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, is partly dependent on Rab3A, while spontaneous uniquantal release is completely independent of it. Here, we studied with intracellular microelectrode methods the acetylcholine (ACh) release at the neuromuscular junction (NMJ) of diaphragm and soleus muscles from Rab3A deficient mice. Surprisingly, we found 20-40% reduction of spontaneous ACh release, measured as miniature endplate potential frequency, but completely intact nerve action potential-evoked release at either high- or low-rate stimulation or during recovery from intense release. ACh release induced by hypertonic medium or α-latrotoxin was also unchanged, indicating that the pool of vesicles that is immediately available for release is unaltered at the Rab3A deficient NMJ. These results indicate a selective role of Rab3A in spontaneous transmitter release at the NMJ for which the closely related Rab3B, -C, or -D isoforms apparently do not, or incompletely, compensate 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 the mouse NMJ, argues against this hypothesis.

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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 review, see Lin and Scheller, 2000; Sudhof, 2004). Many of these processes are subjected 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 traffic (for review, see Darchen and Goud, 2000). It is thought that the Rab3A, B, C and D 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; Fischer von Mollard et al., 1994; Star et al., 2005). So far, two putative GTP-Rab3 binding partners have been demonstrated, rabphilin (Shirataki et al., 1993) and Rab Interacting Molecule (RIM) 1α/2α (Wang et al., 2000).

Transgenic deletion in mice showed that Rab3A is not of crucial importance for nervous system function, since these knock-out (KO) mice are viable and do not show overt behavioural abnormalities (Geppert et al., 1994). Detailed analyses of mutant and KO Rab3A mice, however, revealed mild anomalies in exploration behaviour and sleep (Kapfhamer et al., 2002; D’Adamo et al., 2004). Electrophysiological analyses of cultured and brain-slice hippocampal synapses from Rab3A-deficient mice suggested a modulatory role for Rab3A, selective for evoked but not spontaneous release, by showing unchanged or increased initial release, increased paired-pulse facilitation, and a more pronounced rundown at high-rate stimulation, compared to wild-type (Geppert et al., 1994; Geppert et al., 1997). Rab3A-D proteins appear to act mutually compensatory since each single KO mouse is viable and fertile, but the Rab3A-D quadruple KO mouse shows postnatal lethality (Schluter et al., 2004). Analysis of transmitter release at embryonic autaptic cultures from Rab3A-D quadruple deficient hippocampal neurons showed unchanged spontaneous release properties (Schluter et al., 2004), confirming the finding of unchanged spontaneous release at Rab3A single KO synapses (Geppert et al., 1997). However, an about 30% decrease in evoked transmitter release was revealed, resulting from a decrease in release probability (Schluter et al., 2004). From K⁺-evoked transmitter release studies at Rab3A KO synaptosomes it appeared that recruitment of synaptic vesicles to active zones after vigorous stimulation is impaired (Leenders et al., 2001).

The modulatory role of Rab3A on evoked transmitter release seems to differ amongst synapse types, as illustrated by different effects of Rab3A deletion on long-term potentiation of synaptic strength in different types of hippocampal excitatory
synapses (Geppert et al., 1994; Castillo et al., 2002). Possibly, differential expression of compensatory Rab3B-D proteins is of importance here.

Here, we investigate a possible role of Rab3A on the release of ACh from motor nerve terminals at the neuromuscular junction (NMJ). It is of importance to understand the roles of neuro-exocytotic proteins at the neuromuscular junction (NMJ). At this peripheral synapse, transmitter release is tightly regulated, in order to maintain successful contraction of muscle fibres, which is crucial for the organism. Defective function of Rab3A and other presynaptic proteins may underlie human congenital presynaptic myasthenic syndromes, where reduced ACh release leads to paralysis (Maselli et al., 2001; Maselli et al., 2003a), alike the auto-immune Lambert-Eaton myasthenic syndrome where presynaptic Ca\textsubscript{2+} channels are targeted by auto-antibodies (Kim and Neher, 1988).

