

Review

Mitochondrial Dysfunction and Nicotinamide Dinucleotide Catabolism as Mechanisms of Cell Death and Promising Targets for Neuroprotection

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Both acute and chronic neurodegenerative diseases are frequently associated with mitochondrial dysfunction as an essential component of mechanisms leading to brain damage. Although loss of mitochondrial functions resulting from prolonged activation of the mitochondrial permeability transition (MPT) pore has been shown to play a significant role in perturbation of cellular bioenergetics and in cell death, the detailed mechanisms are still elusive. Enzymatic reactions linked to glycolysis, the tricarboxylic acid cycle, and mitochondrial respiration are dependent on the reduced or oxidized form of nicotinamide dinucleotide [NAD(H)] as a cofactor. Loss of mitochondrial NAD⁺ resulting from MPT pore opening, although transient, allows detrimental depletion of mitochondrial and cellular NAD⁺ pools by activated NAD⁺ glycohydrolases. Poly(ADP-ribose) polymerase (PARP) is considered to be a major NAD⁺ degrading enzyme, particularly under conditions of extensive DNA damage. We propose that CD38, a main cellular NAD⁺ level regulator, can significantly contribute to NAD⁺ catabolism. We discuss NAD⁺ catabolic and NAD⁺ synthesis pathways and their role in different strategies to prevent cellular NAD⁺ degradation in brain, particularly following an ischemic insult. These therapeutic approaches are based on utilizing endogenous intermediates of NAD⁺ metabolism that feed into the NAD⁺ salvage pathway and also inhibit CD38 activity. © 2011 Wiley-Liss, Inc.

Key words: NAD catabolism; CD38; mitochondria; cell death; acute neurodegenerative disease

The mechanisms of acute and chronic neurodegenerative diseases are not understood in detail. However, several lines of evidence suggest the involvement of mitochondrial dysfunction and bioenergetic failure (for review see Fiskum, 2000, 2004; Kristal et al., 2004;

Kristian, 2004; Beal, 2005; Sullivan et al., 2005; Stavrovskaya and Kristal, 2005; Yang et al., 2008; Dumont et al., 2010; Morais and De Strooper, 2010). Although the significance of the mitochondrial role in cell death is well established, the underlying mechanisms remain unclear. One of the extensively studied aspects of mitochondrial dysfunction is a phenomenon called the *mitochondrial permeability transition* (MPT). The MPT is characterized by opening of an inner membrane channel permeable to solutes with molecular masses of approximately 1,500 Da or lower (for review see Zoratti and Szabo, 1995; Bernardi, 1999; Bernardi et al., 2001; Crompton et al., 2002; Halestrap et al., 2002). A prolonged MPT results not only in dissipation of the mitochondrial electrochemical hydrogen ion gradient and swelling of mitochondria but also depletion of pyridine nucleotides from the matrix (Di Lisa et al., 2001; Kristian and Fiskum, 2004). Loss of mitochondrial nicotinamide adenine dinucleotide (NAD⁺) is one of the most detrimental outcomes of MPT. This is because NAD⁺ is an essential cofactor in most enzymatic reactions supporting fundamental mitochondrial functions, including oxidative phosphorylation and enzymatic reactions of the tricarboxylic acid cycle (TCA cycle; Fig. 1). Thus

