

Role of Adenosine A1 Receptors in Remote Ischemic Preconditioning Protection

Papel de los receptores A1 de adenosina en el efecto protector del preconditionamiento isquémico remoto

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ABSTRACT

Background: Adenosine is involved in classical preconditioning acting especially through adenosine A1 and A3 receptors.

Objective: The objective of our study was to evaluate whether remote ischemic preconditioning (rIPC) activates adenosine A1 receptors before ischemia or at the onset of reperfusion to reduce myocardial infarct size.

Methods: Isolated rat hearts were subjected to 30-min ischemia and 60-min reperfusion (I/R). In a second group, a rIPC protocol (3 cycles of hindlimb ischemia/reperfusion) was performed. Infarct size was measured with triphenyltetrazolium staining.

Results: Remote IPC significantly decreased infarct size. This effect was abolished when DPCPX (A1 receptor blocker) or L-NAME (nitric oxide synthesis inhibitor) were administered during reperfusion.

Conclusions: We demonstrated in the isolated rat heart that rIPC reduces myocardial infarction by activation of the adenosine A1 receptor at the onset of the reperfusion period. This protective effect would be also mediated by the activation of nitric oxide synthase during reperfusion.

Key words: Myocardial Infarction - Ischemic Preconditioning, Myocardial/methods - Receptor, Adenosine A1 - Receptor, Adenosine A3

RESUMEN

Introducción: Es conocido que la adenosina está involucrada en el mecanismo de preconditionamiento isquémico clásico, actuando a través de los receptores A1 y A3.

Objetivos: Evaluar si el preconditionamiento isquémico remoto (rIPC) activa los receptores de adenosina A1 antes de la isquemia o en la reperusión y, de ese modo, reduce el tamaño del infarto de miocardio.

Material y métodos: Corazones aislados de rata fueron sometidos a 30 minutos de isquemia y 60 minutos de reperusión (I/R). En otro grupo de ratas, se realizó un protocolo de rIPC. El tamaño del infarto se midió con trifenil de tetrazolio.

Resultados: El rIPC disminuyó significativamente el tamaño del infarto. Este efecto fue abolido cuando se administró DPCPX (bloqueante del receptor A1) o L-NAME (inhibidor de la síntesis de óxido nítrico) durante la reperusión.

Conclusiones: Empleando un modelo de corazón aislado de rata demostramos que el rIPC reduce el tamaño del infarto de miocardio mediante la activación del receptor A1 de adenosina al inicio de la reperusión miocárdica. Este efecto protector también estaría mediado por la activación de la enzima óxido nítrico sintasa durante la reperusión.

Palabras claves: Infarto de miocardio - Precondicionamiento isquémico miocárdico/métodos - Receptor de adenosina A1 - Receptor de adenosina A3

Abbreviations

DPCPX	8-cyclopentyl-1,3-dipropylxanthine	NO	Nitric oxide
I/R	Ischemia/reperfusion	NOS	Nitric oxide synthase
L-NAME	NG-nitro-L-arginine methyl ester		

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INTRODUCTION

Remote ischemic preconditioning (rIPC) is a cardioprotective phenomenon whereby brief ischemia/reperfusion episodes of an organ or tissue render the heart resistant to a subsequent prolonged ischemic episode. (1)

Remote IPC has been well studied in the last 25 years. However, certain aspects of its mechanism of action are still unknown. Some authors have demonstrated that transfer of the cardioprotective signal from the peripheral organ to the target organ involves humoral factors, (2), neuronal pathways, (3, 4) or a neurohumoral interaction. (1) In a previous study, we have shown that rIPC activates cardiac protection signals before myocardial ischemia. (5) However, this study did not evaluate cellular or molecular changes occurring during myocardial reperfusion.

Adenosine might activate the mechanism of rIPC in tissues located remotely from the heart; (6, 7, 8) nevertheless, it is unclear whether rIPC increases the local adenosine concentration in the remote tissue/organ and stimulates the neurogenic pathway, (9) or participates directly on the heart. Different authors have confirmed the participation of adenosine before myocardial ischemia, (9, 10) but the role of this nucleoside during reperfusion in hearts subjected to rIPC has not been studied yet.

Therefore, the purpose of this work was to evaluate the role of adenosine A1 receptors on the cardioprotective effects of rIPC, considering its activation before myocardial ischemia or at the onset of reperfusion.

METHODS

Sprague Dawley rats (300-350 g) were used. The experimental procedures were approved by the Animal Care Research Committee of Universidad de Buenos Aires (Protocol #2948/10, in accordance with the Guide for the Care and Use of Laboratory Animals published by the USA National Institute of Health (NIH).

