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NAVIGATING NOVEL MECHANISMS OF CELLULAR PLASTICITY WITH THE NAD^+ PRECURSOR AND NUTRIENT NICOTINAMIDE

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1. ABSTRACT

Interest in neuroprotectants for the central nervous system continues to garner significant attention. Nicotinamide, the amide form of niacin (vitamin B3), is the precursor for the coenzyme ß-nicotinamide adenine dinucleotide (NAD^+) and is considered to be necessary for cellular function and metabolism. However, recent work has focused on the development of nicotinamide as a novel agent that is critical for modulating cellular plasticity, longevity, and inflammatory microglial function. The ability of nicotinamide to preserve both neuronal and vascular cell populations in the brain during injury is intriguing, but further knowledge of the specific cellular mechanisms that determine protection by this agent is required. The capacity of nicotinamide to govern not only intrinsic cellular integrity, but also extrinsic cellular inflammation rests with the modulation of a host of cellular targets that involve protein kinase B, glycogen synthase kinase-3β (GSK-3β), Forkhead transcription factors, mitochondrial dysfunction, poly(ADP-ribose) polymerase, cysteine proteases, and microglial activation. Intimately tied to the cytoprotection of nicotinamide is the modulation of an early and late phase of apoptotic injury that is triggered by the loss of membrane asymmetry. Identifying robust cytoprotective agents as nicotinamide in conjunction with the elucidation of the cellular mechanisms responsible for cell survival will continue to solidify the development of therapeutic strategies against neurodegenerative diseases.

2. INTRODUCTION

2.1. Nicotinamide: a nutrient that is not just for breakfast anymore

Nicotinamide, the amide form of niacin (vitamin B3), is the precursor for the coenzyme ß-nicotinamide adenine dinucleotide (NAD^+) and is considered to be necessary for cellular function and metabolism. Nicotinamide is provided in grain, in live stock food products, and in commercially available nutrient supplements. In combination with other livestock dietary supplements, nicotinamide can increase protein production and has been demonstrated to enhance milk production while limiting fat percentage in midlactation cows (1). As a cellular nutrient, nicotinamide can improve growth potential and cell viability in a variety of cell populations. For example, nicotinamide can promote the maturation of fetal cells (2), the proliferation and differentiation of embryonic stem cells to yield insulin-producing cells (3) and enhance an adaptive response to physical and chemical agents in mouse bone marrow cells that consists of an error-free DNA repair mechanism (4).

In regards to cellular energy metabolism, nicotinamide is utilized by the body for cellular metabolism through the generation of adenosine triphosphate in the mitochondrial electron transport chain (5). The coenzyme

NAD is a ubiquitous biological molecule that participates in several metabolic reactions involving energy metabolism. Nicotinamide, as an NAD^+ precursor, can be directly utilized by cells to synthesize NAD^+ (6). Nicotinamide participates in energy metabolism through the tricarboxylic acid cycle by utilizing NAD^+ in the mitochondrial respiratory electron transport chain for the production of ATP, DNA synthesis, and DNA repair (7-9). As a result, nicotinamide can maintain cellular homeostasis and energy requirements through its ability to yield NAD^+.

3. THE INTIMATE LINK BETWEEN NAD^+ AND ITS PRECURSOR NICOTINAMIDE

Nicotinamide and nicotinic acid are genetically described as niacin (Vitamin B_3). Nicotinamide is essential for the synthesis of the coenzymes NAD^+ and nicotinamide adenine dinucleotide phosphate ($NADP^+$). Both nicotinamide and nicotinic acid can be acquired either through synthesis in the body or through a dietary source (10). The predominant form of niacin in dietary plant sources is nicotinic acid that is rapidly absorbed through the gastrointestinal epithelium. Nicotinamide is obtained through the conversion of nicotinic acid in the liver or through the hydrolysis of NAD^+. Once nicotinamide is obtained in the body, it is utilized to synthesize NAD^+ (6).

As a coenzyme, NAD^+ plays host to a number of significant physiological functions. In addition to its role in energy metabolism and the mitochondrial respiratory electron transport chain that is involved in the production of ATP, NAD^+ has a critical role in the repair of DNA. Poly (ADP-ribose) polymerase (PARP) is a DNA binding protein and is associated with DNA repair and cell survival (11). DNA degradation results in the formation of DNA strand breaks that lead to the activation of PARP. PARP catalyses the synthesis of poly (ADP-ribose) from its substrate NAD^+, which stimulates the process of DNA repair (12). Furthermore, NAD^+ also can regulate gene transcription. Clock:BMAL1 and NPAS2:BMAL1 are heterodimeric transcription factors that control gene expression as a function of the light-dark cycle. The DNA-binding activity of the Clock:BMAL1 and NPAS2:BMAL1 heterodimers is closely regulated by the redox state of NAD^+ (13). In regards to life span extension, the NAD^+-dependent histone deacetylase known as silent information regulator 2 (Sir2) can facilitate life span duration provided increased levels of NAD^+ are made available to Sir2 (14).

On the flip side, loss of NAD^+ has been associated with cell injury. Increased activation of PARP leads to an extensive turnover of NAD^+ and a significant reduction in NAD^+ levels. Exposure to bleomycin, a DNA-cleaving chemotherapy agent can activate nuclear PARP resulting in a sustained NAD^+ depletion and subsequent tissue injury (15). In neuronal cell populations, zinc toxicity has been associated with the loss of NAD^+ and ATP (16). In contrast, prevention of NAD^+ depletion during enhanced PARP activity has been demonstrated to prevent cellular lysis during oxidative stress (17).

Mitochondrial stores of NAD^+ also have been associated with cellular injury. Oxidative stress can trigger the opening of mitochondrial membrane permeability transition pore (18-21) and subsequently result in the release of NAD^+ from mitochondria (18). During cardiac ischemia and reperfusion injury, opening of the mitochondrial permeability transition pore leads to a significant loss of mitochondrial NAD^+ stores and subsequent cell injury. Yet, maintenance of NAD^+ stores during this ischemic injury can prevent cell death (18). During conditions of oxidative stress and energy depletion in neurons, poly(ADP-ribosylation) activation and loss of NAD^+ stores in mitochondria have been shown to lead to apoptotic injury. Restoration of NAD^+ content in mitochondria with liposomal NAD^+ prevents neuronal injury (22).

Given the detrimental cellular ramifications of NAD^+ depletion, both acute and chronic neurodegenerative diseases have been linked to the loss of NAD^+ stores. Parkinson's disease is chronic progressive neurodegenerative disease that is characterized by the loss of dopaminergic neurons in the substantial nigra. In animal models of Parkinson's disease that employ 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine as a model of central nigrostriatal dopamine neurotoxicity, subsequent depletion of NAD^+ and adenine triphosphate have been associated with neuronal loss (23). In patients with Alzheimer's disease, both PARP and poly(ADP-ribose) can be detected in the frontal and temporal cortex more frequently than in controls, suggesting that increased levels of functional PARP enzyme are present to result in a significant consumption of NAD^+ stores (24). Interestingly, a limited pilot study suggested that administration of nicotinamide adenine dinucleotide (NADH) in patients with Alzheimer type dementia may show improvement in their cognitive function (25). Furthermore, evidence exists that overactivation of PARP, with resulting consumption of NAD^+, plays a significant role during acute cerebral ischemia (26).

