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Nicotinamide riboside restores cognition through an upregulation of proliferator-activated receptor-γ coactivator 1α regulated β-secretase 1 degradation and mitochondrial gene expression in Alzheimer's mouse models

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ABSTRACT

Nicotinamide adenine dinucleotide $(NAD)^+$, a coenzyme involved in redox activities in the mitochondrial electron transport chain, has been identified as a key regulator of the lifespan-extending effects, and the activation of NAD^+ expression has been linked with a decrease in beta-amyloid (Aβ) toxicity in Alzheimer's disease (AD). Nicotinamide riboside (NR) is a NAD^+ precursor, it promotes peroxisome proliferator-activated receptor-γ coactivator 1 (PGC)-1α expression in the brain. Evidence has shown that PGC-1α is a crucial regulator of Aβ generation because it affects β-secretase (BACE1) degradation. In this study we tested the hypothesis that NR treatment in an AD mouse model could attenuate Aβ toxicity through the activation of PGC-1α-mediated BACE1 degradation. Using the Tg2576 AD mouse model, using in vivo behavioral analyses, biochemistry assays, small hairpin RNA (shRNA) gene silencing and electrophysiological recording, we found (1) dietary treatment of Tg2576 mice with 250 mg/kg/day of NR for 3 months significantly attenuates cognitive deterioration in Tg2576 mice and coincides with an increase in the steady-state levels of NAD^+ in the cerebral cortex; (2) application of NR to hippocampal slices (10 μM) for 4 hours abolishes the deficits in long-term potentiation recorded in the CA1 region of Tg2576 mice; (3) NR treatment promotes PGC-1α expression in the brain coinciding with enhanced degradation of BACE1 and the reduction of Aβ production in Tg2576 mice. Further in vitro studies confirmed that BACE1 protein content is decreased by NR treatment in primary neuronal cultures derived from Tg2576 embryos, in which BACE1 degradation was prevented by PGC-1α-shRNA gene silencing; and (4) NR treatment and PGC-1α overexpression enhance BACE1 ubiquitination and proteasomal degradation. Our studies suggest that dietary treatment with NR might benefit AD cognitive function and synaptic plasticity, in part by promoting PGC-1α-mediated BACE1 ubiquitination and degradation, thus preventing Aβ production in the brain.

1. Introduction

Nicotinamide adenine dinucleotide $(NAD)^+$ has been identified as a key regulator in the lifespan-extending effects of calorie restriction in a number of species. Numerous studies have suggested that NAD^+ mediates multiple major biological processes, including calcium homeostasis, energy metabolism, mitochondrial functions, cell death, and aging in various tissues including brain. Increasing evidence has suggested that NAD^+ might play important roles in metabolic processes in the brain, and has effects on brain functioning such as neurotransmission, learning, and memory. Recent studies have shown that the activation of NAD expression has been linked with a decrease in the amyloid toxicity in Alzheimer's disease (AD) animal models (Kim et al., 2007; Qin et al., 2006), in which it might relate to the interactions with the expression of peroxisome proliferator-activated receptor-γ coactivator 1 (PGC)-1α (Nemoto et al., 2005) and through the activation of neuronal NAD-dependent deacetylase sirtuin-1 (SIRT1) activation (Qin et al., 2006; Rodgers et al., 2005). It has been shown that during metabolic stress conditions such as the fasting state, hypoxia, NAD+ levels, and SIRT1 protein levels are increased, leading to deacetylation of PGC-1α, subsequently increasing the

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expression of PGC-1α, promoting gluconeogenic transcriptional program (Rodgers et al., 2005), consequently protecting the mitochondrial energy metabolism. Thus, the benefits of the NAD^+ stimulating cell survival have raised the hope that using pharmacological agents to increase NAD^+ concentrations might provide therapeutic benefits in delaying the onset and slowing the progression of AD dementia.

Nicotinamide riboside (NR) is a NAD precursor, which is converted to NAD through action of human *NrK1* and *NrK2* genes in the de novo fashion (Bieganowski and Brenner, 2004; Bieganowski et al., 2003). Evidence shows that NR treatment increases intracellular NAD^+ concentration and improves NAD^+-dependent activities in the cell by increasing silent mating-type information regulation 2 (Sir2)-dependent gene silencing and longevity via nicotinamide riboside kinase (NRK) 1-dependent NAD^+ synthesis (Belenky et al., 2007). Thus it is possible that the exogenous application of NR is capable of promoting the biosynthesis of NAD, thus promoting the beneficial effects of NAD (Braidy et al., 2008). Excitingly, it has been reported that treatment with nicotinamide prevents cognition in AD transgenic mice via a mechanism involving sirtuin inhibition and reduction of tau phosphorylation (Green et al., 2008). However, the role of NR in the beta-amyloid (Aβ) deposition in AD brain is still not clear.

