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Effects of A₁ adenosine receptor antagonism against ischaemia-reperfusion damage and coronary microcirculation in isolated working rat hearts

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Previous studies suggest that A₁ adenosine receptor antagonists may prevent myocardial reperfusion injury by means of an inhibition of superoxide anion release from neutrophils, leukocyte activation and platelet aggregation. We tested the hypothesis of a blood-independent endothelial protection following A₁ adenosine receptor antagonism with DPCPX (1,3 dipropyl,8-cyclopentylxanthine) during ischaemia-reperfusion damage.

Isolated working rat hearts were used and submitted to 10 and 20 min global ischaemia in order to assess functional alterations, necrosis enzyme and purine release in coronary effluent, arrhythmias, heart weight changes, ultrastructural morphometry and microvascular permeability by FITC-albumin diffusion technique. DPCPX (100 mM) was administered to perfusion buffer before ischaemia. In untreated hearts we detected a significant reduction of functional parameters, associated with a significant enzyme and purine release, myocardial oedema and ultrastructural damage. In DPCPX-treated hearts functional and histological damage was significantly reduced compared to controls. Moreover, a significant reduction of postischaemic endothelial hyperpermeability (FITC-albumin diffusion, $p < 0.02$) and ultrastructural damage was observed.

Our data suggest that A₁ adenosine receptor antagonism significantly reduces ischaemia-reperfusion damage in isolated, crystalloid perfused rat hearts by direct reduction of endothelial damage and postischaemic hyperpermeability. *J Clin Basic Cardiol* 1999; 2: 99–104.

Key words: adenosine receptors, DPCPX, coronary microcirculation, reperfusion damage, endothelium, working heart

Several experimental and clinical studies [1, 2] support the hypothesis that adenosine could reduce cardiac ischaemia-reperfusion damage (myocardial ischaemia or infarction, cardiac surgery), whether by an A₁ (myocytic localization) or A₂ (endothelial localization) or A₃ adenosine receptor activation. This effect may be related to reduction of myocardial oxygen consumption (A₁ receptor mediated), platelet aggregation, neutrophil adhesion and activation and moreover regulation of vascular tone and permeability (A₂ receptor mediated). Although cardioprotective effects exerted by adenosine A₁ receptor agonism against ischaemic damage were previously reported [1], recent studies [3, 4] evidenced oppositely better protection against ischaemia-reperfusion injury by inducing an A₁ receptor adenosine blockade in cat lung and heart. The mechanism of this protection is still unknown but it could probably be related to A₂ receptor stimulation that produces coronary vasodilatation, inhibition of superoxide anion release from neutrophils, reduction of adherence of activated neutrophils to endothelial cells and platelet aggregation. The final result is a reduction of postischaemic endothelial damage and microvascular hyperpermeability. We hypothesized that the cardioprotection against reperfusion injury following A₁ receptor adenosine blockade may occur independently of plasmatic factors or haematic corpusculate elements and that it is related to a direct endothelial protection. For these reasons we assessed, in isolated crystalloid-perfused working rat hearts, the effects of A₁ receptor adenosine blockade on postischaemic endothelial damage and coronary microcirculation.

Materials and methods

Adult male Wistar rats ($n = 60$) were used and the experimental protocol was performed according to the Guidelines of the American Physiological Society. Rats were anaesthetized by an air-ether mixture. After injection of 1000 IU heparin into the femoral vein, the hearts were quickly excised, placed in Krebs-Henseleit (KH) cold buffer (4 °C) and, then, perfused according to the working heart technique [5]. The perfusion buffer used in the control group was a modified KH solution (NaCl 108 mM, NaHCO₃ 25 mM, KCl 4.8 mM, KH₂PO₄ 1.2 mM, MgSO₄ 1.2 mM, CaCl₂ 2.5 mM, Glucose 11 mM) bubbled with a mixture of 95 % oxygen and 5 % carbon dioxide and constantly maintained at 37 °C and pH 7.4. Preload (height of atrial chamber) and afterload (height of aortic chamber) were set at 20 and 72 cm respectively. In order to provide an A₁ adenosine receptor blockade we added 100 nM DPCPX (1,3-dipropyl,8-cyclopentylxanthine) to KH solution at the beginning of experimental procedures.

Hearts were divided into the following groups according to experimental protocol (Figure 1):

A. Control ($n = 20$): hearts perfused in working heart mode for 100 min.

B. 10 min ischaemia ($n = 20$): hearts subjected, after 20 min stabilization in working heart mode, to 10 min global normothermic no-flow ischaemia, 10 min Langendorff and 60 min working heart reperfusion.

C. 20 min ischaemia ($n = 20$): hearts subjected, after 20 min stabilization in working heart mode, to 20 min global normothermic ischaemia, 10 min Langendorff and 50 min working heart reperfusion.