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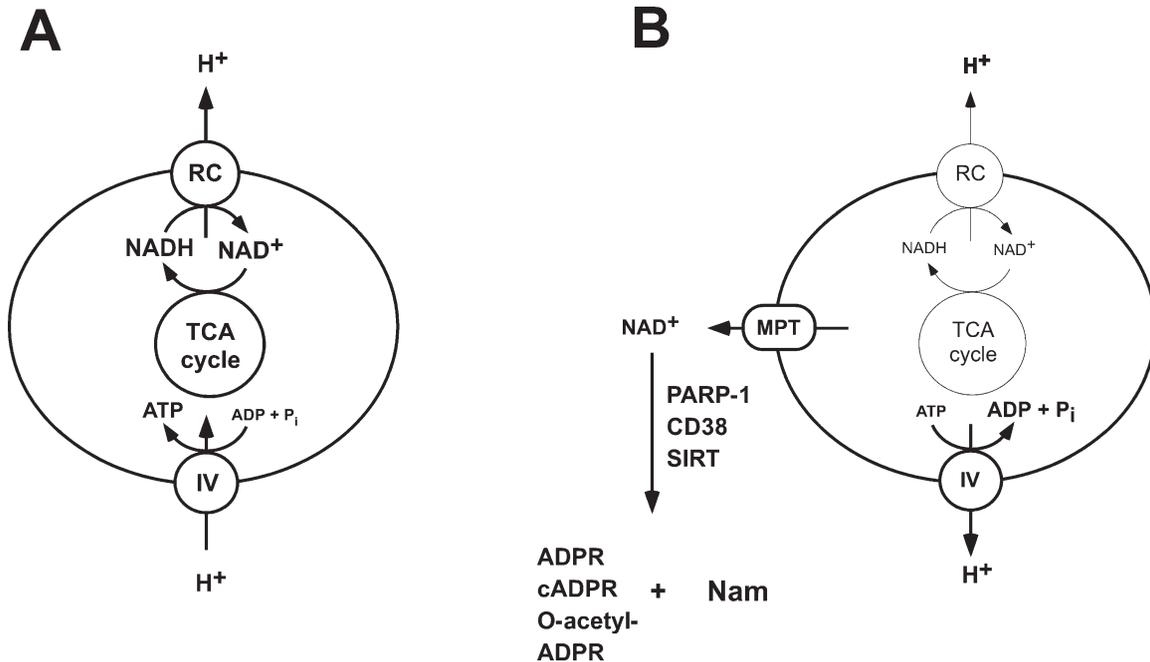


Fig. 1. Diagram illustrating mitochondrial permeability transition (MPT)-induced loss of matrix NAD^+ as a mechanism of inhibition of mitochondrial metabolic and respiratory functions. **A:** NADH donates electrons to respiratory chain complexes (RC) that generate a hydrogen ion gradient across the inner mitochondrial membrane. The NAD^+ is reduced back to NADH by intramitochondrial dehydrogenases and is an essential cofactor for enzymatic reactions within the tricarboxylic acid cycle (TCA). The energy stored in the hydrogen ion gradient drives the ATP-synthase that generates ATP from ADP and phosphate (P_i). **B:** Opening of the MPT leads to translocation of ma-

trix NAD^+ into the cytosol, where it can be hydrolyzed by NAD^+ glycohydrolases (PARP-1, CD38, and SIRT). The products of NAD^+ hydrolysis are ADP-ribose derivatives plus nicotinamide (Nam). Marked reduction in intramitochondrial NAD(H) levels leads to inhibition of respiratory functions and TCA cycle reactions. Furthermore, because of the MPT pore opening, the mitochondria become depolarized, reversing the ATP synthase. Although the MPT pore closes before mitochondria lose their morphological integrity, a significant loss of NAD^+ will prevent normal oxidative phosphorylation.

when NAD^+ is lost, respiration is inhibited, even in the presence of sufficient substrate, and the mitochondria become incapable of ATP synthesis. In fact, they actively consume ATP by reversing the ATP synthase in a futile attempt to maintain the electrochemical gradient across the inner membrane. This results in a loss of cellular metabolic integrity and can potentially lead to cell death.

MPT IN NEUROLOGICAL DISEASES

It is commonly accepted that the activation of MPT results from the interaction and conformational changes of several mitochondrial proteins (for review see Crompton, 2003). This process is catalyzed by cyclophilin D (cypD), a matrix peptidyl-propyl cis-trans isomerase (PPIase). The activity of this enzyme is inhibited by the immunosuppressant compound cyclosporin A (CsA; Crompton et al., 1988; Halestrap and Davidson, 1990; Bernardi, 1992).

The significant role of cypD in controlling MPT and in the function of MPT in brain pathology was examined by using transgenic mice in which the cypD-encoding gene had been eliminated (Basso et al., 2005; Baines et al., 2005; Nakagawa et al., 2005). Mitochondria

from cypD null mice displayed normal respiratory functions but had a striking desensitization to Ca^{2+} -induced damage (Basso et al., 2005; Nakagawa et al., 2005). However, the PPI activity of cypD is not necessarily required for MPT induction, since the MPT pore opening can be triggered by calcium overload in mitochondria isolated from cypD null mice (Basso et al., 2005).

An important role for MPT in mechanisms associated with ischemic brain damage is clearly supported by the dramatic reduction in infarct volume following focal ischemia in cypD knockout animals (Schinzel et al., 2005). Furthermore, cypD deficiency improved mitochondrial and synaptic function by increasing mitochondrial resistance to amyloid- β protein toxicity in transgenic Alzheimer's disease-type mice (Du et al., 2009) and protected axons in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis (Forte et al., 2007). However, absence of cypD in the Huntington's disease R6/2 transgenic mouse model did not show any protective effect regardless of a significant increase in calcium uptake capacity by brain mitochondria in these animals (Perry et al., 2010).

