Vascular Pharmacology 73 (2015) 96–103

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Altered expression of P2Y2 and P2X7 purinergic receptors in the isolated rat heart mediates ischemia–reperfusion injury

Miriam Granado [a], Sara Amor [a], Juan José Montoya [b], Luis Monge [a], Nuria Fernández [a], Ángel Luis García-Villalón [a,*]

[a] Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma, 28029 Madrid, Spain
[b] Universidad Alfonso X el Sabio, Villanueva de la Cañada, 28691 Madrid, Spain

ARTICLE INFO

Article history:
Received 15 January 2015
Received in revised form 26 May 2015
Accepted 8 June 2015
Available online 10 June 2015

Keywords:
Ischemia–reperfusion
Purinergic receptors
P2Y2, P2X7
Langendorff

ABSTRACT

The aim of this study is to analyze the expression of purinergic receptors in the heart after ischemia–reperfusion, and their possible role in ischemia–reperfusion injury. Rat hearts were perfused according to the Langendorff technique and subjected to 30 min ischemia followed by 15 min reperfusion. Ischemia–reperfusion reduced the gene expression and protein content of purinergic receptors of the P2Y2 subtype, and increased the gene expression and protein content of the P2X7 subtype. Treatment with the agonist of the P2Y2 subtype 2-thio-UTP and with the antagonist of the P2X7 subtype Brilliant Blue improved myocardial function parameters, reduced cell death and increased the myocardial expression of antiapoptotic markers after ischemia–reperfusion. These results suggest that the myocardial expression of the protective P2Y2 subtype of purinergic receptors is reduced, whereas that of the harmful subtype P2X7 subtype is increased during coronary ischemia–reperfusion. This may contribute to myocardial injury in this condition.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Cardiac ischemia is one of the most important diseases in developed countries. Cell injury during ischemia and posterior reperfusion may be mediated by oxidative stress and calcium overload, leading to necrosis and/or apoptosis and impairment of cardiac function [23]. Several strategies are being developed to reduce cell death [23], but at this moment none has successfully provided a cardioprotective therapy that can be applied to clinical practice. This may be attributed, in part, to the fact that the mechanisms of ischemia–reperfusion injury are still incompletely understood. Ischemia–reperfusion may affect both myocardial function and coronary vascular function injury [16]. Coronary vascular dysfunction may enhance myocardial impairment by interfering with blood flow recovery during reperfusion, and myocardial injury may compromise coronary blood flow by compression of blood vessels and/or release of vasoactive factors.

Purinergic signalling has attracted intense attention, with an exponential growth of publications in the last twenty years [1]. Purinergic P2 receptors are activated by extracellular nucleotides, such as ATP and UTP, and may be involved in neurotransmission, epilepsy, pain, hemostasis and gastrointestinal function [2]. As purinergic receptors are expressed in myocardial tissue [21,31] and coronary blood vessels [19,26,34], and ATP and UTP are released during myocardial ischemia [32,33], it is probable that they also may play a role in ischemia–reperfusion pathophysiology.

Both P2X and P2Y families of purinergic receptors have been explored for their implication in myocardial ischemia, but the results are inconclusive. P2Y2 subtype has been shown to be protective during ischemia–reperfusion in mouse heart [4,32] and cultured cardiomyocytes [12]. However, in other study P2Y2 receptors increased cell death during hypoxia in cultured cardiomyocytes, whereas P2Y4 were protective [5]. Regarding the P2X subtypes, it has been found that overexpression of the P2X4 subtype may improve myocardial ischemia in mouse [27]. P2X7 receptors may be detrimental in mouse heart [20], and cultured cardiomyocytes [5], however a P2X7 agonist may be protective in rat [30].

Although expression of purinergic receptors has been studied in control conditions, it is not known whether this expression may be altered during ischemia–reperfusion. The hypothesis of this study is that changes in the expression and/or activity of protective or harmful receptors may contribute to the mechanisms of injury in this condition. The protein levels of the subtypes which are expressed at significant levels in the rat heart (P2Y1, P2Y2, P2Y14, P2X4, P2X5 and P2X7 [21]) have been assessed after ischemia–reperfusion in the rat heart. As P2Y2 and P2X7 receptors were altered during ischemia–reperfusion, the effects of specific agonists and antagonists of these subtypes on

* Corresponding author at: Department of Physiology, Faculty of Medicine, Universidad Autónoma de Madrid, C/ Arzobispo Morcillo n°2, 28029 Madrid, Spain.
E-mail address: angeluis.villalon@uam.es (Á.L. García-Villalón).

http://dx.doi.org/10.1016/j.vph.2015.06.003
1537-1891/© 2015 Elsevier Inc. All rights reserved.

myocardial function, cell death and markers of apoptosis and anti-apoptosis were also studied.

2. Methods

2.1. Experimental set-up

In the present study male Sprague–Dawley rats were used (300–350 g body weight). All the experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council of the National Academies, Eight Edition, 2011) and with all relevant laws and regulations, and reported in compliance with ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines. The use of these animals was also approved by the Animal Care and Use Committee of the Universidad Autónoma de Madrid. The hearts were removed from the rats under anesthesia with i.p. sodium pentobarbital (100 mg/kg) and following i.v. injection of heparin (1000 UI). Next, the ascending aorta was cannulated and the heart was subjected to retrograde perfusion with Krebs–Henseleit buffer (115 mM NaCl, 4.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 11 mM glucose) equilibrated with 95% oxygen and 5% carbon dioxide to a pH of 7.3–7.4. Perfusion was initiated in a Langendorff heart perfusion apparatus at a constant flow rate of 11–15 ml/min to provide a basal perfusion pressure of approximately 70 mm Hg. 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 mm Hg, both connected to ICU Medical transducers. 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 computer using the PowerLab/8e data acquisition system (AD instruments, Colorado Springs CO).