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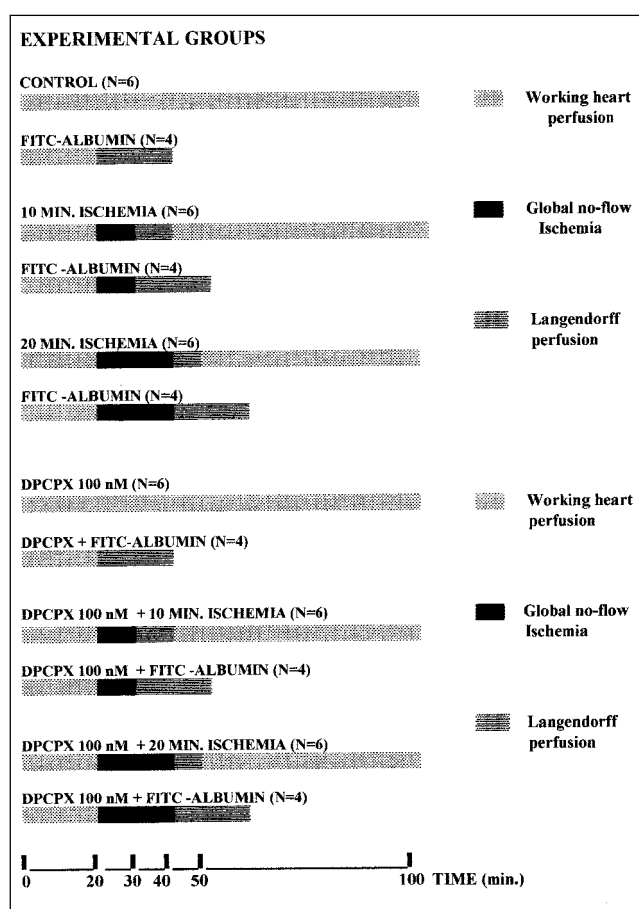


Figure 1. Experimental protocol

The experiments were carried out by perfusing hearts either with normal KH ($n = 10/\text{group}$) or with KH plus DPCPX 100 nM ($n = 10/\text{group}$).

Evaluation of heart weight

The hearts were weighed using an analytical scale (Sartorius Mod. 2842) at the beginning and the end of the experiment; results were expressed as heart weight gain (%): heart weight (gr.) before/heart weight after (gr.) $\times 100$. In addition, three hearts from each group were dried at 105 °C for 24 hours and re-weighed to compute wet weight (ww)/dry weight (dw) ratio (ww/dw).

Left ventricular function (LVF) evaluation

We assessed LVF by measuring the following haemodynamic parameters: aortic pressure, heart rate and aortic and coronary flows. Aortic pressure (mmHg) was monitored by membrane transducer (TNF-R, Viggo-Spectramed, TNF, Oxnard, CA) connected to the aortic cannula, heart rate (bpm) was calculated with an epicardial electrocardiogram (Cardioline Studio 350/1, Remco Italy, I), aortic and coronary flows (ml/min) were measured, at time intervals, by collecting the overflow from the aortic chamber and the effluent from the heart chamber into graduate cylinders.

Coronary perfusate: release of CPK and purine

Every 10 minutes, the coronary effluent was collected in order to determine creatinephosphokinase (CPK) activity by the method of Bergmeyer and Bernt [6]. We also evaluated at 20, 30, 40, 50, 70 and 100 minutes the purines released in the perfusate (adenosine, inosine, hypoxanthine and uric acid) by

high pressure liquid chromatography (HPLC). The separation took place on Supelcosil LC-18T control column. Pump flow was maintained at a constant value of 1.3 ml/min and the quantities of the different purine compounds were measured in relation to the absorbency at 245 and 292 nm, which were then compared to appropriate standard values. A gradient system with two solvents was used. The solvents were: 100 mM ammonium dihydrogen phosphate at pH 6 (A solvent) and methanol (B solvent). Both solvents had been previously filtered with 0.2 μm filters.

Coronary microcirculation permeability evaluation

Microvascular permeability changes were evaluated by means of fluorescein isothiocyanate-labeled albumin diffusion (FITC-albumin diffusion). After ischaemia (groups B and C) or starting at 20 min perfusion (group A) 4 hearts in each group were perfused in Langendorff mode, with 75 mg FITC-albumin (Sigma, Milano, I) dissolved in 200 ml KH (0.375 mg/ml). In all groups mean perfusion time was 20 min. After FITC-albumin perfusion, hearts were perfused for 3–4 min with KH in order to eliminate intravascular fluorescence. Then, hearts were excised, weighed and ventricles cut into 4–5 blocks. Tissue blocks were rapidly immersed in embedding medium (O.C.T. Compound, Miles, Elkhart, IN) and stored at -80 °C. Ten 5 μm sections were obtained from each tissue block and placed on a marked slide. The slides placed in a dark box for at least 1 hr and then viewed and photographed at 40x magnification under fluorescent light. FITC-albumin accumulation was quantified per section using an image analysis system (Image-Pro plus, Media Cybernetics, Silver Spring, MD). The extravasation of FITC-albumin was measured by determining the changes in integrated optical intensity (IOI) [7]. Areas containing arterioles and post-capillary venules were chosen at random and recorded. For data processing, each experimental frame was digitized into 512 $\times$ 512 pixels. Each pixel was associated with an eight-bit gray scale value (a number between 0 and 255). Gray scale values were measured in the interstitium around vessels and myocytes using an image analysis system. IOI was calculated as a sum of gray scale values in a given area and standardized in 180 $\times$ 180 pixels.