Recently, we demonstrated that there is variability in cypD expression in numerous brain regions and

between different cell types in normal adult mouse brain (Hazelton et al., 2009). Interestingly, high levels of cypD immunoreactivity were observed predominantly in a subpopulation of GABAergic interneurons. In addition to neurons, nonneuronal cells demonstrated positive immunoreactivity to cypD (see also Naga and Geddes, 2007). These were mainly subpopulations of astrocytes and NG2 cells (Hazelton et al., 2009). These data from our laboratory and others suggest that the sensitivity of mitochondria to MPT-inducing conditions can vary among cell types and brain regions. Therefore, it is important to recognize and examine the mechanisms of mitochondrial dysfunction as they relate to specific cell types. To date, there is very little information regarding identification of cell-type specific MPT-related mitochondrial pathology in the brain.

LOSS OF MITOCHONDRIAL NAD⁺ AS A CONSEQUENCE OF PATHOLOGIC MPT

In addition to swelling, MPT also leads to depletion of the matrix pyridine nucleotides (NAD⁺, NADP; Di Lisa et al., 2001; Kristian and Fiskum, 2004). Interestingly, the MPT pore can be activated in isolated mitochondria transiently by weaker or brief stimuli without irreversible damage to their membranes (Crompton et al., 1987; Petronilli et al., 1994; Shalbuyeva et al., 2006). Similar transient MPT activation and cristae remodeling were observed in neurons exposed to short glutamate treatments (Shalbuyeva et al., 2006). Mitochondria can release NAD⁺ via the large-conductance MPT pore without damage to inner and outer membranes. Thus, significant loss of matrix pyridine nucleotides can lead to inhibition of mitochondrial respiration but without irreversible damage to the respiratory complexes or mitochondrial membranes.

CATABOLISM OF CELLULAR AND MITOCHONDRIAL NAD⁺

NAD⁺ is an important cofactor involved in multiple metabolic reactions (Brennan et al., 2006). NAD⁺ and NADH have central roles in cellular metabolism and energy production as electron-accepting and electron-donating cofactors. Furthermore, there are several NAD⁺-dependent enzymes that use NAD⁺ as the substrate for their functions, including the histone deacetylase sirtuin 1 (SIRT1), poly(ADP-ribose) polymerase 1 (PARP1), and ADP-ribosyl cyclase (CD38; Belenky et al., 2007). Therefore, maintenance of normal cellular NAD⁺ levels is essential for tissue bioenergetic metabolism and several cell functions. A prominent role for NAD⁺ catabolism in cell death mechanisms is supported by the observation that, after excitotoxic insult or in *in vivo* models of brain ischemia, epilepsy, and Alzheimer's disease, a significant decrease in total cellular NAD⁺ levels occurs prior to neuronal death (Greene and Greenamyre, 1996; Endres et al., 1997; Mattson, 2004; Liu et al., 2008). It has been proposed

that uncontrolled PARP1 activation might deplete intracellular NAD⁺ and consequently ATP, leading to mitochondrial depolarization and cell death (Pieper et al., 1999; Chiarugi and Moskowitz, 2002; for review see Szabo and Dawson, 1998). However, because the major fraction of NAD⁺ is compartmentalized within the mitochondrial matrix, the significant reduction of cellular NAD⁺ content must be preceded by opening of the MPT pore and translocation of NAD⁺ from the mitochondrial matrix into the cytosol, where it can be degraded by activated NAD⁺ glycohydrolases (see Fig. 1). This notion is supported by data presented by Alano et al. (2004, 2010) showing that NAD⁺ depletion and MPT are sequential and necessary steps in PARP1-mediated cell death. However, there are reports showing localization of PARP in mitochondria that could contribute to mitochondrial NAD⁺ depletion in the absence of MPT (Lai et al., 2008). Recently, it was suggested that not only PARP1 activity contributes to NAD⁺ catabolism but also the activity of NAD⁺-dependent histone deacetylases, particularly SIRT1. It was proposed that these enzymes can compromise neuronal survival because of utilization of NAD⁺ as their substrate (Liu et al., 2009). Although it has been recognized that CD38 is a major NAD⁺ glycohydrolase and has a significant role in the regulation of cellular NAD⁺ levels (Iqbal et al., 2006; Aksoy et al., 2006; Young et al., 2006), no systematic studies have examined the contribution of this enzyme to NAD⁺ catabolism in neurodegenerative diseases.

