Coronary response to diadenosine pentaphosphate after ischaemia–reperfusion in the isolated rat heart

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1. Introduction

Diadenosine polyphosphates (ApnAs) are molecules consisting of two adenosine moieties linked by a chain of two to six phosphate groups, and they may act as extracellular or intracellular mediators.¹ In the central nervous system ApnAs may act as neurotransmitters,² and they are also stored in and released from chromaffin cells³ and platelets.⁴ These substances may produce vasodilatation or vasoconstriction of blood vessels depending on the particular ApnA in question and the prior tone of the arteries. Indeed, in the human⁵ or rat⁶ mesenteric arteries, and in rat renal circulation,⁷ ApnAs produce vasoconstriction if the arteries are at basal resting tone and vasodilatation if the vessel tone is raised. In addition, vasodilatation is weaker and vasoconstriction stronger for ApnAs with longer phosphate chains.⁶ In coronary circulation, ApnAs produce vasodilatation in rats,⁸ guinea-pigs,⁹ pigs,¹⁰ or dogs¹¹ when they are present at nM to μM concentrations, as may exist in plasma under normal conditions.¹²

Coronary ischaemia–reperfusion is a frequent clinical event that may produce dysfunction of coronary vessels. Coronary vascular dysfunction after ischaemia–reperfusion involves reduced vasodilatory and increased vasoconstrictor responses,¹³,¹⁴ and these alterations may underlie the clinical phenomenon of no-reflow, whereby coronary blood flow remains reduced after the reopening of the occluded artery.¹⁵ In addition, platelet activation and platelet-released substances, including ApnAs, may be involved in the pathophysiology of ischaemia–reperfusion.¹⁶ It has been shown that the concentration of ApnAs increases in coronary venous blood during ischaemia¹⁷ and as they can produce vasodilatation or vasoconstriction depending on the condition of the blood vessels, these compounds might participate in the altered coronary regulation associated with this condition. However, the coronary effects of ApnAs after
Diadenosine pentaphosphate and ischaemia

Ischaemia-reperfusion are poorly understood and for example, acidosis, which may occur in the tissue during ischaemia, reduces the vasodilatory effect of diadenosine tetraphosphate (Ap4A) and pentaphosphate (Ap5A).18

The objective of this study was to analyse the effects of ApnAs on coronary blood vessels during ischaemia–reperfusion. Accordingly, the effects of Ap5A on coronary circulation were recorded before and after ischaemia–reperfusion of perfused rat hearts. Ap5A was selected as an ApnA with a long phosphate chain as it is more likely to produce vasoconstriction and therefore, to be involved in the coronary vasoconstriction that frequently occurs after ischaemia–reperfusion. Moreover, Ap5A is produced in the heart and its production increases during heart ischaemia.19

2. Methods

In this study, 75 male Sprague–Dawley rats (weight 300–350 g) were used in experiments carried out 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 applicable laws and regulations. The use of these animals was also approved by the Institute’s Animal Care and Use Committee. The hearts were obtained from the rats after anaesthesia with pentobarbital sodium (40 mg/kg) and injection of heparin (1000 UI). After their removal, the ascending aorta was cannulated and the heart was subjected to retrograde perfusion with Krebs-Henseleit buffer (NaCl 115 mM, KCl 4.6 mM, KH₂PO₄ 1.2 mM, MgSO₄•7H₂O 1.2 mM, CaCl₂ 2.5 mM, NaHCO₃ 25 mM, and glucose 11 mM) 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 of 11–15 mL/min in order to reach a basal perfusion pressure of ~70 mmHg. Both the perfusion solution and the heart were maintained at 37°C. Perfusion coronary pressure was measured through a lateral connection in the perfusion cannula and the left ventricular pressure was measured with a latex balloon inflated to a diastolic pressure of 5–10 mmHg, both connected to Statham transducers. Left ventricular developed pressure (systolic left ventricular pressure minus diasstolic left ventricular pressure), the first derivative of the left ventricular pressure curve (dP/dt) and heart rate were obtained from the left ventricular pressure curve. These parameters were recorded on a Macintosh computer by use of Chart v 3.6/s software and a MacLab/8e data acquisition system (ADInstruments).

