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Neuroinflammation and Proteostasis are Modulated by Endogenously Biosynthesized Neuroprotectin D1

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Abstract Neurodegenerative diseases encompass complex cell signaling disturbances that initially damage neuronal circuits and synapses. Due to multiple protective mechanisms enacted to counteract the onset of neurodegenerative diseases, there is often a prolonged period without noticeable impairments during their initiation. Since severe cognitive deficit or vision loss takes place after that period there is an opportunity to harness endogenous protective mechanisms as potential therapeutic approaches. The activation of the biosynthesis of the docosanoid mediator neuroprotectin D1 (NPD1) is an early response to the upsurge of protein misfolding and other neuroinflammatory events. This overview discusses the potent neuroprotective and inflammation-modulating bioactivity of NPD1. This lipid mediator represents an early response to neurodegenerations, aiming to restore homeostasis.

Keywords Misfolding · Retinal degenerations · Alzheimer's disease · Huntington's disease · Epilepsy · Docosahexaenoic acid · Ataxin-1 · Huntingtin · CAG repeats · APP · Bcl-2 proteins

Introduction

Alzheimer's disease, retinal degenerations, and other neurodegenerative diseases are complex progressive disorders that involve in their pathophysiology multiple signaling dysfunctions that converge on the mitochondria, endoplasmic reticulum stress responses, caspase, and caspase-independent forms of cell damage, all of which lead to synaptic damage and ultimately neuronal cell death [1–4]. Neuroinflammation [5, 6] and protein misfolding [7] are early events in many neurodegenerative diseases. Since the initial stages of these diseases span several years, the identification of the key pathogenic steps as well as potential means to modulate those events are of interest to design protective and/or therapeutic approaches to slow down the initiation and progression of neurodegenerative diseases.

The significance of the selective enrichment in omega-3 essential fatty acids (docosahexaenoyl (DHA) chains of membrane phospholipids, 22C and 6 double bonds) in the nervous system (e.g., synaptic membranes, dendrites and photoreceptors) has remained, until recently, incompletely understood [8–15]. While studying mechanisms of cell survival in neurodegenerations, a docosanoid synthesized from DHA by 15-lipoxygenase-1 was identified [16, 17] and dubbed neuroprotectin D1 (NPD1, 10R,17S-dihydroxydocosa-4Z,7Z,11E,13E,15E,19Z hexaenoic acid). This mediator is a docosanoid because it is derived from a 22C precursor (DHA), unlike eicosanoids, which are derived from the 20C arachidonic acid family of essential fatty acids. Endogenous NPD1 biosynthesis is promptly induced in response to oxidative stress [17, 18], protein misfolding/proteotoxicity [19], seizures [20], brain ischemia reperfusion [16, 21], and by neurotrophins [22]. NPD1 is bioactive in experimental brain damage, oxidative-stressed retinal pigment epithelial (RPE) cells, and in human brain cells exposed to amyloid-β peptide [18]. Thus, NPD1 is a protective sentinel made on demand in early stages of neural injury and one of the very first defenses activated when cell homeostasis is threatened by neurodegenerations [8, 23]. Here, we provide an overview of experimental examples that highlight the specificity and potency of NPD1, spanning beneficial bioactivity during neuroinflammatory and

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proteotoxic events critical during the initiation and early progression of neurodegenerations.

The Expansion of Unstable Translated CAG Ataxin-1 82Q or Huntingtin 72Q Activates Endogenous NPD1 Synthesis

Errors in DNA replication result in pathological, large polyglutamine tracts that impair folding, stability, and bioactivity, often triggering damage, unstable translation, and cell death [23, 24]. The expansion of CAG repeats causes a subset of at least nine neurodegenerative disorders. The expression of ataxin-1 82Q mutants, which cause spinocerebellar ataxia type-1, triggers the endogenous synthesis of NPD1 (Fig. 1). This was ascertained by liquid chromatography–tandem mass spectrometry (LC-MS/MS)-based mediator lipidomics [19]. When huntingtin 72Q was expressed under similar conditions, NPD1 synthesis was also activated [19]. To test the hypothesis that those increases in NPD1 synthesis were protective responses, the addition of NPD1 to singular cultures was explored. The idea to be tested here was that the magnitude of the consequences of expressing the misfolded protein was greater than the protection ability of the endogenously produced NPD1. This possibility was explored by adding exogenous NPD1. Figure 2 displays the remarkable ability of 50 mM NPD1 in exerting anti-apoptotic bioactivity under these conditions. Moreover, when the serpin family growth factor PEDF was added along with 100 mM DHA, protection from apoptosis also took place (Fig. 2). Under these conditions, NPD1 is endogenously made as previously shown under basal conditions [22].