Ultrastructural analysis

Tissue specimens were fixed in 2.5 % glutaraldehyde buffered with PBS (pH 7.4), post-fixed in buffered 1.33 % osmium tetroxide, dehydrated and embedded in Epon 812 Resin. Subsequently, thin sections were cut (30–40 sections/specimen), stained with uranyl acetate and lead citrate, and studied by electron microscopy. An average of 30 fields in each section were examined and photographed at 4500x.

Statistical analysis

All data were expressed as mean $\pm$ SD. Statistical analysis was performed using the two-way analysis of variance (ANOVA) and t-test. The probability of null hypothesis of $< 5\%$ was considered statistically significant.

Results

Heart weight

In non-ischaemic hearts we detected a slight increase of heart weight gain without significant differences between groups (control: $+8 \pm 2\%$, DPCPX 100 nM: $+7.2 \pm 1.5\%$); in ischaemic hearts, DPCPX treatment significantly reduced postischaemic increase of weight gain (10 min ischaemia: control: $+25.5 \pm 4\%$, DPCPX 100 nM: $+12.3 \pm 3.8\%$, $p <$

0.001; 20 min ischaemia: control: $+38 \pm 5.5\%$; DPCPX 100 nM $+15 \pm 4.7\%$, $p < 0.001$). The ww/dw ratio is presented in Figure 2. The ww/dw ratio significantly increased after 10 and 20 min ischaemia; this increase was significantly reduced by DPCPX 100 nM perfusion.

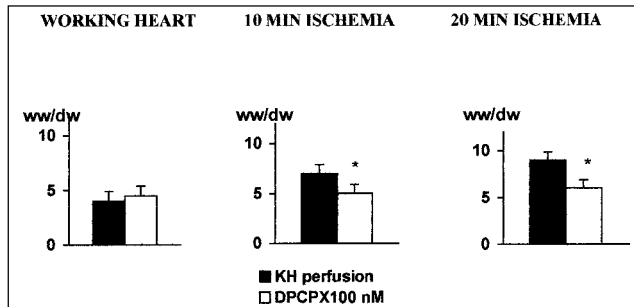


Figure 2. Heart weight changes: wet weight/dry weight (ww/dw) in all experimental groups. Data are expressed as mean and SD: * = $p < 0.01$ vs. KH perfused hearts.

Haemodynamic parameters

The haemodynamic data are reported in Table 1. In non-ischaemic hearts no significant differences were observed according to perfusion buffer used. After 10 and 20 min ischaemia DPCPX-treated hearts have shown a significantly better ventricular performance. As regards coronary flow (Figure 3), in DPCPX-treated hearts a significant improvement was observed during reperfusion whether after 10 or 20 min ischaemia.

Myocardial necrosis enzyme and purine releases

The time-course of myocytic enzyme release is detailed in Table 2. Compared to non-ischaemic hearts, a significant increase of CPK release was noticed in all groups after 10 and 20 min ischaemia. In DPCPX-treated groups this release was significantly reduced. Purine release of all groups is reported in Figure 4. Release of nucleosides (adenosine and inosine) and their metabolites (hypoxanthine and uric acid) was significantly higher in DPCPX-treated hearts, without significant differences between ischaemic and non-ischaemic groups.

Table 1. Haemodynamic data: Systolic aortic pressure, heart rate and aortic flow. Data are reported as mean $\pm$ SD; * = $p < 0.05$, # = $p < 0.01$, ** = $p < 0.001$ (n = 6/group).

Time (min)	CONTROL	DPCPX 100 nM	10 min ischaemia	10 min ischaemia + DPCPX 100 nM	20 min ischaemia	20 min ischaemia + DPCPX 100 nM
Systolic aortic pressure (mmHg)						
20	100(13)	101(9)	98(7)	99(10)	102(9)	101(9)
30	99(8)	98(10)	-	-	-	-
50	97(7)	95(7)	76(9)	90 (11)**	-	-
60	95(8)	93(6)	80(10)	92(8)**	70(7)	90(11)**
80	93(7)	90(6)	84(6)	93(5)**	75(8)	91(10)**
100	90(5)	90(4)	83(4)	91(5)**	74(5)	90(8)**
Heart rate (bpm)						
20	280(10)	305(19)	280(8)	300(10)	280(10)	310(9)
30	280(8)	300(10)	-	-	-	-
50	280(10)	300(10)	280(9)	310(10)	-	-
60	280(10)	300(10)	280(10)	300(10)	270(15)	290(15)
80	280(10)	300(10)	280(10)	300(10)	270(10)	290(10)
100	280(10)	290(15)	280(10)	300(10)	270(10)	290(15)
Aortic flow (ml/min)						
20	60(8)	63 (9)	60(7)	61(10)	59(8)	61(5)
30	59(8)	58(10)	-	-	-	-
50	57(5)	59(4)	44(9)	55(6)**	-	-
60	57(8)	58(6)	47(10)	58(8)#	40(4)	55(7)**
80	56(7)	58(5)	50(6)	60(5)**	45(8)	56(4)**
100	56(5)	58(4)	50(5)	60(5)**	50(6)	57(8)*

Table 2. Release of myocardial necrosis enzymes (mU/ml/grdw): CPK in the effluent perfusate in all groups. Data are expressed as mean $\pm$ SD. * = $p < 0.002$ and ** = $p < 0.001$ vs. controls.

