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MAPK/ERK-dependent Munc18-1 phosphorylation in the neuromuscular junction

FINAL DEGREE PROJECT

Biochemistry and Molecular Biology

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This project has been made based on the results obtained in External Practices at the Unitat d'Histologia I Neurobiologia (UHNEUROB) of the Universitat Rovira I Virgili in Reus, under the supervision of the senior researcher Dr. Maria Angel Lanuza Escolano and the doctoral student Aleksandra Polishchuk.

Abstract

The release of Acetylcholine (ACh) in the neuromuscular junction (NMJ) is a tightly regulated process to ensure a balanced neurotransmission and muscle contraction, as their impairment could lead to neuromuscular disorders. Munc18-1, which is phosphorylated in Ser^{241} by mitogen-activated protein kinase/extracellular-regulated kinase (MAPK/ERK), is a presynaptic protein key in the regulation of the synaptic exocytosis. However, the effect of the phosphorylation of Munc18-1 in Ser^{241} by MAPK/ERK in the release of the ACh in the NMJ remains unknown. In addition, M_2 muscarinic acetylcholine receptor (mAChR) is involved in the activation of MAPK/ERK and, at the NMJ, located in the presynaptic membrane of the nerve terminal, downregulates the ACh release. However, the relation between M_2 and MAPK/ERK at the NMJ has not been studied. The aim of this project is to determine whether the neuromuscular activity regulates the MAPK/ERK pathway in the nerve terminal, and the role of M_2 muscarinic acetylcholine receptor (mAChR) in this regulation. To study the effect of synaptic activity, it was stimulated the phrenic nerve (1Hz, 30min) with or without contraction (abolished by μ-conotoxin GIIIB). Specific inhibitory reagent methoctramine was used to block M_2 mAChRs in diaphragm muscle. The main results obtained from Western blot experiments showed that (1) Presynaptic activity decreases MAPK/ERK-dependent Munc18-1 phosphorylation whereas nerve-induced contraction promotes it; and (2) MAPK/ERK activity and Munc18-1 phosphorylation is modulated through M_2 mAChR in the NMJ. In summary, the results provide evidence that MAPK/ERK-dependent Munc18-1 phosphorylation is differently regulated during presynaptic activity and nerve-induced contraction in the NMJ, and this modulation is performed through the M_2 mAChR.