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Short communication

Reperfusion duration paradox with late myocardial preconditioning in rabbits

Nicolas Lellouche, Renaud Tissier, Rachid Souktani, Jean-François Giudicelli, Alain Berdeaux *, Bijan Ghaleh

Département de Pharmacologie, Faculté de Médecine Paris Sud, INSERM E 00.01, 63, rue Gabriel Péri, 94270 Le Kremlin-Bicêtre, France

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Abstract

In this study, pharmacological late preconditioning was induced in 53 rabbits with an adenosine A_1 receptor agonist (2-chloro-N^6-cyclopentyladenosine, CCPA, 100 μg/kg), or a NO-donor (*S*-nitroso-*N*-acetyl-penicillamine, SNAP, 2.5 μg/kg/min; 75 min) vs. saline as control. Later, after 24 h, rabbits underwent a 30-min coronary occlusion and subsequent reperfusion. After 3 h of coronary artery reperfusion, infarct size was reduced with CCPA (43±4%) and SNAP (27±4%) vs. saline (56±4%). However, after 72 h of coronary artery reperfusion, infarct sizes were similar in all groups, demonstrating an only transient effect of late preconditioning against myocardial infarction. Combined administration of CCPA and SNAP failed to induce sustained cardioprotection.

Keywords: Preconditioning, delay; Myocardial infarction; Adenosine; Nitric oxide (NO) donor

1. Introduction

Brief periods of myocardial ischemia have been demonstrated to induce late preconditioning against myocardial infarction (Marber et al., 1993). This phenomenon can be pharmacologically mimicked by, e.g., nitric oxide donors (Takano et al., 1998b), adenosine A_1 receptor agonists (Baxter et al., 1994; Zhao et al., 2000; Takano et al., 2001) or opioids (Fryer et al., 1999). In most of these studies, infarct size was measured after short durations of coronary artery reperfusion (i.e., not exceeding 3 h). One group also demonstrated beneficial effect of late preconditioning after 72 h of coronary artery reperfusion (Takano et al., 1998a,b, 2001) and confirmed these results using a functional approach (Takano et al., 2000). However, Miki et al. (1999b) reported controversial results in conscious rabbits. These authors demonstrated a cardioprotective effect of ischemic late preconditioning when infarct size was measured after 3 h of coronary artery reperfusion but no longer when it was assessed by histology after 72 h of coronary artery reperfusion. Similar effects have been reported by our group with adenosine A_1 receptor stimulation-induced late preconditioning (Tissier et al., 2002). In the same study, increase in the intensity of this stimulation also failed to prolong cardioprotection up to 72 h of coronary artery reperfusion. Therefore, in order to reach the threshold necessary to achieve a sustained cardioprotection, our hypothesis was that instead of increasing the intensity of late preconditioning stimulus, one should rather try to combine at least two pharmacological stimuli that might exert a synergistic effect. For this purpose, we investigated at 72 h of coronary artery reperfusion the effect of the combination of two well-characterized delayed cardioprotective stimuli, i.e., the stimulation of the nitric oxide pathway using *S*-nitroso-*N*-acetylpenicillamine (SNAP) (Takano et al., 1998b) and that of the adenosine A_1 receptor using 2-chloro-N^6-cyclopentyladenosine (CCPA) (Baxter et al., 1994), on myocardial infarct size in rabbits.

2. Methods

The animal instrumentation and the ensuing experiments were performed in accordance with the official regulations edicted by the French Ministry of Agriculture (approval #A94-043-12).

* Corresponding author. Tel: +33-1-49-59-67-02; fax: +33-1-49-60-00-31.

E-mail address: alain.berdeaux@kb.u-psud.fr (A. Berdeaux).

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2.1. Surgical procedure

Fifty-three male New Zealand rabbits (2–2.5 kg) were anesthetized with a mixture of tiletamine (25 mg/kg, i.v.) and zolazepam (25 mg/kg, i.v.). They were intubated and mechanically ventilated with 100% oxygen. Anesthesia was maintained with pentobarbital sodium (20–30 mg/kg, i.v.). A catheter was positioned in the rabbit's ear marginal artery for arterial pressure measurement (Statham P23ID strain gauge, Statham Instruments, Oxnard, CA, USA). An external electrocardiogram was recorded. A left thoracotomy was performed at the fourth intercostal space under sterile conditions. The pericardium was opened and a 4/0 Prolene suture was passed beneath a major branch of the left coronary artery. The ends of the ligature were passed through a short segment of propylene tubing to form a snare. Regional myocardial ischemia was induced by pulling the snare through the tubing. Ischemia was confirmed by the presence of regional cyanosis of the myocardial surface and ST segment deviation of the electrocardiogram. In all animals, the coronary artery occlusion was performed for 30 min and the snare was released. The chest was closed in layers and a small tube was left in the thorax to evacuate air and fluids after surgery. Rabbits underwent either 3 or 72 h of coronary artery reperfusion (see below). Analgesia was induced with flunixine meglumate.

