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Regulation of Mitochondrial Poly(ADP-Ribose) Polymerase Activation by the β-Adrenoceptor/cAMP/Protein Kinase A Axis during Oxidative Stress

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ABSTRACT

We investigated the regulation of mitochondrial poly(ADP-ribose) polymerase 1 (PARP1) by the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) system during oxidative stress in U937 monocytes. Oxidative stress induced an early (10 minutes) mitochondrial DNA damage, and concomitant activation of PARP1 in the mitochondria. These early events were followed by a progressive mitochondrial oxidant production and nuclear PARP1 activation (by 6 hours). These processes led to a functional impairment of mitochondria, culminating in cell death of mixed (necrotic/apoptotic) type. β-Adrenoceptor blockade with propranolol or inhibition of its downstream cAMP/PKA signaling attenuated, while β-adrenoceptor agonists and cAMP/PKA activators enhanced, the oxidant-mediated PARP1 activation. In the presence of cAMP, recombinant PKA directly phosphorylated recombinant PARP1 on serines 465 (in the automodification domain) and 782 and 785 (both in the catalytic domain). Inhibition of the β-adrenergic receptor/cAMP/PKA axis protected against the oxidant-mediated cell injury. Propranolol also suppressed PARP1 activation in peripheral blood leukocytes during bacterial lipopolysaccharide (LPS)-induced systemic inflammation in mice. We conclude that the activation of mitochondrial PARP1 is an early, active participant in oxidant-induced cell death, which is under the control of β-adrenoceptor/cAMP/PKA axis through the regulation of PARP1 activity by PARP1 phosphorylation.

Introduction

Poly(ADP-ribose) polymerase 1 (PARP1), the major form of the PARP superfamily, is generally viewed as a constitutive nuclear enzyme with physiologic roles in the regulation of DNA repair, chromatin remodeling, and gene transcription (Tulin et al., 2003; Oei et al., 2005; Hottiger et al., 2011; DeVos et al., 2012). Its pathophysiologic overactivation, as a result of oxidative DNA injury, has been implicated in the pathogenesis of several diseases, including neuroinjury, inflammation, and ischemia/reperfusion, and various forms of critical illness (Tulin et al., 2003; Jagtap and Szabo, 2005; DeVos et al., 2012; Szanto et al., 2012; Curtin and Szabo, 2013; Burkle and Virag, 2013). In addition to the nuclear isoform of PARP1 several sets of studies have identified PARP1 in the mitochondrial compartment as well; it appears that, in addition to nuclear PARP1, mitochondrial PARP1 also plays roles in the regulation of various cellular functions—at least in some cell types (Masmoudi et al., 1988; Du et al., 2003; Rossi et al., 2009, reviewed in Burkle and Virag, 2013).

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Protein kinase A (PKA), a cyclic adenosine monophosphate (cAMP)-dependent serine/threonine kinase, is one of the major effector proteins of cAMP and a regulator of cellular function in health and disease (Gancedo, 2013; Taylor et al., 2013; Valsecchi et al., 2013). The purpose of the current study was to determine whether PARP1 activity is regulated by the β-adrenoceptor/cAMP/PKA system during oxidative stress. The results demonstrate an early activation of the mitochondrial form of PARP1, unveil its regulation via phosphorylation by PKA through β-adrenoceptor/cAMP signaling and show the role of these processes in oxidant-induced cell death.

Materials and Methods

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless stated otherwise.

Cell Culture. U937 human monocyte histiocytic lymphoma cells, and C2C12 mouse myoblast cells were obtained from ATCC.