2.2. Experimental procedure

After a 30 min equilibration period with constant flow perfusion, the hearts were exposed to global zero-flow ischemia for 30 min at 37 °C, and reperfused for 15 min at the same flow rate used before ischemia. Time-control hearts were perfused during the same total duration (75 min) without ischemia. Afterwards, the ischemic-reperfused or time-control hearts were removed, and immediately frozen and stored at −80 °C. Some hearts were treated during the ischemia–reperfusion with the specific agonist of P2Y2 purinergic receptors 2-thio-UTP (10 μM), with the specific agonist of purinergic receptors P2X7 BzATP (100 μM), with the specific antagonist of purinergic receptors P2X7 Brilliant Blue (10 μM), and with the non-specific antagonist of P2 receptors suramin (100 μM). These blockers were added to the perfusion solution 10 min before ischemia, and remained during the ischemia and reperfusion. As hearts treated with 2-thio-UTP and Brilliant Blue during ischemia and reperfusion showed differences compared to untreated hearts, these blockers were also tested only during 10 min before ischemia, in which case the blocker was washed out before beginning ischemia, or only during reperfusion, in which case the blocker was added at the beginning of the reperfusion period.

2.3. Tissue homogenization and protein quantification

Heart tissue was homogenized in 500 μl of radioimmunoprecipitation assay lysis buffer with an EDTA-free protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). After homogenization, samples were centrifuged at 14,000 rpm for 20 min at 4 °C. Supernatants were transferred to a new tube and protein concentration was estimated by Bradford protein assay.

2.4. ELISA cell death detection

This photometric enzyme immunoassay for the in vitro quantification of cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes) was performed according to the instructions of the manufacturer (Roche Diagnostics). The same amount of total protein was loaded in all wells and each sample was measured in duplicate (Tecan Infinite M200, Grödig, Austria). The background value was subtracted from the mean value of each sample and all values are referred to the mean value of the control group.

2.5. Immunoblotting

In each assay the same amount of protein was loaded in all wells (75 μg) and resolving gels with different amounts of SDS-acrylamide gels (8–12%) were used depending on the molecular weight of the protein. After electrophoresis proteins were transferred to polyvinylidine difluoride (PVDF) membranes (Bio-Rad) and transfer efficiency was determined by Ponceau red dyeing. Filters were then blocked with Tris-buffered saline (TBS) containing 5% (w/v) non-fat dried milk and incubated with the appropriate primary antibody diluted 1:1000; caspase-3 (Cell Signalling), caspase-8 (Neomarkers), Bcl-2 (Thermo Scientific), Hsp-70(Stressgen Bioreagents), Bax (Thermo Scientific), P2Y2 (Alomone Labs Ltd), and P2X7 (Alomone Labs Ltd). Membranes were subsequently washed and incubated with the corresponding secondary antibody conjugated with peroxidase (1:2000; Pierce, Rockford, IL, USA). Bound peroxidase activity was visualized by chemiluminescence and quantified by densitometry using BioRad Molecular Imager ChemiDoc XRS System. All blots were rehybridized with β-tubuline (Sigma-Aldrich) to normalize each sample for gel-loading variability. All data are normalized to control values on each gel.

2.6. RNA preparation and purification and quantitative real-time PCR

Total RNA was extracted from the myocardium according to the Tri-Reagent protocol [9]. cDNA was then synthesized from 1 μg of total RNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA).

2.7. Quantitative real-time PCR

Purinergic receptors P2Y1, P2Y2, P2Y14, P2X4, P2X5 and P2X7 mRNAs were assessed in heart samples by quantitative real-time PCR. Quantitative real-time PCR was performed by using assay-on-demand kits (Applied Biosystems) for each gene: P2Y1 (00562996), P2Y2 (02070661), P2Y14 (02532502), P2X4(00580949), P2X5(00589966) and P2X7 (00570451). TaqMan Universal PCR Master Mix (Applied Biosystems) was used for amplification according to the manufacturer's protocol in a Step One machine (Applied Biosystems). Values were normalized to the housekeeping gene 18S (Rn01428915). According to manufacturer's guidelines, the ΔΔCT method was used to determine relative expression levels. Statistics were performed using ΔΔCT values.

2.8. Statistical analysis

All parameters are expressed as the mean ± S.E.M. Hemodynamic parameters (coronary perfusion pressure, developed left ventricular pressure, maximal systolic dP/dt and heart rate) were compared before and after ischemia-reperfusion by paired Student's t test, and the genic expression of purinergic receptors was compared in ischemic–reperfused and time-control hearts by unpaired Student's t test. Hemodynamic parameters, total cell death and protein expression of pro- and antiapoptotic markers, were compared in the hearts treated with purinergic agonists or antagonists, with untreated hearts, by one-way ANOVA and Dunnett's test to determine which comparisons are significant. A P value of <0.05 was considered significant.

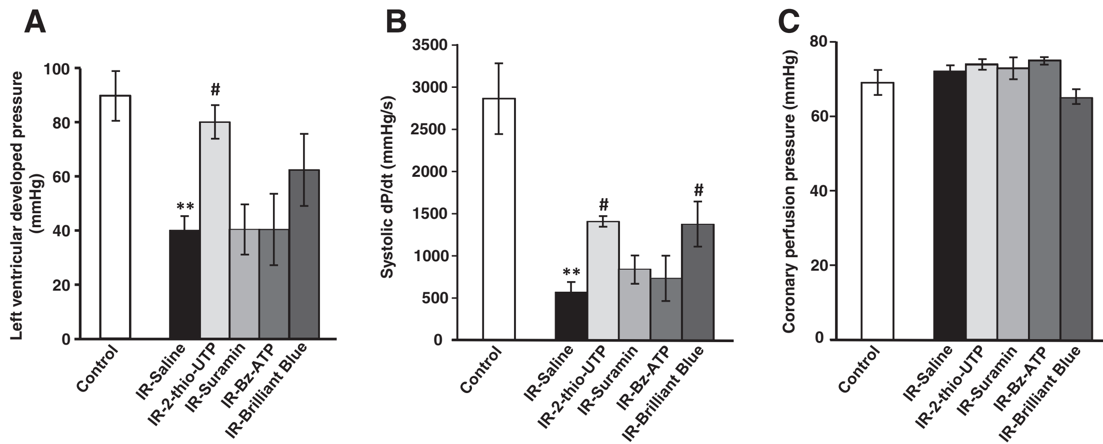

Fig. 1. Left ventricular developed pressure (A), systolic dP/dt (B) and coronary perfusion pressure (C) in perfused hearts in control conditions (n = 5) and after 30 min of ischemia and 15 min of reperfusion (IR) treated with saline (n = 5) or with 2-thio-UTP (10 μM, n = 5), suramin (100 μM, n = 5), BzATP (100 μM, n = 5), or Brilliant Blue (10 μM, n = 6). **P < 0.01 vs. control. #P < 0.05 vs. IR-saline. Values are represented as mean ± S.E.M.