TIME (min)	KH perfusion	DPCPX 100 nM	10 min ischaemia	10 min ischaemia + DPCPX 100 nM	20 min ischaemia	20 min ischaemia + DPCPX 100 nM
10	4 $\pm$ 2	3.7 $\pm$ 2	4.1 $\pm$ 2.2	3.2 $\pm$ 1.9	3.8 $\pm$ 2.1	4.2 $\pm$ 1.6
20	3.5 $\pm$ 1.1	4 $\pm$ 1.5	4 $\pm$ 1.7	3 $\pm$ 1.5	3.5 $\pm$ 2	3.1 $\pm$ 2
30	3 $\pm$ 1	4 $\pm$ 1	---	---	---	---
40	3 $\pm$ 1	4 $\pm$ 1	15 $\pm$ 4	7 $\pm$ 2**	---	---
50	2.5 $\pm$ 1.4	4 $\pm$ 1	9 $\pm$ 3	6 $\pm$ 2*	25 $\pm$ 5.1	11 $\pm$ 4**
60	2 $\pm$ 1	3 $\pm$ 1.5	8 $\pm$ 4	3 $\pm$ 1.4**	18 $\pm$ 4.2	9 $\pm$ 3**
70	2 $\pm$ 1	2.5 $\pm$ 1	9.5 $\pm$ 3.3	3 $\pm$ 1.4**	16 $\pm$ 5	9 $\pm$ 3**
80	3 $\pm$ 1.5	3 $\pm$ 1.5	7 $\pm$ 4	3 $\pm$ 1.4**	12 $\pm$ 4	8 $\pm$ 2.5 *
90	3 $\pm$ 1.5	3 $\pm$ 1.5	7 $\pm$ 4	3.5 $\pm$ 2**	10 $\pm$ 3	6 $\pm$ 2.6**
100	4 $\pm$ 1.1	3.5 $\pm$ 1	8 $\pm$ 3	3.5 $\pm$ 2**	9 $\pm$ 3.8	5 $\pm$ 3*

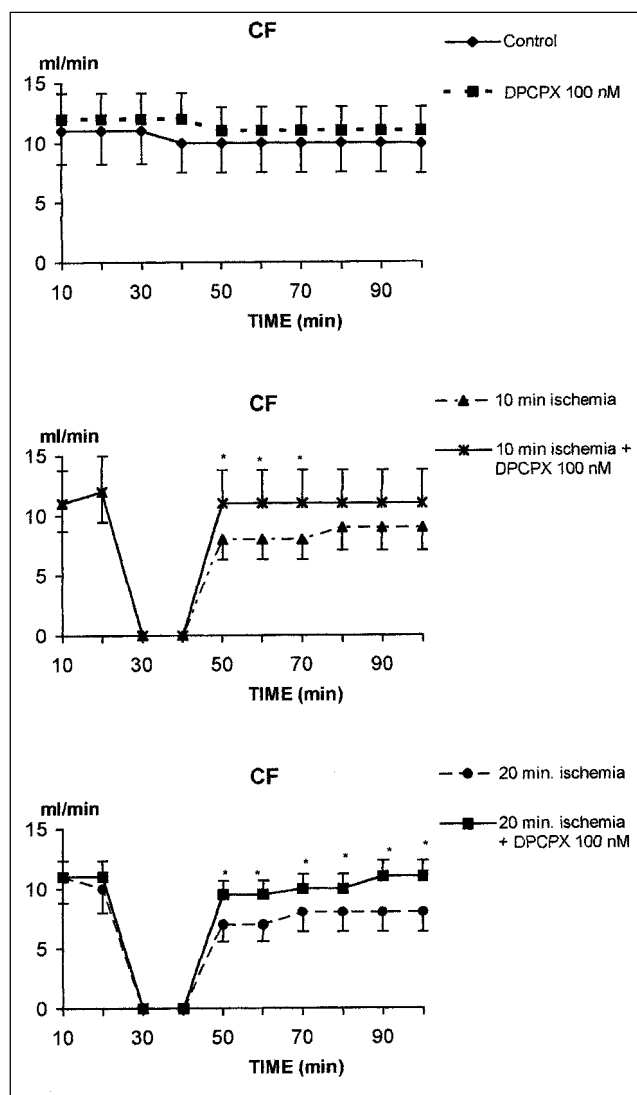


Figure 3. Time course of coronary flow (CF: ml/min) in non-ischaeamic hearts (top) and after 10 (middle) and 20 min. (bottom) ischaemia. Data are expressed as mean and SD; * = $p < 0.01$ vs. KH perfusion.

