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Extracellular NAADP affords cardioprotection against ischemia and reperfusion injury and involves the P2Y11-like receptor

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ABSTRACT

Background and purpose: Extracellular nucleotides may play important regulatory roles within the cardiovascular system and notably in cardioprotection. We aimed to look for a possible pharmacological preconditioning effect of extracellular NAADP ($[NAADP]_e$) against ischemia/reperfusion injury. $[NAADP]_e$ has been recently reported to be a full agonist of the P2Y11 receptor. Therefore, we characterized the involvement of the P2Y11-like receptor in mediating ischemic/reperfusion tolerance induced by $[NAADP]_e$.
Experimental approach: The cardioprotective effects of $[NAADP]_e$ were evaluated in a model of ischemia/reperfusion carried out on Langendorff perfused rat hearts. This model was also instrumented with a microdialysis probe. Furthermore, using isolated cardiomyocytes, we assessed cAMP, inositol phosphate accumulation and prosurvival protein kinases activation induced by $[NAADP]_e$ pretreatement.
Results: Pretreatment with 1 μM $[NAADP]_e$ induced cardioprotective effects with regards to functional recovery, necrosis and arrhythmogenesis ($p < 0.05$). These effects were completely suppressed with NF157, an antagonist of the P2Y11 receptor. Moreover, global ischemia induced a time-dependent increase in interstitial concentration of adenosine, NAADP and UTP. In cardiomyocyte cultures, NF157 suppressed cAMP and inositol phosphate accumulation induced by $[NAADP]_e$. $[NAADP]_e$ induced phosphorylation of ERK 1/2, AKT and its downstream target GSK-3β ($p < 0.05$). These activations were also suppressed by NF157.
Conclusions: Evidence suggests that NAADP signalling at the P2Y11-like receptor affords significant cardioprotection against ischemia/reperfusion injury. Besides adenosine and UTP, microdialysis study supports a potential endogenous role of $[NAADP]_e$.

1. Introduction

During ischemia, numerous effective endogenous mediators have been identified, particularly extracellular nucleotides such as purine and pyrimidine nucleotides [1,2]. They may play important regulatory roles within the cardiovascular system and notably in cardioprotection [1–5]. Recent evidence implicating P2Y receptors in protection of ischemic or reperfused myocardium or ischemic endothelium has been reported [1,3–8].

The P2Y11 receptor is dually coupled to Gs and Gq proteins and is the only one P2Y receptor which has been linked, via a genetic polymorphism, to an increased risk of acute myocardial infarction and elevated levels of C-reactive protein in humans [9]. This polymorphism is also linked to a decrease in maximal response following P2Y11 receptor agonist stimulation [10].

The tissue distribution of the P2Y11 receptor in man includes several tissues relevant for the pathophysiology of myocardial ischemia [5,8,11]. The expression of P2Y11 mRNA has never been characterized because the rodent subtype has not yet been cloned [12]. However, a peptide antigen of the P2Y11-like receptor has been identified in rat cardiac fibroblast [12], in H9C2 rat ventricular cells and in neonatal rat heart cells [6]. A P2Y11-like receptor has been reported to be functionally expressed in mouse heart cells [13].

Even if purine and pyrimidine derivatives as single nucleotides have been the most widely studied P2 agonists, it could be interesting to study the role of extracellular pyridine nucleotides and their metabolites in important regulatory functions [14]. Extracellular nicotinic acid adenine dinucleotide phosphate (NAADP) has been recently reported to be a full agonist of the P2Y11 receptor [15]. Therefore, we particularly focused on the role of extracellular

Abbreviations: I/R, ischemia/reperfusion; LVEDP, left ventricular end-diastolic pressure (mm Hg); NAADP, nicotinic acid adenine dinucleotide phosphate; pEC_{50}, negative logarithm to base 10 of the half maximal effective concentration; RPP, rate-pressure product (mm Hg beat min^{-1}); VF, ventricular fibrillation; VT, ventricular tachycardia.

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NAADP as a potential agonist of the P2Y11-like receptor in triggering cardioprotective effects.

The aim of our study was to investigate the potential role of extracellular NAADP in triggering pharmacological preconditioning against ischemia/reperfusion (I/R) and the involvement of the P2Y11-like receptor in mediating cardioprotection. Microdialysis in isolated perfused rat hearts was used in order to report data of extracellular NAADP and other pyridine nucleotides relating a potential role in pathophysiological implications such as myocardial ischemia. Finally, in order to characterize the functional response following P2Y11-like receptor stimulation by extracellular NAADP, experiments on isolated cardiomyocytes were performed.