Rab3A is localized at transmitter release sites of the mammalian presynaptic NMJ (Mizoguchi et al., 1992), as are many other proteins that have been shown to be involved in exocytosis at central synapses. Previously, we demonstrated an identical silencing effect of Munc18-1 deletion on central synapses and the NMJ (Verhage et al., 2000). However, absence of specific presynaptic proteins does not always yield identical effects at central synapses and the NMJ. For instance, recent analysis of NMJ function in double KO Munc13-1/2 embryos contrasted the finding of completely silenced central synapses (Varoqueaux et al., 2002). A severely reduced but not completely blocked evoked transmitter release was found at the NMJ, together with intact spontaneous release (Varoqueaux et al., 2005).

With intracellular microelectrode methods we measured ACh release at NMJs of nerve-muscle preparations from diaphragm and soleus muscles from Rab3A KO mice. We found 20-40% reduction of the spontaneous ACh release, but unchanged nerve action potential-evoked release at low- as well as high-rate stimulation frequencies, and normal release induced by hypertonic medium or α-latrotoxin. These results suggest a selective role of Rab3A in spontaneous transmitter release at the NMJ for which Rab3B, -C, or -D do not, or incompletely, compensate when Rab3A is deleted. Our study shows that Rab3A deficiency does not endanger successful neuromuscular transmission, leaving the Rab3A gene as an unlikely mutation candidate for causing human congenital presynaptic myasthenic syndromes.

Materials and methods

Mice

The generation and genotyping of the Rab3A KO mice has been described previously (Geppert et al., 1994). Mice were housed under a 12 h light / 12 h dark regime and ad libitum food and water. About 3 months-old animals were killed by CO\textsubscript{2} inhalation, and hemi-diaphragms and soleus muscles with their nerves were rapidly dissected and mounted in Ringer’s solution (see below) at room temperature. All
animal experiments were carried out in agreement with the Dutch law and Leiden University guidelines.

**NMJ electrophysiology**

Intracellular voltage measurements were performed on diaphragm and soleus nerve/muscle preparations from Rab3A KO mice and littermate wild-type controls, pinned out on the bottom of a silicone rubber-lined dish in Ringer's medium containing (mM): NaCl, 116; KCl, 4.5; MgCl$_2$, 1; CaCl$_2$, 2; NaH$_2$PO$_4$, 1; NaHCO$_3$, 23; glucose 11; pH 7.4, gassed with 95 % O$_2$/5 % CO$_2$ at 26-28°C. The nerve was electrically stimulated through either a bipolar electrode (diaphragm) or a suction electrode (soleus) with supramaximal pulses (100 μs duration) from a Master-8 programmable stimulus generator (AMPI, Jerusalem, Israel). Upon stimulation, the nerve terminal synchronously releases a number of ACh quanta that stimulate postsynaptic ACh receptors and thus cause an endplate potential (EPP). Spontaneous release of single quanta results in miniature EPPs (MEPPs). We recorded EPPs and MEPPs by impaling muscle fibres near the NMJ with a 10-20 MΩ glass capillary microelectrode filled with 3 M KCl and connected to a GeneClamp 500B amplifier (Axon Instruments/Molecular Devices, Union City, CA, USA) for amplifying and filtering (10 kHz low-pass) of the signal. Signals were digitized, stored and analyzed (off-line) using a Digidata 1200 interface, Clampex 8.0 and Clampfit 8.0 programs (all from Axon Instruments/Molecular Devices) and routines programmed in Matlab (The MathWorks Inc., Natick, MA, USA). Muscles were incubated with 3.1 μM μ-Conotoxin GIIIB (Scientific Marketing Associates, Herts, UK), a selective blocker of muscle sodium channels, to prevent muscle action potentials. This allowed for the undisturbed recording of EPPs during electrical nerve stimulation (0.3, 20, 30, 40 and 50 Hz). The amplitudes of EPPs and MEPPs were normalized to –75 mV, assuming 0 mV as the reversal potential for ACh-induced current (Magleby and Stevens, 1972), using the formula: $EPP_{normalized} = EPP \times (-75/V_m)$, where $V_m$ is the measured resting membrane potential. The quantal content (i.e. the number of ACh quanta released upon a single nerve impulse) at each NMJ at 0.3 Hz stimulation was calculated by dividing the normalized EPP by the normalized MEPP amplitude. The EPP amplitude was first corrected for non-linear summation, as described before (Plomp et al., 1994). During high-frequent stimulation, EPP amplitudes decrease to a plateau level. This rundown level was determined by calculating the average amplitude of the last 10 EPPs in a 40 Hz train of 1s duration and expressing it as percentage of the amplitude of the first EPP of the train.