After a 15 min equilibration period with constant flow perfusion, diadenosine pentaphosphate (Ap5A) was injected into the perfusion cannula with an infusion pump at a constant rate over 3 min in order to reach a final concentration of 10⁻⁷, 10⁻⁶, and 10⁻⁵ M. In some cases, the coronary arteries were at their basal resting tone and in other cases, coronary arteries were pre-contracted by adding the thromboxane A₂ analogue U46619 (10⁻⁸–10⁻⁷ M) to the perfusion solution 5 min before applying Ap5A. The concentration of U46619 was adjusted in each experiment to reach a perfusion pressure of 120–140 mmHg. After recording the response to Ap5A in control conditions, Ap5A and U46619 were washed out and the hearts were then exposed to global zero-flow ischaemia for 30 min, followed by reperfusion for 15 min at the same flow rate as that before ischaemia. After ischaemia–reperfusion, the response to Ap5A was again recorded at the basal coronary vascular tone or after coronary pre-contraction with U46619. All the times used for ischaemia and reperfusion were chosen on the basis of previous studies,20 which showed they produce decreases in the endothelium-dependent coronary relaxation without modifying endothelium-independent coronary relaxation. In time-control experiments, two successive injections of Ap5A (10⁻⁷, 10⁻⁶ M) were administered, separated by 45 min of perfusion without ischaemia. In addition, in some experiments the Ap5A was only injected after ischaemia–reperfusion in order to test whether the injection before ischaemia modified the subsequent response. In these constant flow experiments the measurement of the perfusion pressure characterized the coronary perfusion resistance. The response to the endothelium-independent vasodilator sodium nitroprusside (10⁻⁸–10⁻⁶ M) was also recorded before and after ischaemia–reperfusion, both at basal vascular tone or after coronary pre-contraction with U46619.

To analyse the mechanisms underlying the effects of Ap5A, the response to this substance was recorded after coronary pre-contraction with U46619 both before and after ischaemia–reperfusion, in the presence and absence of the purinergic P₂X receptor antagonist, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS, 3×10⁻⁶ M), the purinergic P₂Y receptor antagonist, reactive blue 2 (2×10⁻⁶ M), of the nitric oxide synthesis blocker, Nω-nitro-L-arginine methyl ester (L-NAME 10⁻⁴ M), or of the cyclooxygenase blocker, meclofenamate (2×10⁻⁶ M). While L-NAME increased the perfusion pressure, at the concentration used none of the other antagonists modified the perfusion pressure or the heart functional parameters.

To analyse the effects of previous contractile tone on the relaxation of coronary circulation, the response to a single concentration of Ap5A (10⁻⁶ M) or to sodium nitroprusside (10⁻⁷ M) was recorded at four levels of perfusion pressure (approximately 40, 70, 110, and 150 mmHg). The lowest pressure (40 mmHg) was attained by lowering the perfusion flow rate (8–10 mL/min), the intermediate pressure (70 mmHg) was reached by perfusing at the normal flow rate (11–15 mL/min), and the two higher pressures (110 and 150 mmHg) were achieved by perfusing at the normal flow rate with an intermediate (10⁻⁸, 10⁻⁷ M) and high (10⁻⁶ M) concentration of U46619 in the perfusion solution, respectively. This latter concentration (10⁻⁶ M) was maximal, as higher concentrations of U46619 did not increase the perfusion pressure further.