2. Materials and methods

2.1. Compounds and chemical reagents

NAADP and 8,8′-[Carbonylbis[imino-3,1-phenylencecarbonylimino(4-fluoro-3,1-phenylene)carbonylimino]]bis-1,3,5-naphthalenetrisulfonic acid hexasodium salt (NF157) were purchased from Tocris® Bioscience (France R&D Systems Europe, Lille, France). Acetonitrile, methanol and formic acid were purchased from Biosolve (DIEUZE, France). Other chemical compounds were purchased from Sigma® (Saint Quentin Fallavier, France).

2.2. Isolated Heart preparation

Experiments were approved and conducted in conformity with laws and regulations controlling experiments and procedures for animal research in France and the European Convention for the Protection of Vertebrate Animals used in Experimental and Other Scientific Purposes. The study was approved by the local ethics committee. Two-month-old male Wistar rat hearts were prepared according to the non-working Langendorff mode using retrograde perfusion system at constant pressure as previously validated and described [6,16].

2.3. Experiments

All experiments lasted a total of 120 min: t0–20-min of stabilization, t20–t40-min of treatment, t40–t80-min of ischemia then t80–120-min of repefusion. Rat hearts were randomly assigned to 5 groups to receive treatment as follows. The control group was perfused with KHB (t20, 40-min). The groups 2 and 3 were perfused with 0.1 and 1 μM NAADP (t25, 35-min followed with a wash out period of 5-min) respectively. The group 4 was perfused with 1 μM NF157 (t20, 40-min). The group 5, called 1 μM (NAADP + NF157), was perfused with 1 μM NAADP (as group 3) bracketed with 1 μM NF157 (t20, 40-min), a P2Y11 receptor antagonist. Treatment perfusion flow was fixed to 1% of the mean coronary flow.

2.4. Measurements

Evaluation of measurements was done in a randomized blinded manner for all experiments.

2.5. Contractile parameters

The contractile parameters were measured during the whole perfusion period. The difference between systolic pressure (mm Hg) and the left ventricular end-diastolic pressure, an index of contracture (LVEDP, mm Hg) represented the left ventricular developed pressure (LVDP, mm Hg). The heart rate (HR, beats min^{-1}) was measured at the same time. The rate-pressure product (RPP, mm Hg beat min^{-1}) was calculated by multiplying the LVDP and the heart rate.

2.6. Mean coronary flow (MCF)

Before ischemia and during the reperfusion period, the MCF was measured using the perfusate draining out for 1 min and normalized to the heart wet weight (mL min^{-1} g^{-1}).

2.7. Infarct size determination

Myocardial infarct size was determined after 40 min of post-ischemic reperfusion by quantitative image analysis as previously described [6].

2.8. Assessment of arrhythmias throughout reperfusion

Left ventricular pressure traces were analyzed for the incidence and duration of ventricular tachycardia (VT) and fibrillation (VF), as described elsewhere [17].

2.9. Cardiac microdialysis assessment of interstitial nucleotides

To study the kinetics of interstitial nucleotide concentrations (NAADP, nicotinic acid adenine dinucleotide (NAAD), cyclic adenosine diphosphate-ribose (cADPR), β-NAD, Nicotinamide adenine dinucleotide phosphate (NADP), uridine triphosphate (UTP)) and adenosine, a perfused rat heart model ($n = 5$) was instrumented with cardiac microdialysis probe as previously described [1,18]. Dialysates were analysed using ultra-performance liquid chromatography coupled to tandem mass spectrometry detection.

2.10. Cell culture

Neonatal cardiomyocytes were isolated from 1–4-day-old Sprague Dawley rats using the Neonatal Cardiomyocyte Isolation System (Worthington Biochemical Corporation, Lornes Laboratories, Reading, UK) according to the manufacturer's instruction.

