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Mini Review

P2X4 receptor–eNOS signaling pathway in cardiac myocytes as a novel protective mechanism in heart failure

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ABSTRACT

We have demonstrated using immunoprecipitation and immunostaining a novel physical association of the P2X4 receptor (P2X4R), a ligand-gated ion channel, with the cardioprotective, calcium-dependent enzyme endothelial nitric oxide synthase (eNOS). Treatment of murine ventricular myocytes with the P2XR agonist 2-methylthioATP (2-meSATP) to induce a current (mainly Na^+) increased the formation of nitric oxide (NO), as measured using a fluorescent probe. Possible candidates for downstream effectors mediating eNOS activity include cyclic GMP and PKG or cellular protein nitrosylation. A cardiac-specific P2X4R overexpressing mouse line was protected from heart failure (HF) with improved cardiac function and survival in post-infarct, pressure overload, and calsequestrin (CSQ) overexpression models of HF. Although the role of the P2X4R in other tissues such as the endothelium and monocytes awaits characterization in tissue-specific KO, cardiac-specific activation of eNOS may be more cardioprotective than an increased activity of global systemic eNOS. The intra-myocyte formation of NO may be more advantageous over NO derived externally from a donor. A small molecule drug stimulating this sarcolemmal pathway or gene therapy-mediated overexpression of the P2X4R in cardiac myocytes may represent a new therapy for both ischemic and pressure overloaded HF.

Contents

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1. Introduction

Since the original proposal of purinergic transmission by Burnstock [1], the field of purinergic receptors and signaling has grown exponentially. Extracellular ATP and adenosine activating their P2 and P1 purinergic receptors, respectively, mediate a growing number of biological functions. Adenosine (P1) and $P2Y_{12}$ receptors have already become targets for cardiovascular disease [2,3]. P2X receptors (P2XRs) are trimeric ion channels with a molecular weight of each monomer of 43,438 Da (mouse) or 43,369 Da (human). This receptor channel is activated by ATP and its analogues. The subunit composition of a given P2X channel may be homotrimeric, i.e. of the same P2XR protein, or heterotrimeric, which is known to affect the ligand pharmacology. In the current review, we summarized a novel feature of the P2X4R in that the receptor is not just an ion channel, but it also physically associates with the enzyme endothelial nitric oxide synthase (eNOS). In the cardiac myocyte, we show that P2X4R stimulation causes activation of eNOS, as demonstrated by increased nitric oxide (NO) formation, with two structurally different NO-sensitive fluorescent dyes. Functionally, the P2X4R–eNOS pathway is important in mediating cardioprotection in heart failure (HF).

2. Identification and characterization of cardiac myocyte P2X4 receptors

Adult ventricular myocytes from mice, rat and guinea pig show an inward current in response to extracellular ATP [3]. ATP elicited an increase in a non-selective cation current with a reversal potential near 0 mV, which is similar to that of cloned P2X4Rs [4]. In murine ventricular myocytes, the current induced by the P2XR agonist 2-methylthioATP (2-meSATP) is partially insensitive to antagonism by suramin and pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), which is also characteristic of the P2X4R [4]. As an ion channel, the P2X4R conducts current carried by both Na^+ and Ca^{2+}. Although the P2X4R is a calcium permeant channel, the majority of the inward current is carried by Na^+. Initially, it was predicted that this channel would be deleterious in the heart because of the potential calcium overload caused by the influx of Na^+ and Ca^{2+}. Under basal control conditions, mice with cardiac-specific P2X4R overexpression were overtly normal without cardiac hypertrophy or failure as they age [5]. Paradoxically, these mice are protected from HF with improved cardiac function and survival with post-infarct, pressure overload, and calsequestrin (CSQ) overexpression-induced HF [6–8].

