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Relevance of neuronal and glial NPC1 for synaptic input to cerebellar Purkinje cells

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ABSTRACT

Niemann–Pick type C disease is a rare and ultimately fatal lysosomal storage disorder with variable neurologic symptoms. The disease-causing mutations concern NPC1 or NPC2, whose dysfunction entails accumulation of cholesterol in the endosomal–lysosomal system and the selective death of specific neurons, namely cerebellar Purkinje cells. Here, we investigated whether neurodegeneration is preceded by an imbalance of synaptic input to Purkinje cells and whether neuronal or glial absence of NPC1 has different impacts on synapses. To this end, we prepared primary cerebellar cultures from wildtype or NPC1-deficient mice that are glia-free and highly enriched with Purkinje cells. We report that lack of NPC1 in either neurons or glial cells did not affect the excitability of Purkinje cells, the formation of dendrites or their excitatory synaptic activity. However, simultaneous absence of NPC1 from neuronal and glial cells impaired the presynaptic input to Purkinje cells suggesting a cooperative effect of neuronal and glial NPC1 on synapses.

Introduction

Niemann–Pick type C disease (NPC; OMIM #257220) is a rare and ultimately fatal, autosomal recessive lysosomal storage disorder with diverse neurologic symptoms including ataxia and vertical supranuclear gaze palsy (Patterson et al., 2012). The disease is caused by mutations in the genes encoding for Niemann–Pick C1 protein (NPC1) or NPC2, whose dysfunction leads to accumulation of cholesterol and other lipids in the cellular endosomal–lysosomal system (Rosenbaum and Maxfield, 2011; Vance and Peake, 2011). A pathologic hallmark of NPC is the loss of specific types of neurons, namely cerebellar Purkinje cells (PCs), in human patients (Harzer et al., 1978) as well as in mouse (Higashi et al., 1993; Tanaka et al., 1988) and cat models of the disease (March et al., 1997). At present, it is unknown, why NPC1 deficiency causes type-specific neuronal degeneration.

Previous studies reported that NPC1 and NPC2 are located at synapses and in surrounding astrocytic processes (Hu et al., 2000; Karten et al., 2006; Ong et al., 2004; Xu et al., 2011). Neurodegeneration in NPC1-deficient mice and cats was found to start at nerve terminals, axons and dendrites and to progress retrogradely to neuronal somata (March et al., 1997; Ong et al., 2001; Sarna et al., 2003; Zervas et al., 2001). Prominent changes in the levels of different neurotransmitters were observed in the cerebellum of NPC1-deficient mice (Yadid et al., 1998). Together, these observations suggest that NPC1 dysfunction perturbs synaptic activity in PCs (Paul et al., 2004) in a cell-autonomous manner (Ko et al., 2005) or with a contribution from degenerating glial cells (German et al., 2002). To test these hypotheses, we took advantage of a glia- and serum-free cerebellar culture preparation from postnatal mice that is highly enriched with PCs thanks to antibody-based cell selection (Buard et al., 2010). These primary cultures allowed us for the first time to test, whether the lack of NPC1 in neurons or glial cells affects the level of synaptic activity in PCs. Our results show that the absence of NPC1 from either neurons or glial cells left the excitability of PCs, the formation of dendrites or their synaptic activity unaffected, whereas simultaneous NPC1 deficiency in both cell types impaired synaptic input to PCs, possibly at the presynaptic level.

Results

To study the relevance of NPC1 for synaptic activity in cerebellar PCs, we used a serum- and glia-free cerebellar culture preparation from postnatal mice that is enriched with PCs (Buard et al., 2010). The enrichment is accomplished by an immunopanning protocol, which selects first for L1CAM-positive cerebellar neurons and then for Thy1-positive PCs (Buard et al., 2010). The yield of L1CAM-positive cerebellar neurons per NPC1-deficient mouse was reduced

Abbreviations: EPSCs, excitatory postsynaptic currents; NPC, Niemann–Pick type C disease; PCs, Purkinje cells.

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(mean ± SD; 1.01 ± 0.76 × 10^6 cells) compared to cells isolated from wildtype littermates (1.99 ± 1.34 × 10^6 cells; 4 preparations; $p = 0.07$, Wilcoxon matched pairs test), possibly due to a lower number of granule cells. As described previously (Buard et al., 2010), cultures of L1CAM- and Thy1-selected cells comprise granule cells, GABAergic interneurons and PCs, the latter of which can be reliably recognized by the large size of their somata. After one week in defined medium, the neurons formed an extensive network of neurites (Fig. 1A). Cytochemical staining with filipin, a fluorescent antibiotic that binds to unesterified cholesterol, revealed that PCs isolated from mutant mice but not from wildtype animals showed marked intracellular staining (Fig. 1). A similar distribution was observed in primary cultures of glial cells that were prepared from NPC1-deficient mice (Fig. 1). This indicated that cultured PCs and glial cells lacking NPC1 maintain the characteristic intracellular accumulation of cholesterol that they show in vivo (Reid et al., 2004).