4. ENHANCING CELL SURVIVAL AND POTENTIAL LONGEVITY THROUGH NICOTINAMIDE

More recently, the focus on nicotinamide has shifted from a nutrient vital for cellular function to a cytoprotectant that is crucial for neuronal and vascular cell survival as well as inflammatory modulation. The illustration that nicotinamide can prevent cellular injury is not unique and has previously been reported for a variety of experimental models (Table 1). In pancreatic islet cells, nicotinamide prevents cellular injury during free radical exposure (27). In *in vitro* studies, nicotinamide blocks hydrogen peroxide induced necrosis in human β-cells (28). Administration of nicotinamide in non-obese diabetic mice also prevents apoptosis in β-cells resulting during cyclophosphamide injections and delays the development of diabetes (28). In human bronchial epithelial cells, nicotinamide protects against sulfur and nitrogen mustard induced cytotoxicity (29). Clinical studies also support a

Table1. Cytoprotective studies of nicotinamide

Nicotinamide and Cell Injury	Outcome	Reference
Neurons		
1 mM, 15 min prior to a 24 or 48-hour period of incubation with 0.1~1 mM t-BuOOH in a human cortical neuronal cell line	Cell survival ↑ Protein p53↓	35
12.5 mM, 1 h prior to or 2~ 6 h following NO or OGD exposure in hippocampal neurons	NAD^+ ↑ PARP activity ↓ DNA fragmentation ↓ PS exposure ↓	32, 33, 34
1 mM incubate with 40 μM zinc for 4 hours in mouse cortical neurons	NAD^+ ↑ Cell death↓	16
100~1000 mg/kg, sc, immediately or 1000 mg/kg 2~6 h after ip 60 mg/kg MNU in rats and mice	Photoreceptor cell loss ↓	36, 37
125 ~1000 mg/kg, ip, within 6 h after MCAO in Wistar rats	Cerebral infarct volume↓ Neurological deficit scores ↓	44
500~750 mg/kg, iv, at 2 h after MCAO in SHR or Fischer 344 rats	Cerebral infarct volume↓	45
500 mg/kg, ip or iv at 2 h after MCAO in Wistar rats	Cerebral infarct volume↓ Neurological deficit scores ↓ Sensory and motor behavior↑	43
500 mg/kg, ip, at 1.5 h after MCAO in Wistar rats	ATP and NAD^+ content↑ PARP activity↓	214
500 mg/kg, ip, 30 min after spinal cord injury	Grey matter damage ↓	46
Endothelial cells (ECs) 12.5 mM, 1 h prior to or 2~ 6 h following NO exposure in cerebral microvascular ECs	Cell survival↑ PS exposure↓ DNA Fragmentation ↓ PARP cleavage↓	41
12.5 mM, 1 h prior to a 12-hour period of anoxia in cerebral microvascular ECs	Cell survival↑ PS exposure↓ DNA Fragmentation↓	32
Cardiomyocytes 3 mM 10 min prior to oxidant stress in rat ventricular myoblasts	Mitochondrial respiration ↑ Cell necrosis ↓	58
Microglia		
12.5 mM, 1 h prior to OGD deprivation	PS exposure ↓ Activation of Akt↑	34
β-cells 500 mg/kg, ip, 15 min before 150 mg/kg cyclophosphamide (ip) daily for two weeks	Apoptotic cells ↓	28

EC: endothelial cell; ip: intraperitoneal injection; iv: intravenous injection; MCAO: middle cerebral artery occlusion; MNU: N-methyl-N-nitrosourea; NAD: nicotinamide adenine dinucleotide; OGD: oxygen-glucose deprivation; PARP: poly(ADP-ribose) polymerase; PS: phosphatidylserine; sc: subcutaneous injection; t-BuOOH: t-butyl hydroperoxide; PCNA: proliferating cell nuclear antigen; ↑ : increase; ↓ : decrease; h = hour; min = minutes.

role for nicotinamide in the treatment of a variety of disorders, such as the resolution of lactic acidemia in the MELAS syndrome (30) with NADH:ubiquinone oxidoreductase (31).

4.1. Nicotinamide and neurons

In neurons, nicotinamide offers protection against nitric oxide (NO) (20), anoxia (32), and oxygen glucose deprivation (33, 34) in primary cultures rat hippocampal neurons (Figure 1). In cortical neurons, nicotinamide antagonizes cell injury during free radical generating toxins such as tertiary butylhydroperoxide (35). Nicotinamide also can protect both rod and cone photoreceptor cells against N-methyl-N-nitrosourea toxicity (36, 37) as well as against glycation end products in all layers of the retina (38). In animal studies, nicotinamide prevents neuronal degeneration against trauma (39), oxidative stress (20, 32, 34, 40, 41), transient cerebral ischemia (42-44), permanent focal cerebral ischemia (43-45), and spinal cord injury (46, 47). Nicotinamide can directly protect against both neuronal necrosis and apoptosis mechanisms of injury and prevent brain damage through reducing DNA fragmentation during ischemic reperfusion injury (20, 32, 34, 41, 48, 49).

4.2. Nicotinamide and vascular cells

In addition to neuronal populations, nicotinamide also fosters vascular cell survival. Cerebral microvascular endothelial cells (ECs) that line microvessels are important in maintaining homeostasis in the central nervous system. Cerebral microvascular ECs not only control vascular reactivity and autoregulation of cerebral blood flow, but also limit access of potentially harmful blood-borne substances to the brain by forming functional tight junctions in the