To analyse the effects of treatment with Ap5A on coronary blood flow and myocardial function, hearts were perfused with Ap5A during reperfusion after ischaemia. In these experiments, the hearts were equilibrated by perfusing for 15 min, and then they were subjected to 30 min of ischaemia and reperfused for a further 15 min. Ap5A (10⁻⁵ M) was added at the beginning of the reperfusion and it remained present throughout the reperfusion period. Other protocols were also employed whereby the hearts were perfused during reperfusion in the presence of both Ap5A (10⁻⁵ M) and PPADS (10⁻⁷ M), where they were subjected to ischaemia–reperfusion without any prior exposure to drugs, or when they were perfused with Ap5A (10⁻⁶ M) for 15 min after 45 min of perfusion without ischaemia. During all these experiments, the perfusion pressure was maintained at 70 mmHg to detect the changes in coronary flow produced by Ap5A and/or ischaemia.

The coronary vascular response is expressed as the mean (± SEM) change in perfusion pressure and the coronary responses before and after ischaemia–reperfusion were compared with the paired Student’s t-test. The responses to the antagonists were compared with the responses in their absence using one-way ANOVA followed by Dunnet’s t test. A probability of <0.05 was considered significant.

The substances used were: P₁,P₅,di(adenosine-5') pentaphosphate pentaammonium salt (diadenosine pentaphosphate, Ap5A); pyridoxalphosphate-6-azo(benzene-2,4-disulfonic acid) tetrasodium salt (PPADS); 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); 9,11-dideoxy-11α,9β-epoxymethanoprostaglandin F₂α (U46619); Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), meclofenamic acid sodium salt (meclofenamate), all obtained from Sigma.

3. Results

In hearts perfused at the basal coronary resting tone (n = 13, Figure 1) the coronary perfusion pressure did not
change, while after 30 min ischaemia and 15 min of reperfusion the left ventricular developed pressure ($P < 0.001$), maximal $dP/dt$ ($P < 0.001$) and heart rate ($P < 0.05$) diminished. Following coronary pre-contraction with U46619 prior to ischaemia–reperfusion ($n=15$, Figure 1), the coronary perfusion pressure and the left ventricular developed pressure was higher than in hearts perfused at resting pressure, while the maximal $dP/dt$ and heart rate remained similar. After ischaemia–reperfusion, in the hearts during coronary pre-contraction with U46619, coronary perfusion pressure, left ventricular developed pressure, and heart rate were not significantly different to that before ischaemia–reperfusion, although the maximal $dP/dt$ was lower ($P < 0.05$).

In time-control hearts perfused at basal coronary resting tone ($n=5$, Figure 1), coronary perfusion pressure, left ventricular developed pressure, maximal $dP/dt$, and heart rate were similar at the beginning of the experiment and 45 min later.

At basal coronary tone before ischaemia–reperfusion, injection of Ap5A ($10^{-7}$–$10^{-5}$ M, $n=5$) into the hearts produced a small, transient increase in perfusion pressure that was followed by a marked reduction in perfusion pressure (Figure 2), as well as a reduction in left ventricular

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**Figure 1** Diagram showing the experimental protocol, the times at which the infusions were applied, and the values of the haemodynamic parameters at the time of the first and the second infusion of diadenosine pentaphosphate. CPP, coronary perfusion pressure; LVDP, left ventricle developed pressure; $MdP/dt$, maximal $dP/dt$; and HR, heart rate.

**Figure 2** Maximum initial coronary contraction (positive values) and the more slowly developing coronary vasorelaxation (negative values) are shown as a function of the concentration of diadenosine pentaphosphate ($10^{-7}$–$10^{-5}$ M) applied to the coronary perfusion solution. Measurements were taken repeatedly 45 min apart in control experiments (top panel), as well as before and after a 30 min total ischaemia followed by 15 min of reperfusion (bottom panel, left and right, respectively). Values are the mean ($\pm$ SEM) of five experiments. *; ** Statistically significant ($*P < 0.05; **P < 0.01$) with respect to the control.
developed pressure, maximal dP/dt, and heart rate (Table 1). After ischaemia–reperfusion, the vasoconstriction in response to Ap5A increased while vasodilatation diminished in comparison to the response before ischaemia–reperfusion (Figure 2). In contrast, the reductions in left ventricular developed pressure, maximal dP/dt, and heart rate were similar (Table 1).

