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Research report

Nampt is required for long-term depression and the function of GluN2B subunit-containing NMDA receptors

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ABSTRACT

Nicotinamide adenine dinucleotide (NAD+) is an essential coenzyme/cosubstrate for many biological processes in cellular metabolism. The rate-limiting step in the major pathway of mammalian NAD+ biosynthesis is mediated by nicotinamide phosphoribosyltransferase (Nampt). Previously, we showed that mice lacking Nampt in forebrain excitatory neurons (*CamKIIαNampt*$^{-/-}$ mice) exhibited hyperactivity, impaired learning and memory, and reduced anxiety-like behaviors. However, it remained unclear if these functional effects were accompanied by synaptic changes. Here, we show that *CamKIIαNampt*$^{-/-}$ mice have impaired induction of long-term depression (LTD) in the Schaffer collateral pathway, but normal induction of long-term potentiation (LTP), at postnatal day 30. Pharmacological assessments demonstrated that *CamKIIαNampt*$^{-/-}$ mice also display dysfunction of synaptic GluN2B (NR2B)-containing *N*-methyl-D-aspartate receptors (NMDARs) prior to changes in NMDAR subunit expression. These results support a novel, important role for Nampt-mediated NAD+ biosynthesis in LTD and in the function of GluN2B-containing NMDARs.

1. Introduction

Nicotinamide adenine dinucleotide (NAD+) is an essential coenzyme/cosubstrate in multiple metabolic reactions, including glycolysis and oxidative phosphorylation (Houtkooper et al., 2010; Lunt and Vander Heiden, 2011; Pellerin, 2008; Stein and Imai, 2012). While there are several pathways of NAD+ biosynthesis, most mammalian cells generate NAD+ from nicotinamide (Houtkooper et al., 2010; Stein and Imai, 2012). The rate-limiting step of this pathway is performed by nicotinamide phosphoribosyltransferase (Nampt) (Revollo et al., 2004). Recently, we found that mice lacking Nampt in forebrain excitatory neurons of the hippocampal CA1 subregion and cortical layers II/III (*CamKIIαNampt*$^{-/-}$ mice) exhibited hyperactivity, impaired learning and memory, and reduced anxiety-like behaviors at 2–3 months of age (Stein et al., 2014). And yet, at postnatal day (P) 60, *CamKIIαNampt*$^{-/-}$ mice had intact tetanic long-term potentiation (LTP), a form of synaptic plasticity considered to be an electrophysiological correlate of learning and memory (Foster, 2012). Both spatial memory deficits and decreased expression of the immediate early genes *Egr1*and *Arc*, which were exhibited by *CamKIIαNampt*$^{-/-}$ mice, have been strongly linked to LTP (Davis et al., 2003; Jeffery and Morris, 1993; Kubik et al., 2007; Tombaugh et al., 2002). Thus, it was surprising that the spatial memory performance and loss of immediate early gene expression in *CamKIIαNampt*$^{-/-}$ mice were not intrinsically linked with LTP. Our findings raised an important question: does loss of Nampt in forebrain excitatory neurons have any synaptic manifestations?

Little is known regarding the importance of NAD+ for synaptic transmission. Besides LTP, another form of synaptic plasticity is long-term depression (LTD), or a persistent decrease in synaptic strength (Foster, 2012; Kemp and Manahan-Vaughan,

Abbreviations: CA1, cornu ammonis region 1; fEPSP, field excitatory postsynaptic potential; HFS, high frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; LFS, low frequency stimulation; NAD+, nicotinamide adenine dinucleotide; Nampt, nicotinamide phosphoribosyltransferase; NMDAR, *N*-methyl-D-aspartate receptor; P, postnatal day.

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2007; Zorumski and Izumi, 2012). Consolidation of hippocampal-dependent memory has been linked to *N*-methyl-D-aspartate receptor (NMDAR) dependent LTD in CA1 (Brigman et al., 2010; Ge et al., 2010; Wong et al., 2007). NMDAR activation modulates induction of LTP and LTD (Zorumski and Izumi, 2012) and occurs during conditions of energy deprivation, such as hypoxia and hypoglycemia (Corbett and Nurse, 1998; Crepel et al., 2003; Godukhin et al., 2002; Hsu and Huang, 1997; Tekkok and Krnjevic, 1995). NMDARs are tetrameric receptors consisting of two obligatory NR1 (GluN1) subunits and two regulatory subunits, usually a combination of GluN2A (NR2A) and GluN2B (NR2B) (Zorumski and Izumi, 2012). Previous work found that mice lacking GluN2B function behave similarly to *CamKIIαNampt*$^{-/-}$ mice (Stein et al., 2014), with hyperactivity (Badanich et al., 2011; Higgins et al., 2003; von Engelhardt et al., 2008), memory impairments (Brigman et al., 2010; Ge et al., 2010), and reduced levels of anxiety-like behaviors (Barkus et al., 2010; Brigman et al., 2010; von Engelhardt et al., 2008).

Here, we show that *CamKIIαNampt*$^{-/-}$ mice exhibit a specific defect in induction of LTD in the Schaffer collateral pathway. Moreover, *CamKIIαNampt*$^{-/-}$ mice display insensitivity to pharmacological inhibition of synaptic GluN2B-containing NMDARs. These functional changes occur prior to decreased expression of hippocampal NMDARs and in the absence of changes in basal transmission or presynaptic mechanisms. Together, the constellation of phenotypes that we report in *CamKIIαNampt*$^{-/-}$ mice informs our understanding of the effects of NAD^+ depletion, a form of energy deprivation, on synaptic plasticity and function of NMDARs.