Microvascular permeability changes

Endothelial permeability changes of coronary microcirculation are reported in Figure 5. DPCPX treatment induced a light increase of perivascular fluorescence in non-ischaeamic hearts, whereas the IOI unit value was unchanged with respect to controls. In ischaemic hearts, DPCPX administration significantly reduced FITC-albumin diffusion (Figure 6).

Ultrastructural data

In non-ischaeamic hearts myocardial ultrastructure (fibers, mitochondria, nuclei) was well preserved without significant differences between KH and DPCPX-perfused groups. After 10 and 20 min ischaemia we detected a significant degree of interstitial oedema, mitochondria damage and fiber disruption; the nuclei presented an irregular edge mainly due to mitochondrial compression; myofibrils were disarrayed by mitochondrial compression and swelling. Myocytes swelling of and a moderate dilation of the interfibril spaces were also noticed. After 20 min ischaemia the endothelium of the capillary appeared significantly damaged, it presented extensive swelling, blebs extended from the endothelial cells into the

lumen, which was partially obstructed by numerous membrane-bound vesicles. In DPCPX-treated hearts ultrastructural ischaemic damage of myocardium was significantly reduced; particularly, an evident decrease of interstitial oedema, mitochondria swelling and endothelial damage was detected (Figure 7).

Discussion

Structural and functional integrity of the coronary endothelium represents the most important factor involved in the regulation of capillary-stitium exchange. Previous studies have shown that microvascular permeability changes are observed during and after an ischaemic event, even after a short-time ischaemia, well-known to be unable to induce myocytes and/or endothelium morphological alterations [2, 8, 9]. Endothelial permeability changes may be related to a reduction of endothelial nitric oxide production, oxygen free-radical-mediated damage, endothelial cell hypercontraction, enlargement of tight junctions and endothelial lesions [10, 11]. Adenosine production is able to modulate postischaemic hyperpermeability [2, 11–14] by reducing intracellular Ca^{++} concentration (A_1 receptors mediated effects), platelet aggregation, leukocyte-endothelium adhesion and by modulating vascular tone and permeability (A_2 receptors mediated effects).

When administered before ischaemia, a selective A_1 adenosine receptor antagonist DPCPX attenuated ischaemia-reperfusion injury of the rat heart after 10 and 20 min global normothermic ischaemia and reperfusion. Haemodynamic data, enzymatic release and ultrastructural morphometry supported this effect. Thus, our data suggest that the modulation of A_1 adenosine receptors activity is an important factor in the injury process after ischaemia and reperfusion, and this cardioprotection occurs also in absence of blood perfusion (granulocytes, monocytes, platelets, complement factors and plasma proteins). If the mechanisms of this protection against reperfusion injury are still unknown, the antagonism of A_1 adenosine receptors probably promotes the interaction between adenosine and A_2 receptors, induces coronary vasodilatation (prearterioles resistance reduction), endothelial protection and reduction of postischaemic hyperpermeability. Moreover, this preservation of coronary microcirculation may be due to an increase of nitric oxide production and intracellular cAMP. Neely et al. [3] also reported that the effect of A_1 adenosine receptor inhibitors is related to receptor selectivity and affinity, in fact, DPCPX is more effective in reducing infarct size in feline heart with respect to bamifylline and xanthine amine congeners that have a lower A_1 -receptor selectivity; in rats, intravenous administration of DPCPX blocked the selective A_1 adenosine receptor agonist, cyclopentyladenosine induced bradycardia with little effect on the A_2 adenosine receptor agonist, CGS 21680 induced decrease in blood pressure at doses of 0.03 to 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$, and it was suggested that DPCPX is 1000-fold more selective for A_1 adenosine receptors *in vivo*. The way that adenosine, via A_1 and/or A_2 receptors, exerts its cardioprotective effects is not fully understood. With regard to A_1 or A_2 adenosine receptors stimulation conflicting data are reported [15–19]. This discrepancy may be due either to the haemodynamic side effects of adenosine (with a decrease in coronary perfusion pressure) or, in part, to interspecies differences or to experimental protocols used [18–19]. Moreover, the pathophysiology of reperfusion damage and myocardial stunning is multifactorial and it is not possible to determine the most important factor involved in postischaemic damage. Probably, in our model, with fixed pre- and afterload and in absence of blood perfusion, the main factor involved is