CD38 IS A MAJOR NAD⁺ GLYCOHYDROLASE

CD38 is an ectoenzyme that uses NAD⁺ to generate cyclic ADP-ribose (cADPR) or ADP-ribose, and nicotinamide (Nam). CD38 can also use NADP to generate nicotinic acid dinucleotide phosphate (NAADP; for review see Malavasi et al., 2008). These products then act as potent second messengers that release calcium from intracellular stores (Galione, 1993). CD38 is highly expressed in the brain as well as in a variety of blood cells, including T cells, B cells, monocytes, and platelets. Although the enzyme is located mainly in the cellular plasma membrane, immunogold staining also revealed CD38 localization in the outer mitochondrial membrane, nuclear envelop, and rough endoplasmic reticulum (ER) membranes (Yamada et al., 1997). The intracellular localization of CD38 was confirmed by detecting NADase activity in mitochondrial, microsomal, and nuclear membrane fractions (Aksoy et al., 2006). Therefore, it is most likely that cADPR generated by plasma membrane CD38 may be released in the extracellular space and/or transferred in the intracellular space (Franco et al., 1996). Alternatively, cADPR synthesized inside the cells may function as a modulator to control the intracellular Ca²⁺ homeostasis via ryanodine receptors (Lee, 1997).

This enzyme was originally identified as a human lymphocytic surface antigen whose activity is required for

proper lymphocyte chemotaxis (Reinherz et al., 1980). Expression of CD38 in the brain can be found in specific populations of neurons, as well as astrocytes and microglia (Yamada et al., 1997; Mayo et al., 2008). Immunohistochemistry showed labeling of the plasma membrane and cell organelles such as ER, mitochondria, and nuclear envelope. Interestingly, immunolabeling was more intense in astrocytes compared with neurons (Yamada et al., 1997). Little is known about regulation of ADP-ribosyl cyclase or CD38 expression in neuronal cells. Astrocytes overexpress CD38 when cocultured with neurons as a result of glutamate released from activated neurons (Bruzzone et al., 2001). Formation of cADPR is enhanced by nitric oxide (NO) and cyclic GMP (cGMP; Galione, 1993). NO and cGMP are formed in response to neurotransmitters, so the cADPR level may be indirectly controlled by receptors through a cascade that culminates in activation of cytosolic ADP-ribosyl cyclase by cGMP-dependent protein phosphorylation (Clementi et al., 1996; Willmott et al., 1996). These findings are also supported by data showing protein kinase C (PKC)- and protein kinase A (PKA)-dependent activation of CD38 in microglia (Franco et al., 2006).

We detected NAD⁺ glycohydrolase activity in isolated synaptosomes and also in intact brain mitochondria (Balan et al., 2009), confirming localization of CD38 also in outer mitochondrial membranes (see also Boyer et al., 1993; Ziegler, 2000; Di Lisa et al., 2001). Interestingly, the NAD⁺ glycohydrolase activity appeared to be much higher in nonsynaptic mitochondria compared with mitochondria isolated from synaptosomes (Masmoudi et al., 1988). Insofar as nonsynaptic mitochondria represent mitochondria from both neurons and non-neuronal brain cells (e.g., astrocytes, oligodendroglia and microglia), this finding is in agreement with the report that astrocytes and microglia are the main CD38-expressing cells in brain (Salmina et al., 2008). Taken together, these data suggest that NAD⁺ depletion can occur more rapidly in astrocytes following ischemic insult, compromising the ability of astrocytes to support neuronal functions. Interestingly, the NAD⁺ catabolic activity is higher in brain regions that are vulnerable to ischemic insult (Fig. 2). Furthermore, the CD38 NAD⁺ glycohydrolase activity is significantly increased in post-ischemic tissue, and the immunohistochemistry shows overexpression of this enzyme preferentially in neuroglial cells (Fig. 2). Thus, the data suggest that the increased CD38 NADase activity is at least partially the result of higher expression of this enzyme in postischemic tissue.

Only a very few studies have examined the mechanisms of CD38 activation. In murine mesangial cells or cardiomyocytes, the activation of CD38 by angiotensin (ANG) II involves ANG II type 1 receptor, phosphoinositide 3-kinase, protein tyrosine kinase, and phospholipase C- γ 1 (Kim et al., 2008). In astrocytes the CD38 activation by β -adrenergic stimulation is transduced via G proteins (Hotta et al., 2000). Finally, in microglia, lipopolysaccharide induces phosphorylation of CD38, mediated by multiple protein kinases (PKC and PKA),

resulting in significant enhancement of CD38 ADPR cyclase activity (Franco et al., 2006).