2.9. Drugs

The following substances were used: 2-thio-UTP sodium salt (Tocris), 2′(3′)-O-(4-Benzoylbenzoyl)adenosine 5′-triphosphate triethylammonium salt (BzATP, Sigma), Brilliant blue G (Sigma), and suramin sodium salt (Sigma).

3. Results

3.1. Hemodynamic parameters

Ischemia–reperfusion in untreated hearts (n = 5) reduced developed left ventricular pressure (39 ± 5 vs. 86 ± 13 mm Hg, P < 0.01) and maximal systolic dP/dt (585 ± 136 vs. 2744 ± 535 mm Hg/s, P < 001), without modifying coronary perfusion pressure (72 ± 2 vs. 68 ± 6 mm Hg) or heart rate (129 ± 52 vs. 197 ± 20 beats/min). None of these parameters were modified in the time-control experiments (not shown, n = 5). In the ischemic hearts treated with 2-thio-UTP (n = 5) during ischemia and reperfusion, developed left ventricle pressure (79 ± 6, P < 0.05 mm Hg) and maximal systolic dP/dt (1419 ± 61 mm Hg/s, P < 0.05) were higher compared with untreated ischemic hearts (Fig. 1A and B). However, 2-thio-UTP did not modify developed left ventricle pressure or maximal systolic dP/dt when applied only before ischemia (n = 4) or only during reperfusion (n = 5) (Fig. 2A and B), and it did not modify coronary perfusion pressure in any case (Figs. 1C and 2C). In the ischemic hearts treated with Brilliant Blue during ischemia and reperfusion (n = 6) maximal systolic dP/dt (1387 ± 267 mm Hg/s, P < 0.05) was higher, compared with untreated ischemic hearts (Fig. 1A), and developed left ventricle pressure tended to be higher although the difference was not significant (Fig. 1B). Both maximal systolic dP/dt and developed left ventricle pressure were increased by treatment with Brilliant Blue only during reperfusion (n = 6), but not modified by treatment only before ischemia (n = 5) (Fig. 2A and B). However, coronary perfusion pressure was increased

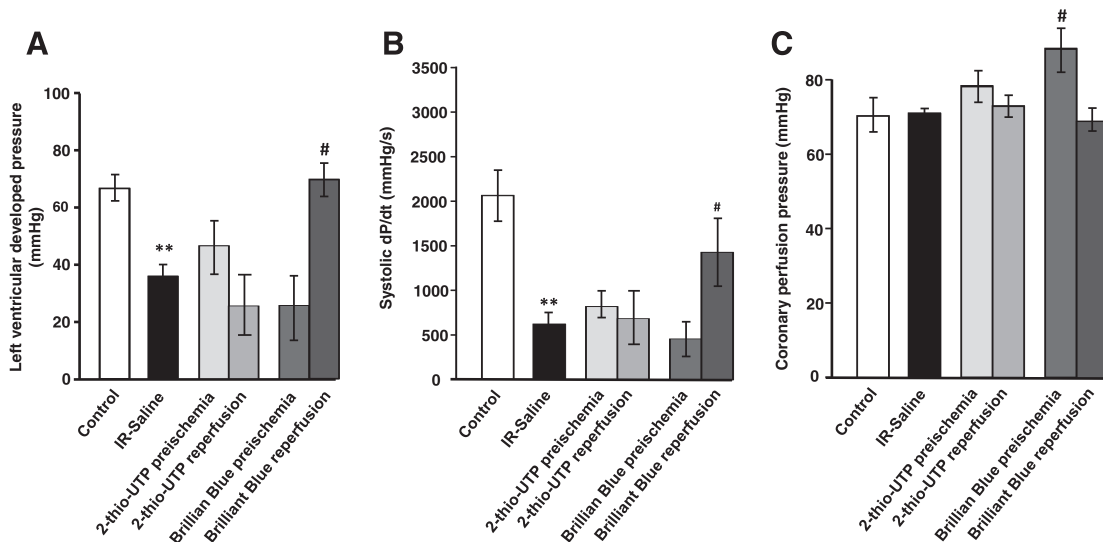

Fig. 2. Left ventricular developed pressure (A), systolic dP/dt (B) and coronary perfusion pressure (C) in perfused hearts in control conditions (n = 5) and after 30 min of ischemia and 15 min of reperfusion (IR) treated with saline (n = 5) or with 2-thio-UTP or Brilliant Blue (10 μM, n = 6), before (preischemia, n = 4 and n = 5, respectively) or after ischemia (reperfusion, n = 5 and n = 6, respectively). **P < 0.01 vs. control. #P < 0.05 vs. IR-saline. Values are represented as mean ± S.E.M.

