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Research Report

# Rab3A deletion selectively reduces spontaneous neurotransmitter release at the mouse neuromuscular synapse

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ABSTRACT

Rab3A is a synaptic vesicle-associated GTP-binding protein thought to be involved in modulation of presynaptic transmitter release through regulation of vesicle trafficking and membrane fusion. Electrophysiological studies at central nervous system synapses of Rab3A null-mutant mice have indicated that nerve stimulation-evoked transmitter release and its short- and long-term modulation are partly dependent on Rab3A, whereas spontaneous uniquantal release is completely independent of it. Here, we studied the acetylcholine (ACh) release at the neuromuscular junction (NMJ) of diaphragm and soleus muscles from Rab3A-deficient mice with intracellular microelectrode methods. Surprisingly, we found 20–40% reduction of spontaneous ACh release but completely intact nerve action potential-evoked release at both high- and low-rate stimulation and during recovery from intense release. The ACh release induced by hypertonic medium was also unchanged, indicating that the pool of vesicles for immediate release is unaltered at the Rab3A-deficient NMJ. These results indicate a selective role of Rab3A in spontaneous transmitter release at the NMJ which cannot or only partly be taken over by the closely related Rab3B, Rab3C, or Rab3D isoforms when Rab3A is deleted. It has been hypothesized that Rab3A mutation underlies human presynaptic myasthenic syndromes, in which severely reduced nerve action potential-evoked ACh release at the NMJ causes paralysis. Our observation that Rab3A deletion does not reduce evoked ACh release at any stimulation rate at the mouse NMJ, argues against this hypothesis.



## 1. Introduction

Exocytosis of neurotransmitter from synaptic vesicles involves a complex interplay of many presynaptic proteins that regulate vesicle transport, docking, priming, fusion, and recycling (for a review, see Lin and Scheller, 2000; Sudhof, 2004). Many of these processes are subject to modulation, tuning synaptic signaling to ensure proper neuronal network function. Rab3A seems one important modulating factor and is a member of the Rab family of GTP-binding proteins with a general function in intracellular trafficking (for a review, see Darchen and Goud, 2000). It is thought that the Rab3A, Rab3B,