NAD⁺ METABOLISM: GLIA AND NEURON SPECIFICITY

The distribution of NAD⁺ in cells and the locations of NAD⁺ synthesis have recently received new consideration. NAD⁺ can be generated in cells by de novo synthesis from tryptophan, or it can be resynthesized from nicotinamide (Nam) via a salvage pathway (Fig. 3). Recently, a third vitamin precursor of NAD⁺ was discovered: nicotinamide riboside (NR) that is taken up by cells and phosphorylated to nicotinamide monophosphate (NMN) by NR kinases (Nr1 and Nr2; Bieganski and Brenner, 2004; Belenky et al., 2009). NMN is then adenylated to form NAD⁺ by nicotinamide nucleotide adenylyltransferase (Nmnat; Fig. 3; for review see Belenky et al., 2007). Nmnat has three isoforms; Nmnat-1 is localized to nuclei (Berger et al., 2005), Nmnat-2 is in Golgi, and Nmnat-3 is present in mitochondria (Berger et al., 2005). Thus, it can be inferred that Nmnat activity is required to complete all salvage and de novo pathways of NAD⁺ biosynthesis and that mammalian cell NAD⁺ is compartmentalized. Cellular fractionation studies have shown that mitochondria maintain relatively high NAD⁺ concentrations and that mitochondrial NAD⁺ does not readily leak across the inner membrane (Di Lisa and Ziegler, 2001). In contrast, the majority of cytosolic NAD⁺ is made within the nucleus of cells and then redistributed to the cytosol by passive diffusion through nuclear pores (Berger et al., 2005). The efficiency of de novo and salvage pathways is greater in glia compared with neurons (Ruddick et al., 2006). Downstream intermediates of NAD⁺ biosynthesis and NAD⁺ itself could provide a potent delay in neuronal Wallerian degeneration assays (Araki et al., 2004; Sasaki et al., 2006). Wallerian degeneration refers to the ordered process of axonal degeneration and occurs when the axon is severed from the cell body (Glass et al., 1993). Addition of nicotinic acid (Na) or nicotinamide (Nam) to these neuronal explants failed to delay Wallerian degeneration unless the salvage pathway enzymes Nampt or Nmnat were overexpressed. Furthermore, Nampt1, which initiates NAD⁺ biosynthesis by using Nam, is transcriptionally induced after sciatic nerve transection in mice, in which glia are present. Additionally, downstream enzymes for NAD⁺ biosynthesis (Nampt and Nmnat) are located almost exclusively in glial cells (Kohler et al., 1988; but see Zhang et al., 2010). These studies clearly revealed the rate-limiting nature of the Nam-Nampt pathway in controlling NAD⁺ biosynthesis specifically in neurons (Sasaki et al., 2006). Because the NAD⁺ salvage pathway enzymes are expressed mainly in glia, they may play important roles in both synthesis of NAD⁺ from Na or Nam and delivery of NAD⁺ to neurons in vivo. However, neurons preferentially use NR as a precursor to maintain intracellular NAD⁺ levels (Sasaki et al., 2006).