Table 1 Reduction (%) of left ventricular developed pressure, systolic dP/dt, and heart rate produced in rat hearts perfused at basal coronary tone by injection of diadenosine pentaphosphate ($10^{-7} - 10^{-5}$ M) before (control) and after ischaemia–reperfusion. Values are means ± SEM of 5–6 experiments

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<th>Control</th>
<th>Ischaemia–reperfusion</th>
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<tr>
<td></td>
<td>$10^{-7}$ M</td>
<td>$10^{-6}$ M</td>
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<tr>
<td></td>
<td>$10^{-7}$ M</td>
<td>$10^{-6}$ M</td>
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<tr>
<td>Left ventricular developed pressure</td>
<td>11 ± 2</td>
<td>20 ± 6</td>
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<tr>
<td>Maximal dP/dt</td>
<td>10 ± 4</td>
<td>16 ± 3</td>
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<tr>
<td>Heart rate</td>
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<td>1 ± 1</td>
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<tr>
<td></td>
<td>1 ± 1</td>
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<td>1 ± 1</td>
<td>12 ± 3</td>
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<td></td>
<td>0</td>
<td>10 ± 4</td>
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When hearts were pre-contracted with U46619 and treated with PPADS ($n = 5$), the contraction in response to Ap5A was less than in pre-contracted hearts not exposed to PPADS, both before and after ischaemia, whereas relaxation was not modified. After ischaemia–reperfusion in the presence of PPADS the relaxation was weaker and the contraction was stronger than before ischaemia–reperfusion in the presence of PPADS (Figure 4).

When hearts were subjected to coronary pre-contraction with U46619 and treated with reactive blue 2 ($n = 5$), the contraction to Ap5A was stronger than in hearts pre-contracted but not exposed to reactive blue 2. In addition, relaxation was nearly abolished both before and after ischaemia. After ischaemia–reperfusion in the presence of reactive blue 2, the contraction was higher than that before ischaemia–reperfusion in the presence of reactive blue 2 for Ap5A $10^{-7}$ M, lower for $10^{-6}$ M, and similar for $10^{-5}$ M (Figure 4). The response to Ap5A, before or after ischaemia–reperfusion was not significantly modified by exposing hearts pre-contracted with U46619 to L-NAME ($n = 5$, Figure 4).

When subjected to coronary pre-contraction with U46619, meclofenamate treatment before ischaemia–reperfusion ($n = 6$) did not modify contraction or relaxation in response to Ap5A, although after ischaemia–reperfusion it diminished the contraction and increased the relaxation to this diadenosine (Figure 4).

At basal tone, injection of sodium nitroprusside ($10^{-8}$ – $10^{-6}$ M) into the hearts produced vasodilatation, which was significantly reduced after ischaemia–reperfusion ($10^{-8}$ M, $2 ± 1$ vs. $7 ± 2$ mmHg; $10^{-7}$ M, $6 ± 1$ vs. $28 ± 5$ mmHg, $P < 0.05$; $10^{-6}$ M, $10 ± 2$ mmHg vs. $32 ± 6$, $P < 0.05$; $n = 4$). Moreover, the relaxation induced by sodium nitroprusside was similar before and after ischaemia–reperfusion in hearts pre-contracted with U46619 ($10^{-5}$ M, $14 ± 4$ vs. $13 ± 2$; $10^{-7}$ M, $36 ± 7$ vs. $53 ± 10$; $10^{-6}$ M, $51 ± 7$ vs. $58 ± 11$; $n = 6$).