It has been shown that PGC-1α also plays an important role in energy metabolism by regulating mitochondrial function in different tissues. The expression of PGC-1 has been found significantly decreased in Alzheimer's brains, and it is involved in the Aβ pathological generation by affecting the processing of amyloid precursor protein (APP), at least partially through enhancing the α-secretase activity (Qin et al., 2009; Wu et al., 2006). Recently, our group and others reported that 1 of the mechanisms in which the PGC-1 decreases the Aβ burden is also involved in the regulation of the F-Box (FbX)2-E3-ligase-mediated β-secretase (BACE1) degradation (Gong et al., 2010; Katsouri et al., 2011) as it does in other E3 ligases in the ubiquitin system in other tissues. Encouraged by the effects of NAD on promoting the PGC-1 expression, in this study, we tested the hypothesis that exogenous treatment of NR might reduce the Aβ burden in AD brain via enhancing PGC-1α expression, which increases BACE1 ubiquitination, degradation, and improves mitochondrial metabolism. Our study provides a novel therapeutic strategy for the treatment of AD.

2. Methods

2.1. Animals

Tg2576 mice were crossed with PGC-1$\alpha^{-/-}$ mice (Qin et al., 2009) and generated PGC-1$\alpha^{-/-}$/Tg2576 mice. Animals were backcrossed at least 10 generations onto normal C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME, USA). All experiments were approved by the Mount Sinai School of Medicine Animal Care committees.

2.2. Primary neuronal cell culture

Tg2576 mouse primary neuronal cell cultures were prepared from the brains of 14.5-day-old embryos bred from wild type C57BL/6 females crossed with Tg2576 males, as described previously (Gong et al., 2010). Briefly, after isolation, cerebral hemispheres were placed into Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) and 1× penicillin-streptomycin. Brain tissue was dissociated and the single cells were suspended in Dulbecco's modified Eagle's medium supplemented with 10% FBS and 1× penicillin-streptomycin. Cells were seeded into precoated 12-well plates (BD Biosciences) at a concentration of 8×10^5 cells per well. After 30 minutes of incubation in a tissue culture incubator, the cells were changed to neural basal medium (Invitrogen) supplemented with 0.5 mM L-glutamine (Cellgro), 1 × B-27 (Invitrogen), and 1× penicillin-streptomycin (Invitrogen). The cells were kept in an atmosphere of 95% air and 5% CO_2. After 7 more days of incubation, cells were used for the treatment.

2.3. BACE1 activity measurements and quantification of amyloid peptides by enzyme-linked immunosorbent assay

The measurement of the Aβ levels has been described previously (Gong et al., 2004). Briefly, levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in primary cultured Tg2576 neurons infected with various adenoviral vectors were determined using sandwich type enzyme-linked immunosorbent assay (ELISA) (Biosource International, Camarillo, CA, USA). The background from control medium (transfected with the adeno-green fluorescent protein [GFP] vector) was subtracted from the sample values.

2.4. NR treatment and behavioral assessment

Seven- to 8-month-old Tg2576 mice were treated with 250 mg/kg/day NR; control Tg2576 mice were treated with saline. Treatments started at approximately 5–6 months of age, and lasted until 10–11 months of age. Mice had their cognitive functions assessed by the object recognition protocol. Mice were first placed in an apparatus and allowed to explore an object. After a certain interval, the mouse was returned to the apparatus, which contained the familiar object and a novel object (Bevins and Besheer, 2006). The time which the mouse spent on the novel object was calculated and was compared between treated and control groups. After behavioral assessment, mice were sacrificed, and their brains were dissected. One hemisphere was snap frozen for subsequent assessment of Aβ-specific ELISA as discussed above. The other hemisphere was fixed in formaldehyde for subsequent stereological quantitative assessments of neuritic plaque pathology, as previously described (Green et al., 2008; Wang et al., 2008). In parallel, control studies using age-, sex-, and strain-matched wild type (WT) mice, were conducted to evaluate the potential effect of NR treatment on cognitive function in the absence of Aβ neuropathology.

2.5. Western blot

Cells and tissues were lysed in either radioimmunoprecipitation assay (RIPA) buffer lysis buffer or Cell Signaling Lysis Buffer supplemented with protease inhibitors. Fifty to 100 μg of protein lysate was then run on sodium dodecyl sulfate polyacrylamide gel electrophoresis. Proteins were transferred to a nitrocellulose transfer membrane (Whatman). The membranes were blocked in 5% fat-free milk for 1 hour, then incubated in primary antibody for 1 hour, horseradish peroxidase (HRP)-conjugated secondary antibody for 1 hour, and developed in enhanced chemiluminescence (ECL) substrate.

2.6. Hippocampal slice preparation and electrophysiology recording

We cut four hundred μm brain slices from Tg2576 mice, and WT littermates, and maintained them in an interface chamber at 29 °C for 90 minutes before recording, as previously reported (Gong et al., 2006). The bath solution consisted of 124.0 mM NaCl, 4.4 mM KCl, 1.0 mM Na_2HPO_4, 25.0 mM $NaHCO_3$, 2.0 mM $CaCl_2$, 2.0 mM $MgSO_4$, and 10.0 mM glucose. The stimulating electrode, a bipolar tungsten electrode, was placed at the level of the Schaeffer collateral fibers, whereas the recording electrode, a glass electrode filled with bath solution, was placed at the level of the CA1 stratum radiatum. Basal synaptic transmission was assayed by plotting the stimulus voltages against slopes of field excitatory postsynaptic potentials. For the