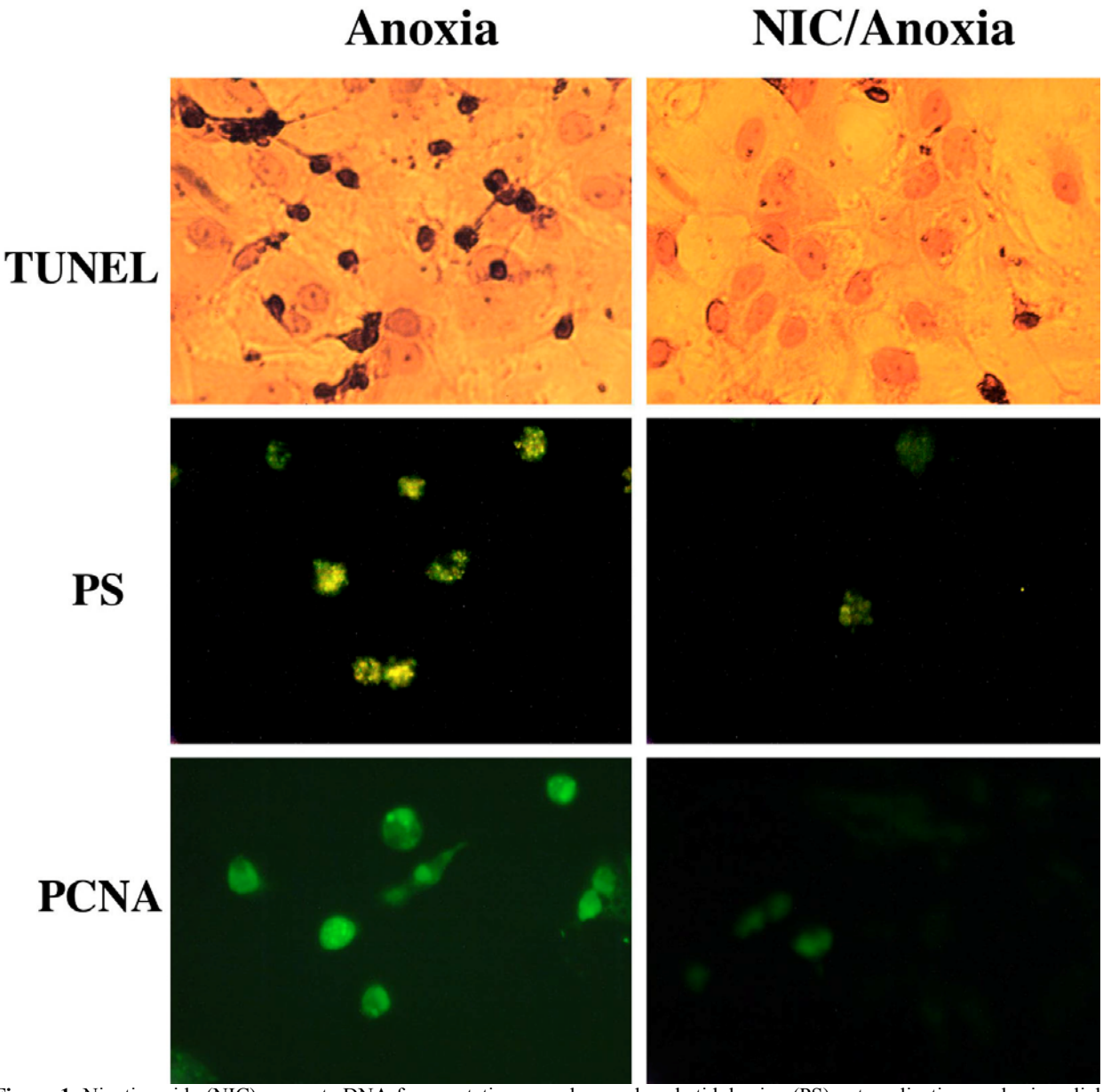

Figure 1. Nicotinamide (NIC) prevents DNA fragmentation, membrane phosphatidylserine (PS) externalization, and microglial activation during anoxia. Following a 4 hour period of anoxia in neurons, DNA fragmentation and PS exposure were determined 24 hour later using the TUNEL assay and annexin V phycoerythrin labeling respectively. Representative microscopy fields of neurons were imaged with transmitted light for TUNEL and fluorescence images were obtained using 490 nm excitation and 585 nm emission wavelengths for PS. Pretreatment with NIC (12.5 mM) 1 hour prior to anoxia decreased DNA fragmentation and membrane PS externalization significantly during anoxia. For microglial activation, media from neurons 24 hours following a 4 hour period of anoxia was applied to pure cultures of microglia for 3 hour. Twelve hours later, microglial activation was assessed through the expression of proliferating cell nuclear antigen (PCNA). Representative images illustrate that PCNA expression was significantly increased in microglia treated with media from anoxia exposed neurons, but was significantly less in microglia exposed to media from NIC treated neurons.

blood-brain barrier (50). Injury in ECs can lead to the active destruction of the endothelium and precipitate both acute and chronic vascular degeneration that destroys cortical function (51) and precipitate neurodegenerative disorders, such as Alzheimer's disease and cerebral ischemia (52, 53). During different models of oxidative stress in ECs, nicotinamide can prevent both early manifestations of apoptotic injury that involve membrane phosphatidylserine (PS) residue exposure as well as late apoptotic injury during nuclear DNA degradation (19, 54).

In the peripheral vascular system, nicotinamide prevents oxidative stress mediated vascular failure during endotoxic shock (55). Nicotinamide also impacts vascular

physiology and can influence arteriolar dilatation and blood flow, although the effects on vascular flow may be tissue specific and pertain primarily to neoplastic disorders (56). Nicotinamide can promote vascular survival during endotoxic shock (55) and also maintain EC membrane integrity during oxygen radical exposure (57). Nicotinamide also is believed to be responsible for the preservation of endocardial EC function during models of oxidative stress and chronic increases in left ventricular cardiac load (58, 59). As a result, studies with nicotinamide in both neuronal and EC populations suggest that nicotinamide functions as a "broad spectrum" cytoprotectant that may prevent injury in the nervous system at both a neuronal and a vascular level.

4.3. Nicotinamide extends beyond neuronal and vascular cells during inflammatory injury

Nicotinamide's ability to modulate cellular function appears to be broad in nature. The agent not only modulates intrinsic cellular function in neuronal and vascular cells, but also facilitates extrinsic cell homeostasis through microglial activation and the control of cytokine release. Microglia are monocyte-derived immunocompetent cells that enter the central nervous system during embryonic development and function similar to peripheral macrophages. During microglia activation, the phagocytic removal of apoptotic cells within the central nervous system play an important role during development, tissue homeostasis, and host defense. The removal of injured cells and foreign microorganisms can be considered to be beneficial for the preservation of cellular physiological homeostasis.

There exist several potential mechanisms that may regulate the phagocytosis of cells that have entered the apoptotic pathway. Some studies identify the generation of annexin I and membrane PS exposure that appears to be necessary to connect an apoptotic cell with a phagocyte (60). Secreted factors by either apoptotic or phagocytic cells, such as milk fat globule-EGF-factor 8 (61), fractalkine (62), and lipid lysophosphosphatidylcholine (63) also have been shown to assist with the phagocytic removal of injured cells.

A common denominator that appears to be critical for the removal of apoptotic cells is the translocation of membrane PS residues from the inner cellular membrane to the outer surface (21, 64, 65). During normal cellular function, the phospholipids of the plasma membrane are asymmetrical with the outer leaflet of the plasma membrane consisting primarily of choline-containing lipids, such as phosphatidylcholine and sphingomyelin, and the inner leaflets consisting of aminophospholipids that include phosphatidylethanolamine and PS. The disruption of membrane phospholipid asymmetry leads to the externalization of membrane PS residues and serves to identify cells for phagocytosis (21, 49, 66, 67). In some cases, the externalization of membrane PS residues is dependent upon reduced aminophospholipid translocase activity (68) and activation of a phospholipid scramblase that may be calcium independent (69). During apoptotic injury, ATPase-dependent activity is significantly reduced in a cell. This severely limits the ability of the 120-Da magnesium-dependent ATPase that is responsible for the maintenance of PS on the inner leaflet of the cell membrane to function. As a result, the inhibition of the ATP-dependent aminophospholipid translocase during cellular injury can play a significant role in PS externalization (70).

Expression of the phosphatidylserine receptor (PSR) on microglia works in concert with cellular membrane PS externalization. Neurons exposed to free radical injury can lead to the induction of both microglial activation and microglial PSR expression. Treatment with an anti-PS receptor neutralizing antibody in microglia prevents this microglial activation (71, 72). In addition, application of PS could directly result in microglial activation that was blocked by PSR neutralizing antibody (21, 71), suggesting that both PS exposure in target cells and PSR expression in microglia are necessary for microglial recognition of apoptotic cells in the nervous system. Recognition of cellular membrane PS by the PS-specific receptors on microglia may require cofactors, such as Gas6. The protein Gas6 binds to negatively charged phospholipids, such as membrane PS, through calcium dependent lipid binding domains and may be necessary for membrane PS to dock with PSRs (73). In addition, microglia recognition of injured neurons through membrane PS mediated mechanisms also may involve other agents, such as integrin and lectin (74).

Although vital for both cellular homeostasis as well as host defense mechanisms, microglia can sometimes aggravate a cellular insult. Studies with microglia stimulated by phorbol myristate acetate have demonstrated the release of superoxide radicals. Application of scavenger agents for reactive oxygen species, such as superoxide dismutase or deferoxamine mesylate, in the presence of activated microglia can prevent cellular injury. These studies suggest that oxidative stress generated by microglia can be responsible for cellular injury (75). Activated microglia up-regulate a variety of surface receptors and yield significant pro-inflammatory and neurotoxic factors, such as tumor necrosis factor (TNF) and interleukin-1ß, free radicals such as NO and superoxide (76), and fatty acid metabolites such as eicosanoids that can precipitate cell death (77).

The analysis of brain tissue in patients who have succumbed to neurodegenerative disorders has supported the premise that microglia also may lead to the progression of some neurological disorders. In Huntington's disease and amyotrophic lateral sclerosis, significant microglial activation has been reported in regions of the nervous system that are specific for these disease entities (78, 79). During cerebral ischemia, activation of microglia can parallel the induction of cellular apoptosis and correlate well with the severity of the ischemic insult (80). In patients with Alzheimer's disease, microglial cells co-localize with the perivascular deposits of Aβ. In addition, microglial activation has been observed to occur in concert with the evolution of amyloid plaques (81). The generation of oxidative stress by microglia during Aβ deposition

suggests that microglia may play an important role in the pathogenesis of Alzheimer's disease. The secretion of cytokines by microglia also may represent another source of cytotoxicity for microglia. Microglia produce a variety of cytokines in response to toxic stimulation, such as interleukins and TNF. TNF-α production by microglia may be linked to neurodegeneration by increasing the sensitivity of neurons to free radical exposure (82).