When hearts were perfused at different pressures, the relaxation to Ap5A increased with the perfusion pressure across the entire range of pressures studied ($37$–$150$ mmHg). However, the initial contraction to Ap5A increased with perfusion pressures in the range $37$–$114$ mmHg while it fell at the highest perfusion pressure examined ($150$ mmHg, Table 2). The relaxation to sodium nitroprusside increased at perfusion pressures between $40$ and $113$ mmHg, although the relaxation at $143$ mmHg was similar to that at $113$ mmHg (Table 2).
At a constant perfusion pressure of 70 mmHg, during the first 5 min of reperfusion after ischaemia (n = 5) there was less coronary flow in the hearts treated with Ap5A (10^{-5} M) than in untreated ones (4.1 ± 0.6 vs. 7.1 ± 0.7 mL/min, n = 5, P < 0.05), whereas after 10 and 15 min of reperfusion coronary flow was similar in both groups (7.2 ± 0.6 vs. 7.9 ± 0.7 and 8.1 ± 0.4 vs. 7.7 ± 0.6 mL/min, respectively). The hearts treated with both Ap5A and PPADS during reperfusion (n = 5) had a similar coronary flow to untreated hearts at 5, 10, and 15 min (7.6 ± 0.9, 8.0 ± 0.8, and 7.9 ± 0.7 mL/min for 5, 10, and 15 min). In addition, after ischaemia–reperfusion the left ventricular developed pressure (37 ± 1 mmHg, P < 0.05), dP/dt (508 ± 42 mmHg/s, P < 0.05), and heart rate (163 ± 28 b.p.m., P < 0.05) in untreated hearts was lower than before ischaemia, and these parameters were no different in treated and untreated ischaemic hearts. Finally, the coronary flow in non-ischaemic hearts treated with Ap5A (22.9 ± 3.8, 25.5 ± 3.7, and 25.9 ± 3.5 mL/min at 5, 10, and 15 min after beginning Ap5A injection, n = 4) was greater than before injection or than in treated or untreated ischaemic–reperfused hearts.

4. Discussion

The results we present here suggest that Ap5A may have vasoactive effects on coronary circulation and that these effects may be altered after ischaemia–reperfusion. Thus, in normal conditions, the main effect of Ap5A on coronary circulation is likely to be vasodilatory, although it can cause a small transient contraction both when hearts are

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**Figure 4** The extent of the initial contraction (positive values) and of the more slowly developing relaxation (negative values) in rat hearts in response to diadenosine pentaphosphate (10^{-6} M) during coronary precontraction with U46619 (10^{-2} M), before (control), after ischaemia–reperfusion, and in the presence or absence (untreated) of PPADS (3 x 10^{-5} M), reactive blue 2 (2 x 10^{-6} M), L-NAME (10^{-4} M), or meclofenamate (2 x 10^{-6} M). Values are the mean (±SEM) of 5-6 experiments and the statistical significance shown is between control and ischaemic–reperfused hearts (*P < 0.05; **P < 0.01) or between treated and untreated hearts (†P < 0.05; ††P < 0.01).

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**Table 2** Maximums of coronary initial contraction and slow relaxation to diadenosine pentaphosphate (10^{-6} M) and relaxation to sodium nitroprusside (10^{-7} M) produced in rat hearts perfused at different initial perfusion pressures

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<tr>
<th>Initial perfusion pressure (mmHg)</th>
<th>Diadenosine pentaphosphate</th>
<th>Sodium nitroprusside</th>
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<tr>
<td>37 ± 1</td>
<td>75 ± 2</td>
<td>114 ± 5</td>
</tr>
<tr>
<td>3 ± 1</td>
<td>5 ± 3</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>18 ± 3</td>
<td>43 ± 3</td>
<td>74 ± 8</td>
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Values are means ± SEM of five experiments.