2.11. Intracellular D-myo-inositol 1 phosphate (IP) and cyclic adenosine monophosphate (cAMP) accumulation assay

Neonatal rat cardiomyocytes in culture were stimulated for 1 h, in 24-well plates for inositol-phosphate accumulation assay, or 30-min, in 6-well plates for cAMP assay, at 37 °C with extracellularly applied concentrations of NAADP in the absence or presence of 10 μM NF157, added 15-min before NAADP. IP accumulation was determined using an IP-One ELISA (Cisbio, Bagnols/Cèze, France) Assay, following the manufacturer's instruction. Intracellular cAMP accumulation was determined as previously described [19].

2.12. Western blot

Primary neonatal cardiomyocytes, plated in 6-well plates, were stimulated for 15-min with 10 μM extracellular NAADP in the absence or presence of 10 μM NF157, added 15-min before NAADP. Cells were extracted and aliquots containing equal amounts of denatured protein (40 μg) were separated by 10% SDS–PAGE, transferred to 0.22 mm nitrocellulose membranes (Sigma) and incubated with specific antibodies.

2.13. Statistical analysis

Statistical analyses were performed using IBM-SPSS statistics software version-20.0. All values are expressed as mean ± sem of experiments. $p < 0.05$ was considered to be statistically significant.

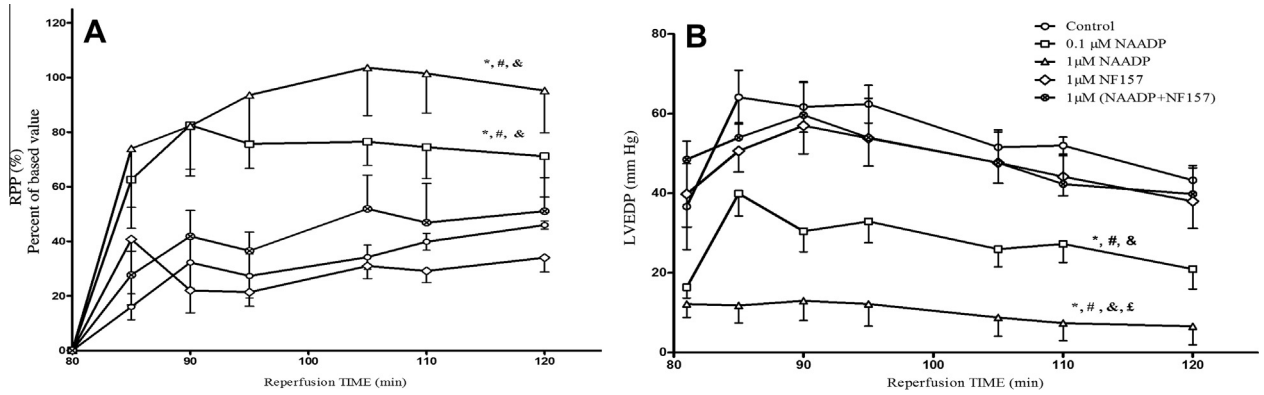

Fig. 1. NAADP improves postischemic contractile function. Effect of NAADP with or without NF157, an antagonist of the P2Y11 receptor, on postischemic of the time course of changes in Rate Product Pressure (RPP) (%) normalized to baseline value before pretreatment (A) and on recovery of Left Ventricular End Pressure (LVEDP) (mm Hg) (B). Data are means ± sem (n = 6/group). *$p < 0.05$, compared to control group; #$p < 0.05$, compared to 1 µM (NAADP + NF157) group; & $p < 0.05$, compared to 1 µM NF157 group; £$p < 0.05$, compared to 0.1 µM NAADP group.