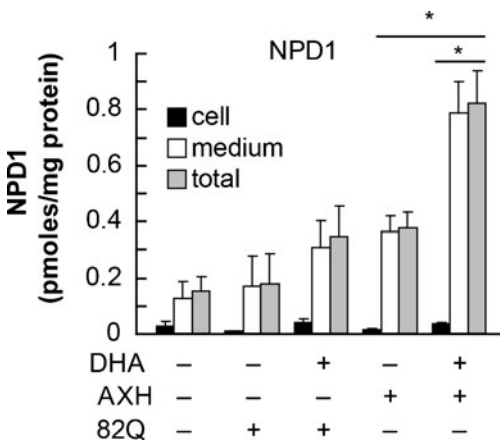

Fig. 1 Endogenous NPD1 biosynthesis is enhanced upon expression of ataxin-1 82Q in RPE cells. Primary human RPE cells were transfected with 82Q. Cells (*black bars*), NPD1 content in media (*white bars*) and total (*gray bars*) were measured by LC MS-MS with or without the addition of DHA. *$p<0.005$ (figure modified and published with permission from [19])

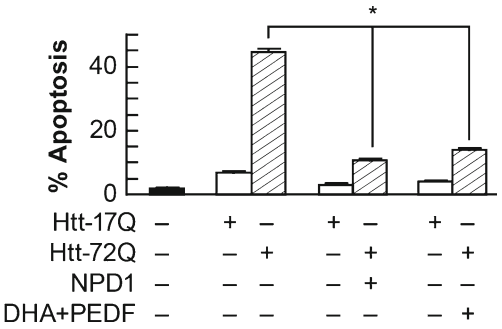

Fig. 2 NPD1 prevents huntingtin-17Q-induced apoptosis in ARPE-19 cells. ARPE-19 cells transfected with an expression construct containing htt-72Q were treated with 50 nM NPD1 or DHA (100 nM) along with PEDF (10 ng/mL). Apoptosis percentage was calculated by dividing pyknotic over the total count of cells. Results are averages±SD. * $p<0.0005$ (figure modified and published with permission from [19])

NPD1-Attenuated Prototoxicity of CAG Repeat-Containing Proteins

NPD1 also decreased phospho-Ser-776 in ataxin-1. We speculate that in agreement with our previous findings that NPD1 may work by increasing PP2A activity. Thus, the lipid mediator may counteract PP2A inhibition, allowing the 82Q form to be de-phosphorylated and cleared or relocated into the spliceosome. The fact that Anp32 was proposed to have a stronger interaction with the expanded form rather than with the wild-type ataxin-1 makes this protein an excellent target candidate for NPD1 signaling. Thus, in addition to the expansions in the polyglutamine tract, AXH has an important role in the functionality of ataxin-1. AXH, a self-folding domain present in ataxin-1, is responsible for the protein–protein interactions between ataxin-1 and other transcription factors, such as the capicua homolog CIC protein. The sequestration of the complex partners formed by ataxin-1 by its inactive counterpart may be involved in the loss of function observed in neurodegenerations. Brother of ataxin-1 (Boat), another member of the AXH domain-containing protein family, is an example of the proposed loss of function. Boat is an in vivo binding partner of ataxin-1 that is also affected by the malfunction of ataxin-1 82Q. Therefore, the expression of AXH alone in our cells resulted in increased apoptosis. Furthermore, AXH expression aggravated the cytotoxicity induced by ataxin-1 82Q. Unlike the sequestration scenario, in which the complexes are formed but are inactive, AXH induces toxicity in this case by increasing disassembly of the complex, thus promoting inactivation of its partners. NPD1 signaling promotes survival by modulating a set of genes that homeostatically control cell fate and regulate proteostasis. NPD1 reversed the toxicity of ataxin-1 82Q as well as of huntingtin 72Q in our cells [19]. Since protein misfolding and proteotoxic stress take place in early stages of several neurodegenerative diseases, we have explored these events as

possible NPD1-targets in cell culture models (human RPE cells and primary neuronal mix cultures). We have studied the expansion of unstable translated CAG repeats that encode polyglutamine tracts that cause spinocerebellar ataxia type 1 and Huntington's disease, which are ataxin-1 poly-Q and huntingtin poly-Q.