MEPPs were also recorded shortly after exposure of preparations to hypertonic medium (0.5 M sucrose Ringer), assessing the pool of ACh vesicles ready for immediate release (Stevens and Tsujimoto, 1995). Furthermore, MEPPs were recorded after application of 2.5 nM α-latrotoxin (Alomone Laboratories, Jerusalem, Israel).
Statistical analyses
The data is given as mean ± S.E.M, with n representing the number of muscles tested. At least 30 MEPPs and EPPs were recorded at each NMJ. From the mean NMJ values, a muscle mean value was calculated (at least 10 NMJs were sampled per muscle per experimental condition), which was used to calculate a genotype group mean value. Four to six mice were used per genotype, from each mouse one dia-phragm and one soleus muscle was analyzed. Possible differences between the mean values of genotype groups were tested with Student’s t-test and were considered statistically significant if the p-value was <0.05.

Results
Spontaneous ACh release is reduced at Rab3A deficient NMJs
Spontaneous ACh release at diaphragm NMJs from Rab3A KO mice and wild-type littermates was electrophysiologically determined by recording MEPPs, the spontaneous uniquantal release events. At diaphragm NMJs, a decrease of 19% in MEPP frequency was observed in Rab3A KO NMJs, compared to wild-type (1.24 ± 0.06 s⁻¹ and 1.54 ± 0.08 s⁻¹, respectively, n=6 mice, p<0.05, Figure 1A). The amplitude of the MEPPs was similar for both genotypes (0.94 ± 0.09 mV at wild-type, and 1.00 ± 0.06 mV at Rab3A KO NMJs, n=6, p=0.5, Figure 1C, D). Similarly, the MEPP kinetics (rise times, decay times and half-widths) were unchanged (data not shown, Figure 1D), indicating that there were no changes in postsynaptic ACh receptor function due to the Rab3A deficiency. The diaphragm muscle consists of a mixed population of fast- and slow-twitch fibres, which are known to have different NMJ characteristics (Bewick, 2003). To investigate whether Rab3A deletion has differential effects on slow-twitch fibres we also investigated spontaneous ACh release at NMJs of the soleus muscle, which exclusively consists of slow-twitch fibres. Again, we found a decreased MEPP frequency (~40%) in Rab3A KO NMJs compared to wild-type (1.63 ± 0.11 s⁻¹ and 2.77 ± 0.28 s⁻¹, respectively; n=6, p<0.001, Figure 1A). This decrease was more pronounced than that observed at diaphragm NMJs (p<0.01, Figure 1B). MEPP kinetics as well as amplitudes (~0.65 mV, n=6, p=0.6, Figure 1C, D) were again not different from wild-type. Thus, in contrast to central synapses (Hughes et al., 1999), Rab3A deletion reduces spontaneous transmitter release at NMJs, in particular those of slow-twitch muscles.

Unaltered size of readily releasable vesicle pool
The observed reduction in spontaneous ACh release may be caused by a reduction of the number of transmitter vesicles that are ready for release. In order to probe for this vesicle pool (Stevens and Tsujimoto, 1995) we added hypertonic medium (0.5 M sucrose-Ringer) and measured the resulting elevated MEPP frequency. No
difference between genotypes was observed (diaphragm: 53.6 ± 6.5 s⁻¹ at wild-type and 58.3 ± 9.3 s⁻¹ at Rab3A KO NMJs, n=5-6 mice, p=0.68; soleus: 53.9 ± 6.9 s⁻¹ at wild-type and 52.8 ± 9.6 s⁻¹ at Rab3A KO NMJs, n=5 mice, p=0.61, Figure 1E, F).

Figure 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.
This result suggests unaltered size of the readily releasable vesicle pool at Rab3A KO NMJs.