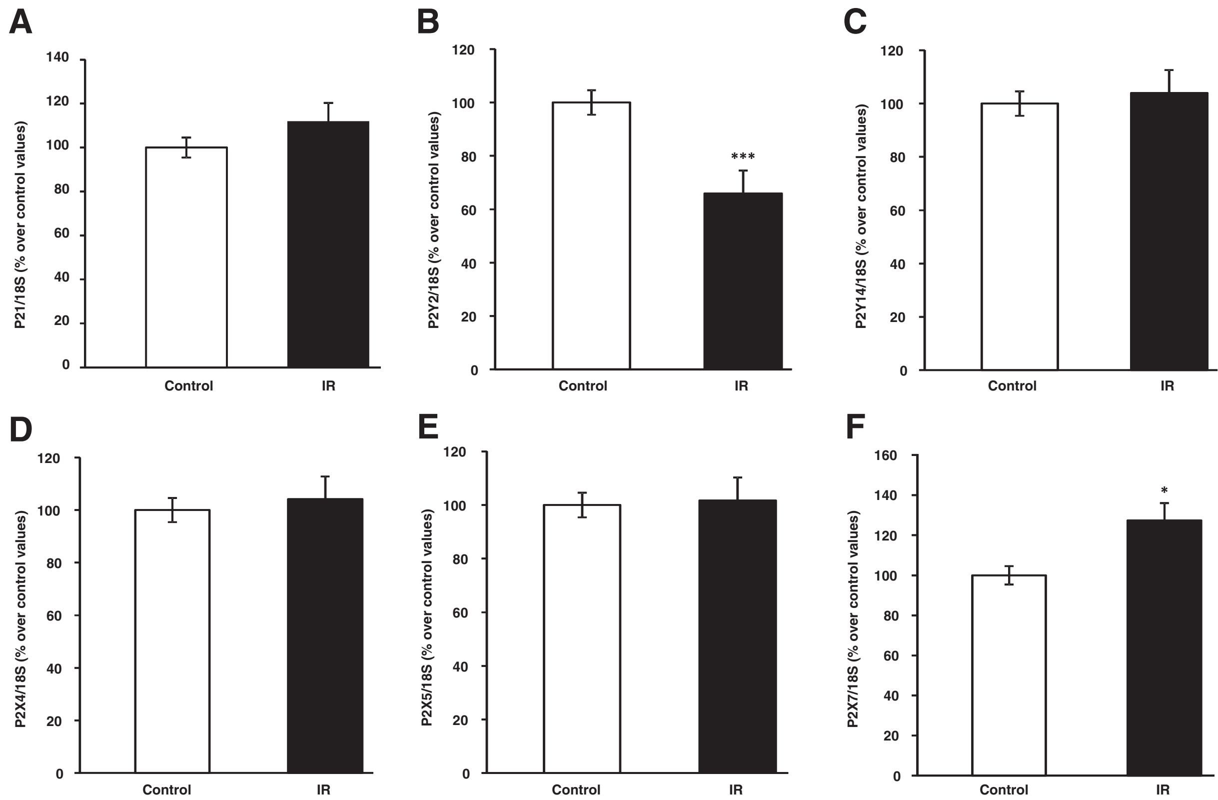

Fig. 3. Gene expression of P2Y1 (A), P2Y2 (B), P2Y14 (C), P2X4 (D), P2X5 (E) and P2X7 (F) purinergic receptors in control hearts (n = 5) and after 30 min of ischemia and 15 min of reperfusion (IR, n = 5). ***P < 0.001 vs. control. *P < 0.05 vs. control. Values are represented as mean ± S.E.M.

by treatment before ischemia (Fig. 2C). Suramin (n = 5) or BzATP (n = 5) did not modify maximal systolic dP/dt, developed left ventricle pressure, or coronary perfusion pressure (Fig. 1), and heart rate was not modified by any treatment (not shown).

3.2. Purinergic receptors gene and protein expression

Purinergic receptors P2Y1, P2Y1, P2X4, and P2X5 (Fig. 3A, C, D and E respectively) mRNA levels were unchanged in ischemic hearts compared to non-ischemic hearts. Ischemia–reperfusion significantly decreased both P2Y2 mRNA levels (P < 0.001, Fig. 3B) and protein content (P < 0.001, Fig. 4B), and significantly increased P2X7 gene expression (P < 0.05, Fig. 4C) and protein content in myocardial tissue (P < 0.05, Fig. 4A) compared to controls.

3.3. Total cell death

Ischemic hearts without treatment showed increased total cell death in the myocardium compared to control hearts (P < 0.05; Fig. 5). Treatment with the specific P2Y2 receptor agonist 2-thio-UTP,

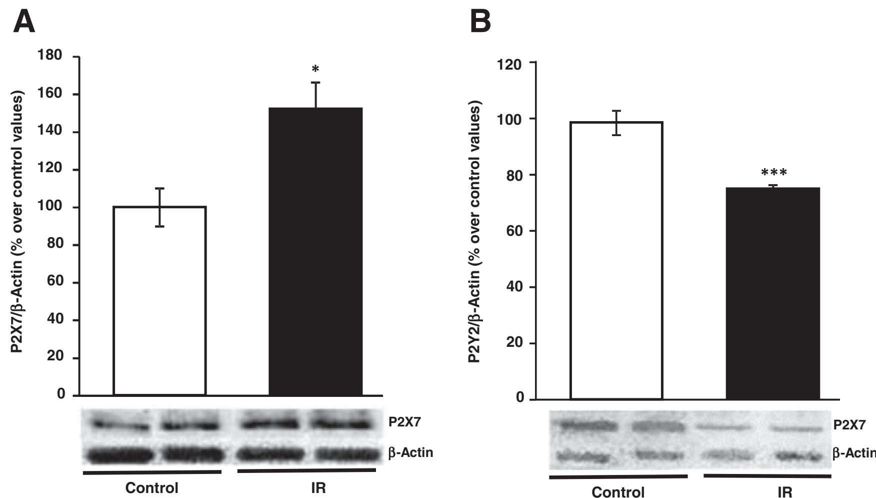

Fig. 4. Protein levels of P2Y2 (A) and P2X7 (B) purinergic receptors in control hearts (n = 5) and after 30 min of ischemia and 15 min of reperfusion (IR, n = 5). *P < 0.05 vs. control. ***P < 0.001 vs. control. Values are represented as mean ± S.E.M.

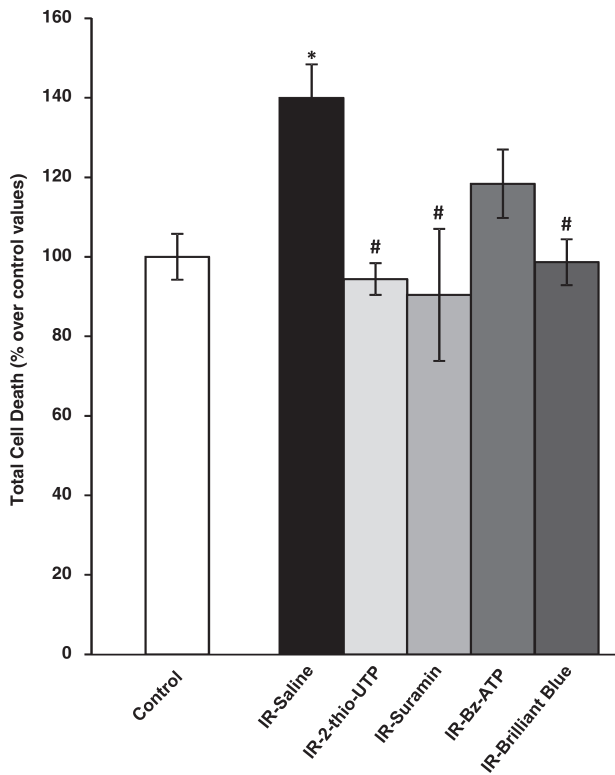

Fig. 5. Total cell death in the myocardium in control hearts (n = 5) and after 30 min of ischemia and 15 min of reperfusion (IR) treated with saline (n = 5), or with 2-thio-UTP (10 μM, n = 5), suramin (100 μM, n = 5), BzATP (100 μM, n = 5), or Brilliant Blue (10 μM, n = 6).*P < 0.05 vs. control; # P < 0.05 vs. IR-saline.

with the specific P2X7 receptor antagonist Brilliant Blue or with the non-specific antagonist of P2 receptors suramin during IR prevented IR-induced increase in total cell death (P < 0.05 for all). On the contrary treatment or with the P2X7 receptor specific agonist BzATP had no effect.