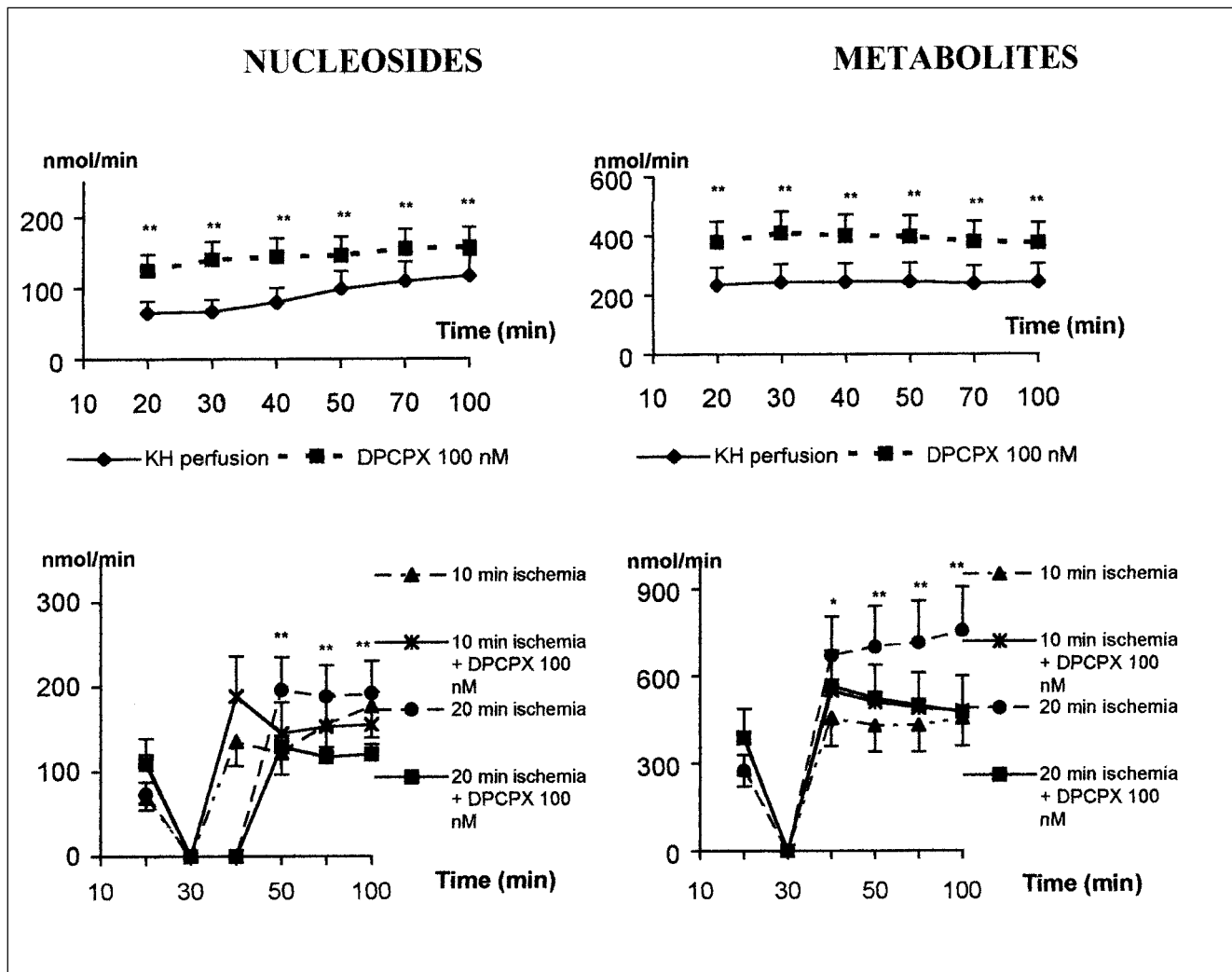


Figure 4. Purine release ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{gr.dw}^{-1}$). Time course of nucleosides (adenosine, inosine) and metabolites (hypoxanthine and uric acid) in all groups. Data are expressed as mean and SD. * = $p = 0.05$; ** = $p < 0.001$.

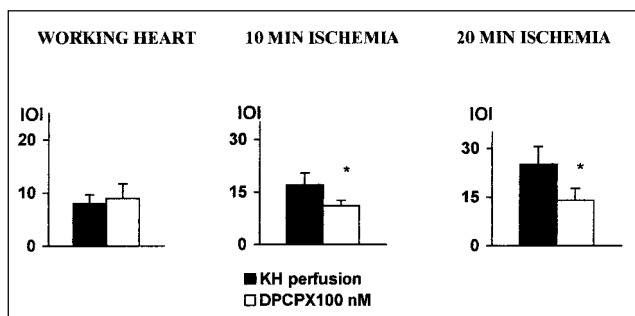


Figure 5. Effects of DPCPX on microvascular permeability. Quantitative assessment of FITC-albumin extravasation expressed as IOI (integrated optical intensity; see Material and methods) units; data are expressed as mean and SD. * = $p < 0.02$ vs. KH.

the endothelial damage and the microcirculatory dysfunction, against which the A_2 stimulation mediated by DPCPX-induced A_1 receptor blockade exerts the best cardioprotective effect.