2.2. Measurement of the risk area and infarct size

After completion of reperfusion, animals received heparin and sodium pentobarbital (50 mg/kg, i.v.). Potassium chloride was administered i.v. to induce cardiac arrest. The hearts were excised. The ascending aorta was cannulated and perfused (120 mm Hg) retrogradely with saline followed by Evans blue (1%). The left ventricle was cut into five to six slices. These slices were weighed and incubated in 1% triphenyltetrazolium chloride (TTC, Sigma, Poole, UK) in a pH 7.4 buffer during 15 min at 37 °C. Slices were fixed overnight in 10% formaldehyde and then photographed with a digital camera. Using a computerized planimetric program (Scion Image, Scion, Frederick, MD, USA), the area at risk and the infarcted zones were quantified. The area at risk was identified as the non-blue region and was expressed as a percentage of the left ventricle weight. Infarcted area was identified as the TTC-negative zone and was expressed as a percentage of the area at risk.

2.3. Experimental protocol

The protocol was realized during 2 consecutive days, i.e., 24 h apart. On day 1, rabbits were randomized into "Control", "SNAP" and "CCPA" groups and received an intravenous (ear vein) administration of either saline (bolus), *S*-nitroso-*N*-acetyl-penicillamine (SNAP, 2.5 μg/kg/min; 75 min), or 2-chloro-N^6-cyclopentyladenosine (CCPA, 100 μg/kg, bolus), respectively. These doses were chosen on the basis of previous reports (Baxter et al., 1994; Takano et al., 1998b). On day 2, all animals underwent the 30-min protocol of coronary artery occlusion followed by subsequent reperfusion which lasted either 3 or 72 h. In order to investigate whether the combination of both drugs was able to induce a stronger and more sustained cardioprotection, another group of rabbits received, 24 h before the 30-min coronary artery occlusion, the SNAP infusion followed 5 min later by the administration of CCPA. In this group so-called SNAP+CCPA, infarct size was assessed after 72 h of coronary artery reperfusion.

2.4. Statistical analysis

Data are reported as mean±S.E.M. Comparisons were performed among all groups at one duration of coronary artery reperfusion using a one-way analysis of variance and Fisher's protected least-significant difference test if necessary. Moreover, infarct sizes (expressed as percentage of the left ventricle weights) were plotted against area at risk sizes and an analysis of covariance was performed to detect differences among groups. Significant differences were determined as $P<0.05$.

3. Results

3.1. Hemodynamic

On day 1, baseline values of heart rate and mean arterial pressure were not significantly different in all groups. Intravenous administrations of saline and perfusion of SNAP did not significantly affect heart rate and mean arterial pressure but administration of CCPA decreased both heart rate and mean arterial pressure (averaging −18% and −20%, respectively, in CCPA and SNAP+CCPA groups) as compared to the corresponding baseline value. On day 2, heart rate and mean arterial pressure were not significantly different between Control, SNAP, CCPA and SNAP+CCPA groups at baseline, during coronary artery occlusion and reperfusion.

3.2. Infarct sizes after 3 h of coronary artery reperfusion

The sizes of the area at risk (27±2%, n=12; 27±4%, n=6 and 31±2%, n=7, respectively) were not significantly different among Control, SNAP and CCPA groups at 3 h of coronary artery reperfusion. As illustrated in Fig. 1 (upper panel), infarct sizes were significantly decreased in SNAP and CCPA as compared to Control groups. Because infarct size can be influenced by the size of the area at risk (Ytrehus et al., 1994), the effects of SNAP and CCPA were also investigated by plotting these two parameters expressed as percentage of the left ventricle weight. As shown in Fig. 1 (lower panel), the infarct size/area at risk regression line was shifted downwards by SNAP and CCPA as compared to

Control. The analysis of covariance demonstrated similar significant differences between SNAP and CCPA as compared to Control groups.