Nicotinamide may prevent inflammatory cell demise through extrinsic cellular mechanisms that involve both membrane PS exposure as well as cytokine release. Nicotinamide can prevent cellular membrane PS externalization in both neurons and ECs during a variety of insults that involve anoxia, free radical exposure, and oxygen-glucose deprivation (20, 41, 48, 49). Nicotinamide may regulate membrane PS exposure and microglial activation through activation of protein kinase B, also known as Akt (34) (Figure 1). The protein Akt can modulate the spatial regulation of actin assembly, suggesting a relationship between Akt and the coordination of cytoskeletal organization (83). In addition, Akt appears to be a necessary component for the modulation of membrane PS externalization and prevent microglial activation (21). Microglial activation and proliferation can occur during oxidative stress (21, 71, 72, 84). Activation of Akt can prevent membrane PS exposure on injured cells and block the activation of microglia that are exposed to media taken from cells that overexpress active, phosphorylated Akt during cellular injury (21, 72). As a corollary to this work, use of an antibody to the PSR demonstrates that membrane PS residue exposure is both necessary and sufficient to induce microglial activation and proliferation (21, 71). The work supports the premise that nicotinamide through mechanisms that involve Akt can regulate microglial activation and proliferation through the modulation of membrane PS exposure on cells and conceivably prevent the shedding of membrane PS residues that is known to occur during apoptosis (85). In addition to targeting the activity of membrane PS exposure and microglial activation, nicotinamide may also directly address cellular inflammation by inhibiting several pro-inflammatory cytokines, such as interleukin-1ß, interleukin-6, interleukin-8, tissue factor, and TNF-α (86-89). Nicotinamide also has been shown to depress interferon-gamma-induced class II major histocompatibility complex expression on ECs (90).

4.4. The physiological and toxic sides of nicotinamide

Nicotinamide possesses a variety of cellular functions. Nicotinamide serves as an anxiolytic (91), increases brain choline levels (92), and functions as an endogenous ligand for benzodiazepine receptors (93). In addition to neuronal cell populations, nicotinamide can influence cerebrovascular EC function. Early studies have suggested that the agent can protect against vascular thermal injury and increase capillary density (94). Nicotinamide also can alter EC major histocompatibility complexes (95), inhibit EC intracellular adhesion molecule expression (96), and modulate the production of TNF in ECs (95).

Yet, nicotinamide functions in a specific concentration range. Administration of nicotinamide in a concentration of 12.5 mM in cell culture offers significant protection against both anoxic and NO injury in neurons and ECs (20, 32, 48, 49). This concentration of nicotinamide is similar to other cytoprotective concentrations with nicotinamide (39) and parallels nicotinamide concentrations employed in clinical studies that have demonstrated no detrimental effects on the vascular system (97). In animal models, intraperitoneal injections of nicotinamide of 500 mg/kg, but not greater, are able to significantly reduce transient focal cerebral ischemia (43). Furthermore, pre-existing conditions, such as hypertension or diabetes, can raise or lower the concentration of nicotinamide that is necessary to achieve cytoprotection (45).

Although the cellular pathways that may determine the specific concentration range for the cytoprotective ability of nicotinamide have not been fully determined at this time, the toxicity of nicotinamide can occur under a variety of circumstances. Combination therapy with nicotinamide and methamphetamines can prolong toxic symptoms related to serotonin release, such as hyperthermia (98). Exposure to elevated concentrations of nicotinamide can inhibit the function of rat pancreatic beta-cells, decrease DNA content of adult rat pancreatic islet cells, and induce cell death in fetal rat pancreatic islet cells (99). In other experimental models, nicotinamide has been shown to result in the release of choline that may precipitate neuronal injury when levels of choline become excessive (92). In addition, cellular mechanisms modulated by nicotinamide that normally offer protection may, at times, lead to cellular injury. Administration of nicotinamide in concentrations less than 20 mM can promote activity of the DNA repair enzyme PARP. Yet, concentrations of nicotinamide greater than 20 mM have been shown to inhibit PARP function and lead to apoptosis (100). Increased clinical consumption of nicotinamide has been suggested to possibly increase genomic instability through PARP-1 inhibition and may result in tumorigenesis (9). Other studies that have observed toxicity with increased concentrations of nicotinamide have suggested that injury may be a result of a secondary metabolic acidosis generated by nicotinamide (101).

4.5. Nicotinamide and the aging process

Recent work has identified nicotinamide as an agent that can influence lifespan and may reverse the aging process in some cell populations. Nicotinamide has been shown to lead to the reversion of aging phenotypes in human diploid fibroblasts in terms of cell morphology and senescence-associated beta-galatosidase activity through the possible modulation of histone acetyltransferase activity (102). Yet, other work has shown that nicotinamide, as an NAD^+ precursor, can negatively influence lifespan of cells and longevity of the body through regulating the *Sir2* gene in the salvage cycle pathway (8).

The *Sir2* gene belongs to a family of genes which is a highly conserved group in the genomes of organisms ranging from archaebacteria to eukaryotes (103, 104). The

encoded Sir2 protein is involved in several diverse processes ranging from the regulation of gene silencing to DNA repair. The Sir2 protein also plays a critical role in transcriptional silencing, genome stability, longevity and cell viability (105) and has been shown to deacetylate histone H3 and H4 in the presence of NAD (106). Duplication of a gene in C. elegans that is most homologous to yeast Sir2 can confer a lifespan that is extended by up to fifty percent when compared to controls (107). Seven human homologs of Sir2 termed SIRT1-7 exist and have functions closely associated with the maintenance of cell integrity and survival (108). SIRT1, 2 and 3 have NAD-dependent protein deacetylase activities. SIRT1 is a nuclear protein that can deacetylate histones or p53 (109). SIRT2 can co-localize with microtubules and deacetylate tubulin (110). SIRT3 is a mitochondrial NAD-dependent deacetylase that is located in the mitochondrial matrix (111).

Interestingly, SIRT1 (Sir2α), as a human homologue of Sir2, is intimately linked with the modulation of cellular apoptotic pathways. The Sir 2 protein is associated with nicotinamide and pyrazinamidase/ nicotinamidase 1 (PNC1), an enzyme that deaminates nicotinamide. SIRT1 can regulate the activity of the p53 tumor suppressor via an NAD-dependent deacetylation of p53 protein and inhibit p53-dependent programmed cell death (PCD) (112, 113). SIRT1 also represses the activity of the transcription factor FOXO3a and prevents the induction of PCD through FOXO3a activation (114, 115).

Nicotinamide appears to be capable of decreasing cell longevity through Sir2. Nicotinamide can strongly inhibit Sir2 and its closest human homologue SIRT1. Nicotinamide is believed to directly block cellular Sir2 by intercepting an ADP-ribosyl-enzyme-acetyl peptide intermediate with regeneration of NAD+ (transglycosidation) (116). Recent investigations suggest that physiological concentrations of nicotinamide noncompetitively inhibit both Sir2 and SIRT1 *in vitro*. The degree of inhibition by nicotinamide (IC_{50} < 50 microm) is equal to or better than the most effective known synthetic inhibitors of this class of proteins, suggesting that nicotinamide is a physiologically relevant regulator of Sir2 enzymes (113).