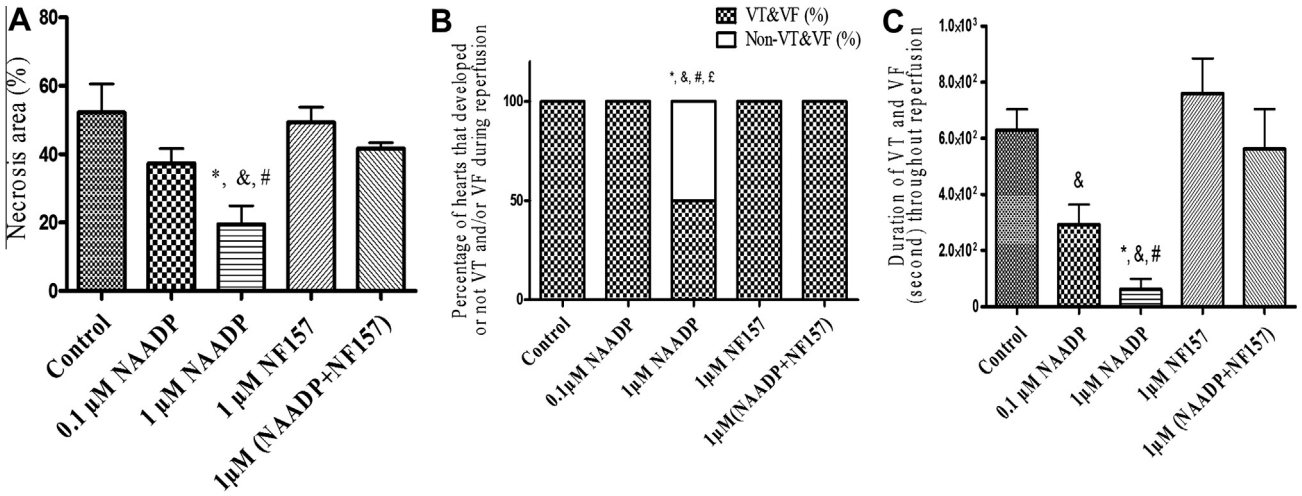

Fig. 2. NAADP reduces infarct size and post-ischemic arrhythmogenesis. Variation of necrosis area following treatment with extracellular concentrations of NAADP associated or not with the P2Y11 receptor antagonist 1 µM NF157 (A). Photomicrographs were quantified and necrosis area was expressed as percentage of black pixels relative to total pixels. Variation of duration of VF and/or VT following pre-treatment with extracellular concentrations of NAADP associated or not with the P2Y11 receptor antagonist 1 µM NF157: Incidence (B) and duration (C) of ventricular arrhythmias were recorded throughout the 40-min of reperfusion. Data are means ± sem ($n = 6$/group). *$p < 0.05$, compared to control group; #$p < 0.05$, compared to 1 µM (NAADP + NF157) group; & $p < 0.05$, compared to 1 µM NF157 group; £$p < 0.05$, compared to 0.1 µM NAADP group.

More details of materials and methods are described in Supplementary material.

3. Results

3.1. Pre-ischemic function

Baseline functional data are provided (Supplementary data-Table 1). Functional parameters were in the same order of magnitude compared to our previously published data for the same model [6,16]. At baseline HR, MCF, RPP were comparable between all groups (Supplementary data-Table 1).

3.2. NAADP improves postischemic contractile function

Data corresponding to 40-min of reperfusion are represented in Fig. 1. Global effect on RPP and LVEDP did not significantly differ between 1 µM NF157, 1 µM (NAADP + NF157) and control groups (Fig. 1). As shown in Fig. 1, and the 1 µM NAADP significantly increased RPP throughout reperfusion compared to all the other groups except the 0.1 µM NAADP group ($p < 0.05$). Hearts treated with 0.1 µM NAADP exhibited a lower increase in RPP than 1 µM NAADP but a higher increase in RPP compared to other groups ($p < 0.05$) (Fig. 1A). These beneficial effects were accompanied by a decrease in LVEDP in both groups during reperfusion ($p < 0.05$; Fig. 1B).

NAADP sped up contractile recovery. The velocity of contractile recovery (RPP (%.min^{-1})), calculated between t80-min and t90-min, was equal between hearts treated with 0.1 µM NAADP and 1 µM NAADP (8.26 ± 0.06 %.min^{-1}, 8.22 ± 0.07 %.min^{-1}, respectively; Fig. 1A). In the steady state, between t90-min and t120-min, hearts treated with 1 µM NAADP exhibited higher levels of RPP (%) than hearts treated with 0.1 µM NAADP ($p < 0.05$; 95.22 ± 3.75% vs. 76.08 ± 1.86%).

The beneficial effects of 1 µM NAADP in reducing LVEDP and increasing RPP were suppressed with the 1 µM NF157, a P2Y11 antagonist ($p < 0.05$) (Fig. 1).

3.3. NAADP reduces infarct size

Infarct size did not significantly differ between 1 µM NF157, 1 µM (NAADP + NF157) and control groups (Fig. 2A). 1 µM NAADP significantly reduced infarct size compared to control group and compared to 1 µM NF157 group. However, 0.1 µM NAADP failed

to decrease significantly infarct size compared to control group (Fig. 2A).

