduced the left ventricular developed pressure and maximal dP/dt before but not after ischemia–reperfusion, without significantly modifying left ventricular end-diastolic pressure or heart rate (Table 2). The coronary perfusion pressure, left ventricular developed pressure, left ventricular end-diastolic pressure, maximal dP/dt and heart rate were similar in hearts irrespective of whether they were treated with MRS2179, both before and after ischemia–reperfusion (Table 1).

Ap3A response in hearts treated with L-NAME

Before ischemia–reperfusion, L-NAME treatment (10^{-4} mol/L) attenuated the vasodilatory response to the lowest concentration of Ap3A used (10^{-7} mol/L, $n = 6$; Figure 3), whereas after ischemia–reperfusion, the same dose of L-NAME attenuated the vasodilatory response to the two highest concentrations of Ap3A (10^{-6} and 10^{-5} mol/L). Ap3A did not significantly alter left ventricular developed pressure, left ventricular end-diastolic pressure, maximal dP/dt or the heart rate after L-NAME treatment, either before or after ischemia–reperfusion (Table 2). Coronary perfusion pressure, left ventricular developed pressure, left ventricular end-diastolic pressure, maximal dP/dt and heart rate were similar in the hearts irrespective of whether they were treated with L-NAME, both before and after ischemia–reperfusion (Table 1).

Ap3A response in hearts treated with glibenclamide

In hearts precontracted with U46619, glibenclamide treatment (10^{-5} mol/L) before ischemia–reperfusion attenuated the vasodilatory effect of the lowest concentration of Ap3A used (10^{-7} mol/L, $n = 6$; Figure 3). In precontracted hearts after ischemia–reperfusion, glibenclamide attenuated the vasodilatory response to all concentrations of Ap3A used. In hearts treated with U46619 and glibenclamide, Ap3A significantly increased left ventricular developed pressure and maximal dP/dt without modifying left ventricular end-diastolic pressure or the heart rate, both before and after ischemia–reperfusion (Table 2). Coronary perfusion pressure, left ventricular end-diastolic pressure, left ventricular developed pressure, maximal dP/dt and heart rate were similar in hearts treated with and without glibenclamide, both before and after ischemia–reperfusion (Table 1).

P-eNOS and eNOS content in the myocardium

No differences in eNOS protein levels were observed between control and Ap3A-treated myocardia. However, P-eNOS protein content was significantly higher in Ap3A-treated than in untreated myocardia ($P < 0.05$) (Figure 4).

Discussion

Our results suggest that Ap3A promotes coronary vasodilation and that this effect is impaired after ischemia–reperfusion. The vasodilatory effect of Ap3A in normal conditions was similar to that described for Ap4A in the

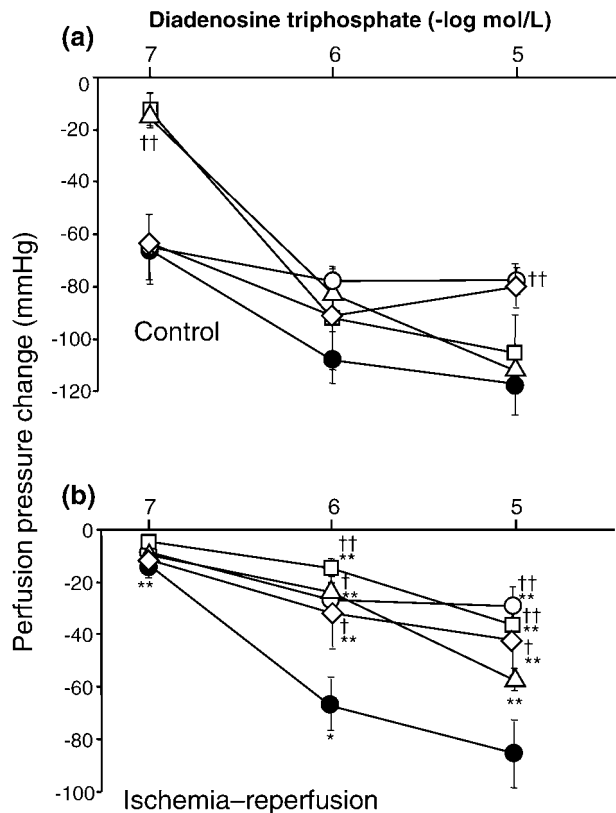


Figure 3 Coronary vasodilation induced by diadenosine triphosphate (Ap3A; 10^{-7} – 10^{-5} mol/L) in perfused rat hearts during coronary precontraction with U46619, before (a) and after (b) ischemia–reperfusion, in the presence or absence of reactive blue 2 (2×10^{-6} mol/L), MRS2179 (10^{-5} mol/L), L-NAME (10^{-4} mol/L) or glibenclamide (10^{-5} mol/L). Untreated (•); +reactive blue (○); +MRS2179 (◇); +L-NAME (Δ). Values represent the mean ($\pm$ SEM) of 5–6 experiments. ** $P < 0.01$, control versus ischemic-reperfused hearts. † $P < 0.05$, †† $P < 0.01$, treated versus untreated hearts. L-NAME, *N*-omega-nitro-L-arginine methyl ester; Ap3A, diadenosine triphosphate

canine heart,¹⁴ for Ap3A, Ap4A, Ap5A and Ap6A in the guinea pig heart,¹² and for Ap4A and Ap5A in pig coronary arteries¹³ and rabbit coronary circulation.¹¹ We previously demonstrated that Ap5A in control conditions induces coronary vasoconstriction followed by vasodilation.¹⁸ By contrast, Ap4A induces vasodilation in control hearts but triggers a transient vasoconstrictor response after inhibition of purinergic P2y receptors,¹⁷ while Ap3A induced vasodilation only (present study). Taken together, these findings suggest that diadenosines with a longer phosphate chain induce vasoconstriction, while those with a shorter chain elicit vasodilator responses alone.²²

