ABSTRACT

NEUROMUSCULAR TRANSMISSION IN A NATURALLY OCCURRING MOUSE MUTANT OF THE β SUBUNIT OF THE NEURONAL CALCIUM CHANNEL

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Entry of Ca2+ through voltage gated calcium channels (VGCCs) into nerve terminals is a necessary step coupling the action potential to release of acetylcholine (ACh). VGCCs are heteromultimeric complexes of α1, α2δ, and β subunits, and sometimes γ subunits. The specific α1-β combination assembled determines the channel properties. The mouse mutant lethargic (lh) has severe neurological defects due to a mutation that deletes α1 subunit interaction domain of the β4 subunit. β4 normally associates with the α1A subunit of the P/Q-type VGCCs, and has a major role in stabilizing the final α1A subunit conformation and targeting it to the cell membrane. Loss of the β4 subunit could alter the channel characteristics and localization of α1A. The overall goal of this dissertation was to test the hypothesis that disruption of the β4 subunit affects the function of the α1A subunit of the P/Q-type VGCCs.

Electrophysiological recordings were performed at neuromuscular junctions (NMJs) of adult lh and wild type (wt) mice. The quantal content and phrenic nerve evoked release showed a significant decrease in lh with respect to wt. The frequency of spontaneous release of ACh also decreased significantly, although the reduction was only evident when Ca2+ was replaced by Sr2+ or Ba2+ as charge carriers. The amplitude of spontaneous release was not affected by this mutation, implying that each vesicle contains approximately the same amount of ACh in wt and lh mice. These results are due

to a significantly slower process of neurotransmitter vesicles release, as confirmed by FM1-43 staining method.

There are specific VGCCs antagonists that can be used to determine the contribution of the different types of VGCCs in nerve-stimulated ACh release from motor nerve terminals. ω-agatoxin IVA and SNX-482, specific antagonists for P/Q- and R-type VGCCs respectively, significantly reduced the quantal content in adult lh mice. Immunolabeling of VGCC subunits revealed an increase in α1E, β1 and β3, but no apparent change in the levels of α1A at adult lh neuromuscular junctions. Therefore, lh animals control ACh release by P/Q- and R-type VGCCs.

The studies of this dissertation provide evidence for: 1) decreased nerve-evoked ACh release in lh mice, 2) slowed vesicle release process in lh mice, 3) increased level of β1 and β3, compensating for the lack of β4 subunit, and 4) P/Q- and R-type VGCC involvement in release of ACh from motor nerve terminals.