As regards the higher purines level in coronary perfusate observed in non-ischaemic DPCPX-treated hearts, several hypotheses are possible. First, it may be the direct conse-

quence of the A_1 receptors blockade; it is reported that A_1 receptors exert an inhibitory influence on membrane molecular mechanisms that control purine release via phospholipase A_2 prostaglandin system [20–21]; for this reason, the A_1 receptors blockade with DPCPX, by abolishing the inhibitory effect of A_1 receptors increases the purine release also in absence of an ischaemic period. Second, the higher purine levels may represent an index of metabolic activation secondary to the inhibition of A_1 receptor mediated negative inotropic and chronotropic stimuli. This release seems to have a pathogenic role in the protective effect of DPCPX; moreover, various studies [1, 14–18, 21] reported that adenosine modulates, at micromolar concentrations endothelial and myocytic reperfusion damage. The observation that, after ischaemia, total purine release only increased in the control group may be the consequence of the detected increase of cellular damage, in other words, the alteration of cellular membrane determines an increase of coronary permeability to purine pool diffusion. For this reason, purine release represents, in hearts subjected to 20 min ischaemia, an index of severe membrane damage, as confirmed by ultrastructural findings and microcirculatory alterations.

In *isolated* working rat hearts we discovered first that i) A_1 adenosine receptors antagonism with DPCPX is able to re-

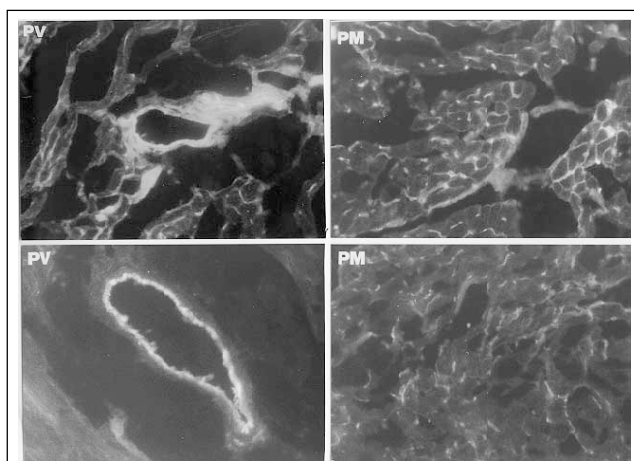


Figure 6: Histological samples (200x, fluorescent light) showing the FITC-albumin diffusion. A: control heart submitted to 20 min global ischaemia, B) effect of 100 nM DPCPX: a significant reduction of perivascular (PV, left) and perimyocytic (PM, right) fluorescence is detected.

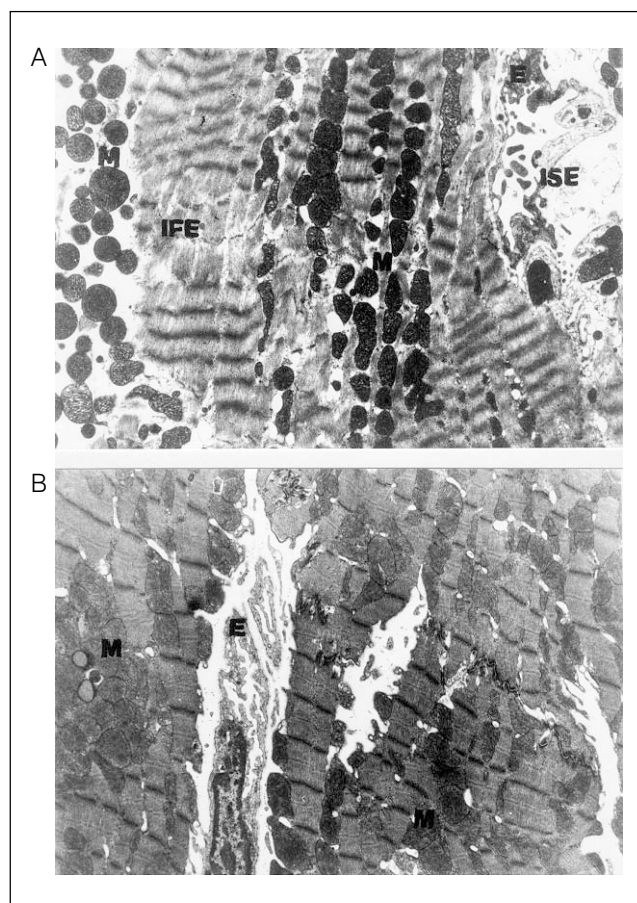


Figure 7: Ultrastructural morphometry (4500x). A: longitudinal section of control rat heart subjected to 20 min. ischaemia and reperfusion; interstitial and interfibrils oedema, severe mitochondria damage and endothelial lesions are detected. B: effect of DPCPX 100 nM: a significant reduction of ultrastructural damage is reported. M: mitochondria, ISE: interstitial oedema, IFE: interfibrils oedema, E: endothelium.

duce reperfusion injury in isolated saline-perfused hearts; ii) this cardioprotection, in absence of blood perfusion, may be also due to direct modulation of endothelial damage of coronary microcirculation and to a reduction of coronary postischaemic hyperpermeability. As regard to a therapeutic approach, A₁ adenosine receptor-mediated side effects, including negative inotropy, bradycardia or asystoly, bronchoconstriction and renal vasoconstriction or A₂ adenosine receptor mediated-hypotension preclude the use of intravenous adenosine, A₁ or A₂ adenosine receptor antagonists. However, A₁ adenosine receptor antagonists may be potentially useful in ischaemic conditions by reducing reperfusion damage and microcirculatory dysfunction.