3.3. Infarct sizes after 72 h of coronary artery reperfusion

The sizes of the area at risk were not significantly different among Control, SNAP and CCPA groups at 72 h of coronary artery reperfusion (27±2%, n=9; 28±4%, n=6 and 34±3%, n=6, respectively). As illustrated in Fig. 2 (upper panel), infarct sizes were not significantly different among Control, SNAP and CCPA groups. The combination SNAP+CCPA elicited nonsignificantly different values of infarct sizes with similar area at risk (35±3%, n=7) as compared to Control group. As shown in Fig. 2 (lower panel), the infarct size/area at risk regression line was similar among the four groups. The analysis of covariance confirmed this result.

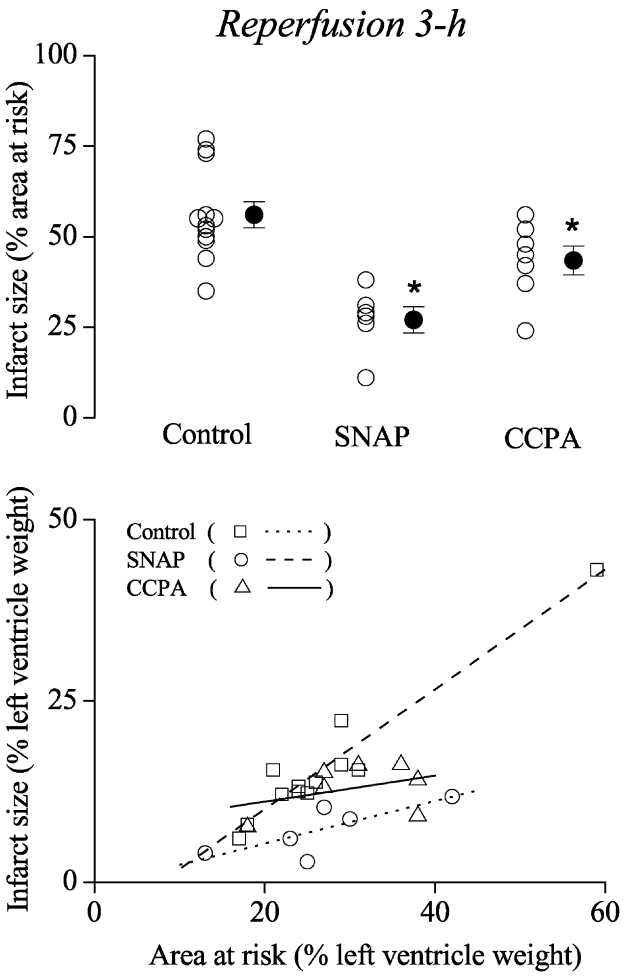

Fig. 1. Upper panel: infarct sizes measured after 3 h of coronary artery reperfusion. Open and closed circles indicate individual values and group means, respectively (SNAP: SNAP-induced late preconditioning; CCPA: CCPA-induced late preconditioning; SNAP+CCPA: combined SNAP and CCPA-induced late preconditioning). *, P<0.05 vs. control group. Lower panel: scatterplots and regression lines of the relationship between the sizes of the infarct and area at risk (expressed as percentage of left ventricular weight).