3.4. Heart content of antiapoptotic proteins Bcl-2 and Hsp-70

Myocardial content of the anti-apoptotic proteins Bcl-2 and Hsp-70 was significantly decreased in ischemic hearts with no treatment compared to control hearts (P < 0.05 for both: Fig. 6A and B respectively). Treatment with the specific P2Y2 receptor agonist 2-thio-UTP or with non-specific antagonist of P2 receptors suramin during IR prevented IR-induced decrease in Bcl-2 (P < 0.05 and P < 0.001 respectively). Likewise, administration of the specific P2X7 receptor antagonist Brilliant Blue during IR prevented IR-induced decrease in Hsp-70 protein content in the myocardium (P < 0.001).

3.5. Heart content of pro-apoptotic proteins caspase-3, caspase-8 and Bax

Myocardial content of the pro-apoptotic proteins caspase-3 and Bax was unchanged between groups (Fig. 7). However caspase-8 content was significantly decreased in Bz-ATP and Brilliant Blue treated hearts compared to untreated ischemic hearts (P < 0.05 for both).

3.6. Bax/Bcl-2 ratio

The Bax/Bcl-2 ratio was significantly increased in ischemic hearts treated with saline compared to controls (P < 0.05), and this increase was only prevented by 2-thio-UTP treatment (P < 0.01) (Table 1).

4. Discussion

The results of this study suggest that purinergic receptors may play an important role in the pathophysiology of ischemia–reperfusion injury. P2X7 receptors may contribute to myocardial impairment by increasing apoptosis, whereas P2Y2 receptors may be protective. Firstly, we studied whether the expression of purinergic receptors were altered in this condition. From the subtypes more expressed in rat myocardium

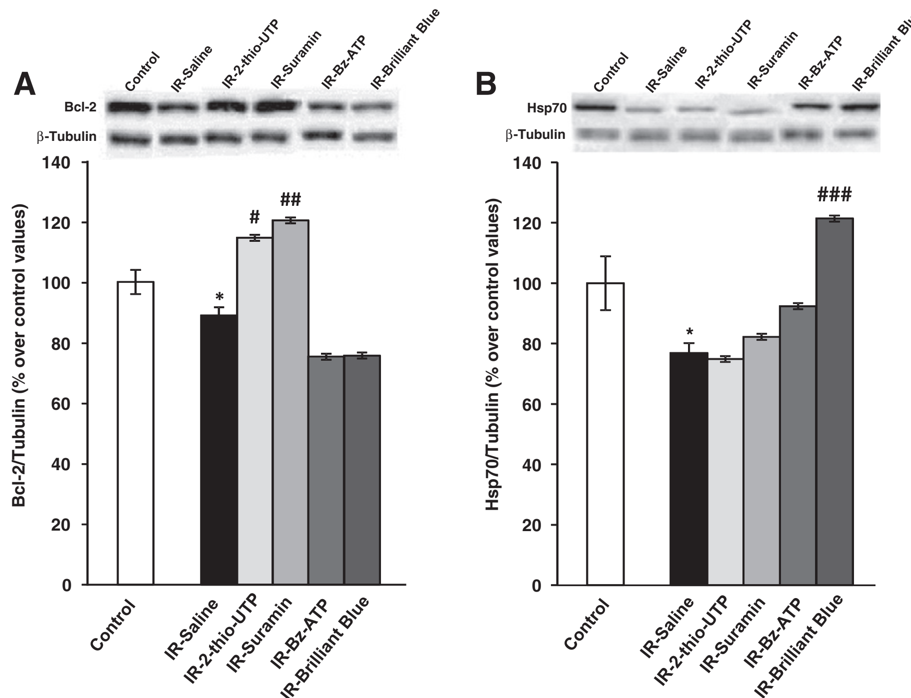

Fig. 6. Levels of Bcl-2 (A) and HSP-70 (B) in control hearts (n = 5) and after 30 min of ischemia and 15 min of reperfusion (IR) treated with saline (n = 5) or with 2-thio-UTP (10 μM, n = 5), suramin (100 μM, n = 5), BzATP (100 μM, n = 5), or Brilliant Blue (10 μM, n = 6).*P < 0.05 vs. control; # P < 0.05, ## P < 0.01, ### P < 0.001 vs. IR-saline. Values are represented as mean ± S.E.M.