References

1. Ely SW, Berne RM. Protective effects of adenosine in myocardial ischemia. *Circulation* 1992; 85: 893–904.
2. Olsson RA, Pearson JD. Cardiovascular purinoceptors. *Physiol Rev* 1990; 70: 761–815.
3. Neely CP, DiPierro FV, Kong M, Greelish JP and Gardner TJ. A₁ adenosine receptor antagonists block ischemia-reperfusion injury of the heart. *Circulation* 1996; 94 (Suppl II): II376–II380.
4. Neely CP, Matot J. A₁ adenosine receptors blocks ischemia-reperfusion injury of the lung. *Am J Physiol* 1995; 268 (Lung Cell Mol Physiol): L1036–L1046.
5. Di Napoli P, Contegiacomo G, Di Crecchio A, Di Muzio M, Tiloca P, Taccardi AA, Maggi A, Barsotti A. Ischemic preconditioning of rat myocardium: effects on postischemic coronary endothelium hyperpermeability and microcirculatory damage. *J Clin Bas Cardiol* 1998; 1: 37–42.
6. Bergmeyer HU, Bemt E. Creatin Kinase: UV-assay manual method. In: Bergmeyer HU (ed). *Methods of enzymatic analysis*. Verlag Chemie, Weinheim, Academic Press, New York, 1974; 601–5.
7. Ramirez MM, Quardt SM, Kim D, Oshiro H, Minniccozi M and Duran WN. Platelet activating factor modulates microvascular permeability through nitric oxide synthesis. *Microvasc Res* 1995; 50: 223–34.
8. Dauber IM, vanBenthuyzen KM, McMurtry IF, Wheeler GS, Lesnefsky EJ, Horwitz LD, Weil JF. Functional coronary microvascular injury evident as increased permeability due to a brief ischemia and reperfusion. *Circ Res* 1990; 66: 986–98.
9. Di Napoli P, Di Crecchio A, Tiloca P, Contegiacomo G, Di Muzio M, Barsotti L, Spoletini L and Barsotti A. Endothelial permeability as factor affecting reperfusion damage. *Int Med* 1997; 5: 1–7.
10. Garcia-Dorado D, Oliveras J. Myocardial oedema: a preventable cause of reperfusion injury. *Cardiovasc Res* 1993; 27: 1555–63.
11. Smits P, Williams SB, Lipson DE, Banitt P, Rongen GA, Creager NM. Endothelial release of nitric oxide contributes to the vasodilator effect of adenosine in humans. *Circulation* 1995; 92: 2135–41.
12. Haselton FR, Alexander JS, Mueller SN. Adenosine decreases permeability of in vitro endothelial monolayers. *J Appl Physiol* 1993; 74 (4): 1581–90.
13. Lasley RD, Mentzer RM. Adenosine improves recovery of postischemic myocardial function via an adenosine A₁ receptor mechanism. *Am J Physiol* 1992; 263 (Heart Circ Physiol 32): H1460–H1465.
14. Lasley RD, Mentzer RM. Protective effects of adenosine in the reversibly injured heart. *Ann Thorac Surg* 1995; 60: 843–6.
15. Wainwright CL, Parratt JR. Effects of R-PIA, a selective A₁ adenosine agonist, on hemodynamics and ischaemic arrhythmias in pigs. *Cardiovasc Res* 1993; 27: 84–9.
16. Li Y, Kloner RA. The cardioprotective effects of ischemic preconditioning are not mediated by adenosine receptors in rat hearts. *Circulation* 1993; 87: 1642–8.
17. Schlack W, Schafer M, Uebing A, Schafer S, Borchard U, Thamer V. Adenosine A₂-receptor activation at reperfusion reduces infarct size and improves myocardial wall function in dog heart. *J Cardiovasc Pharmacol* 1993; 22: 89–96.
18. Kersten JR, Orth KG, Pagel PS, Mei DA, Gross GJ, Warltier DC. Role of adenosine in isoflurane-induced cardioprotection. *Anesthesiology* 1997; 86: 1128–39.
19. Tsuchida A, Thompson R, Olsson RA, Downey JM. The anti-infarct effect of an adenosine A₁-selective agonist is diminished after prolonged infusion as is the cardioprotective effect of ischemic preconditioning in rabbit heart. *J Mol Cell Cardiol* 1994; 26: 303–11.
20. Di Iorio P, Battaglia G, Ciccarelli R, Ballerini P, Giuliani P, Poli A, Nicoletti F, Caciagli F. Interaction between A₁ adenosine and Class II metabotropic glutamate receptors in the regulation of purine and glutamate release from rat hippocampal slices. *J Neurochemistry* 1996; 67: 302–9.
21. Shryock JC, Belardinelli L. Adenosine and adenosine receptors in the cardiovascular system: biochemistry, physiology and pharmacology. *Am J Cardiol* 1997; 79 (12A): 2–10.

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