Beneficial effects of 1 μM NAADP in reducing the infarct size were suppressed with the 1 μM NF157, a P2Y11 antagonist (Fig. 2A).

3.4. NAADP reduces post-ischemic arrhythmogenesis

All hearts exhibited VT and/or VF, compared with only 50% of hearts treated with 1 μM NAADP (Fig. 2B; $p < 0.05$).

Duration of ventricular arrhythmias did not significantly differ between 1 μM NF157, 1 μM (NAADP + NF157) and control groups (Fig. 2C). 1 μM NAADP significantly reduced duration of ventricular arrhythmias. Hearts treated with 0.1 μM NAADP exhibited significant lower duration of ventricular arrhythmias compared to hearts treated with 1 μM NF157.

Beneficial effects of 1 μM NAADP in reducing the duration of ventricular arrhythmias were completely suppressed with 1 μM NF157 (Fig. 2C). 1 μM NAADP pre-ischemic treatment significantly reduced the incidence and duration of ventricular arrhythmias which could be associated, *in vivo*, with lethal prognosis.

3.5. Effects of ischemia on myocardial interstitial concentration of NAADP, NAAD, cADPR, β-NAD, NADP, UTP and adenosine

We assessed nucleotides and adenosine concentration increase in the interstitial compartment during global ischemia (Table 1). Before ischemia (basal value), we were able to detect all above-mentioned nucleotides and adenosine. As shown in Table 1, except for NAAD, a NAADP metabolite, global ischemia induced a significant increase of all other analytes ($p < 0.05$ compared to basal value). A maximum concentration was observed during the last 10 min of global ischemia. During reperfusion, a delayed recovery to basal values was observed for adenosine, UTP and NAADP. The fact that the ratio of NAAD to NAADP decreased slowly whereas NAADP concentration increased in the same time is a good testimony of the stability of extracellular NAADP. To validate our data, the kinetic data for adenosine and UTP, during ischemia, were in the same order of interstitial concentrations compared to previously published data [1,18].

3.6. IP and cAMP accumulation reflecting Gq and Gs subunits activity induced by extracellular NAADP

Extracellular NAADP induced a concentration-dependent increase of cAMP and IP-one intracellular concentrations (Fig. 3). EC_{50} was 8.190 μM for cAMP production and 1.2 μM for IP-one production (Fig. 3). These results were in the same order of magnitude compared to previously published data [20,21]. 10 μM NF157 were able to inhibit cAMP and IP-one production induced by 100 μM extracellular NAADP (Fig. 3C). 10 μM NF157 concentration was chosen as a concentration ensuring 90 % inhibition of P2Y11 receptor activity [20].

Table 1
Ischemia induced an increase of myocardial interstitial concentration of NAADP, cADPR, NAD, NADP, UTP and adenosine.

Interstitial microdialysis ($n = 5$)	Before ischemia	Global ischemia				Reperfusion		
	−10-min	+10-min	+20-min	+30-min	+40-min	+10-min	+20-min	+30-min
[NAADP] μM	0.017 ± 0.005	0.039 ± 0.013	0.12 ± 0.070	0.22 ± 0.043*	0.633 ± 0.180*	0.05 ± 0.003*	0.017 ± 0.004	0.016 ± 0.003
[NAAD] μM	0.01 ± 0.002	0.027 ± 0.005	0.03 ± 0.009	0.05 ± 0.02	0.1 ± 0.05	0.017 ± 0.003	0.006 ± 0.002	0.03 ± 0.0002
NAAD/NAADP	0.75	0.70	0.26	0.22	0.16	0.32	0.34	0.22
[cADPR] μM	0.013 ± 0.006	0.011 ± 0.002	0.02 ± 0.005	0.025 ± 0.006	0.11 ± 0.036*	0.026 ± 0.009	0.02 ± 0.010	0.013 ± 0.005
[NAD] μM	0.019 ± 0.004	0.036 ± 0.007	0.056 ± 0.020	0.07 ± 0.040	0.24 ± 0.080*	0.037 ± 0.012	0.04 ± 0.009	0.015 ± 0.0001
[NADP] μM	0.018 ± 0.009	0.049 ± 0.015	0.065 ± 0.026	0.11 ± 0.050	0.22 ± 0.060*	0.046 ± 0.011	0.019 ± 0.008	0.02 ± 0.009
[UTP] μM	0.04 ± 0.010	0.11 ± 0.030	0.21 ± 0.080	0.41 ± 0.100*	0.42 ± 0.110*	0.23 ± 0.050*	0.06 ± 0.020	0.04 ± 0.020
[Adenosine] μM	0.30 ± 0.140	0.31 ± 0.170	1.31 ± 0.140*	5.893 ± 1.580*	18.30 ± 4.580*	15.13 ± 4.90*	2.98 ± 0.870*	0.40 ± 0.100

* $p < 0.05$ compared to the basal values (± sem) or before ischemia.