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perfused at their basal coronary resting tone and when coronary arteries are pre-contracted. This is in accordance with studies of Ap4A, which produces vasodilatation in the canine heart, of Ap3A, Ap4A, Ap5A, and Ap6A that produce vasodilatation in the guinea pig heart, and of Ap4A and Ap5A that produce vasodilatation in pig coronary arteries and in rabbit coronary circulation. However, the effect of ApnA on blood vessels may depend on their previous contractile tone. Indeed, Ap5A in human renal arteries, and Ap3A, Ap4A, Ap5A, and Ap6A in rat mesenteric arteries produced vasoconstriction when the arteries were at the basal tone and vasodilatation when they were pre-contracted. In contrast, we found that the response was predominantly vasodilatation in both cases. This may reflect the differences in experimental design, since the coronary arteries of the hearts perfused under basal conditions in our preparations may have some degree of tone. Conversely, in isolated vascular segments basal contractile tone is likely to be very low and under these latter conditions, the vasoconstrictor effects of APnA will probably be more evident. We have seen that isolated segments of the anterior descending coronary artery of the rat did not relax on exposure to Ap5A unless they were pre-contracted (unpublished results). In addition, Ap5A also reduced myocardial contractility and heart rate, in agreement with the negative inotropic effects of Ap4A found in the dog and guinea-pig heart, and of Ap3A, Ap4A, and Ap5A in guinea-pig hearts.

After ischaemia–reperfusion, the relaxation provoked by Ap5A was milder and vasoconstriction augmented, both in hearts at the basal tone and in those with increased vascular tone. At basal tone, the reduced vasodilatation in the heart may in part be non-specific, as the relaxation produced by sodium nitroprusside was also milder. These reductions may be due to differences in the contractile tone of the coronary vasculature, particularly since our results suggest that the increase in contractile tone enhances, and that a reduction in contractile tone impairs, the relaxation in response to both Ap5A and sodium nitroprusside. In the hearts perfused at basal resting tone, there may be changes in the contractile status of the coronary vessels that reduce the amount of tone available for relaxation, which could be due to metabolites released from the working or hypoxic myocardium. However, when vascular tone was increased with U46619, the relaxation produced by sodium nitroprusside was not altered after ischaemia–reperfusion, while the relaxation to Ap5A was reduced and contraction increased. Pre-contracting with U46619 may increase coronary tone and eliminate any possible differences in tone between control and ischaemia–reperfusion, thereby abolishing the non-specific constraint on relaxation to sodium nitroprusside but not the specific impairment in relaxation to Ap5A. Thus, the coronary effects of AP5P may change after ischaemia–reperfusion, increasing the contractile effects while impeding its vasodilatory effects.

The coronary constrictor and dilatory effects of Ap5A may be mediated by different receptors. The antagonist of purinergic receptors of the P2x subtype, PPADS, reduced contraction while leaving relaxation unaffected, whereas the antagonist of purinergic P2y receptors, reactive blue 2, reduced the relaxation and augmented contraction. Therefore, coronary vasoconstriction in response to Ap5A may be mediated by purinergic P2x receptors and coronary vasodilatation may be mediated by purinergic P2y receptors, in agreement with other studies. For example, in rat mesenteric arteries the vasodilator effect of Ap2A and Ap3A was mediated by purinergic P2y receptors, and the vasoconstrictor effect of Ap4A, Ap5A, and Ap6A by purinergic P2x receptors. In human mesenteric arteries the vasoconstriction in response to Ap4A and Ap5A was controlled by purinergic P2x receptors, and the relaxation to Ap4A was mediated by P2y receptors but not that in response to Ap5A. In rat renal circulation, P2x receptors mediate vasoconstriction in response to Ap4A, Ap5A, and Ap6A. Moreover, Ap5A inhibits ATP-sensitive potassium channels and adenylylate kinase activity, which could also contribute to Ap5A vasoconstriction.