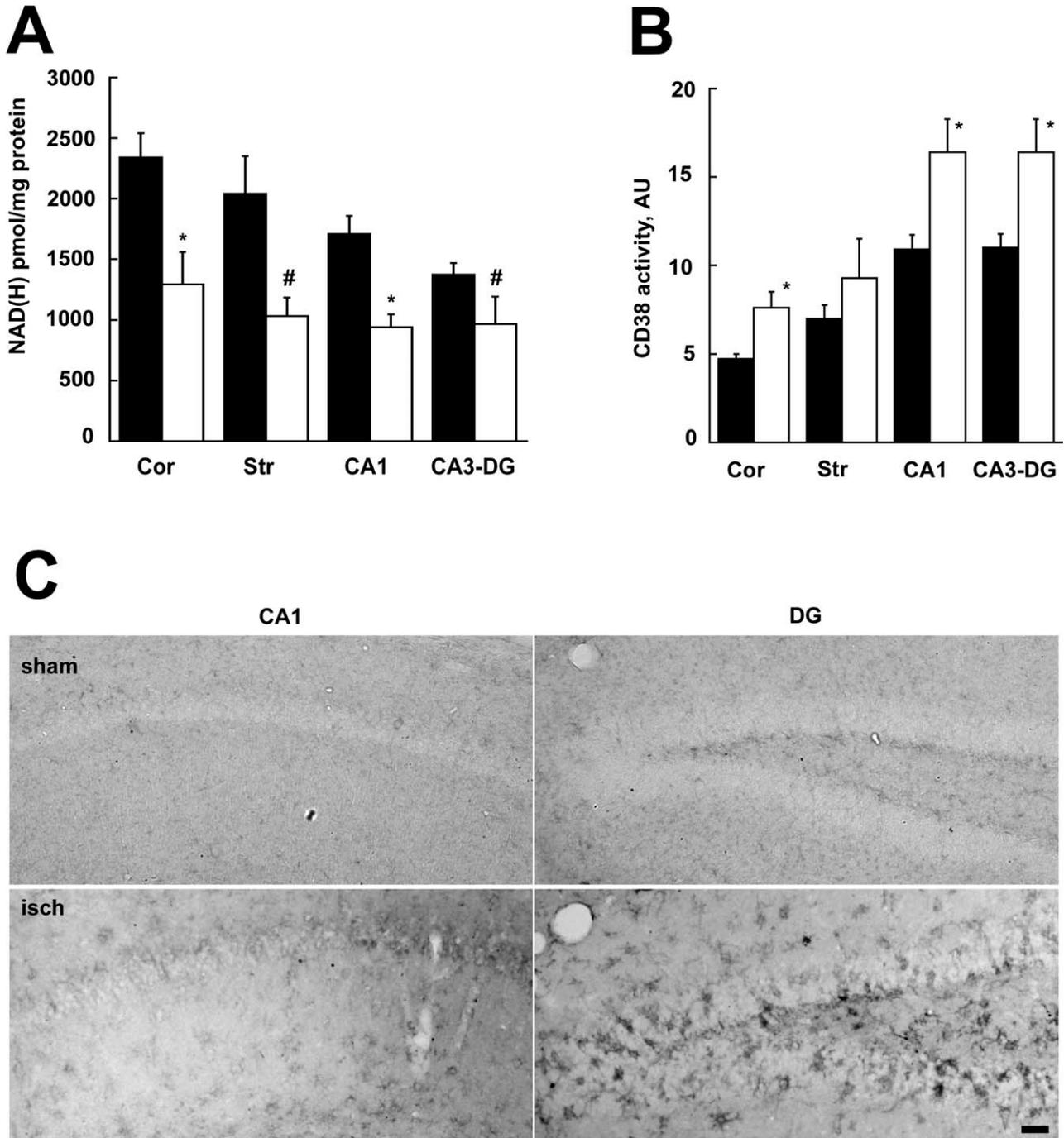


Fig. 2. Ischemia-induced changes in tissue NAD(H) levels and CD38 NADase activity and immunoreactivity. Brains from sham-operated animals or animals subjected to 10 min of global cerebral ischemia and 24 hr of reperfusion were used to determine the tissue NAD(H) levels in different brain regions. The parietal cortex; striatum; and CA1, CA3, and DG subregions of the hippocampus were dissected. After perchloric acid extraction, the NAD(H) content was deter-

mined (A). Similarly, the tissues of corresponding brain regions from sham or postischemic animals were homogenized, and after a low-speed spin the CD38 activity in the supernatant was determined (B). C shows CD38 immunoreactivity in control and postischemic tissue (left: CA1 subregion; right: dentate gyrus of the hippocampus). #*P* < 0.05, **P* < 0.01 compared with the corresponding sham group (*n* = 6). Scale bar = 100 μm.