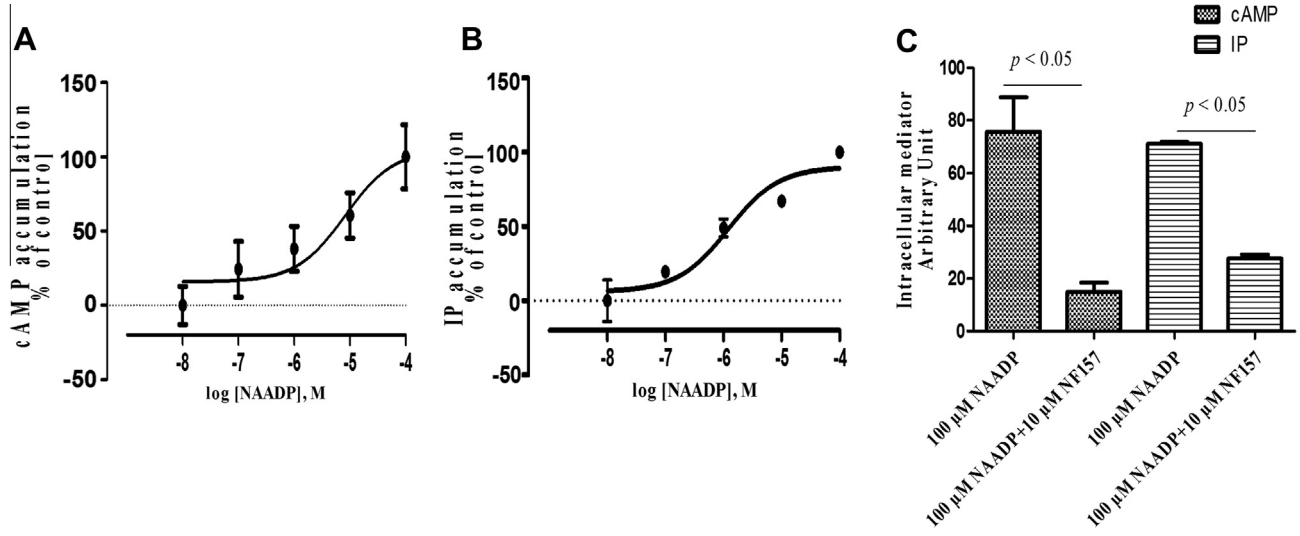

Fig. 3. Metabotropic response following extracellular NAADP stimulation. Extracellular NAADP concentration–response curve for cAMP (A) and IP (B) accumulation in rat cardiomyocytes. The effect of 10 μM NF157, a P2Y11 receptor antagonist, on cAMP and IP accumulation induced by 100 μM extracellular NAADP (C). Data are means ± sem ($n = 4$). pEC50 [NAADP] = 5.087, CI95 [6.038-4.130], for cAMP accumulation. pEC50 [NAADP] = 5.917, CI95 [6.511-5.324], for IP accumulation.

3.7. NAADP induces prosurvival protein kinases activation

As shown in Fig. 4, 10 μM Extracellular NAADP stimulation phosphorylated ERK 1/2, Akt and its downstream target GSK-3β ($p < 0.05$ compared to control group). NF157 pretreatment abolished prosurvival protein kinases activation induced by 10 μM extracellular NAADP (Fig. 4). Non phosphorylated protein kinase (ERK 1/2, Akt) and B-actin were non-significant between groups (data not shown).

4. Discussion

Using a pharmacological preconditioning protocol [6,16], with a 10-min perfusion of NAADP, followed by a 5-min drug-free perfusion before a prolonged *I/R*, hearts rapidly recovered post-ischemic contractile function and displayed attenuated contracture, infarct size and arrhythmogenesis. 1 μM NF157, a suramin-derived P2Y11 receptor antagonist [20], suppressed the beneficial effects induced by 1 μM NAADP. During the trigger phase of pharmacological preconditioning, none of the NAADP-pretreated hearts developed arrhythmias.