Western Blotting Analysis. Western blotting analysis was carried out as previously described (Gerö et al., 2013a) using anti-PARP1 antibody (Cell Signaling Technology, Beverley, MA), anti-β-receptor antibody (Abcam, Cambridge, MA), anti-protein kinase A (R&D Systems, Minneapolis, MN), anti-β-actin–horseradish peroxidase (HRP) conjugate (Santa Cruz Biotechnology, Inc., Dallas, TX), anti-rabbit-IgG (Cell Signaling Technology), and anti-mouse-IgG (Cell Signaling Technology). PARP1 activity was quantified by detection of its product, poly

ABBREVIATIONS: cAMP, cyclic adenosine monophosphate; DDA, 2,5-dideoxi-adenosine; FSK, forskolin; H_2O_2, hydrogen peroxide; HRP, horseradish peroxidase; ISP, isoproterenol; LC, liquid chromatography; LDH, lactate dehydrogenase; LPS, bacterial lipopolysaccharide; NAD^+, nicotinamide adenine dinucleotide; PAR, poly(ADP-ribose); PARG, poly(ADP-ribose) glycohydrolase; PARP1, poly(ADP-ribose) polymerase 1; PCR, polymerase chain reaction; PJ34, *N*-(6-oxo-5,6-dihydrophenanthridin-2-yl)-(*N*,*N*-dimethylamino)acetamide hydrochloride; PKA, protein kinase A; PLA, proximity ligation assay; PP, propranolol; siRNA, small interfering RNA.

subsequent cell death response (Tann et al., 2011; Guicciardi et al., 2013; Virag et al., 2013; Dorn, 2013). In the current experimental model, early oxidant-mediated mitochondrial PARP1 activation, as well as other mitochondrial events, such as a progressive mitochondrial oxidant production (Fig. 2A) and mitochondrial electron transport defects (Fig. 11), probably contribute to the cell death. The results of the current research demonstrate that these processes are attenuated by blocking the early-onset PKA activation and the subsequent PARP1 activation. The working model outlined in Fig. 12 shows one possible interpretation of the findings of the current report: the cAMP/PKA axis is stimulated by β-adrenoceptor agonists (such as isoproterenol), cAMP analogs (such as 8-bromo-cAMP), and by adenylyl cyclase activators (such as forskolin), leading to mitochondrial PARP1 phosphorylation and increased PARP activity. Moreover, the cAMP/PKA axis is inhibited by β-adrenoceptor antagonists (such as propranolol), by adenylyl cyclase inhibitor (such as DDA) and by inhibition/downregulation of PKA (by the pharmacological inhibitor Rp-cAMP or by genetic silencing of PKA) leading to the inhibition of mitochondrial PARP1 phosphorylation and decreased PARP activity. In the Fig. 12, mitochondrial PKA is shown to be stimulated by cytosolic cAMP. It must be pointed out that multiple lines of data indicate that the cytosolic and mitochondrial cAMP pools do not communicate in most cases. At the same time, several sets of data also indicate that cell membrane and cytosolic signals can elevate intramitochondrial PKA activity, followed by phosphorylation of intramitochondrial proteins (reviewed in Lefkimmiatis and Zaccolo, 2014). Yet another possibility may be the extramitochondrial phosphorylation of PARP1, followed by its transport into the mitochondria, even though the early time course of extranuclear PARP1 phosphorylation (see Fig. 9) tends to speak against this possibility.

Although many additional details of the underlying mechanisms remain to be explored, the current findings may have implications for a number of cellular processes that are known to be regulated by PARP1, including cellular metabolism and DNA repair, given the fact that both of these processes can be regulated by the β-adrenoceptor/cAMP system (Carlucci et al., 2008; Cho and Juhnn, 2012; Valsecchi et al., 2013; Lefkimmiatis and Zaccolo, 2014). However, these aspects remain to be directly investigated in further studies. The current findings may also provide a mechanistic explanation for our previously observed clinical/translational findings (Olah et al., 2011) showing that treatment of patients with severe burn injury with propranolol suppresses PARP1 activation in endothelial cells and tissue-resident mononuclear cells. Further studies are needed to determine whether modulation of PARP1 phosphorylation and its consequent catalytic activity by various drugs targeting the β-adrenoceptor/cAMP/PKA system may be used for therapeutic modulation of PARP1 in various pathophysiologic conditions associated with oxidative stress.

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Authorship Contributions

Participated in research design: Szabo, Brunyanszki and Szczesny.
Conducted experiments: Brunyanszki, Coletta, Oláh, Szczesny.
Performed data analysis: Brunyanszki, Szczesny.
Wrote or contributed to the writing of the manuscript: Szabo, Brunyanszki, Szczesny.

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