Surgical procedure

Rats were anesthetized with urethane (1.5 g/kg) followed by intubation and ventilation with a mixture of ambient air and oxygen, using a 683 Harvard respirator. The animals

were then randomly assigned to the different experimental groups (Figure 1). Following group allocation, the left femoral artery was dissected free of surrounding tissue to perform the rIPC protocol.

After completing the in vivo protocols (see later), the animals were sacrificed with an overdose of urethane. The heart was removed and mounted by the aortic root in a perfusion system for isolated organ, according to the Langendorff technique, and stabilized for 20 minutes.

Experimental groups (Figure 1)

1. I/R (n=8): Rats were anesthetized and the femoral artery was dissected and exposed. After a 30-minute monitoring period, the hearts were removed and perfused according to the Langendorff technique. After 15 minutes of stabilization, a 30-minute global ischemia was performed, followed by 60 minutes of reperfusion.
2. rIPC (n=8): Once the rats were anesthetized and the femoral artery was dissected and exposed, the animals were preconditioned with a 5-min ischemia - 5-min reperfusion protocol by left femoral artery occlusion. Then the hearts were subjected to the same protocol as in the I/R group to induce myocardial infarction.
3. rIPC + DPCPX (n=6): 5 minutes before the onset of the rIPC protocol, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 100 $\mu\text{g}/\text{kg}/\text{IP}$), a selective adenosine A1 receptor blocker was administered. Infarction was induced as previously explained.
4. rIPC + DPCPX (R) (n=6): The same protocol as in the rIPC group was used, except that 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 1 μM) was administered during the first 10 min of reperfusion.
5. rIPC + L-NAMETM (n=7): The same protocol as in the rIPC group was used, except that NG-nitro-L-arginine methyl ester (L-NAME, 100 mmol/l), a nitric oxide synthesis inhibitor, was administered during the first 10 min of reperfusion.

Infarct size

After the 60-minute reperfusion period in the Langendorff system, the hearts were frozen and sliced from apex to base in 2-mm thickness transverse sections, incubated during 20 minutes in 1% triphenyltetrazolium chloride (pH 7.4; 37° C) and then immersed in 10% formaldehyde. With this technique, viable myocardial zones stain red, while infarct zones will remain unstained. Finally, sections were scanned and

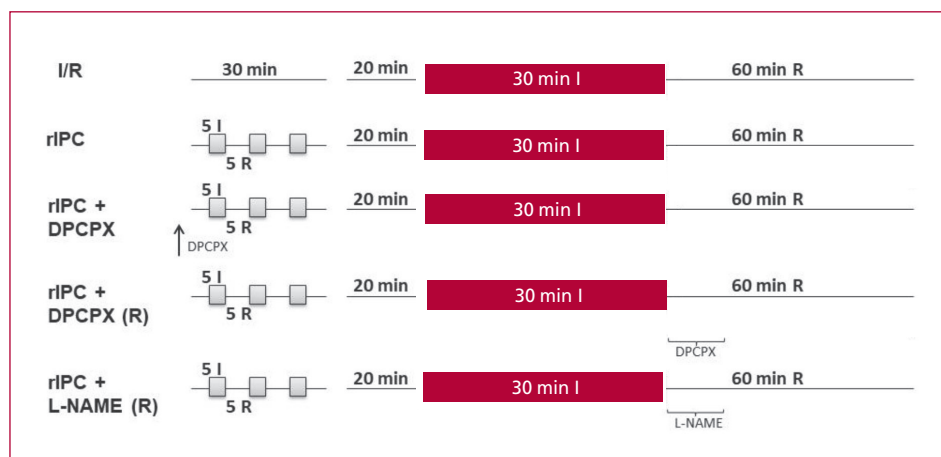
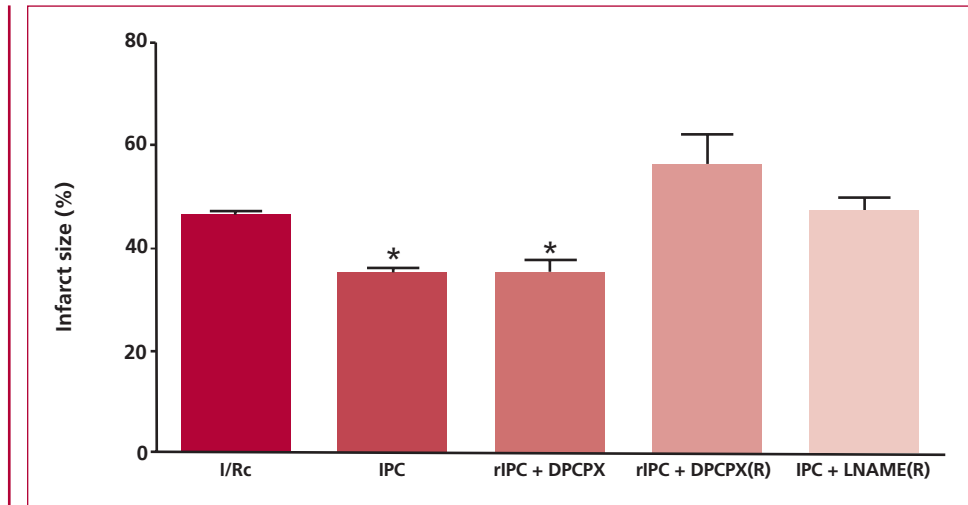