Cardiac microdialysis was used to study the increase of interstitial nucleotides (NAADP, NAAD, cADPR, β-NAD, NADP, UTP) and adenosine concentrations during ischemia. As previously shown [1,18], we confirm that UTP and adenosine are released during ischemia, and we report for the first time a significant increase in extracellular concentrations of β-NAD and its metabolites such as NADP, NAADP and cADPR during heart ischemia. Besides adenosine, it is noteworthy that the observed increase in extracellular NAADP concentration was the most significant among the nucleotides tested.

In cardiomyocyte cultures, extracellular NAADP was able to trigger cAMP and IP3 endogenous production. This metabotropic response was suppressed by NF157 which confirms the existence of a functional P2Y11-like receptor in rat cardiomyocytes. Our data confirm, as reported by Moreschi et al. [15], that extracellular NAADP is an agonist of the P2Y11 receptor. In cardiomyocyte cultures, a pretreatment during 15 min with 10 μM extracellular NAADP, a concentration higher than half maximal effective concentration (EC_{50}) for cAMP and inositol phosphate accumulation, phosphorylated the prosurvival protein kinases ERK 1/2, Akt and its downstream target GSK-3β. These phosphorylations were suppressed by NF157, implying that the P2Y11-like receptor might be responsible for the NAADP-induced cell activation of the prosurvival pathways in cardiomyocyte cultures. These results suggest that cardiomyocytes are directly sensitive to extracellular stimulation of NAADP and even to a paracrine effect of interstitial NAADP.

Elevation of cAMP and IP3 may lead to arrhythmogenic alterations in Ca^{2+} signaling [22,23]. Ischemic preconditioning (IPC) protects against reperfusion arrhythmias, contractile dysfunction and myocardial infarction [24] despite transient elevation of intracellular cAMP [25] and IP3 [26] during the trigger phase. Blockade of cAMP [25] or IP3 [26] production during the trigger phase of IPC suppresses the cardioprotective effects. It has been reported that transient cell-permeable cAMP analogue treatment triggers rapid activation of PKA and induces cardioprotective effect against I/R [27]. Juhaszova et al. [28] also reported that PKA activates MEK/ERK 1/2 pathway and phosphorylates GSK-3β. Transient IP3 production has been shown to mediate cardioprotective effects induced by morphine [29]. IP production is linked to Gq activity following P2Y11 receptor stimulation [11] and Gq subunit is able to induce AKT activation [30]. Like IPC [25,26], NAADP is able to induce cAMP and IP elevation during the trigger phase of preconditioning. Our data support that stimulation of the P2Y11 receptor, coupled to Gs and Gq subunits, is able to induce phosphorylation of ERK 1/2, AKT and their downstream target GSK-3β. These prosurvival protein kinases are phosphorylated and involved during the triggering phase of cardiac preconditioning and in cardioprotection against I/R injury [16,28,31,32].

The cardioprotective effect of P2Y11 receptor stimulation has not been pointed out in previously reported studies [3,4,8,33]. Ninomiya et al. (2002a), using the rate pressure product as a main end point in a model of ischemic preconditioning, reported that endogenous ATP with endogenous adenosine triggered protection. These beneficial effects seem mediated by P2Y and adenosine receptors. ATP is an agonist of several purinergic and pyrimidinergic