Yet, during nicotinamide depletion, Sir2 is activated and employs PNC1 to regulate cell longevity. Increased expression of PNC1 has been found to be both necessary and sufficient for lifespan extension during calorie restriction (105). Nicotinamide and PCN1 are intimately linked in controlling cell life span. PNC1 can stimulate Sir2 histone deactylase activity by preventing the accumulation of nicotinamide through its conversion to nicotinic acid in the NAD^+ salvage pathway. Overexpression of PNC1 has been demonstrated to suppress the inhibitory effect of exogenous nicotinamide on silencing, life span, and transcriptional repression of Sir2. As a result, PNC1 can positively regulate Sir2-mediated silencing and longevity by preventing the accumulation of intracellular nicotinamide (117).

5. NICOTINAMIDE AND APOPTOTIC INJURY

Apoptosis, also termed PCD, is a primary component of cellular injury in both neuronal and vascular cell populations. Apoptotic injury is believed to contribute significantly to a variety of neurological disorders such as ischemic stroke (51, 67), dementia (118), Alzheimer's disease (119), Parkinson's disease (120), and spinal cord injury (121, 122). Circumstances such as the lack of trophic support, exposure to neurotoxins, and the induction of oxidative stress and DNA damage can become critical for the precipitation of PCD (123).

Membrane PS exposure and DNA fragmentation are two functionally independent processes that lead to PCD. The biological role of membrane PS externalization can vary in different cell populations. In many cell systems, membrane PS externalization can become a signal for the phagocytosis of cells (21, 66, 71, 124). In the nervous system, cells expressing externalized PS may be removed by microglia. An additional role of membrane PS externalization in the vascular cell system is the activation of coagulation cascades. The externalization of membrane PS residues in ECs can promote the formation of a procoagulant surface (64, 65, 125). In contrast to the early externalization of membrane PS residues, the cleavage of genomic DNA into fragments is a delayed event that occurs late during PCD (65, 126-129).

A variety of enzymes responsible for DNA degradation have been differentiated based on their ionic sensitivities to zinc (130) and magnesium (131). In addition, DNA degradation can proceed through several mechanisms that involve calcium/magnesium - dependent endonucleases such as DNase I (132), the acidic, cation independent endonuclease (DNase II) (133), cyclophilins (134), and the 97 kDa magnesium - dependent endonuclease (135). These studies have been extended to demonstrate that modulation of endonuclease activity directly influences cell survival in the nervous system (136, 137). Three separate endonuclease activities are present in neurons. They are a constitutive acidic cation-independent endonuclease, a constitutive calcium/magnesium-dependent endonuclease, and an inducible magnesium dependent endonuclease (136). The inducible magnesium-dependent endonuclease may be unique for the nervous system (136). The physiologic characteristics of the magnesium dependent endonuclease, such as a pH range of 7.4-8.0, a dependence on magnesium, and a molecular weight of 95-108 kDa, are consistent with a recently described constitutive 97 kDa endonuclease in non-neuronal tissues, but the endonuclease in the nervous system is inducible rather than constitutive in nature.

An ideal cytoprotectant would prevent not only DNA degradation, but also membrane PS exposure to provide greater overall protection for both neuronal and vascular cell populations (49, 138). Nicotinamide provides protection against PCD in neurons and ECs through the prevention of both DNA fragmentation and the inhibition of membrane PS exposure (20, 32, 48, 49). Studies with nicotinamide demonstrate a wide spectrum of

cytoprotection in addition to preventing passive cellular destruction during necrosis (44). At one level, nicotinamide yields immediate cytoprotection through the maintenance of an intact genomic DNA. At another level, nicotinamide can maintain membrane PS asymmetry and provide a more long-term protection by inhibiting the destruction of cells by phagocytes (20, 41, 48). Application of nicotinamide during anoxia, oxygen-glucose deprivation, and NO exposure can prevent the early exposure of membrane PS residues and also inhibit the later stages of genomic DNA destruction (20, 36, 41). Potentially more significant, nicotinamide prevents membrane PS exposure in ECs (41, 49). Exposure of membrane PS residues during EC injury can lead to the loss of anticoagulant membrane components, the propagation of the coagulation process, antibody-dependent aggregations, and cellular inflammation (125, 139). Thus, nicotinamide, through the prevention of EC membrane PS exposure, may enhance an organism's ability to prevent a procoagulant state and lower the risk for diseases such as stroke and arteriosclerosis.

An important caveat to cell injury focuses upon the initial stages of apoptotic death, namely membrane PS residue exposure, and whether this is reversible in nature (20, 140, 141). Investigations that examine the efficacy of cytoprotectants during cerebral ischemia have supported the premise that cellular PCD is reversible. For example, the application of growth factors (142), benzothiazole compounds (143-145), metabotropic glutamate receptor agonists (141, 146-148), and enhanced Bcl-2 expression (149) have been shown to either prevent or reverse membrane and nuclear changes associated with PCD.

Some of these studies, such as those with the metabotropic glutamate system, employ a technique that offers the ability to follow the progressive externalization of membrane PS residues in *living* cells over time (65, 150, 151). These studies provide a significant advantage over more conventional techniques employed to assess PCD, such as terminal deoxyUTP nick end labelling (TUNEL) or transmission electron microscopy (152, 153). Procedures that rely on tissue fixation lack the ability to assess dynamic changes in PCD in individual cells. As an alternative, more sensitive techniques have been developed to monitor the induction and change in PCD in individual living cells over a period of time (65, 150, 151). The method employs the reversible labelling of annexin V to exposed PS residues of cells undergoing PCD. By exploiting the dependence of annexin V on cellular calcium to bind to exposed membrane PS residues, one can reversibly label individual cells over time. During the induction of PCD, such as following an injury paradigm, progressive externalization of membrane PS residues occurs that is independent of the loss of cellular membrane integrity (150).

The use of post-treatment strategies with nicotinamide in studies using reversible labelling of annexin V in living cells illustrate that PCD is reversible rather than being a fixed, committed cellular pathway that results in cellular injury. During post-treatment studies, nicotinamide can reverse an initial progression of membrane PS inversion and maintain the suppression of PS exposure over a 24 hour period (20, 32, 48, 49). These results suggest that apoptotic injury, at least along the pathway that involves membrane PS exposure, is dynamic and reversible in nature (20, 32, 48, 49). As a result, nicotinamide may impart an additional advantage for cell survival and function.

6. NICOTINAMIDE PARTNERS WITH SEVERAL CELLULAR ENTITIES

6.1. Nicotinamide, Akt, FOXO3a, and GSK-3β

Modulation of cell function, integrity and survival by nicotinamide occurs at a series of cellular pathways. Initially, nicotinamide may be dependent upon the activation of protein kinase B, also known as Akt. Akt is a critical protein that promotes growth and survival in several cell systems and functions through regulating the activity of its downstream targets. Akt is phosphorylated and activated through the phosphoinositide 3 kinase (PI 3-K) pathway. Once recruited to the plasma membrane, PI 3-K phosphorylates glycerophospholipid phosphatidylinositol 4,5-bisphosphate and yields phosphatidylinositol 3,4 bisphosphate (PIP2) and phosphatidylinositol 3,4, 5 trisphosphate (PIP3). In the cytosol, Akt translocates to the cell membrane as a result of its binding to PIP2 and PIP3 and becomes activated through phosphorylation by phosphoinositide-dependent kinase 1 (154).

Increased activity of Akt can provide protection against neuronal and vascular injury. Maximal activity of Akt is achieved through phosphorylation by phosphoinositide-dependent kinase 1 at Ser^{473} to confer protection against genomic DNA degradation (53, 155, 156) and membrane PS exposure (21, 53, 71). During injuries involving excitotoxicity (157), free radical exposure (71, 158), hypoxia (53), or trauma (159), phosphorylation of Akt is increased. The ability of Akt to function as an anti-apoptotic agent is dependent upon the activity of several substrates, such as Bad, caspase 9, IκB kinase α, the Forkhead transcription factor (FOXO3a, FHKRL1), and glycogen synthase kinase 3β (GSK-3β). The activation of Akt plays a principal role in the control of PCD through the inactivation of its pro-apoptotic substrates.