Fig. 1. Representative diagram of experimental protocols. I: Ischemia. R: Reperfusion. rIPC: Remote ischemic preconditioning. DPCPX: 8-cyclopentyl-1,3-dipropylxanthine; L-NAME: NG-nitro-L-arginine methyl ester. (R): Drugs applied at the onset of reperfusion.

Fig. 2. Infarct size, expressed as percentage of left ventricular area. Remote IPC significantly reduced infarct size and this effect was abolished by the administration of DPCPX and L-NAME at the onset of reperfusion. I: Ischemia. R: Reperfusion. rIPC: Remote ischemic preconditioning, DPCPX: 8-cyclopentyl-1,3-dipropylxanthine; L-NAME: NG-nitro-L-arginine methyl ester. (R): Drugs applied at the onset of reperfusion. *: $p < 0.05$ vs. I/R.



viable and infarcted areas measured (Image Pro Plus 4.5). Infarct size was expressed as percentage of left ventricular area.

RESULTS

Figure 2 shows infarct size induced by 30-minute global ischemia followed by 60-minute reperfusion. In the I/R group, infarct size was $44.6 \pm 2.2\%$, while rIPC decreased it to $35.6 \pm 1.2\%$ ($p < 0.05$ vs. I/R group), evidencing the significant protection of rIPC. The administration of DPCPX before the rIPC protocol did not influence the effects of this cardioprotective strategy on infarct size. However, blockade of the adenosine A1 receptor at the onset of reperfusion, completely eliminated the beneficial effect of rIPC, resulting in infarct size of $46.4 \pm 3.3\%$ ($p < 0.05$ vs. rIPC).

The administration of L-NAME at the onset of reperfusion completely abolished the rIPC effect. In this case, infarct size was $46.4 \pm 2.8\%$, thus showing the participation of nitric oxide in the mechanism of rIPC.

DISCUSSION

In this study we investigated the role of the adenosine receptor in the mechanism of rIPC, analyzing its activation before myocardial ischemia or at the onset of reperfusion. We have also demonstrated that the activation of adenosine A1 receptors during early reperfusion is associated with nitric oxide (NO) synthesis, as L-NAME administration abolished the beneficial effect of rIPC.

Pell et al. demonstrated that only one cycle of renal preconditioning reduced myocardial infarct size in an in vivo rabbit model. This cardioprotective effect was abolished by 8-(p-sulfophenyl)-theophylline (8-SPT), a non-selective adenosine receptor blocker, which suggests its participation in the mechanism of acute myocardial preconditioning. (9) Takaoka et al. also described that renal rIPC significantly reduces infarct size and improves myocardial energy metabolism in rabbit hearts subjected to I/R, through the activation of adenosine receptors. (10). Dong et al showed

that rIPC induces cardioprotective effects against I/R injury, and that this was eliminated following the resection of the femoral nerve and the administration of DPCPX, suggesting the participation of adenosine in the remote tissue and the activation of the neural pathway of preconditioning. (7) However, none of these studies assessed the participation of adenosine receptors before myocardial ischemia and at the onset of reperfusion. In the present investigation we show the importance of adenosine A1 receptor activation only during reperfusion, to achieve cardioprotection.

Nitric oxide can modulate mitochondrial permeability transition pore opening (mPTP) in isolated mitochondria. (11) The mPTP is a non-specific channel inducing mitochondrial inner membrane depolarization. This produces ATP depletion, which improves the colloidal osmotic pressure in the mitochondrial matrix leading to swelling and mitochondrial external membrane rupture. (12) The physiological concentration of NO can inhibit the mPTP through mechanisms related with S-nitrosylation. (11) Several studies have demonstrated that NO production can protect the heart against I/R injury. (13) In the present study, we observed that rIPC cardioprotection was abolished by L-NAME, suggesting the participation of NO in rIPC.

In conclusion, with this investigation we demonstrate that in the isolated rat heart, rIPC limits infarct size through the activation of adenosine A1 receptors during early reperfusion. The results of this work also indicate the participation of the NOS enzyme in the mechanism of rIPC. Further studies investigating the underlying myocardial transduction pathways of rIPC are necessary to explain the final effector leading to protection after activation of the A1 receptor.

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