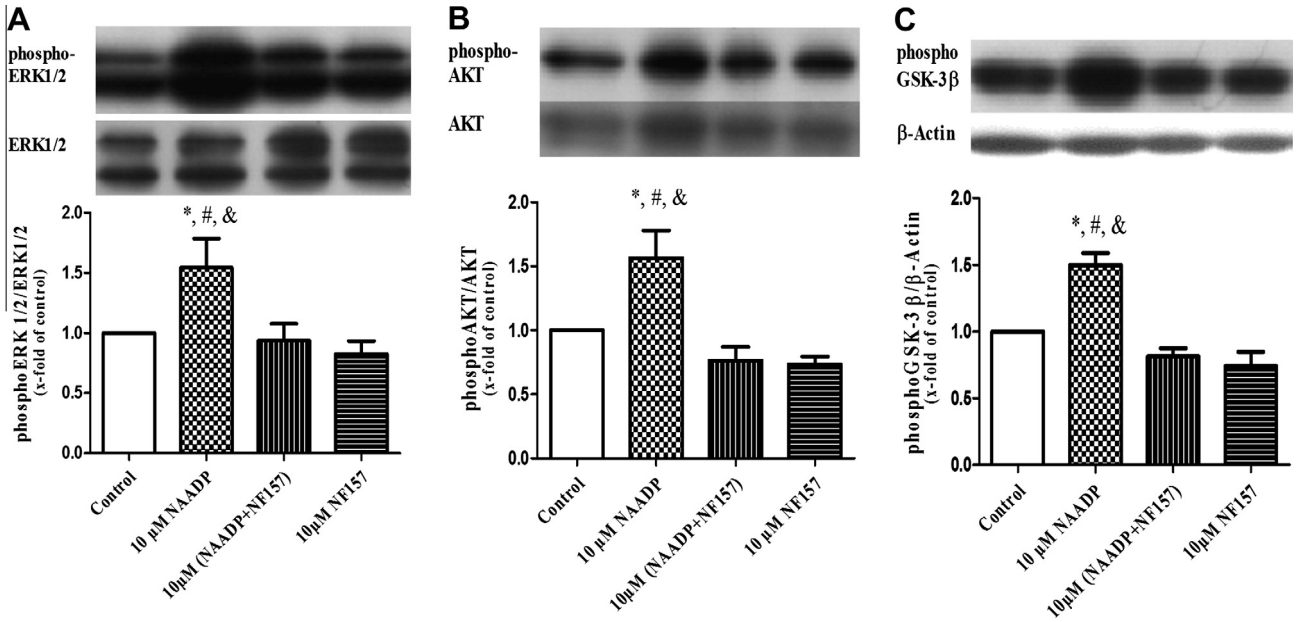

Fig. 4. Prosurvival protein kinases activation induced by extracellular NAADP. Using a normoxic newborn rat cultured cardiomyocytes, (A) phosphorylation of ERK 1/2 (thr 202/tyr 204), (B) AKT (ser 473) and (C) its downstream target GSK-3β (ser 9) (respectively normalized to non-phosphorylated ERK 1/2, AKT and β-Actin) induced by stimulation of 10 μM extracellular NAADP in the absence or presence of 10 μM NF157, a P2Y11 receptor antagonist added 15-min before NAADP, was assessed by immunoblotting utilizing specific antibodies. Data are means ± sem ($n = 5$/group). $^{*}p < 0.05$, compared to control group; $^{\#}p < 0.05$ compared to 10 μM (NAADP + NF157) group; & $p < 0.05$, compared to 10 μM NF157 group.

receptors such as P2Y1, P2Y2, P2Y4, P2Y11 and P2Y12 receptors [11]. Like UTP, ATP has been reported as a full agonist of P2Y11 [11]. Wee et al. [1] excluded a beneficial role of P2Y1 stimulation against I/R. Millart et al. [6] have suggested a cardioprotective effect following dual P2Y11 and P2Y6 receptor stimulation before a sequence of I/R. Our present results suggest a beneficial role of P2Y11-like receptor stimulation before I/R. Consequently, the beneficial role reported for ATP associated with adenosine [3], and for UTP [4,7,33], could partially involve the P2Y11-like receptor stimulation. Finally, the protective role of UTP and ATP and endogenous mediators, such as NAADP, capable of stimulating the P2Y11-like receptor could play a complementary role in triggering an I/R tolerance. P2Y receptors have been reported to mediate ATP prosurvival pathways and to reduce ischemia-induced apoptosis in endothelial cell [8]. Moreover the P2Y11 receptor has been shown to mediate the beneficial effects of extracellular β-NAD in bone marrow-derived human mesenchymal stem cells, including proliferation and migration [34].

In conclusion, extracellular pre-treatment with NAADP triggered ischemic-reperfusion tolerance. Our results support a beneficial role of P2Y11-like receptor stimulation induced by low extracellular NAADP concentrations against ischemia and reperfusion injury. Extracellular NAADP was able to induce intracellular cAMP and IP_3 production and activation of prosurvival protein kinases during the trigger phase of cardiac preconditioning. Beside its well-known intracellular activity [15], NAADP is released during ischemia suggesting that extracellular NAADP may act as a paracrine survival factor, prolonging cardiomyocytes lifespan during ischemic events.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.03.089.

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