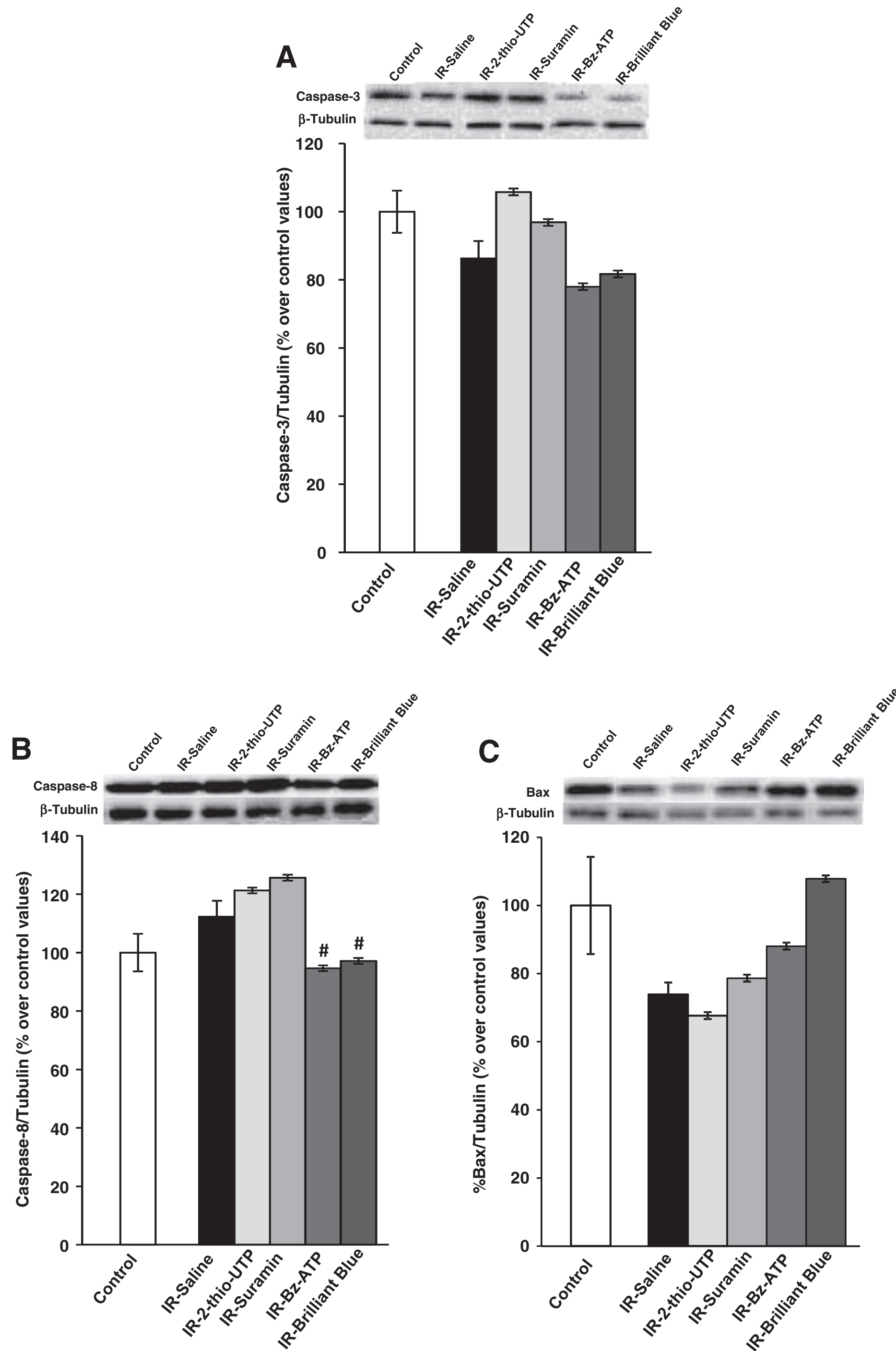

Fig. 7. Levels of caspase 3 (A), caspase 8 (B) and Bax (C) in control hearts (n = 5) and after 30 min of ischemia and 15 min of reperfusion (IR) treated with saline (n = 5) or with 2-thio-UTP (10 μM, n = 5), suramin (100 μM, n = 5), BzATP (100 μM, n = 5), or Brilliant Blue (10 μM, n = 6). Values are represented as mean ± S.E.M. # P < 0.05 vs. IR-saline.

according to literature (P2Y1, P2Y2, P2Y14, P2X4, P2X5 and P2X7; [21]) only two, P2Y2 and P2X7, were altered by ischemia–reperfusion. As these subtypes are also described as having protective or harmful effects on the myocardium [4,12,20,30,32] we hypothesized that those might probably be involved in the pathophysiology of ischemia–reperfusion, and therefore we studied the effects of agonists and antagonists of these particular receptors. Our results suggest that purinergic receptors of the P2X7 subtype may be detrimental for the myocardial tissue during ischemia–reperfusion, as the reduction in ventricular contractility was partially reverted with the specific antagonist of that subtype

Table 1
Bax/Bcl-2 ratio in control hearts (n = 5) and after 30 min of ischemia and 15 min of reperfusion (IR) treated with saline (n = 5), or with 2-thio-UTP (10 μM, n = 5), suramin (100 μM, n = 5), BzATP (100 μM, n = 5), or Brilliant Blue (10 μM, n = 6).*P < 0.005 vs. control; ##P < 0.01 vs. IR-saline.

	Controls	IR-saline	IR-2-thio-UTP	IR-Bz APT	IR-Suramine	IR-Brilliant Blue
Ratio Bax/Bcl-2	0.89 ± 4.7	1.14 ± 0.05*	0.73 ± 0.09 ##	0.96 ± 0.13	1.16 ± 0.09	1.14 ± 0.13

Brilliant Blue. Brilliant blue was effective when applied during ischemia and reperfusion and also when applied only during reperfusion, suggesting that the harmful effect of P2X7 receptors takes place during the reperfusion period. The reduction in ventricular contractility by P2X7 receptors may be mediated by an increase of cardiomyocyte apoptosis, as Brilliant Blue also reduced cell death, increased the expression of the antiapoptotic marker Hsp-70 and decreased caspase-8 protein levels. As the ratio Bax/Bcl-2 was unchanged in Brilliant Blue treated hearts, the protective effect of Brilliant Blue on myocardial apoptosis could be mediated by both an overexpression of the anti-apoptotic marker Hsp-70 and/or to a decrease on apoptotic extrinsic pathway. The P2X7 agonist BzATP did not increase further the functional impairment or cell apoptosis during ischemia–reperfusion, which may be due to these receptors are already activated by endogenous nucleotides in this condition. Purinergic P2X7 receptors have been found to increase apoptosis in intestinal [6] and skin [10] cancer cells, vascular smooth muscle myocytes [3] and retina [28].

Interestingly, the hearts treated with Brilliant Blue before ischemia showed increased coronary vascular resistance after ischemia. P2X7 receptors are expressed in blood vessel cells [17,22,24,25] therefore they might have a protective preconditioning effect circumscribed to the coronary vascular wall, although its effect on the myocardial cells would be mainly detrimental during reperfusion. It has been described that endothelial P2X7 receptors may induce release of nitric oxide [8,22], which is involved in preconditioning of the vascular ischemia–reperfusion injury [16].

On the other hand, purinergic P2Y2 receptors may be protective during ischemia–reperfusion, as the agonist of these receptors 2-thio-UTP increased myocardial contractility, reduced cell death and increased expression of the antiapoptotic maker Bcl-2 and the Bax/Bcl-2ratio. Thus, the antiapoptotic effect of 2-thio-UTP seems to be mediated by inactivation of intrinsic apoptotic pathway. As Brilliant Blue and 2-thio-UTP increased different antiapoptotic markers, P2X7 and P2Y2 receptors may influence different antiapoptotic pathways, in negative and positive direction respectively. Moreover, the protective effect of 2-thio-UTP was not observed when this blocker was applied only before ischemia or only during reperfusion, suggesting that P2Y2 receptors are protective only during ischemia. Our results agree with others, which found a protective effect of P2Y2 receptors in mouse heart in vitro [32] and in vivo [4], and in cultured rat cardiomyocytes [12].