Several potential mechanisms may underlie the effects of ApnAs in blood vessels. First, ApnAs may activate P2 purinergic receptors, which mediate vasoconstriction and vasodilation via P2x and P2y, respectively. Purinergic P2y receptors have been proposed to mediate the vasodilatory effects of Ap4A in mesenteric arteries, both in humans²³ and rabbits,²⁴ that of Ap3A in rat mesenteric arteries,²⁵ and that of Ap5A and Ap4A in rat coronary arteries.^{18,19} NO is also implicated in the APnA response, although its role may vary between animal species. For example, NO is involved in Ap4A-mediated coronary vasodilation in dogs,¹⁴ but not in the effects of Ap3A, Ap4A, Ap5A or

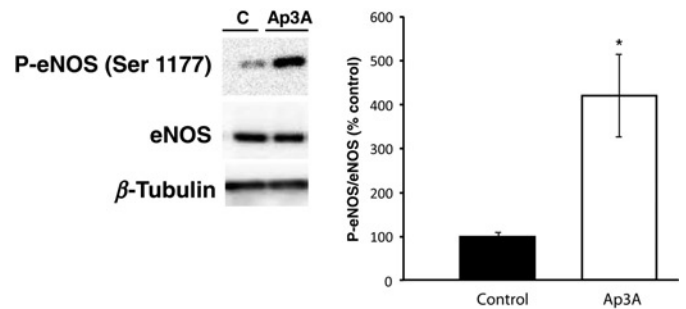


Figure 4 Endothelial nitric oxide synthase (eNOS) and phosphorylated endothelial nitric oxide synthase (P-eNOS) protein content in the myocardia of perfused rat hearts treated with ($n = 4$) or without (control, $n = 4$) Ap3A (10^{-5} mol/L) for 15 min. *Significant difference as compared with control group ($P < 0.05$). Ap3A, diadenosine triphosphate

Ap6A on coronary circulation in guinea-pigs.¹² Moreover, NO is implicated in the vasodilatory effects of Ap4A but not Ap3A in rabbit coronary circulation.¹¹ Potassium channels may also be involved in the effects of Ap4A and Ap5A. Both drugs inhibit K_{ATP} potassium channels,²⁶ which may contribute to its vasoconstrictor effect, whereas K_{ATP} potassium channels in pig coronary arteries²⁷ are activated by Ap4A. The present findings suggest that all three mechanisms are involved in Ap3A-induced vasodilation of rat coronary circulation, as this effect was attenuated by reactive blue 2, MRS2179, L-NAME and glibenclamide. However, in control conditions, reactive blue 2 and MRS2179 only antagonized the highest concentration of Ap3A used, suggesting that purinergic P2y receptors mediate the vasodilator effects of high Ap3A concentrations. The specific P2y1 antagonist, MRS2179, had similar effects on Ap3A-induced coronary relaxation as reactive blue 2, a general P2y antagonist, suggesting that purinergic receptors of the P2y1 subtype are involved in this response. L-NAME and glibenclamide only attenuated the effects of the lowest concentration of Ap3A used, suggesting that potassium channels and NO release are predominantly activated by low Ap3A concentrations. Moreover, NO release may be induced by the phosphorylation of eNOS, the levels of which increased after Ap3A treatment. Endothelial NO may be specifically involved in the vasodilatory effects of short-chain diadenosines, as the vasodilation of rat mesenteric arteries in response to Ap3A is endothelium-dependent, unlike that in response to Ap4A or Ap5A.²⁵ Thus, the increase in NO release induced by Ap3A may be related to the stronger vasodilatory effects of short-chain diadenosines.

Ap3A-induced coronary relaxation was attenuated after ischemia–reperfusion and indeed, ischemia–reperfusion attenuates the vasodilatory effects of Ap5A¹⁸ and Ap4A.¹⁹ Moreover, ischemia–reperfusion impairs both NO-dependent²⁸ and K_{ATP} potassium channel-dependent relaxation²⁹ in the perfused rat heart, suggesting that impairment of these mechanisms is involved in the decreased vasodilatory response to Ap3A observed following ischemia–reperfusion. Functional impairment of P2y receptors may also contribute to this effect, as previously described for Ap4A¹⁸ and Ap5A.¹⁹

Our findings indicate that the no-reflow phenomenon, whereby coronary blood flow does not recover after ischemia-reperfusion, may be related to the impaired vasodilatory effects of Ap3A following ischemia-reperfusion. In agreement with previous findings,^{18,19} coronary vasodilation in response to a ApnA was disrupted after ischemia-reperfusion, an effect that may contribute to the reduced coronary blood flow in this condition.

In summary, the present findings demonstrate that ischemia-reperfusion impairs coronary vasodilation induced by Ap3A. Decreased purinergic P2y receptor function, reduced activation of K_{ATP} potassium channels and/or reduced release of NO in coronary blood vessels contribute to this effect, which may be implicated in the pathophysiology of ischemia-reperfusion.

Author contributions: ALG-V, NF, LM and MG performed the experiments on perfused hearts; GC-T and JCF performed the Western blot experiments; ALG-V wrote the manuscript; and GD participated in the study design, analysis and interpretation of the data and review of the manuscript.

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