Seizure or Experimental Stroke Triggers NPD1 Synthesis and this Docosanoid Attenuates Damage

Epileptogenesis as a model to explore mechanisms that sustain neuronal network integrity under adverse conditions shows that NPD1 is a protective mediator candidate. Using LC-MS/MS-based mediator lipidomic analysis shows that NPD1 synthesis increases during seizures in the hippocampus [20] and that when administered this docosanoid during pharmacologically induced epileptogenesis it elicited a remarkable attenuation of pathological brain oscillations. Moreover, in experimental stroke when DHA is systemically injected it exerts potent protection with concomitant NPD1 synthesis in the penumbra (Fig. 3). This effect reflects modulatory bioactivity of aberrant neuronal networks that lead to spontaneous recurrent seizures. Thus, docosanoid-mediated signaling rescues neuronal network disruptions.

NPD1 is Reduced in Alzheimer's Disease Brains and Redirects APP Processing to Non-Amyloidogenic Pathway

Early stages of AD display a reduced content of NPD1 [25, 26], and 15 lipoxigenase-1 expression is decreased in AD [25]. This enzyme is key for NPD1 synthesis. The silencing of this enzyme in human retinal pigment epithelial cells leads to selective rescue from oxidative stress-induced apoptosis only by NPD1. Since this enzyme catalyzes conversion of arachidonic acid in several eicosanoids, the addition of 12-HETE, 15-HETE, and of