**Unchanged nerve action potential-evoked ACh release**

We measured ACh release at diaphragm NMJs evoked by low-frequent (0.3 Hz) electrical stimulation of the phrenic nerve (Figure 2A). EPP amplitudes of wild-type and Rab3A KO NMJs were similar (25.4 ± 1.4 and 26.2 ± 0.9 mV, respectively, n=6 mice, p=0.6). The calculated quantal content, the number of ACh quanta re-
Figure 3. No differences in high-rate nerve stimulation-evoked ACh release between Rab3A KO and wild-type NMJs.

Equal EPP rundown level (mean value of the 21-35th EPP in the train, expressed as percentage of the amplitude of the first EPP), upon 20, 30, 40 or 50 Hz nerve stimulation at Rab3A KO and wild-type NMJs from diaphragm (A) or soleus (B) muscles. Data obtained from n=5-6 mice, 10-15 NMJs per muscle. Averaged 40 Hz EPP rundown profiles from diaphragm (C) or soleus (D) muscles, with examples of individual EPP trains as insets. Kinetics and level of recovery form EPP rundown was equal for Rab3A and wild-type NMJs of diaphragm (E) and soleus (F) NMJs, as assessed with a protocol applying single nerve stimuli at 1 or 5 s intervals.
leased upon nerve stimulation was similar (40.9 ± 2.1 at wild-type, and 38.9 ± 1.8 at Rab3A KO NMJs, n=6 mice, p=0.5). At soleus muscle NMJs, we found a somewhat decreased (23%) quantal content at Rab3A KO NMJs, but this reduction was not statistically significant (p=0.06, Figure 2B). The EPP amplitudes were slightly smaller in Rab3A KO NMJs, but again this difference was not statistically significant (26.7 ± 1.5 mV in wild-type, and 22.6 ± 1.6 mV in Rab3A KO NMJs, n=5-6 mice, p=0.09, Figure 2B). These results indicate that low-rate evoked release is not dependent on Rab3A presence.

In view of the more pronounced rundown of evoked transmitter release at high-rate stimulation at central synapses (Geppert et al., 1994), we studied the ACh release at NMJs upon 1 s trains of 20, 30, 40 and 50 Hz stimulation. At all stimulation frequencies, EPP amplitudes ran down to a similar level in both Rab3A KO and wild-type diaphragm NMJs, with EPP amplitudes at the plateau phase of the stimulus train being about 80-85% of the first EPP in the trains (p=0.48-0.70, n=6 mice, Figure 2C, E). Similarly, at soleus NMJs, high-rate stimulation resulted in equal EPP run-down level in both genotypes for all tested frequencies (to about 72-78%, of the first EPP in the trains, p=0.30-0.61, n=5-6 mice, Figure 3A-D). Thus, Rab3A deletion does not affect high-rate evoked ACh release at NMJs.

Normal recovery from high-rate ACh release

Rab3A may be important for replenishment of the readily releasable vesicle pool after intense transmitter release. Therefore, we monitored the recovery of the EPP amplitude after a 2 s train of 40 Hz stimuli, by giving single stimuli with intervals of either 1 or 5 s, starting 1 s after the end of the 40 Hz train. No differences in recovery rates were observed between Rab3A and wild-type NMJs, of either diaphragm or soleus NMJs (Figure 3E).

α-Latrophotoxin-induced transmitter release

As an alternative means to evoke transmitter release, we applied 2.5 nM α-latrophotoxin to diaphragm NMJs. This toxin binds to presynaptic receptors and evokes Ca²⁺-dependent and -independent transmitter release by insertion of ion-pores and stimulation of secondary messengers (Ushkaryov et al., 2004), resulting in high frequency MEPPs at the NMJ. However, the MEPP frequency was not statistically significantly different between genotypes, 120.8 ± 23.4 s⁻¹ in wild-type NMJs, compared to 88.3 ± 13.9 s⁻¹ in Rab3A NMJs (n=4-5 mice, p=0.25).