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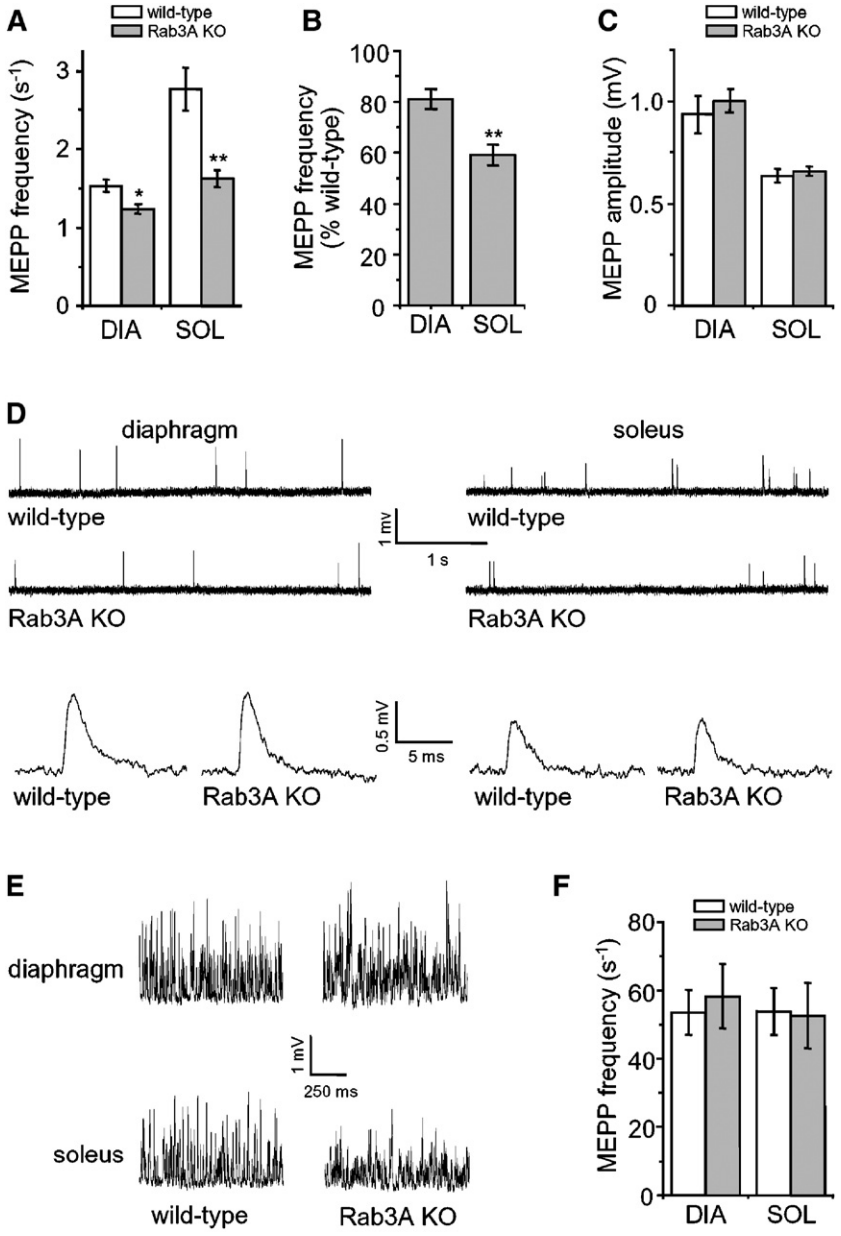


**Fig. 1 – Deletion of Rab3A reduces spontaneous transmitter release at mouse diaphragm and soleus NMJs. (A) Mean MEPP frequency at NMJs from diaphragm (DIA) and soleus (SOL) from wild-type and Rab3A KO mice. (B) The reduction of MEPP frequency is more pronounced at soleus than at diaphragm Rab3A KO NMJs. (C) No difference in MEPP amplitude between Rab3A KO and wild-type NMJs of either diaphragm or soleus muscles. (D) Typical examples of 4-s recording traces of MEPPs, at diaphragm (left) and soleus (right) NMJs. Lower traces are typical examples of individual MEPPs. (E) Typical examples of 1-s recording traces of MEPPs measured in the presence of hypertonic medium (0.5 M sucrose-Ringer). (F) No difference in mean values of MEPP frequency measured in the presence of 0.5 M sucrose-Ringer. Data obtained from $n$ = 5–6 mice, 10–15 NMJs per muscle. Error bars represent SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$.**

Rab3C, and Rab3D subfamily, of which Rab3A is the most abundant in the brain (Geppert et al., 1994), plays a role in mammalian exocytosis. Rab3A is a GTP/GDP-binding protein which, in the GTP-state, is associated with the synaptic vesicle membrane but becomes detached upon GTP-to-GDP hydrolysis during or shortly after the exocytotic event (Fischer von Mollard et al., 1991, 1994; Star et al., 2005). So far, three putative GTP-Rab3-binding partners have been identified, rabphilin (Shirataki et al., 1993), Rab-interacting molecule (RIM) 1α/2α (Wang et al., 2000), and synapsin-I (Giovedi et al., 2004).

Transgenic deletion in mice showed that Rab3A is not of crucial importance for nervous system function because these knockout (KO) mice are viable and do not show overt behavioral abnormalities (Geppert et al., 1994). Detailed analyses of mutant and KO Rab3A mice, however, revealed mild anomalies in exploration behavior and sleep (D'Adamo et al., 2004; Kapfhamer et al., 2002). Electrophysiological analyses