Akt can phosphorylate Bad, a pro-apoptotic Bcl-2 family member, thereby inhibiting the pro-apoptotic activity of Bad (160)}. Bad can bind to Bcl-x_L, an anti-apoptotic Bcl-2 family member, to release Bax from Bcl-x_L and promote apoptosis. The phosphorylation of Bad by Akt also can promote the binding of Bad with the cytosolic protein 14-3-3 to release Bcl-x_L and allow it to block apoptosis (161). Bcl-2 and Bcl-x_L prevent Bax translocation to the mitochondria, maintain the mitochondrial membrane potential, and prevent the release of cytochrome c from the mitochondria (162). Nicotinamide can promote the phosphorylation of Bad during oxidative stress (33). This phosphorylation of Bad by nicotinamide is blocked by inhibitors of the PI 3-K pathway, suggesting that nicotinamide phosphorylates Bad through a PI 3-K/Akt mediated pathway. In addition, Akt

may promote cell survival through the inhibition of p53 transcriptional activity (156) that may be regulated by nicotinamide. Activation of p53 can promote the expression of Bax to result in apoptotic cell death (163). Nicotinamide has been shown to either directly limit the expression of p53 (35) or prevent an NAD-dependent p53 deacetylation induced by Sir2α (164)}.

A significant downstream substrate of Akt activation includes FOXO3a (165). FOXO3a can result in PCD in cerebral granule neurons, sympathetic neurons and other cell types in a transcription-dependent manner following its translocation to the nucleus (166-168). Phosphorylation of FOXO3a by Akt leads to the association of FOXO3a with 14-3-3 protein and retention of FOXO3a in the cytoplasm, rendering it unable to regulate its target genes in the nucleus and thus inhibiting apoptosis. Activation of FOXO3a has been demonstrated to disrupt mitochondrial membrane potential ($\Delta\Psi_m$) and may result in cytochrome c release (169). During oxidative stress in neurons, an initial inhibitory phosphorylation of FOXO3a at the regulatory phosphorylation sites (Thr^{32} and Ser^{253}) (168, 170) results within 3 or 6 hours post injury (34). Yet, loss of phosphorylated FOXO3a expression occurs within a subsequent 12 hour period (34). Nicotinamide may derive its neuroprotective capacity through two separate mechanisms of post-translational modification of FOXO3a by maintaining not only inhibitory phosphorylation of FOXO3a, but also preserving the integrity of total FOXO3a and phosphorylated FOXO3a over a 12 hour period. The loss of both FOXO3a phosphorylation and the integrity of this transcription factor may function as a significant precipitant of neuronal injury. FOXO3a proteolysis occurs during cell injury yielding an amino-terminal (Nt) fragment that can become biologically active (171). During cell injury and caspase-dependent cleavage of Akt1 (172), it is the activation of FOXO3a Nt fragments that become available and result in apoptotic cellular injury. Nicotinamide, through both the promotion of extended phosphorylation of FOXO3a at regulatory sites that possess high affinity for Akt and the inhibition of the proteolytic cleavage of FOXO3a, may prevent apoptotic cell injury (34).

Glycogen synthase kinase-3 is a serine/threonine kinase that also is a substrate of Akt. Of the two isoforms of GSK-3 termed GSK-3α and GSK-3β, the latter is specifically expressed in the central nervous system. Akt can phosphorylate GSK-3β at Ser^{9} and inactivate the enzyme (173). In contrast, phosphorylation of GSK-3β at Thr^{216} results in an enhanced activity of the enzyme, which can occur during neuronal degeneration (174). GSK-3β plays a significant role in the regulation of apoptosis. GSK-3β phosphorylates a variety of substrates that play vital roles in cellular survival, such as modulation of the eukaryotic initiation factor 2B and the microtubule – associated protein tau. In addition, GSK-3β can regulate the transcription factors cAMP response element binding protein, c-myc, c-jun, and β-catenin. The inactivation of GSK-3β can result in the prevention or reduction in apoptotic injury in neurons (175), vascular smooth muscle cells (176), and cardiomyocytes (177). In contrast, GSK-3β has been demonstrated to precipitate cellular injury during oxidative stress and lead to caspase 3 activation and cytochrome c release (178). Initial studies with nicotinamide suggest that GSK-3β activity is blocked through the modulation of Akt to further cellular survival (34).

6.2. Nicotinamide and mitochondrial dysfunction

Downstream from the modulation of Akt and its substrates, protection by nicotinamide is closely associated with the maintenance of $\Delta\Psi_m$. Maintenance of $\Delta\Psi_m$ becomes critical for cellular survival. Loss of $\Delta\Psi_m$ through the opening of the mitochondrial permeability transition pore represents a significant determinant for cell injury and the subsequent induction of the apoptotic cascade (19, 20, 179). Oxidative stress through free radical generation leads to the opening of the mitochondrial permeability transition pore and the release of cytochrome c into the cytosol (180). Mitochondria are a significant source of superoxide radicals that are associated with oxidative stress. Blockade of the electron transfer chain at the flavin mononucleotide group of complex I (NADPH ubiquinone oxidoreductase) or at the ubiquinone site of complex III (ubiquinone-cytochrome c reductase) results in the active generation of free radicals which can impair mitochondrial electron transport and enhance free radical production (181, 182).

The pro-apoptotic member Bax can precipitate the release of cytochrome c (183). Once Bax is translocated to mitochondrial membrane from cytosol, it undergoes conformational alteration resulting in its insertion into the mitochondrial membrane to facilitate cytochrome c release. Bax forms clusters with the formation of Bax multimers that appear to be a prerequisite for cytochrome c release (184). Subsequent release of cytochrome c results in the oligomerization of apoptotic protease activating factor-1 (Apaf-1) and promotes the allosteric activation of caspase 9 by forming the Apaf-1 apoptosome (185). Caspase 9 can subsequently activate caspase 3 (185) as well as caspase 1 through the intermediary caspase 8 (186). Together, caspase 1 and caspase 3 lead to both DNA fragmentation and membrane PS exposure (53, 65, 185).

Administration of nicotinamide prevents this depolarization of the mitochondrial membrane (41, 48, 49). Studies have suggested that nicotinamide acts directly at the level of mitochondrial membrane pore formation to prevent the release of cytochrome c. Pretreatment of neurons or ECs with either nicotinamide alone or in combination with the mitochondrial permeability transition pore inhibitor cyclosporin A (187, 188) prior to an injury paradigm can equally prevent mitochondrial membrane depolarization. The absence of a synergistic response with the addition of cyclosporin A suggests that nicotinamide functions by directly inhibiting mitochondrial membrane pore formation during cellular injury. Additional work during studies that involve oxygen-glucose deprivation demonstrate that nicotinamide maintains $\Delta\Psi_m$ and prevents the release of cytochrome c (34). Interestingly, nicotinamide appears to act directly at the level of mitochondrial membrane pore formation to prevent

cytochrome c release. Nicotinamide can prevent the chemical induction of mitochondrial membrane depolarization during exposure to either *tert*-butylhydorperoxide or atractyloside (34).

The precise pathways that are necessary for nicotinamide to modulate mitochondrial membrane pore formation require further analysis. Intimately associated with the disruption in $\Delta\Psi_m$ and the release of cytochrome c into the cytosol during neuronal injury is the induction of cysteine protease activity. Oligomerization of Apaf-1 with cytochrome c is critical for the allosteric activation of caspase 9 (185). Although some "anti-apoptotic" proteins, such as erythropoietin (19) and heat-shock protein 70 (189, 190), appear to modulate both Apaf-1 expression and cytochrome c release, protection through nicotinamide remains independent from Apaf-1 (34).