Discussion

We have examined the effect of Rab3A deficiency on ACh release at the NMJ and found that spontaneous uniquantal release, but not the release evoked by either low- or high-rate electrical stimulation, hypertonic medium or α-latrophotoxin, is reduced.
These results indicate that Rab3A has a specific role in the mechanism underlying spontaneous transmitter release at the NMJ, which is not compensated for by other Rab3 family members. The absence of effects on evoked ACh release indicates that Rab3A is either not involved in the underlying mechanism, or that other Rab3 family members fully compensate for its loss. Together these results show that Rab3A deletion does not endanger successful neuromuscular transmission.

The present finding of reduced spontaneous ACh release at NMJs due to Rab3A deletion is surprising in view of the central synapse studies (on primary cultured Rab3A-deficient hippocampal neurons) that showed unaltered frequency of spontaneous synaptic events (Geppert et al., 1997; Schluter et al., 2004). Although developmental reasons cannot be ruled out (the central studies used embryonic material, while we studied NMJs from adult mice), the discrepancy most likely follows from more fundamental differences between hippocampal synapses and NMJs. It may be that expression of Rab3B, C, and D isoforms is lower at the NMJ, so that their compensatory action upon Rab3A deletion is less pronounced. Alternatively, the specific composition of the neuro-exocytotic release machinery at the NMJ may differ from hippocampal synapses, in that it is more dependent on Rab3A for spontaneous release. For instance, the Rab3A binding partners rabphilin and/or RIM1α may be relative highly expressed at NMJs. About half of the spontaneous uniquantal ACh release at the mammalian NMJ is caused by Ca\(^{2+}\) influx via Ca\(_{\text{v}2.1}\) channels, which apparently undergo some opening at resting membrane potential (Plomp et al., 2000; Kaja et al., 2005). Rab3A might have some (indirect) stimulatory effect on the opening of such channels. In this respect it is of interest that NMJs of some Ca\(_{\text{v}2.1}\)-mutant mice show altered spontaneous ACh release without having altered low-rate evoked ACh release (Plomp et al., 2000; Kaja et al., 2004; Kaja et al., 2005).

The similar response of Rab3A KO and wild-type NMJs to α-latrotoxin and hypertonic medium application indicate that it is not likely that the reduced MEPP-frequency in Rab3A KO NMJs results from a decrease in the number of vesicles in the readily releasable vesicle pool. Unchanged vesicle pool-size was also found at Rab3A KO hippocampal synapses (Geppert et al., 1997).

Postsynaptic MEPPs caused by spontaneous uniquantal ACh release at NMJs do not influence the postsynaptic response to nerve stimulation-evoked release (EPPs), nor do they trigger muscle contraction by themselves (Wood and Slater, 2001). Thus, the selective reduction of spontaneous uniquantal ACh release at Rab3A deficient NMJs does not acutely endanger the successful transmission of impulses at the NMJ. However, it may have indirect effects in the longer term. At central synapses, miniature events have recently been shown to inhibit postsynaptic protein synthesis (Sutton et al., 2004). If a similar mechanism exists at the NMJ, reduced spontaneous release at Rab3A KO NMJs would lead to upregulated postsynaptic protein synthesis, possibly affecting ACh receptor expression or homeostatic
mechanisms regulating presynaptic transmitter release via retrograde messengers (Plomp et al., 1992). For hippocampal synapses it has been hypothesized that transmitter quanta for spontaneous and nerve stimulation-evoked release originate from functionally distinct vesicle pools (Sara et al., 2005), although findings at another synapse seem not in agreement (Lou et al., 2005). If, however, such separate pools would also exist at NMJs, it might be speculated on the basis of the present results that Rab3A is selectively involved in trafficking of the vesicle pool that fuels spontaneous ACh release at the NMJ.

The reduction of spontaneous ACh release in Rab3A KO mice was more pronounced at soleus than at diaphragm NMJs. This may be related to the difference in muscle fibre types: the soleus is a slow-twitch muscle, while the diaphragm is a muscle with mixed slow- and fast-twitch fibre composition (Prakash et al., 1996; Bewick, 2003). NMJs on slow- and fast-twitch fibres differ in structure and function, those on slow fibers being less elongated, more fatigue-resistant, and having a lower initial ACh release level (Bewick, 2003). These differences are probably related to differences in stimulation frequency and history of use, possibly affecting local expression of neuro-exocytotic proteins. It may be that Rab3A (as well as Rab3B, C, and D) expression level is differentially regulated at motor nerve terminals on different muscle fibre types, leading to the observed difference in magnitude of the reduction of spontaneous ACh release. In a recent study we showed that neuro-exocytotic proteins other than Rab3A also have differential roles at NMJs of different fibre types: in mice deficient for α-neurexins we found differential reducing effects on ACh release at soleus and diaphragm NMJs (Sons et al., 2006).