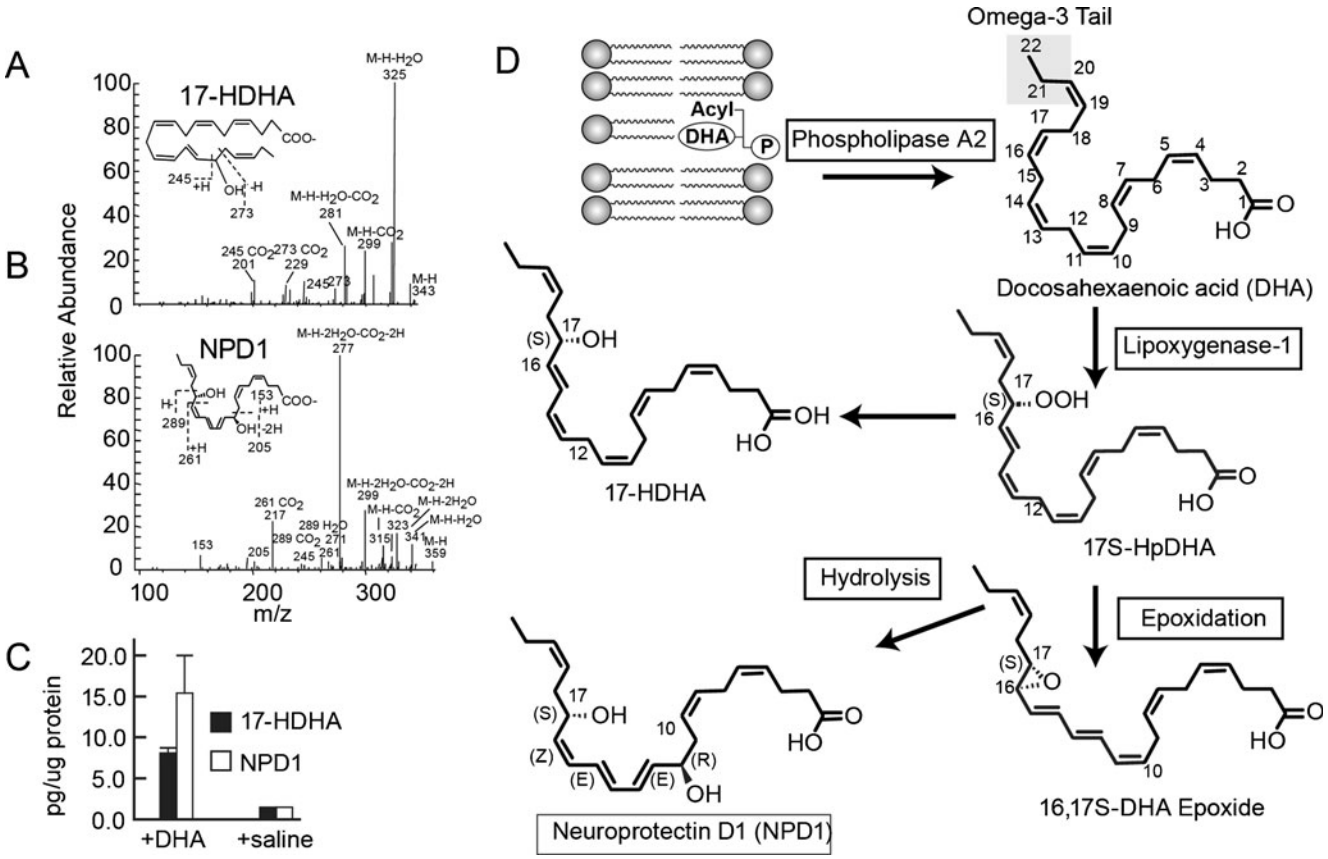

Fig. 3 The characterization and quantification of 17-HDHA and NPD1 in the ipsilateral penumbra 3 days after MCAo. **a**–**b** The fragmentation pattern is depicted and **c** the quantification is presented. The increased content of 17-HDHA and of NPD1 in the ipsilateral penumbra in animals injected with DHA is consistent with the activation of the biosynthesis of NPD1. **d** Enzyme-mediated oxygenation of DHA for the biosynthesis of NPD1. Phospholipase A2 releases DHA from the second C position of phospholipids during brain ischemia-reperfusion. 15-Lipoxygenase-1 catalyzes the synthesis of 17S-H(p)DHA, which in turn is converted to a 16(17)-epoxide and then is enzymatically hydrolyzed to NPD1 (figure modified and published with permission from [33])

protective lipoxin A4 fails to rescue 15 lipoxigenase-1 deficient cells from oxidative stress-induced cell death [27]. We have therefore explored the significance of NPD1 in cellular models that recapitulate part of the Alzheimer's pathology. Human neurons and astrocytes challenged by amyloid-β or by overexpressing APPsw (double Swedish mutation that causes familial forms of the disease) show that NPD1 downregulates amyloidogenic processing of amyloid-β precursor protein (Fig. 4). NPD1 switches off pro-inflammatory gene expression (TNF-α, COX-2, and B-94-TNF-α inducible pro-inflammatory element), and promotes neural cell survival. Moreover, anti-amyloidogenic processing by NPD1 targets α- and β-secretases and peroxisome proliferator-activated receptor γ activation [18]. Currently, we are using imaging matrix-assisted laser desorption/ionisation-time of flight mass spectrometry to further unravel the lipidome in specific brain regions.