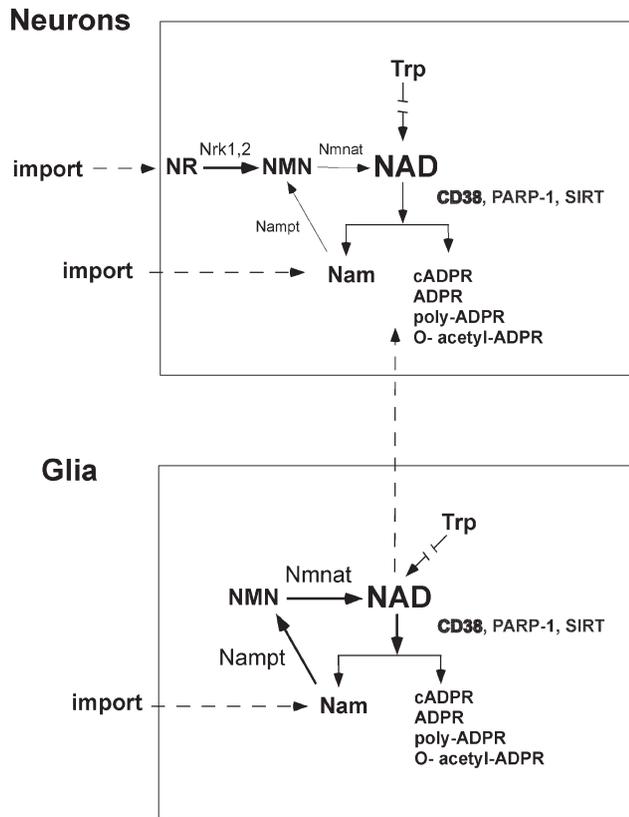


Fig. 3. Schematic diagram illustrating NAD⁺ biosynthesis and catabolism in neurons and glia. Two metabolic pathways can generate NAD⁺. The de novo pathway synthesizes NAD⁺ in eight steps from tryptophan (Trp). The salvage pathway utilizes nicotinamide (Nam), a product of NAD⁺ glycohydrolases (CD38, PARP-1, sirtuins). Nam is converted to nicotinamide monophosphate (NMN) by nicotinamide phosphoribosyltransferase (Nampt). NMN is then converted to NAD⁺ by nicotinamide nucleotide adenyltransferase (Nmnat). The salvage pathway can also utilize nicotinamide riboside (NR) that is converted to NMN by NR kinases (Nrk1, -2). Because Nmnat is preferentially localized in glia, Nam administration supports NAD⁺ generation mainly in nonneuronal cells, and the neurons depend on NAD⁺ supplied by glia. NR can directly support NAD⁺ biosynthesis in neurons.

OVERACTIVATION OF CD38 CAN PLAY A SIGNIFICANT ROLE IN ACUTE AND CHRONIC NEURODEGENERATIVE PATHOLOGY

Activation of CD38 can lead to rapid and almost complete tissue NAD⁺ depletion (Balan et al., 2010). The importance of this enzyme in controlling the cellular NAD⁺ pools was confirmed in CD38 null mice that showed 10–20-fold higher tissue NAD⁺ levels compared with wild-type animals (Aksoy et al., 2006). As mentioned above, CD38 plays an important role in the immune system response because of its high level of expression in dendritic cells and regulation of their activation. Both astrocytes and microglia show abundant CD38 expression, so, in addition to NAD⁺ glycohydro-

lyase activity, this enzyme plays an important role in astrocyte and microglial activation (Franco et al., 2006; Mayo et al., 2008; Kou et al., 2009). Microglia, the resident immune cells of the CNS, enter the CNS during the early postnatal period (Kershman, 1939). After entering the CNS, these cells disseminate through the parenchyma and transform into resting microglia.

After acute brain injury, microglia become activated (for review see Heneka et al., 2010). CD38 participates in microglial activation that gradually transforms these cells into motile, secretory, and potentially cytotoxic phagocytes (Hoffmann et al., 2003). Thus, overactivation of CD38 not only can lead to potentially catastrophic depletion of cellular NAD⁺ pools but also promotes pathologic activation of neuroglia that can culminate in a chronic inflammatory response and aggravation of brain tissue damage.

NAD⁺ PRECURSORS AS THERAPEUTIC COMPOUNDS FOR NEURODEGENERATION

Several strategies can be utilized to inhibit the reduction in tissue NAD⁺ levels during pathologic conditions. Insofar as the majority of cellular NAD⁺ is localized in mitochondria and opening of the MPT pore leads to release of NAD⁺ from the matrix to the cytosol, MPT inhibition can prevent mitochondrial and consequently cellular NAD⁺ depletion. However, even transient opening of the MPT pore leading to NAD⁺ leak from mitochondria can have adverse effects on mitochondrial functions, particularly respiration. It has been shown that ischemia diminishes brain mitochondrial respiration (Almeida et al., 1995; Kuroda et al., 1996; Canevari et al., 1997; Anderson et al., 1999). Interestingly, inhibition of mitochondrial respiration following transient ischemia was observed at the end of the ischemic period, which was followed by partial recovery and development of secondary failure (Kuroda et al., 1996; Kristian et al., 1998). This reduction in mitochondrial respiratory capacity may be the result of transient matrix NAD⁺ depletion. Experiments in our laboratory support the concept that ischemia/reperfusion can result in extensive catabolism of tissue NAD⁺ (Balan et al., 2010) that may be at least partially responsible for mitochondrial respiratory inhibition. We assessed the NAD⁺ levels in brain subregions following transient forebrain ischemia at 24 hr of recovery before an imminent cell death occurred. In all vulnerable areas, the tissue NAD(H) levels were significantly reduced (Fig. 2). However, mitochondrial respiratory dysfunction can also be due to pyruvate dehydrogenase complex activity inhibition (Martin et al., 2005; Vereczki et al., 2006; Richards et al., 2006).