We next tested, whether the lack of NPC1 affected the level of synaptic activity in PCs using whole-cell patch-clamp recordings. In the absence of glial cells, PCs from wildtype and mutant mice showed a similarly low level of synaptic activity with inhibitory postsynaptic currents occurring even more rarely than excitatory postsynaptic currents (Figs. 2, 3). Based on our previous finding that glial cells strongly increase synaptic input to PCs (Buard et al., 2010), we cultured PCs with glial cells and tested whether the lack of NPC1 in glial cells affected synaptic activity in PCs. Glial cells with or without NPC1 enhanced significantly the frequency and the size of spontaneous excitatory postsynaptic currents (EPSCs) in wildtype PCs (Fig. 3). In these cocultures, the level of

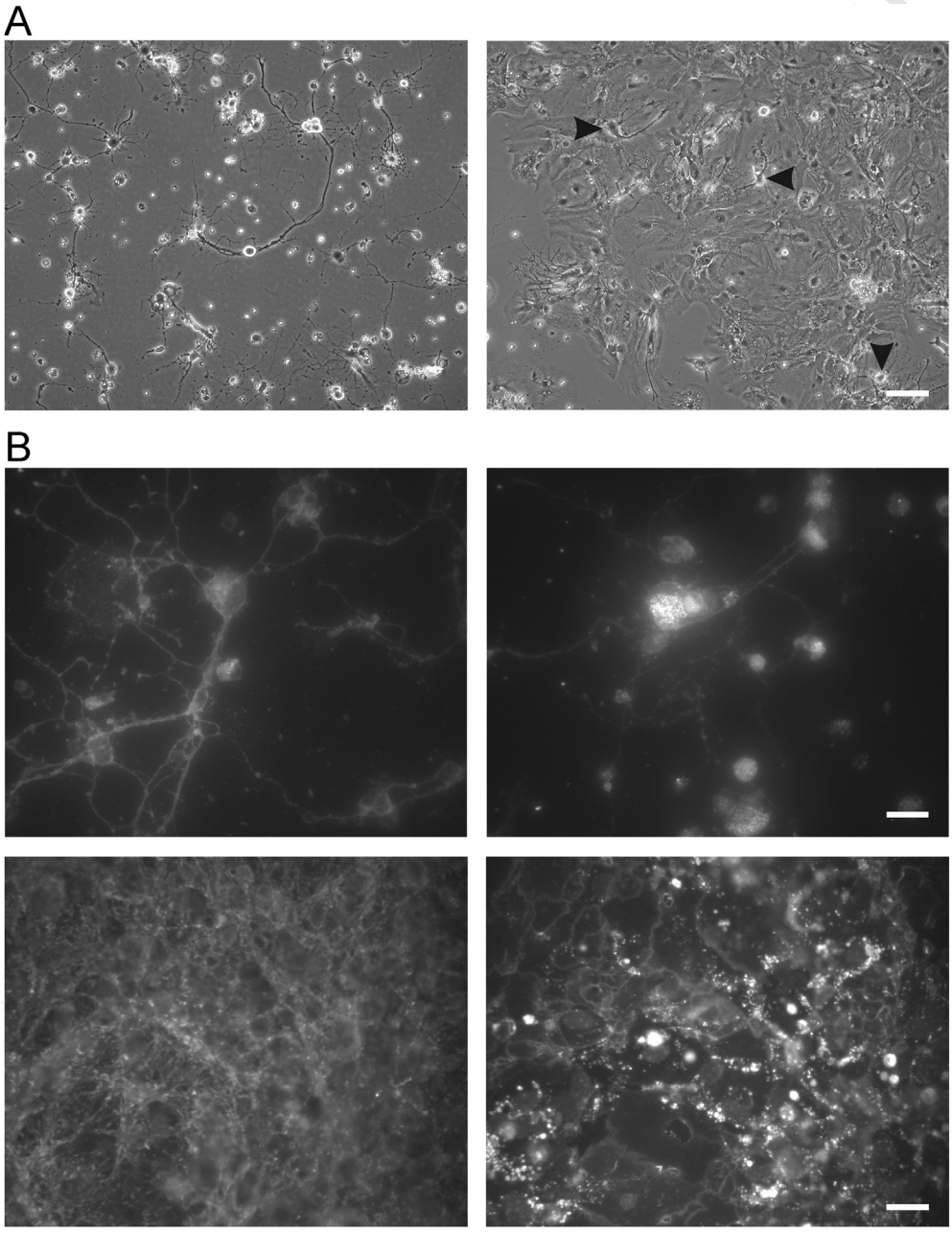

Fig. 1. Intracellular accumulation of cholesterol in cultured Purkinje cells and glial cells from NPC1-deficient mice. A, phase-contrast micrographs of PCs from wildtype mice that were enriched by immunopanning and cultured for seven days in chemically defined medium in the absence (left) or presence (right) of cerebellar glial cells. Black arrowheads indicate somata of PCs growing in coculture. Scale bar: 80 μm. B, Fluorescence micrographs of PCs (top) and glial cells (bottom) from wildtype (left) and mutant (right) mice that were cultured for one week in defined medium and then stained with filipin to reveal the distribution of unesterified cholesterol. Cells from mutant mice show intracellular accumulation of cholesterol. Scale bar top: 20 μm, bottom: 50 μm.

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