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PATHOLOGICAL CHOLESTEROL METABOLISM FAILS TO MODIFY ELECTROPHYSIOLOGICAL PROPERTIES OF AFFLICTED NEURONES IN NIEMANN-PICK DISEASE TYPE C

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Abstract—Niemann-Pick disease type C (NPC) is a recessive inherited neurovisceral lipid storage disease characterized by progressive motor impairment and a loss of neurones including those integrated into the motor system. One of the key neuropathological findings is the intracellular accumulation of lysosomes enriched with free cholesterol. This accumulation is due to impaired transport proteins named NPC1 (approx. 95% of the cases) or NPC2 (approx. 5%) responsible for the transport of endocytosed cholesterol from lysomes to plasma membranes. The perturbed lipid-transport in NPC cells leads to an altered lipid composition of the plasma membrane. Available evidence suggests that the lipid matrix influences the electrophysical properties of ion channels in membranes. We therefore evaluated whether electrophysiological properties of NPC neurones differ from healthy neurones. Both, acute brain slices and primary neuronal cell cultures from wildtype and NPC mice, a well-established mouse model for the Niemann-Pick type C disease, were used for a comparison of electrophysiological properties like resting membrane potential, input resistance, action potential amplitudes and synaptic properties of the neurones. In addition we optically recorded the changes of intraneuronal calcium levels elicited by depolarization. Our results show that the characteristics of ion channels in NPC neurones do not differ significantly from wildtype neurones. We therefore conclude that gross alterations of the electrophysiological properties of neurones will probably not initiate or substantially contribute to the development of the motor impairment or other neurological signs of NPC.

Key words: Niemann-Pick disease type C, neurones, electrophysiology, calcium homeostasis, cholesterol.

Niemann-Pick type C (NPC) is an inherited neurovisceral, lysosomal storage disease and shows a broad clinical spectrum with marked neurological symptoms including ataxia, dysarthria, dysphagia and progressive dementia. The disease originates from mutations in either the *NPC1* or *NPC2* locus (Carstea et al., 1997; Naureckiene et al., 2000). The encoded proteins (NPC-1, NPC-2 or HE1) are normally involved in the intracellular lipid/cholesterol transport, shuttling lipoprotein-derived, endocytosed cholesterol and sphingolipids between late endosomal/lysosomal compartments and plasma membrane. NPC cells of different organs including the brain accumulate lipid-enriched lysosomes. Especially for neurones these accumulations have fatal effects (Zervas et al., 2001). A progressive loss of Purkinje cells in the cerebellum and neurones in the basal ganglia, thalamus and brain stem mark the brain of human cases (Harzer et al., 1978; Elleder et al., 1985). Also cytoskeletal changes have been detected in neurones, resembling neurofibrillary tangles known as an important pathohistological hallmark in Alzheimer's disease (Suzuki et al., 1995). However, although the initial cause for the disease is well known, details about the associated pathophysiology are still lacking. It has been reported that the cholesterol content of the plasma membrane in NPC-cells differs from normal cells, which was explained by a distortion in cellular cholesterol synthesis and uptake (Wojtanik and Liscum, 2003). Cholesterol is an essential lipid compound in the plasma membrane of eucaryotic cells. It determines the fluidity of the membrane by influencing the phospholipid composition (Leppimaki et al., 2000) and saturation of the fatty acid moieties (Ntambi, 1999). Recently it was shown that cholesterol content of membranes influences the properties of ion channels in the plasma membrane, e.g. voltage-gated calcium channels (Lundbaek et al., 1996, 2004; Jennings et al., 1999) and consequently the capacity of cellular responses (Glenson et al., 1991; Sparrow et al., 1999; Mitter et al., 2003; Ohm et al., 2003). The perturbed cholesterol distribution in NPC neurones may thus influence their electrophysiological properties and calcium homeostasis with impact on their function and survival. To test this hypothesis we examined neurones of BALB/cNctr-npc1N (NPC) mice, a well described animal model for the NPC disease (Loftus et al., 1997). Using acute brain slices from NPC and wildtype (WT) animals we performed intracellular recordings with sharp electrodes. Patch clamp analysis and optical measurements of depolarization-induced calcium responses were carried out in dissociated primary neurone cultures from genotyped animals.

EXPERIMENTAL PROCEDURES

Animals

Breeding pairs of BALB/cNctr-npc1N mice were purchased from Jackson Laboratories (Bar Harbor, MA, USA). This strain contains a mutation in the NPC1 locus (Loftus et al., 1997). Animals were

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Abbreviations: ACSF, artificial cerebrospinal fluid; AP, action potential; $(Ca^{2+})_i$, intracellular free calcium concentration; $IPSP_A$, early inhibitory postsynaptic potentials; $IPSP_B$, late inhibitory postsynaptic potentials; NPC, Niemann-Pick disease type C; PBS, phosphate-buffered saline; R_M, input resistance; WT, wildtype.