3. Regulation of P2X receptors in diseased hearts

A question arises as to whether cardiac P2X receptors are regulated during pathological conditions. For example, are these receptors up- or down-regulated in disease states such as heart failure? As we gain understanding of the role of cardiac P2X receptors, their regulation in diseased hearts may have implications for the progression of the disease. In our previous study, the expression of the cardiac P2X4 receptor is increased in the CSQ mice-overexpressing model of hypertrophy and dilated cardiomyopathy. The P2X agonist-stimulated current is also

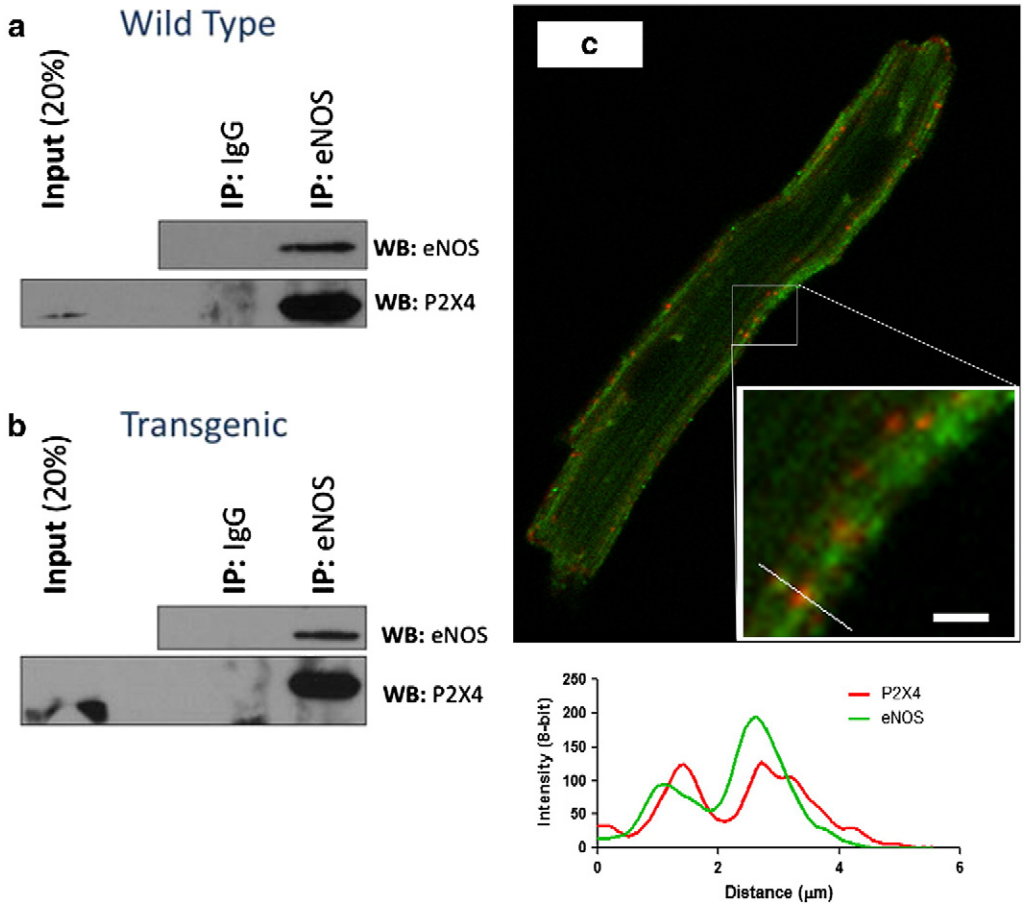

Fig. 1. P2X4R and eNOS physically associate with each other as evidenced by co-immunoprecipitation and co-localization in cardiac ventricular myocytes of WT and P2X4R Tg mice. (a) WT myocyte lysates were incubated with anti-eNOS antibody or with non-specific IgG as control. The isolated complex was probed with eNOS (top panel) and P2X4 (bottom panel) antibodies using western blotting. In a control experiment, eNOS co-immunoprecipitated itself. Co-immunoprecipitation of P2X4R with eNOS antibody (lane 3) but not with control IgG (lane 2) was shown. (b) Same experiment as in (a) conducted in P2X4-Tg myocytes. (c) Immunostaining of eNOS (green), P2X4R (red), and merged image was shown for a P2X4R-overexpressing Tg cardiac myocyte.
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