Another approach to maintaining cellular NAD⁺ levels following an ischemic insult is administration of NAD⁺ precursors to facilitate NAD⁺ generation by the salvage pathway or to reduce ROS-induced DNA damage that results in extensive PARP1 activity and NAD⁺ depletion. It was shown that administration of nicotina-

amide (Nam) increases tissue NAD^+ levels (Klaidman et al., 1996; Yang et al., 2002; Sadanaga-Akiyoshi et al., 2003; for review see Bogan and Brenner, 2008). Nam rapidly penetrates the blood–brain barrier (Spector and Kelley, 1979) and was demonstrated to improve energetics following ischemia or oxidative stress (Ayoub et al., 1999; Mokudai et al., 2000; Ayoub and Maynard, 2002; Sakakibara et al., 2002; Yang et al., 2002). The mechanisms of Nam's protective effect are still elusive; however, it was reported to exert a number of pharmacological effects, including prevention of ATP depletion (Klaidman et al., 1996, 2003; Yang et al., 2002), inhibition of PARP-1 (Klaidman et al., 1996; Szabo and Dawson, 1998; Yang et al., 2002), lipid peroxidation (Mukherjee et al., 1997; Klaidman et al., 2001; Chong et al., 2002), antiinflammatory activity (Ungerstedt et al., 2003), and prevention of apoptosis (Klaidman et al., 1996; Mukherjee et al., 1997). Thus, Nam crosses the blood–brain barrier and is converted to NAD^+ in the brain. At present, however, it is not known whether Nam is an NAD^+ precursor in neuronal or nonneuronal cells (Spector and Johanson, 2007).

Nicotinamide mononucleotide (NMN) and the recently discovered nicotinamide riboside (NR) are alternative precursors for NAD^+ biosynthesis that are utilized by the NAD^+ salvage pathway. Interestingly, although NMN-utilizing enzymes are intracellular, the NMN-generating enzyme (Nampt) is also found in sera and is being considered as a diagnostic biomarker for inflammation (Luk et al., 2008). NMN application leads to increases in cellular NAD^+ levels by a one-step enzymatic reaction in which NMN is converted to NAD^+ by Nmnat (Belenky et al., 2007). Furthermore, NMN has been shown to delay Wallerian degeneration (Sasaki et al., 2006). Measurement of NMN content in subcellular fractions revealed that the nucleotide is highly enriched in mitochondria, suggesting intramitochondrial NAD^+ synthesis (Formentini et al., 2009). NMN may also inhibit CD38 NAD^+ glycohydrolase activity (Snell et al., 1984; Balan et al., 2010), reducing NAD^+ and ATP depletion in cells undergoing PARP-1 hyperactivation and significantly delaying cell death (Formentini et al., 2009). However, it is unclear how NMN enters cells and whether it is transported as a nucleoside (NR; Belenky et al., 2007). Regardless, all the indications are that NMN has great therapeutic potential (Araki et al., 2004; Sasaki et al., 2006). Our data show a remarkable protective effect of NMN against CD38-dependent NAD^+ degradation (Balan et al., 2010). Given the high concentrations of the enzyme Nampt and NMN in plasma, the Nam protective mechanisms could be via NMN, in that administered Nam or at least part of the Nam can be converted to NMN in the blood.

CONCLUSIONS

It is widely accepted that mitochondria play a central role in many neurological diseases. However, the mechanisms and cell-type-specific contribution of MPT-

dependent mitochondrial damage to brain damage is unknown. Apart from the possibility that glial and neuronal mitochondria have different sensitivity to calcium-induced damage, there are cell-type-specific differences in NAD^+ levels, NAD^+ biosynthesis, and NAD^+ catabolism under pathologic conditions. In addition to PARP1 activation, cellular NAD^+ can be profoundly depleted by activation of the CD38 enzyme. Because CD38 is also engaged in regulation of the immune response in the brain and can affect the inflammation triggered by pathologic conditions, cell-type-specific targeting of CD38 inhibition can offer new therapeutic targets for the treatment of acute or chronic neurodegenerative diseases.

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