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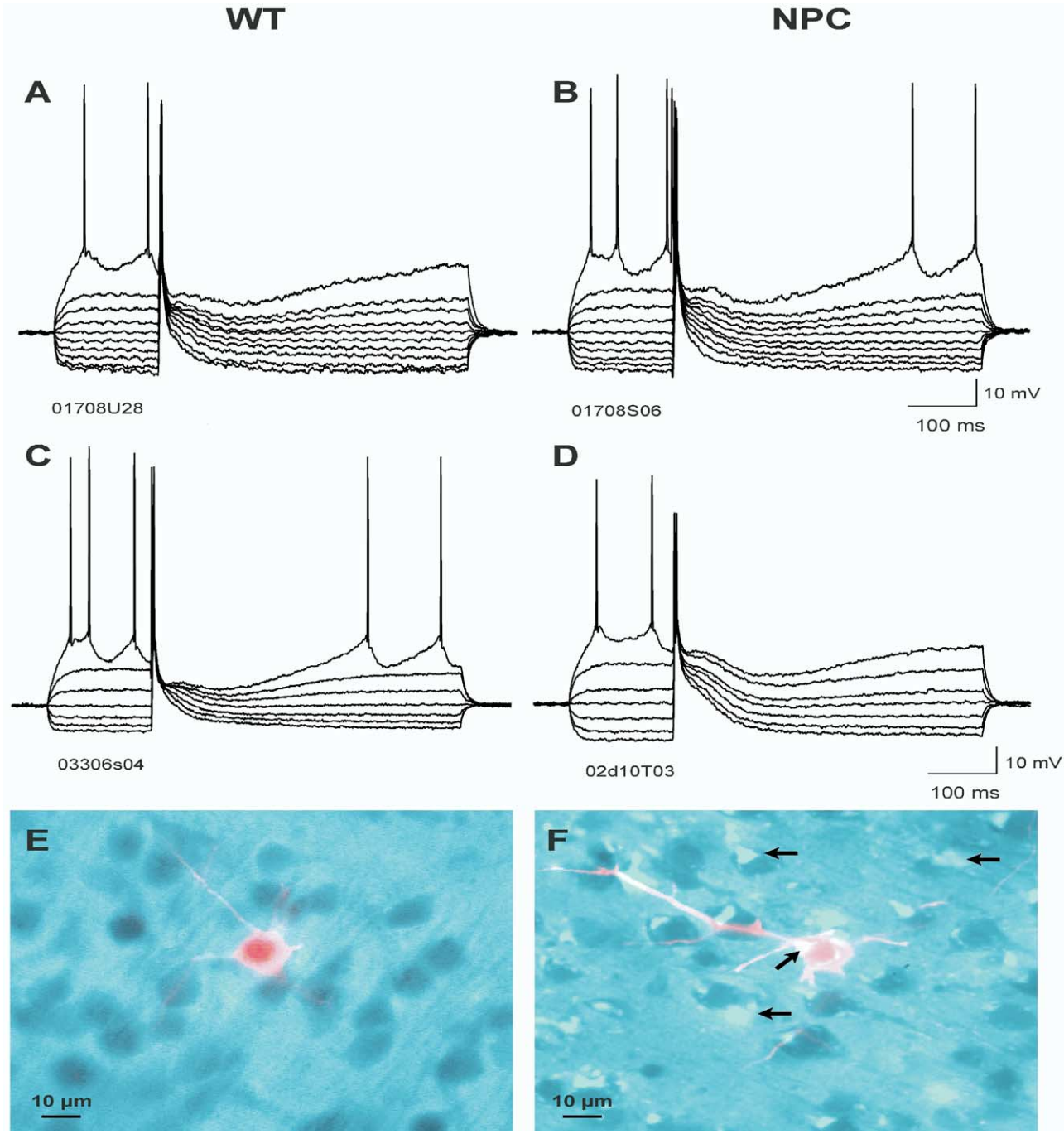

Fig. 1. Electrophysiological characteristics of NPC and WT littermates. Sharp electrode recordings from slices of WT (A, C, E) and NPC animals (B, D, F). (A) Superimposed traces of the membrane potential during injection of families of currents (0.1 nA increment) and synaptic stimulation. This neurone had a resting membrane potential of −74 mV, an AP amplitude of 98 mV and an neuronal R_M of 35 MΩ. The reversal potentials of the $IPSP_A$ and $IPSP_B$ were −65.7 and −78.1 mV, respectively. (B) Superimposed traces of the membrane potential during injection of families of currents (0.1 nA increment) and synaptic stimulation. This neurone had a resting membrane potential of −75 mV, an AP amplitude of 99 mV and an neuronal R_M of 43 MΩ. The reversal potentials of the $IPSP_A$ and $IPSP_B$ were −67.3 and −81.2 mV, respectively. Note, both neurones from WT and NPC slices exhibit the typical regular firing on sufficient depolarization before the stimulus and the two types of inhibition elicited by orthodromic stimulation (first row). A similar behavior is obtained in neurones recorded with biocytin containing electrodes, second row (0.2 nA current injections). The traces shown in the middle row were obtained from the neurones depicted below. The bottom row shows micrographs of recorded and biocytin-filled neurones after staining with avidin–Texas Red and filipin for cholesterol. Note that all NPC neurones show bright filipin fluorescence; inclusions are indicated by arrows (F). Scale bars=10 µm.