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Research Report

# Glutamatergic neurotransmission in a mouse model of Niemann–Pick Type C Disease

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ABSTRACT

Niemann–Pick Type C Disease (NPCD) is a progressive neurodegenerative disorder characterized by accumulation of free cholesterol, sphingomyelin, glycosphingolipids (GSLs) and sphingosine in lysosomes, mainly due to a mutation in the NPC1 gene. One of the main symptoms in NPCD patients is hyperexcitability leading to epileptic activity, however, the pathophysiological basis of this neural disorder is not yet well understood. Here we studied the excitatory neurotransmission in the hippocampus of BALB/c NPC1NIH (NPC1–/–) mice, a well-described animal model of the disease. We report that hippocampal field potential population spike (fPS), as well as paired pulse ratio, is enhanced in NPC1–/– with respect to Wild Type (WT). To evaluate the contribution of glutamate receptor activity in the enhanced fPS observed in mutant mice, we recorded slices treated with glutamate receptor agonists alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and Kainate (KA). We found that a prolonged application of KA and AMPA in NPC1–/– mice do not induce the dramatic decrease of synaptic transmission observed in WT hippocampal slices suggesting a functional impairment of presynaptic KA receptors and an imbalance of AMPA receptor exo/endocytosis. In line with electrophysiological data, we also found notable differences in calcium influx during KA and AMPA bath application in NPC1–/– hippocampal culture as compared with WT. Nevertheless in synaptosomal membranes, Western Blot analysis didn't reveal any modification in protein expression levels of KA and AMPA receptor subunits.

All together these data indicate that in mutant mice the hyperexcitability, that is at the basis of the insurgence of seizures, might be due to the enhanced glutamatergic neurotransmission caused by an altered KA and AMPA receptor functioning.



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## 1. Introduction

Niemann–Pick Type C Disease (NPCD) is an autosomal recessive disorder (1:150,000 of newborns) caused by mutations in the NPC1 gene (~95% of the cases) or NPC2 gene (~5% of the cases). NPC1 gene encodes a transmembrane protein of the acidic compartment and is involved in the movement of cholesterol and lipids within cells. NPC1 has been suggested to facilitate endocytic transport, lysosomal cholesterol and fatty acid efflux (Carstea et al., 1997).

A typical feature of NPCD is the broad range of lipids that accumulate, including sphingomyelin, cholesterol, glycosphingolipids (GSLs) and, unusually, sphingosine (Zervas et al., 2001). This trend, observed in many tissues, leads to compromised liver and spleen function as well as widespread neurological deficits, such as ataxia, dystonia, seizures, and reduced cognitive abilities up to dementia, which are eventually responsible for premature death. The mechanism involved in this lipid storage, as well as how it causes neurodegeneration has yet to be clarified. There is an increasing interest in the effects of NPCD on neuronal function in the brain, which is also due to several discoveries directly and indirectly related to NPCD. Expression of a wild type copy of the NPC1 gene in a central nervous system restricted manner rescued the wasting, ataxia, neurodegeneration, sterility, and early fatality of NPC1-deficient mice (Pentchev et al., 1995). This makes the brain of prime importance in this disorder and points to neurodegeneration as a primary event rather than a consequence of visceral abnormalities. The autonomous nature of the brain pathology is consistent with studies showing that the vast majority of cholesterol synthesis and metabolism in the central nervous system occurs de novo, in the absence of uptake, since plasma lipoproteins, including low density lipoprotein–cholesterol complexes, do not cross the blood–brain-barrier (Turley and Dietschy, 1997).

Cholesterol has been proposed to play an important role in synapse organization and activity, and is crucial to the formation of membrane microdomains, or lipid rafts, thought to be crucial for a variety of cellular functions. Indeed, several human diseases of the nervous system depend on impairment (increase or decrease) of lipid metabolism and transport (Valenza et al., 2007; Vance, 2006). In a previous paper (Frank et al., 2008) we demonstrated that cholesterol depleted neurons exhibit an impaired AMPA/Kainate and NMDA receptor-mediated synaptic transmission and plasticity. Therefore, loss of a correct dynamic of cholesterol–sphingolipids-enriched microdomains, caused by an imbalance in lipid trafficking due to the NPC1 gene mutation, might have a key role in neuronal dysfunction and the consequent clinical pathologies such as seizures.