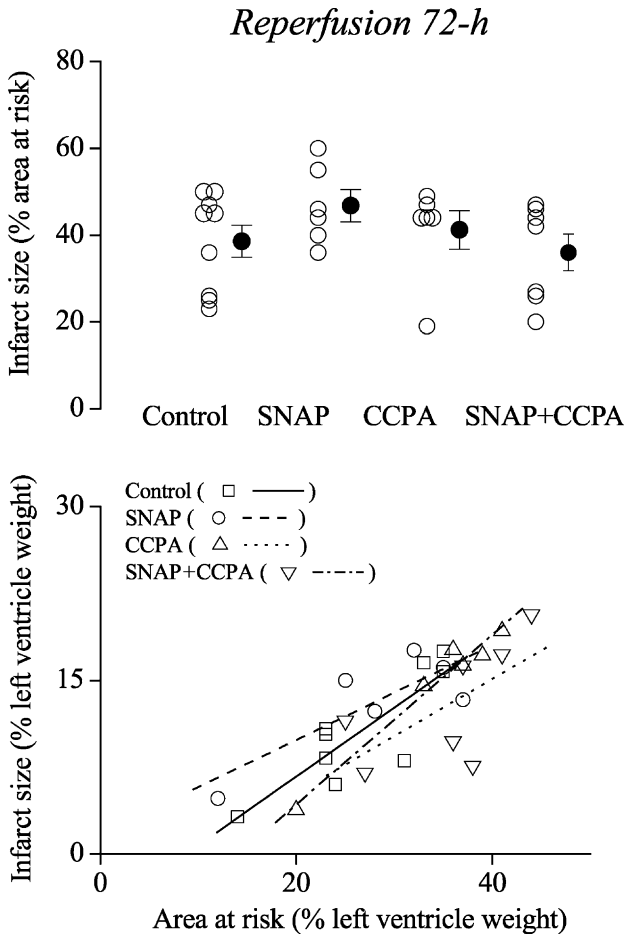

Fig. 2. Upper panel: infarct sizes measured after 72 h of coronary artery reperfusion. Open and closed circles indicate individual values and group means, respectively (SNAP: SNAP-induced late preconditioning; CCPA: CCPA-induced late preconditioning; SNAP+CCPA: combined SNAP and CCPA-induced late preconditioning). *, P<0.05 vs. control group. Lower panel: scatterplots and regression lines of the relationship between the sizes of the infarct and area at risk (expressed as percentage of left ventricular weight).

4. Discussion

The present results indicate that SNAP—as well as CCPA—induced late preconditioning reduced infarct size after 3 h but not 72 h of coronary artery reperfusion, thus demonstrating a "reperfusion duration paradox". The combination of these two stimuli also failed to limit infarct size after 72 h of coronary artery reperfusion, suggesting that the lack of sustained cardioprotection afforded by late pharmacological preconditioning is not due to an insufficient intensity of the preconditioning stimulus.

The "reperfusion duration paradox" has already been reported with ischemia- (Miki et al., 1999b; Tissier et al., 2002) and adenosine A_1 agonist-induced late preconditioning

(Tissier et al., 2002). In these studies, myocardial infarction after 72 h of coronary artery reperfusion was assessed using an accurate histological analysis and similar pattern was demonstrated using TTC-technique (Tissier et al., 2002). The present results further extend this "reperfusion duration paradox" with SNAP-induced late preconditioning. Our hypothesis was that insufficient preconditioning stimulus could be responsible for this paradox in rabbits, although we previously reported for adenosine A_1 agonist-induced late preconditioning that this was not related to insufficient doses of adenosine A_1 receptor agonist (Tissier et al., 2002). In the present study, the combination of two different late preconditioning protocols, i.e., adenosine A_1 agonist- and nitric oxide donor-induced late preconditioning, was not able to induce a sustained infarct size limitation. Thus, it is clear that, in our experimental conditions, the "reperfusion duration paradox" seems neither related to the nature nor the intensity of the late preconditioning stimulus. Finally, it is unlikely that methodological bias would explain our results since early preconditioning dramatically reduced infarct size in our experimental conditions with both 3 and 72 h of coronary artery reperfusion (data not shown).

The opposing results observed at 3 and 72 h of coronary artery reperfusion have previously been discussed regarding ischemia- (Miki et al., 1999b; Tissier et al., 2002) and adenosine A_1 agonist-induced late preconditioning (Tissier et al., 2002). We cannot exclude that the transient cardioprotection observed in SNAP, CCPA and SNAP+CCPA groups after 3 h of coronary artery reperfusion could be related to a delay in the loss of deshydrogenase enzymes in necrotic tissue. Preserved capillary permeability may delay the subsequent washout of deshydrogenases, which might lead to a transient decrease of the TTC-negative zone. We also cannot rule out that late preconditioning may delay the very early evolution of infarct size. Indeed, maximal infarct size is reached only after the very first hours of coronary artery reperfusion in anesthetized rabbits subjected to 30 min of coronary artery occlusion (Birnbaum et al., 1997). One might speculate that this kinetic is altered with late preconditioning. However, the "reperfusion duration paradox" is controversial considering previous reports from Bolli's group (Takano et al., 1998b, 2001). Rabbit's strain variability might at least in part explain such discrepancies as previously demonstrated in mice (Bao et al., 2000).

One point deserves to be mentioned. Smaller "Control" infarct sizes were observed in the present study after 72 h as compared to 3 h of coronary artery reperfusion. This result is probably explained by technical reasons, i.e., underestimation of infarct size using the TTC-technique after 72 h of coronary artery reperfusion (Miki et al., 1999a; Tissier et al., 2002). Indeed, infarct sizes were similar between 3 and 72 h of reperfusion when histology was performed after 72 h of reperfusion. However, this technique is not suitable after short periods of reperfusion.

In conclusion, an adenosine A_1 agonist and a nitric oxide donor induced significant cardioprotection against myocardial infarction when infarct size was assessed after 3 h of coronary artery reperfusion, but this effect vanished after 72 h. The combination of these two pharmacological stimuli also failed to exert additive or synergistic effects and to elicit a more sustained cardioprotection.

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