Reactive oxygen species also have been postulated as a potential mechanism for the induction of acidosis-induced cellular toxicity (191) and subsequent mitochondrial failure (192). In the nervous system, toxic insults, such as hypercapnia (193), hypoxia (194), glutamate toxicity (195), and NO (137, 151, 196) can result in the disturbance of intracellular pH. In addition, modulation of intracellular pH is physiologically relevant for endonuclease activities during PCD (136, 137, 151). Yet, nicotinamide does not directly prevent the induction of intracellular acidification (20). In addition, nicotinamide cannot prevent cellular injury during intracellular acidification paradigms (20). These studies illustrate that nicotinamide maintains genomic DNA integrity through mechanisms that are independent of intracellular pH.

An attractive pathway that may mediate protection by nicotinamide could involve the stress activated family of mitogen-activated protein (MAP) kinases that includes the p38 kinases and the c-Jun N-terminal kinases (JNKs). These proteins are activated by phosphorylation and play a significant function during cell differentiation, growth, and death (197). Significant activation of p38 and JNK is present in both neurons and ECs during oxidative stress (20, 32, 48, 49). In addition, JNK can promote Bax translocation through phosphorylation of 14-3-3 proteins and lead to cytochrome c release (198). Furthermore, during cellular injury such as with cyanide-induced apoptosis, p38 can modulate Bax translocation from the cytosol to the mitochondria and result in both cytochrome c release and caspase activation (199). Yet, nicotinamide does not alter the activity of either p38 or JNK, suggesting that protection by nicotinamide is independent of the p38 and JNK pathways (20, 32, 48, 49).

It is conceivable that nicotinamide modulates alternate cellular pathways linked to mitochondrial dysfunction. Nicotinamide may stabilize cellular energy metabolism since the maintenance of $\Delta\Psi_m$ is an ATP facilitated process (200) or may inhibit the assembly of the mitochondrial permeability transition pore complex similar to the action of cyclosporin A (201). Another pathway that nicotinamide may employ is through the activation of Akt, since Akt is closely linked to the maintenance of $\Delta\Psi_m$ (72, 202).

6.3. Nicotinamide and caspase activity

Cytoprotection by nicotinamide also may reside with the generation of caspase activity. Caspase activation is responsible for cellular morphological alteration during PCD that includes DNA fragmentation, chromatin condensation, and externalization of membrane PS residues. Subsequent to the loss of $\Delta\Psi_m$ and the release of cytochrome c, induction of caspase activation occurs. The caspases are mammalian homologues of the *C. elegans* cell death genes. Each of the aspartate-specific caspases is synthesized as a proenzyme that is proteolytically cleaved into subunits that form catalytically active heterodimers during development or injury (203).

Caspases can be functionally categorized into three groups. Group I is the cytokine-processing caspases which include caspase 1, 4, 5, 11, 12, and 13. Group II caspases consist of caspase 3, 6, and 7 and are termed executioner or effector caspases that cleave crucial cellular protein substrates leading to cell destruction. Group III members include caspase 2, 8, 9, and 10 and are described as initiator caspases that activate downstream executioner caspases resulting in an amplification of caspase activity (204). An extrinsic and an intrinsic caspase activation pathway can each lead to PCD. The extrinsic pathway is initiated by death receptor activation on the cell surface and results in enhanced caspase 8 and 10 activities. As a result, Bid is cleaved by caspase 8 and translocates to mitochondria to release cytochrome c through the Bax subfamily of Bcl-2 proteins. This leads to the subsequent activation of executioner caspases. The intrinsic pathway is mediated by caspase 9 following the release of mitochondrial cytochrome c. Cytochrome c binds to Apaf-1 followed by activation of caspase-9 (205). The active caspase 9 can then activate executioner caspases 3 and 7.

In both neuronal and vascular cell populations, nicotinamide can prevent specific caspase activity. The caspases 1 and 3 have each been linked to the independent apoptotic pathways of genomic DNA cleavage and cellular membrane PS exposure (19, 84, 186). The ability of nicotinamide to modulate these caspases appears to play a significant role in its cellular protection. Genomic DNA degradation and membrane PS exposure can ensue through the activation of caspase 3 and caspase 1 (186). Caspase 3 becomes a prominent mediator of genomic DNA degradation. Experimental models that use caspase 3 gene deletions or pharmacological inhibition illustrate little or no DNA fragmentation following toxic cellular insults (53, 206).

Modulation of caspase 3 activity by nicotinamide also appears to be closely associated with a unique regulatory mechanism that blocks the proteolytic degradation of phosphorylated FOXO3a by caspase 3. Given that FOXO3a has been shown to be a substrate for caspase 3-like proteases at the consensus sequence $DELD^{304}A$ (171), current work demonstrates that blockade of caspase 3 - like activity prevents the destruction of phosphorylated FOXO3a during oxidative stress (34). In light of the dual capacity of nicotinamide to directly inhibit caspase 3 - like activity and maintain inhibitory

phosphorylation of FOXO3a, investigations suggest that nicotinamide maintains a regulatory "neuroprotective loop" through the independent modulation of caspase 3 and phosphorylated FOXO3a integrity.

In regards to membrane PS exposure, nicotinamide appears to prevent PS externalization primarily through the inhibition of caspase 1 -like activity and, to a lesser degree, through other caspases such as 3, 8, and 9 (20, 41, 48, 49). These caspases are also tied to the direct activation and proliferation of microglia (21, 71, 72). Caspase 1 is believed to be principally responsible for the externalization of membrane PS residues in several cell systems through the digestion of cytoskeletal proteins, such as fodrin and to be responsible for microglial phagocytosis (65, 207).

Nicotinamide also can modulate caspase 8, and caspase 9 - like activities. These cysteine proteases are associated with the independent apoptotic pathways of genomic DNA cleavage and cellular membrane PS exposure (141, 186). Caspase 9 is activated through a process that involves the cytochrome c -Apaf-1 complex (185, 208). Yet, independent of Apaf-1, nicotinamide can significantly prevent cell injury by inhibiting caspase 9 - like activity directly (34). In addition, caspase 8 serves as an upstream initiator of executioner caspases, such as caspase 3, and also leads to the mitochondrial release of cytochrome c (209, 210). Following caspase 8 and caspase 9 activation, caspase 3 directly leads to genomic DNA degradation. As a result, nicotinamide appears to function at both intrinsic and extrinsic pathways to prevent caspase activation and promote cellular integrity with maintenance of membrane PS asymmetry (20, 41, 48, 49).

6.4. Nicotinamide, PARP, and metabolism

Poly(ADP-ribose) polymerase (PARP) is a nuclear protein that binds to DNA strand breaks and cleaves NAD^+ into nicotinamide and ADP-ribose (211). During DNA repair, ADP-ribose is polymerized onto nuclear proteins that include histones and transcription factors at DNA strand breaks (212). Yet, excessive PARP activity may be detrimental to cellular function. Augmented PARP activation leads to a rapid depletion of its sole substrate NAD^+ and lowered ATP production. As a cell consumes ATP in an effort to replenish NAD^+, this results in a cellular energy crisis that precipitates cell death. A significant increase in PARP activity also can promote NF-κB-driven transcription and microglial activation leading to the overexpression of pro-inflammatory and adhesion molecules (213).

Nicotinamide may provide cellular protection through the maintenance of PARP integrity and the preservation of cellular energy reserves. Nicotinamide concentrations of at least 1 mM have been shown to provide sufficient stores of NAD^+ during PARP activation (214). Nicotinamide can prevent PARP degradation and allow for DNA repair through the direct inhibition of caspase 3 – like activity (20, 41). In addition, nicotinamide can inhibit the activity of PARP during ischemic cell injury (44).