In the hearts treated with PPADS, the relaxation after ischaemia–reperfusion remained low when compared with that observed in the same hearts before ischaemia. However, in the hearts treated with reactive blue 2, only contraction in response to the lower concentration of Ap5A increased after ischaemia–reperfusion, whereas the contraction in untreated hearts in response to all the concentrations studied increased after ischaemia–reperfusion. PPADS or reactive blue 2 did not modify the coronary perfusion pressure, suggesting that changes in basal tone are not involved in the effects of these antagonists on the response to Ap5A. Therefore, the changes in the response to Ap5A after ischaemia–reperfusion may be due to a dampened response to the activation of purinergic P2y receptors and not to an increase in the response to purinergic P2x receptor activation, particularly since blocking of P2y receptors reduced these changes after ischaemia–reperfusion but not blockage of P2x. During ischaemia–reperfusion, the receptors in all cell types of the heart could to some extent be altered by degradation or changes in their expression. Also, ATP or ADP, which may be released from damaged post-ischaemic heart tissue, may desensitize purinergic receptors.

It is known that ischaemia–reperfusion may produce endothelial dysfunction and therefore reduce the endothelium-dependent relaxation mediated by nitric oxide. However, the relaxation induced by Ap5A in our experimental conditions may not be mediated by nitric oxide as it was not affected by L-NAME. Thus, nitric oxide reduction may not be involved in the impaired relaxation to AP5P observed after ischaemia–relaxation. Participation of nitric oxide in response to APnAs may vary, since while it might mediate the vasodilatation provoked by Ap4A in the canine heart, it may not be involved in the vasoconstrictor effects of Ap5A, Ap4A, and Ap5A in the guinea-pig coronary circulation. Indeed, in the rabbit heart nitric oxide may participate in the relaxation to Ap4A but not that to Ap3A.

In control conditions, prostanoids do not seem to be involved in the effects of Ap5A in coronary circulation, because the cyclooxygenase inhibitor meclofenamate does not modify vasodilatation or vasoconstriction in response to this substance. However, the inhibition of prostanoic synthesis with meclofenamate after ischaemia–reperfusion increased relaxation and reduced contraction. Hence, after ischaemia–reperfusion there appears to be some production of vasoconstrictor prostanoids that may contribute to the increased vasoconstriction and impaired vasodilatation produced by Ap5A. In coronary ischaemia–reperfusion a change in arachidonic acid metabolism may provoke vasoconstrictor prostanoid production, as observed in goat or pig coronary circulation.
A frequent complication of clinical coronary ischaemia-reperfusion is the phenomenon of no-reflow, by which the reduction in coronary blood flow persists after reperfusion. This phenomenon may be related to impaired vasodilatation and/or to increased vasoconstriction produced by substances that affect the coronary vasculature during ischaemia. While we found that Ap5A predominantly induces vasodilatation in control conditions, this response switches to vasoconstriction after ischaemia-reperfusion. If perfusion pressure remains constant in non-ischaemic hearts, treatment with Ap5A markedly increases coronary blood flow, in accordance with the hypothesis that this substance produces mainly vasodilatation in control conditions. However, after ischaemia treatment with Ap5A reduced rather than increase coronary flow during the first minutes of reperfusion. Therefore, Ap5A released during reperfusion may have a harmful effect on cardiac recovery by further reducing coronary blood flow. Conversely, treatment with PPADS improved coronary flow in ischaemic-reperfused hearts treated with Ap5A and thus, blockade of purinergic P2X receptors or cyclo-oxygenase inhibitors, found that the coronary vasoconstrictor response to Ap5A was even higher locally, in the proximity of activated platelets, and thus, Ap5A may indeed be involved in the increased vasoconstriction in this condition.

The effects of Ap5A observed here were observed at concentrations that might be present in the plasma during ischaemia-reperfusion. Indeed, ApnA concentrations may be even higher locally, in the proximity of activated platelets, and thus, Ap5A may indeed be involved in the increased vasoconstriction associated with this condition. We also found that the coronary vasoconstrictor response to Ap5A after ischaemia-reperfusion may be impaired by antagonists of purinergic P2X receptors or cyclo-oxygenase inhibitors, suggesting that these agents could be useful in the treatment of alterations to coronary perfusion in this condition.

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