2. Materials and methods

2.1. Animals

Mice were bred and maintained as described (Stein et al., 2014). Briefly, *Nampt*$^{flox/flox}$ mice (Rongvaux et al., 2008) were crossed to *CamKIIαCre* mice [The Jackson Laboratory, Stock #005359, T29–1 (Tsien et al., 1996)] to generate *CamKIIαNampt*$^{-/-}$ mice. In all experiments, control mice were age-matched littermates. Since no sex differences were observed, both male and female mice were used. All animal procedures were approved by the Washington University Animal Studies Committee, Division of Comparative Medicine, Washington University School of Medicine, St. Louis, MO, and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised 1996. All efforts were made to minimize the number of animals used and their suffering.

2.2. Reagents

Chemicals were purchased from Sigma or Tocris (St. Louis, MO). Drugs were freshly dissolved in artificial cerebrospinal fluid (ACSF) at the time of experiment and administered by bath perfusion as noted in the text. The concentrations and durations of drug administration were based on prior studies indicating that the agents are effective at altering synaptic transmission or synaptic plasticity as administered.

2.3. Western blotting

Protein was isolated and analyzed as previously described (Stein et al., 2014). Briefly, protein extracts (15–50 μg) were prepared from acutely isolated mouse hippocampi flash frozen in liquid nitrogen, and stored at −80 °C until use. Membranes were incubated with primary antibodies in Tris-buffered saline containing 0.1% Tween 20 (TBST) overnight at 4 °C. Primary antibodies used: Gapdh (1:1000; Millipore, CB1001, 6C5), Nampt (1:3000; Alexis Biochemicals, ALX-804-717-C100, mouse), GluN2B (1:1000; NeuroMab, N59/36, mouse).

2.4. Quantitative real-time RT-PCR

RNA was isolated and analyzed as previously described (Stein et al., 2014). Briefly, mouse hippocampi were flash frozen in liquid nitrogen and stored at −80 °C. Total RNA was isolated from the hippocampus using the RNeasy kit (Qiagen) and reverse-transcribed into cDNA with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Quantitative real-time RT-PCR was conducted with the TaqMan Fast Universal PCR Master mix and appropriate TaqMan primers in the GeneAmp 7500 fast sequence detection system (Applied Biosystems). Relative expression levels were calculated for each gene by normalizing to levels of Gapdh and then to a control.

2.5. Immunohistochemistry

Mice were anesthetized by intraperitoneal injection of ketamine and xylazine, and perfused transcardially through the left ventricle with cold phosphate buffer (0.1 M, pH 7.4) followed by a phosphate-buffered solution of 4% paraformaldehyde (PFA). Brains were postfixed with 4% PFA overnight, equilibrated in 15% sucrose overnight, equilibrated in 30% sucrose overnight, frozen, and stored at −80 °C until sectioning. 30 μm coronal sections in a 1 in 8 series were made by cryostat and stored at −30 °C in cryoprotectant until use. Every eighth section was processed. Tissue sections were incubated in 50% formamide in 2× saline/sodium citrate (SSC) at 65 °C for 2 h and incubated with 3% H_2O_2 for 15 min to remove endogenous peroxidase activity. Tissue sections were incubated in blocking/permeabilization solution containing 10% normal goat serum, 1% bovine serum albumin (BSA), and 0.3% Triton-X in PBS for 45–60 min prior to 24 or 48 h of incubation with primary antibodies in 5% normal goat serum and 0.1% Triton-X in PBS at 4 °C at the following concentrations: Iba1 (1:500; Wako, #019–19741, rabbit), Gfap (1:1000; Millipore, MAB360, mouse), Nampt (1:1000; Alexis Biochemicals ALX-804-717-C100, mouse), GluN2B (1:10; NeuroMab, N59/36, mouse). Antibody specificity was determined by lack of staining after omission of primary or secondary antibodies. Alexa647 (1:200), Alexa488 (1:200), or Cy3 (1:400) conjugated-secondary antibodies (Jackson ImmunoResearch) diluted in 2% normal goat serum, 1% BSA, and 0.1% Triton-X in PBS were added for 2 h at room temperature. Detection of Nampt and GluN2B was performed using the TSA-Plus kit (PerkinElmer, Boston, MA). Nuclei were stained with 4,6-diamidino-2-phenylindole (Sigma) for 10 min at room temperature. High-magnification (20×, 0.8DICII or 40× oil 1.3DICII) microscopic imaging was performed using a Zeiss Axioimager.Z1 or an Olympus NanoZoomer 2.0-HT. Images were taken in z-stacks of 1 μm steps through the range of tissue section immunoreactivity. ImageJ was used to 3D render z-stacks.

2.6. Hippocampal slice physiology

Hippocampal slices were prepared from postnatal day 30 (P30) mice using standard methods (Stein et al., 2014). Mice were anesthetized with isoflurane and decapitated. Right hippocampi were rapidly dissected and placed in ACSF containing (in mM): 124 NaCl, 5 KCl, 2 $MgSO_4$, 2$CaCl_2$, 1.25 NaH_2PO_4, 22 $NaHCO_3$, 10 glucose, gassed with 95% O_2–5% CO_2 at 4–6 °C, and sectioned transversely into 400 μm slices using a rotary slicer. Slices were incubated in gassed ACSF for at least 2 h at 30 °C. Experiments were performed in a submersion-recording chamber at 30 °C with continuous perfusion of ACSF (2 ml/min). Extracellular recordings were obtained from the CA1 apical dendritic region (stratum radiatum) for analysis of field excitatory postsynaptic potentials (fEPSPs) using 2 M