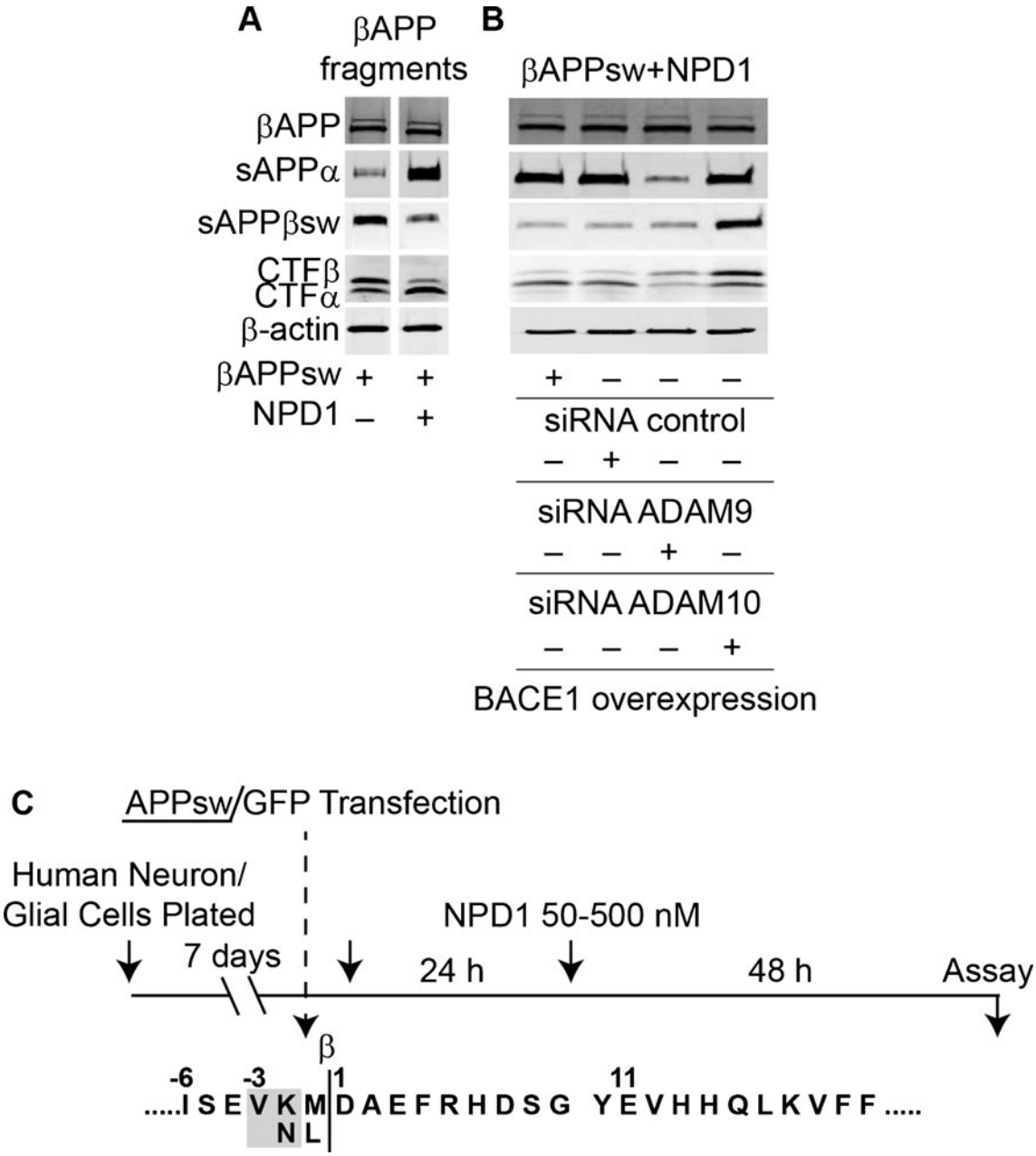

Fig. 4 NPD1 shifts βAAP processing to a non-amyloidogenic pathway. **a** Control or HNG cells over-expressing βAPPsw were treated with increasing concentrations (0, 50, 100, 500 nM) of NPD1 for 48 h and subjected to Western blot detection of holo-βAPP (βAPP holoenzyme), sAPPα, sAPPβsw, CTFα, and CTFβ in comparison to β-actin levels in the same sample. **b** Quantification of gel bands in **a** analyzing βAPP fragments with increasing doses of NPD1. Results are means±SEM (n=4); *p<0.01 vs. βAPPsw control

The Abundance of Anti-Apoptotic BCL-2 Proteins is Positively Modulated by NPD1, whereas Pro-Apoptotic BCL-2 Proteins are Negatively Regulated

The availability of anti-apoptotic BCL-2 proteins is positively modulated by NPD1, whereas pro-apoptotic BCL-2 proteins are negatively regulated, as is microglial activation. NPD1 modulates the protein phosphatase PP2A that targets S62- Bcl-xl. In turn Bcl-xl heterodimerizes with BAX, thus decreasing the availability of this pro-apoptotic BCL-2 protein and leading to cytoprotection [28]. Overall, oxidative stress consequences are attenuated [29, 30]. Moreover, in a model of the wet form of age-related macular degeneration, it was found that NPD1 attenuates choroidal neovascularization [31]. Additionally, microglial activation towards a highly ramified phenotype is induced by NPD1 under these conditions [32].

This cell survival cascade and the events that sustain neuronal network homeostatic integrity involves multiple checkpoints and signaling networks that include restoring proteostasis during protein misfolding/proteotoxicity. NPD1 regulation of upstream events such as cell survival, neuroinflammatory signaling, and transcription in turn promotes homeostatic regulation of synaptic and neural circuitry integrity.

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