Low-rate nerve stimulation-evoked ACh release was unaltered at Rab3A deficient NMJs. Again, as for spontaneous release, this finding seems at variance with data obtained from central synapses from Rab3A KO mice. In hippocampal synapses, basal electrical stimulation-evoked release was found unchanged (brain slices, Geppert et al., 1994) or increased (cultured neurons, Geppert et al., 1997). Furthermore, at the Rab3A KO NMJ we did not observe increased rundown of transmitter release upon repetitive stimulation as observed at hippocampal synapses (Geppert et al., 1994). We excluded a role for Rab3A in recovery from high-rate evoked release by showing that EPP amplitudes of wild-type and Rab3A NMJs returned with similar time-course to similar levels after a 2 s 40 Hz stimulus train. Similarly, recovery from hypertonic stimulation-induced transmitter release was shown unchanged at hippocampal synapses (Geppert et al., 1997), indicating that Rab3A is not generally involved in recovery of the readily releasable vesicle pool and evoked transmitter release. However, a role of Rab3A in vesicle trafficking upon recovery of K+ depolarization-evoked transmitter release has been proposed in a study of Rab3A deficient forebrain synaptosomes (Leenders et al., 2001).
ACh release at diaphragm NMJs from Rab3A KO mice has been studied to some extent by Hirsh and colleagues (Hirsh et al., 2002). In that study, spontaneous ACh release was found reduced by ~30%, although this change was not statistically significant. Furthermore, it was reported that low-rate evoked ACh release was unchanged, which we confirmed in the present study. However, a less pronounced rundown of EPPs was shown at 50 Hz nerve stimulation at Rab3A deficient NMJs, which we could not reproduce at any of the high-rate stimulation frequencies used in the current study. One reason for this apparent discrepancy may be that Hirsh et al. in their experiments used d-tubocurarine to reduce the EPPs to subthreshold levels in order to prevent the triggering of muscle action potentials. Besides blocking postsynaptic ACh receptors, d-tubocurarine is known to have a presynaptic effect, possibly mediated by nicotinergic autoreceptors, resulting in increased EPP rundown (Glavinovic, 1979). The reduced EPP rundown at Rab3A deficient NMJs, compared to wild-type, in the presence of d-tubocurarine in the experiments of Hirsh et al. may indicate that Rab3A is in some way involved in the mechanism underlying this presynaptic effect of d-tubocurarine. However, in our experiments using μ-conotoxin as a tool to eliminate muscle action potentials (which has no presynaptic effects and does not interfere with ACh receptors) we failed to observe any effect of Rab3A deletion on EPP rundown. This indicates that increased EPP rundown is not a physiological feature of the Rab3A deficient NMJ.

The present study argues against the hypothesis that Rab3A mutation could underlie paralysis in human congenital presynaptic myasthenic syndromes. Rab3A mice do not show symptoms of muscle weakness, which is in agreement with the lack of effects we observed on nerve stimulation-evoked ACh release at their NMJs. Furthermore, mutational analyses have not yielded any mutation or polymorphism in the Rab3A gene in patients with congenital presynaptic myasthenic syndromes (Maselli et al., 2001; Maselli et al., 2003a). Thus far, only some episodic ataxia type-2 associated mutations in CACNA1A, the gene encoding presynaptic Ca\(_{\text{v}}\)2.1 channels, have been shown to cause presynaptic dysfunction at human NMJs (Maselli et al., 2003b).

Taken together, the present study shows that the synaptic phenotype resulting from Rab3A deletion at the NMJ clearly differs from that observed at central synapses, especially with respect to spontaneous transmitter release, adding further evidence to the view that Rab3A has distinct roles at different synapse types (Castillo et al., 2002; Sudhof, 2004).

References


