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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.