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Research Report

Cellular NAD depletion and decline of SIRT1 activity play critical roles in PARP-1-mediated acute epileptic neuronal death in vitro

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ABSTRACT

Intense poly(ADP-ribose) polymerase-1 (PARP-1) activation was implicated as a major cause of caspase-independent cell death in the hippocampal neuronal culture (HNC) model of acute acquired epilepsy (AE). The molecular mechanisms are quite complicated. The linkage among neuronal death, cellular nicotinamide adenine dinucleotide (NAD) levels, apoptosis-inducing factor (AIF) translocation, SIRT1 expression and activity were investigated here. The results showed that PARP-1 over-activation caused by Mg^{2+}-free stimuli led to cellular NAD depletion which could block AIF translocation from mitochondria to nucleus and attenuate neuronal death. Also, SIRT1 deacetylase activity was reduced by Mg^{2+}-free treatment, accompanied by elevated ratio of neuronal death, which could be rescued by NAD repletion. These data demonstrated that cellular NAD depletion and decline of SIRT1 activity play critical roles in PARP-1-mediated epileptic neuronal death in the HNC model of acute AE.

Abbreviations: Poly(ADP-ribose) polymerase-1, PARP-1; Hippocampal neuronal culture, HNC; Acquired epilepsy, AE; Nicotinamide adenine dinucleotide, NAD; Apoptosis-inducing factor, AIF; Poly(ADP-ribose), PAR; Mitochondrial permeability transition, MPT; Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling, TUNEL; Whole-cell current-clamp, WCC; 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone, DPQ; Cytochrome oxidase subunit IV, COXIV; Spontaneous recurrent epileptiform discharges, SREDs

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1. Introduction

The nuclear enzyme PARP-1 activated by DNA damage cleaves the ADP-ribose moiety from NAD to generate poly(ADP-ribosyl) ation of specific nuclear acceptor proteins, and this process is thought to facilitate DNA repair (Schreiber et al., 2006). However, PARP-1 over-activation by excessive DNA damage can trigger apoptosis or necrotic cell death which depletes a large amount of NAD and ATP. Cumulative evidence suggests that inhibition of PARP-1 has cytoprotective effects against neurotoxicity, central nervous system injury, neurodegenerative disorders, inflammation and epilepsy (Chiarugi and Moskowitz, 2003; Skaper, 2003; Yu et al., 2003). And recent studies showed that PARP inhibitors activated Akt and suppressed JNK and p38 MAPK (Sarszegi et al., 2012; Song et al., 2008). Our previous study also indicated that PARP-1 inhibition could protect neurons against caspase-independent cell death by suppressing Akt-mediated AIF translocation in both vivo and vitro models of epilepsy (Wang et al., 2007b; Yang et al.). In addition, other possibilities have been raised including cellular NAD depletion caused by PARP-1 over-activation and suppression of NAD-dependent processes.

Recent studies showed that NAD depletion is necessary and sufficient for PARP-1-mediated cell death for the following reasons: (1) NAD depletion blocks glycolysis and leads to energy deficit in cortical neurons (Alano et al., 2010). (2) NAD depletion attenuates deacetylase activity of SIRT1 which is implicated in longevity and cellular protection, because NAD is the irreplaceable substrate of SIRT1 (Aksoy et al., 2006). (3) Decrease of NAD levels results in mitochondrial depolarization, mitochondrial permeability transition (MPT) and release of AIF in astrocytes (Alano et al., 2004).

The SIRT1 enzyme is a NAD-dependent deacetylase and ADP-ribosyl transferases involved in numerous fundamental cellular processes including gene silencing, DNA repair and metabolic regulation, which is particularly located in nuclei (Donmez and Guarente, 2010; Pillai et al., 2005). SIRT1 proteins are considered as nuclear targets of redox signaling and function as energy sensors (Cohen et al., 2004). Decrease of SIRT1 deacetylase activity can activate many apoptotic effectors. A protective role of SIRT1 has been demonstrated in neurodegeneration, Huntington's disease and Parkinson's disease (Pasinetti et al., 2011; Wu et al., 2011).