Aim of this study was to evaluate whether excitatory neurotransmission and glutamate receptor function are impaired in the hippocampus of BALB/c NPC1NIH (NPC1−/−) mice, a well-described animal model of NPCD. Since the hippocampus is one of the main brain region involved in the epileptogenesis, we exploit field potential recordings in hippocampal slices, optical measurements of depolarization-induced calcium responses in hippocampal cell cultures and Western Blot analysis on synaptosomal membranes from Wild Type (WT) and NPC1−/− mice. We found that: i) glutamatergic neurotransmission is enhanced in hippocampal slices from NPC1−/− mice; ii) in NPC1−/− hippocampal neurons KA-induced calcium influx is reduced whereas AMPA-induced calcium influx is increased: iii) KA and AMPA receptor subunit expression is not modified in synaptosomal membranes obtained from NPC1−/− hippocampal slices. Overall these results suggest that the hyperexcitability observed in mutant mice might be ascribed to an altered receptor functioning and/or to a compromised intracellular signaling of glutamate receptors.

## 2. Results

### 2.1. *Hyperexcitability of NPC1−/− hippocampal slices*

We first studied the synaptic properties of NPC1−/− neurons by analyzing basal synaptic transmission (BST), input–output response (I/O) and paired-pulse stimulation (PPS)-induced response in hippocampal CA1 pyramidal layer from NPC1−/− and WT mouse slices. Fig. 1 shows that population spike (PS) amplitude in NPC1−/− slices is significantly higher compared to WT, indicative of an enhanced BST, during 1 hour field potential recording (Figs. 1A–B; WT n=4, NPC1−/− n=4). PS values at time 25 min were respectively 146.21±5.062 vs 106.71±12.01 ($p<0.01$) and at time 50 min 144.77±10.83 vs 111.025±9.329 ($p<0.05$). The I/O analysis did not exhibit any statistical significant difference between the curves of the WT and NPC1−/− slices (not shown). To evaluate whether the enhanced BST in NPC1−/− slices was due to an increased glutamate release, we applied a paired pulse facilitation (PPF) protocol. PPF is a short lasting presynaptic alteration in synaptic efficacy determined by neurotransmitter release. A high PPF ratio indicates a high release of neurotransmitter from presynaptic nerve terminals (Tancredi et al., 2000; Zucker and Regehr, 2002). PPF data were obtained at the beginning of the experiments (at virtual time 5) as also showed by the first traces (Fig. 1C).

The analysis of the PS during stimulation with different interpulse intervals (20–40 ms) indicated a significantly higher PPF ratio in NPC1−/− mouse slices (n=6) as compared to WT (n=8) (Figs. 1C–D), thus indicating an increased neurotransmitter release from NPC1−/− presynaptic nerve terminals. Overall, these data indicate that NPC1−/− mice exhibit a condition of hyperexcitability in synaptic activity.

### 2.2. *Enhanced glutamatergic neurotransmission in NPC1 −/− mice*

Synaptic transmission analysis highlighted an increased excitability in NPC1−/− hippocampal slices. Hence, we evaluated the glutamatergic neurotransmission following glutamate agonist application in NPC1−/− slices as compared with WT. In a first series of experiments, we recorded PS amplitudes in the pyramidal cell layer of the CA1 hippocampal region in the presence of KA (1 and 30 μM for 25 min) and during 30 min of washout. As indicated in Figs. 2A(a–f) and B, 30 μM KA perfusion induced a marked potentiation of BST followed by a complete field potential disappearance in WT slices (n=6); on the contrary, in NPC1−/− slices (n=5) BST slightly increased during the perfusion declining afterwards without never disappearing.