Exclusive of PARP activity, the preservation of cellular energy metabolism by nicotinamide may be dependent upon glycolytic metabolism with glyceraldehydes-3-phosphate dehydrogenase (40). In some respects, nicotinamide appears to function as a double-edge sword that can have detrimental effects, since PARP activity and energy depletion become significantly increased over a 24 hour period as a result of nicotinamide administration (215). Furthermore, depletion of PARP activity by nicotinamide has been associated with genomic instability and the increased risk for neoplastic growth in some experimental models (9).

7. FUTURE CONSIDERATIONS FOR NICOTINAMIDE

As a necessary nutrient to maintain cellular homeostasis and metabolism, nicotinamide has "matured" into an agent that possesses both unique and broad functions that directly impact upon cellular plasticity, cellular aging mechanisms, and inflammatory cell modulation (Figure 2). Nicotinamide, a precursor to the co-enzyme NAD^+, interfaces with an array of vital cellular functions that involve stem cell development, energy metabolism, ATP production, DNA repair, and cellular longevity. Cellular protection offered by nicotinamide through the maintenance of genomic DNA integrity and the preservation of membrane PS asymmetry impacts acute cellular injury as well as secondary thrombosis, clot formation, and inflammation.

Nicotinamide fosters cellular function and survival through a series of distinct pathways that involve the serine-threonine kinase Akt and its downstream substrates of FOXO3a, and GSK-3β. Particularly attractive is the capacity of nicotinamide to employ the Akt pathway for protection of cells from inflammatory injury through the direct modulation of cellular membrane PS externalization. Intimately associated with the protective ability of nicotinamide is the maintenance of $\Delta\Psi_m$ and the central modulation of Bax, mitochondrial energy reserves, cytochrome c release, and PARP. Targeting by nicotinamide of specific extrinsic and intrinsic caspase pathways ultimately serve to preserve genomic integrity and prevent early apoptotic injury "tagging" for microglial disposal.

As both a therapeutic agent and investigational tool, nicotinamide holds great promise for the future. Yet, caution must be applied as further development for clinical applications is pursued for neurodegenerative disorders as well as other disease entities. New work must uncover the cellular mechanisms that determine whether a particular concentration of nicotinamide will promote cellular function or ultimately precipitate genomic instability and possible tumorigenesis. In addition, as a precursor to NAD^+, nicotinamide has the potential to significantly improve or deplete cellular energy stores as well as detrimentally alter a cell's life span. As our knowledge of nicotinamide becomes more refined, we should be able to appreciate in greater depth and enthusiasm the role nicotinamide plays during the development, growth, and aging of cells not only in the nervous system, but through the human body.

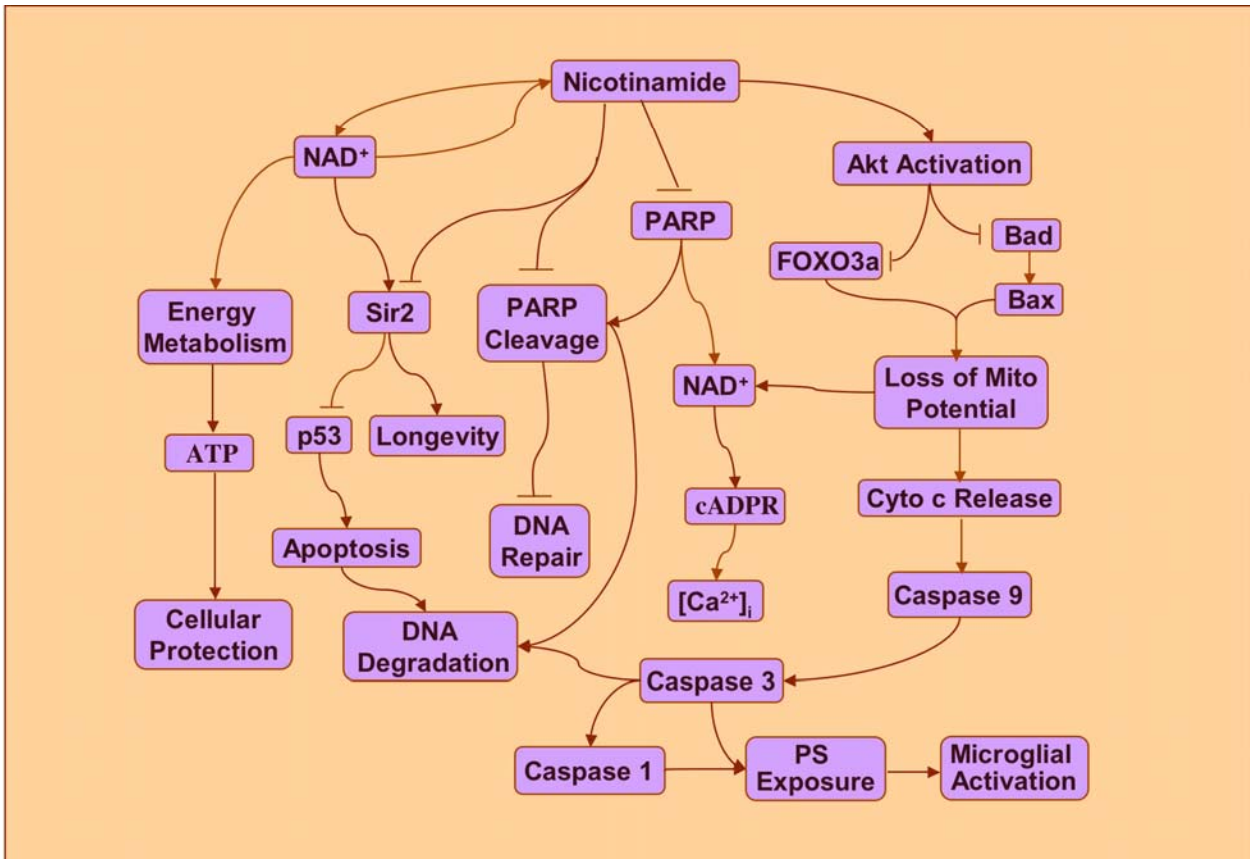

Figure 2. Nicotinamide employs a host of cellular mediators to regulate cellular metabolism, longevity, survival, and inflammatory microglial activation. Nicotinamide promotes cellular function and survival through a series of distinct pathways that involve NAD^+, cell senescence mechanisms, the serine-threonine kinase Akt and its downstream substrates of FOXO3a, and Bad. Closely associated with the protective ability of nicotinamide is the maintenance of $\Delta\Psi_m$ and the central modulation of Bad, Bax, mitochondrial energy reserves, cytochrome c (cyto c) release, and PARP. Targeting by nicotinamide of specific extrinsic and intrinsic caspase pathways ultimately serve to preserve genomic integrity and prevent early apoptotic injury "tagging" for microglial disposal. NAD: β-nicotinamide adenine dinucleotide; cADPR: cyclic-ADP-ribose; Sir2: Silent information regulator 2; mito: mitochondria.

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Abbreviations: ß-nicotinamide adenine dinucleotide (NAD^+), Poly (ADP-ribose) polymerase (PARP), silent information regulator 2 (Sir2), nicotinamide adenine dinucleotide (NADH), endothelial cells (ECs), phosphatidylserine (PS), phosphatidylserine receptor (PSR), tumor necrosis factor (TNF), nitric oxide (NO), protein kinase B (Akt), programmed cell death (PCD), pyrazinamidase/ nicotinamidase 1 (PNC1), phosphatidylinositol 3,4 bisphosphate (PIP2), phosphatidylinositol 3,4, 5 trisphosphate (PIP3), forkhead transcription factor (FOXO3a, FHKRL1), phosphoinositide 3 kinase (PI 3-K), mitochondrial membrane potential ($\Delta\Psi_m$), apoptotic protease activating factor-1 (Apaf-1), mitogen-activated protein (MAP), c-Jun N-terminal kinases (JNKs)

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