The study of P2Y2 receptors is complicated by the current lack of a strongly specific antagonist of P2Y2 receptors commercially available. Although suramin is a potent antagonist of this subtype, it also may block other subtypes [14], so it may be described as an unspecific P2 antagonist. In our study, suramin reduced cell death and increased the antiapoptotic marker Bcl-2, but it did not improve myocardial function, suggesting that this antagonist may be have both beneficial and harmful effects, which could cancel each other at the functional level. Suramin may have antiapoptotic and anti-inflammatory effects in kidney, brain and myocardium [18], and may protect against ischemic injury in kidney [35] and brain [15]. This protection may be mediated by antagonism of purinergic receptors or by other effects of suramin, as this substance also may reduce conexin-mediated membrane permeability [7], inhibit tyrosine kinase [13] and metalloprotease activity [29] and block NF-KappaB activation [11].

During ischemia–reperfusion the concentration of nucleotides such as ATP and UTP in myocardial tissue is increased [32,33]. The present and previous studies suggest that they may have both protective and detrimental effects. However, their net effect will depend on the balance of purinergic receptors expressed in the tissue after ischemia–reperfusion, and this expression has not been studied before in this condition. Our study is the first, to our knowledge, to show that during ischemia–reperfusion expression of P2Y2 protective receptors is reduced, whereas that of harmful P2X7 receptors is increased. This suggests that in this condition the balance is shifted to the pernicious side and the net effect of nucleotides may be detrimental, thus contributing to the mechanisms of cell injury and functional impairment after ischemia–reperfusion. We have also found that P2X7 receptors might have some protective effect on the coronary vasculature, but this is probably overwhelmed during reperfusion as the expression of proapoptotic P2X7 receptors in the myocardium increases after ischemia. The agonists of P2Y2 receptors and/or the antagonists of P2X7 receptors may counteract the reduction and increase of these subtypes, respectively, and have cardioprotective effects during ischemia–reperfusion by enhancing antiapoptotic mechanisms and reducing myocardial cell death. Particularly, P2X7 antagonists may be therapeutically useful as are effective when administered after ischemia, and do not seem to have adverse effects on the coronary circulation when used in this manner.

Acknowledgments

This work was supported by Fondo de Investigaciones Sanitarias (Grant number PS09/00394); and Proyectos UAM-Banco de Santander con EEUU (Convocatoria 2013). We are indebted to María Esther Martínez for her invaluable technical assistance.

References

[1] G. Burnstock, Purinergic signalling: from discovery to current developments, Exp. Physiol. 99 (2014) 16–34.
[2] G. Burnstock, M. Williams, P2 purinergic receptors: modulation of cell function and therapeutic potential, J. Pharmacol. Exp. Ther. 295 (2000) 862–869.
[3] C. Cario-Toumaniantz, G. Loirand, A. Ladoux, P. Pacaud, P2X7 receptor activation-induced contraction and lysis in human saphenous vein smooth muscle, Circ. Res. 83 (1998) 196–203.
[4] R. Cohen, A. Shainberg, E. Hochhauser, Y. Cheporko, A. Tobar, E. Birk, L. Pinhas, J. Leipziger, J. Don, E. Porat, UTP reduces infarct size and improves mice heart function after myocardial infarct via P2Y2 receptor, Biochem. Pharmacol. 82 (2011) 1126–1133.
[5] S. Cosentino, C. Banfi, J.C. Burbiel, H. Luo, E. Tremoli, M.P. Abbracchio, Cardiomyocyte death induced by ischaemic/hypoxic stress is differentially affected by distinct purinergic P2 receptors, J. Cell. Mol. Med. 16 (2012) 1074–1084.
[6] R. Coutinho-Silva, L. Stahl, K.-K. Cheung, Nathalia Enes de Campos, Carolina de Oliveira Souza, D.M. Ojcius, G. Burnstock, P2X and P2Y purinergic receptors on human intestinal epithelial carcinoma cells: effects of extracellular nucleotides on apoptosis and cell proliferation, Am. J. Physiol. Gastrointest. Liver Physiol. 288 (2005) G1024–G1035.
[7] Y. Chi, K. Gao, H. Zhang, M. Takeda, J. Yao, Suppression of cell membrane permeability by suramin: involvement of its inhibitory actions on connexin 43 hemichannels, Br. J. Pharmacol. 171 (2014) 3448–3462.
[8] C.W. Chiao, R.C. Tostes, R.C. Webb, P2X7 receptor activation amplifies lipopolysaccharide-induced vascular hyporeactivity via interleukin-1 beta release, J. Pharmacol. Exp. Ther. 326 (2008) 864–870.
[9] P. Chomczynski, A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples, Biotechniques 15 (532–534) (1993) 536–537.
[10] W. Fu, T. McCormick, X. Qi, L. Luo, L. Zhou, X. Li, B.-C. Wang, H.E. Gibbons, F.W. Abdul-Karim, G.I. Gorodeski, Activation of P2X(7)-mediated apoptosis Inhibits DMBA/TPA-induced formation of skin papillomas and cancer in mice, BMC Cancer 9 (2009) 114.
[11] T. Goto, S. Takeuchi, K. Miura, S. Ohshima, K.-i. Mikami, K. Yoneyama, M. Sato, T. Shibuya, D. Watanabe, E. Kataoka, D. Segawa, A. Endo, W. Sato, R. Yoshino, S. Watanabe, Suramin prevents fulminant hepatic failure resulting in reduction of lethality through the suppression of NF-kappaB activity, Cytokine 33 (2006) 28–35.
[12] E. Hochhauser, R. Cohen, M. Waldman, A. Maksin, A. Isak, D. Aravot, P.S. Jayasekara, Christa E. Müller, K.A. Jacobson, A. Shainberg, P2Y2 receptor agonist with enhanced stability protects the heart from ischemic damage in vitro and in vivo, Purinergic Signal 9 (2013) 633–642.
[13] M. Izikki, O. Mercier, F. Lecerf, L.L. Guin, E. Hoang, Peter Dorfmüller, F.e. Perros, M. Humbert, G. Simonneau, P. Dartevelle, E. Fadel, S. Eddahibi, The beneficial effect of suramin on monocrotaline-induced pulmonary hypertension in rats, PLoS One 8 (2013) e77073.
[14] M.F. Jarvis, B.S. Khakh, ATP-gated P2X cation-channels, Neuropharmacology 56 (2009) 208–215.
[15] A. Kharlamov, S.C. Jones, D.K. Kim, Suramin reduces infarct volume in a model of focal brain ischemia in rats, Exp. Brain Res. 147 (2002) 353–359.
[16] K. Laude, P. Beauchamp, C. Thuillez, V. Richard, Endothelial protective effects of preconditioning, Cardiovasc. Res. 55 (2002) 466–473.
[17] C.J. Lewis, R.J. Evans, P2X receptor immunoreactivity in different arteries from the femoral, pulmonary, cerebral, coronary and renal circulations, J. Vasc. Res. 38 (2001) 332–340.
[18] N. Liu, S. Zhuang, Tissue protective and anti-fibrotic actions of suramin: new uses of an old drug, Curr. Clin. Pharmacol. 6 (2011) 137–142.
[19] M. Malmsjo, M. Hou, T.K. Harden, W. Pendergast, E. Pantev, L. Edvinsson, D. Erlinge, Characterization of contractile P2 receptors in human coronary arteries by use of the