AIF is a mitochondrial flavoprotein that is released in response to death stimuli, inducing nuclear condensation and large-scale DNA degradation. The translocation of AIF from mitochondria to nucleus is identified as a key step in the PARP-1-mediated cell death process (Yu et al., 2006). It has been confirmed that the translocation of AIF is regulated via the PI3k/Akt pathway in PARP-1-mediated neuronal death in both animal and HNC models of epilepsy (Wang et al., 2007b; Yang et al.). In addition, NAD depletion has been proposed as a reason for AIF release (Alano et al., 2004; Andrabi et al., 2006). Therefore, it is necessary to find out whether NAD depletion is another pathway to trigger the translocation of AIF.

In the present study, we investigated the linkage among PARP-1-mediated neuronal death, cellular NAD levels, AIF translocation, SIRT1 expression and activity in the HNC model of acute AE, which is generally accepted as the neuronal culture model of spontaneous seizure discharge (Sombati and Delorenzo, 1995). The results revealed their underlying associations for the first time.

2. Results

2.1. Electrophysiological characteristics of the HNC model of acute AE induced by Mg^{2+}-free treatment

Whole-cell current-clamp (WCC) recordings of control neurons revealed "normal" baseline recordings with spontaneous action potentials (Fig. 1A). During the 3-h Mg^{2+}-free exposure, neuronal firing behavior changed to spontaneous, continuous, high-frequency action potential firing (Fig. 1B). As shown in Fig. 1C, spontaneous recurrent epileptiform discharges (SREDs) were recorded at 24 h after the 3-h Mg^{2+}-free treatment. They are characteristic of the epileptic phenotype and can last for the life of the neurons after the initial insult with Mg^{2+}-free treatment (Sombati and Delorenzo, 1995).

2.2. Mg^{2+}-free treatment depletes cellular NAD and leads to neuronal death

Intense PARP-1 activation has been implicated as a major cause of caspase-independent neuronal death in both vivo and vitro

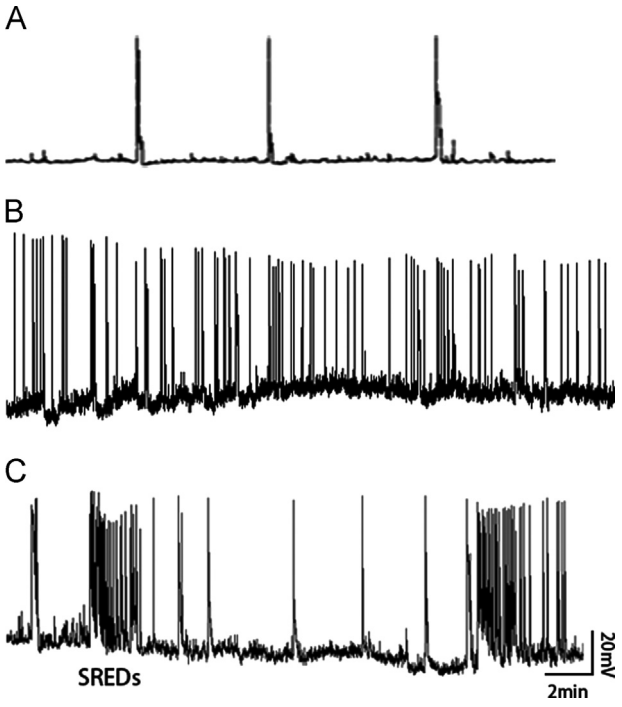

Fig. 1 – Induction of epileptiform discharge activity in HNC: (A) a representative intracellular recording from a neuron of the control group showing spontaneous excitatory and inhibitory postsynaptic potentials and occasional spontaneous action potentials. (B) During the 3-h Mg^{2+}-free exposure, neuronal firing behavior changed to spontaneous, continuous, high-frequency action potential firing. (C) A representative recording from an epileptic neuron 24 h after 3-h Mg^{2+}-free treatment. The pathophysiological state of epileptiform activity in this preparation is evident by the presence of two independent SREDs.