stable pyrimidines uridine 5′-O-thiodiphosphate and uridine 5′-O-3-thiotriphosphate, J. Pharmacol. Exp. Ther. 293 (2000) 755–760.
[20] E. Mezzaroma, S. Toldo, D. Farkas, I.M. Seropian, Van Tassell, W. Benjamin, F.N. Salloum, H.R. Kannan, A.C. Menna, N.F. Voelkel, A. Abbate, The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 19725–19730.
[21] H. Musa, J.O. Tellez, N.J. Chandler, I.D. Greener, M.l. Maczewski, U. Mackiewicz, A. Beresewicz, P. Molenaar, M.R. Boyett, H. Dobrzynski, P2 purinergic receptor mRNA in rat and human sinoatrial node and other heart regions, Naunyn Schmiedebergs Arch. Pharmacol. 379 (2009) 541–549.
[22] S.D. Oliveira, R. Coutinho-Silva, C.L. Silva, Endothelial P2X7 receptors' expression is reduced by schistosomiasis, Purinergic Signal 9 (2013) 81–89.
[23] A.J. Perricone, Vander Heide, S. Richard, Novel therapeutic strategies for ischemic heart disease, Pharmacol. Res. 89C (2014) 36–45.
[24] M. Piscopiello, M. Sessa, N. Anzalone, R. Castellano, F. Maisano, E. Ferrero, R. Chiesa, O. Alfieri, G. Comi, M.E. Ferrero, C. Foglieni, P2X7 receptor is expressed in human vessels and might play a role in atherosclerosis, Int. J. Cardiol. 168 (2013) 2863–2866.
[25] A.N. Ramirez, D.L. Kunze, P2X purinergic receptor channel expression and function in bovine aortic endothelium, Am. J. Physiol. Heart Circ. Physiol. 282 (2002) H2106–H2116.
[26] S.J. Rayment, M.L. Latif, V. Ralevic, S.P. Alexander, Evidence for the expression of multiple uracil nucleotide-stimulated P2 receptors coupled to smooth muscle contraction in porcine isolated arteries, Br. J. Pharmacol. 150 (2007) 604–612.
[27] D. Sonin, S.-Y. Zhou, C. Cronin, T. Sonina, J. Wu, K.A. Jacobson, A. Pappano, B.T. Liang, Role of P2X purinergic receptors in the rescue of ischemic heart failure, Am. J. Physiol. Heart Circ. Physiol. 295 (2008) H1191–H1197.
[28] T. Sugiyama, M. Kobayashi, H. Kawamura, Q. Li, D.G. Puro, M. Kobayshi, Enhancement of P2X(7)-induced pore formation and apoptosis: an early effect of diabetes on the retinal microvasculature, Invest. Ophthalmol. Vis. Sci. 45 (2004) 1026–1032.
[29] A.P.T. Taniguti, Cintia Yuri Matsumura, Lea Rodrigues-Simioni, Humberto Santo Neto, M.J. Marques, Suramin affects metalloproteinase-9 activity and increases $$-dystroglycan levels in the diaphragm of the dystrophin-deficient mdx mouse, Muscle Nerve 46 (2012) 810–813.
[30] D.A. Vessey, L. Li, M. Kelley, P2X7 receptor agonists pre- and postcondition the heart against ischemia–reperfusion injury by opening pannexin-1/$P2X_7$ channels, Am. J. Physiol. Heart Circ. Physiol. 301 (2011) H881–H887.
[31] T.E. Webb, M.O. Boluyt, E.A. Barnard, Molecular biology of P2Y purinoceptors: expression in rat heart, J. Auton. Pharmacol. 16 (1996) 303–307.
[32] S. Wee, J.N. Peart, J.P. Headrick, P2 purinoceptor-mediated cardioprotection in ischemic-reperfused mouse heart, J. Pharmacol. Exp. Ther. 323 (2007) 861–867.
[33] A.-K. Wihlborg, J. Balogh, L. Wang, C. Borna, Y. Dou, B.V. Joshi, E. Lazarowski, K.A. Jacobson, A. Arner, D. Erlinge, Positive inotropic effects by uridine triphosphate (UTP) and uridine diphosphate (UDP) via P2Y2 and P2Y6 receptors on cardiomyocytes and release of UTP in man during myocardial infarction, Circ. Res. 98 (2006) 970–976.
[34] Z. Zhou, D. Merkus, C. Cheng, H.J. Duckers, A.H. Jan Danser, D.J. Duncker, Uridine adenosine tetraphosphate is a novel vasodilator in the coronary microcirculation which acts through purinergic P1 but not P2 receptors, Pharmacol. Res. 67 (2013) 10–17.
[35] S. Zhuang, B. Lu, R.A. Daubert, K.D. Chavin, L. Wang, R.G. Schnellmann, Suramin promotes recovery from renal ischemia/reperfusion injury in mice, Kidney Int. 75 (2009) 304–311.