Further evidence for the role of IP\textsubscript{3}R\textsubscript{1} in regulating subsynaptic gene expression and neuromuscular transmission

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Abbreviations: IP\textsubscript{3}, inositol 1,4,5-triphosphate; IP\textsubscript{3}R\textsubscript{1}, inositol 1,4,5-triphosphate receptor 1; INPP5a, type I inositol 1,4,5-trisphosphate 5-phosphatase; CTB, cholera toxin \(\beta\) subunit; \(\alpha\)BT, \(\alpha\)bungarotoxin; ER, endoplasmic reticulum; ACh, acetylcholine; AChR, acetylcholine receptor; MEPC, miniature end plate current; siRNA, small interference RNA; ctRNA, control RNA; TA, anterior tibialis; 2-APB, 2-aminoethoxydiphenyl borate

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The inositol 1,4,5-triphosphate IP\textsubscript{3}R channel is highly expressed on specialized ER membrane, where it initiates a slow wave of Ca\textsuperscript{2+} release from internal stores, allowing subcellular compartmentalization of Ca\textsuperscript{2+} signals. In skeletal muscle, IP\textsubscript{3}R\textsubscript{1} is also highly concentrated at some myonuclei, particularly near the NMJ. We have reported that in fully developed adult muscle, IP\textsubscript{3}R\textsubscript{1} knockdown by siRNA increases synaptic strength at both pre- and postsynaptic sites of neuromuscular transmission, increasing both the amplitude and frequency of spontaneous quantal events and quantal content, as well as expression of AChR subunits and other NMJ-specific genes. Here, we demonstrate that reducing IP\textsubscript{3}R\textsubscript{1} activity in mouse TA muscle by promoting hydrolysis locally of IP\textsubscript{3}R\textsubscript{1} also amplifies expression of subsynaptic genes and transcription factors. Furthermore, using a retrograde tracer, cholera toxin \(\beta\) subunit, we find that siRNA-mediated silencing of IP\textsubscript{3}R\textsubscript{1} in TA muscle increases vesicle trafficking. These studies suggest that postsynaptic IP\textsubscript{3}R\textsubscript{1} activity regulates synaptic gene expression and neuromuscular transmission.

Internal Ca\textsuperscript{2+} release plays an important role in early synapse formation, neuronal death and neurodegeneration.\textsuperscript{1} The IP\textsubscript{3}R\textsubscript{1} has emerged as a central molecule in mediating neurodegeneration and in underlying some genetic forms of neurodegenerative disease.\textsuperscript{2} Previously, we found that focal iontophoresis of ACh near dissociated, fura-2-loaded muscle fibers induces localized, IP\textsubscript{3}R\textsubscript{1}-dependent Ca\textsuperscript{2+} increases in NMJ to either normal or pathological levels.\textsuperscript{3} Moreover, the discrete localization of IP\textsubscript{3}R\textsubscript{1} near AChRs and in postsynaptic areas surrounding NMJ nuclei is striking, and suggests a role of IP\textsubscript{3}R\textsubscript{1}-mediated internal Ca\textsuperscript{2+} release in synaptic gene expression and synaptic physiology.\textsuperscript{4} We have recently discovered that several developmental events, changes in gene expression and control of homeostatic plasticity at the NMJ, are dependent on the presence of intact functioning IP\textsubscript{3}R\textsubscript{1}. The same IP\textsubscript{3}R\textsubscript{1} channel molecule plays a key role in amplifying pathological Ca\textsuperscript{2+} elevations arising from excessive cholinergic activity.\textsuperscript{5}

Our studies showed that reducing IP\textsubscript{3}R\textsubscript{1}-mediated Ca\textsuperscript{2+} release by siRNA knockdown in C2C12 cells decreases calpain activity and prevents agonist-induced AChR cluster dispersal.\textsuperscript{1} In adult mice, selective knockdown of IP\textsubscript{3}R\textsubscript{1} in TA muscle regulates expression of AChR subunits, other NMJ-specific genes and transcription factors in a pattern resembling muscle denervation.\textsuperscript{1} Moreover, in normal and diseased muscle, siRNA-mediated IP\textsubscript{3}R\textsubscript{1} silencing in TA muscle effectively increases presynaptic neuro-muscular transmission by amplifying MEPC frequency and quantal content.\textsuperscript{1}

Confocal immunofluorescence microscopy shows that the IP\textsubscript{3}R\textsubscript{1}-selective siRNA eliminates NMJ-localized IP\textsubscript{3}R\textsubscript{1}. Here, we report additional data that further highlight the importance of postsynaptic IP\textsubscript{3}R\textsubscript{1} activity in regulation of AChR subunit expression and presynaptic vesicle trafficking in NMJ. The membrane-associated enzyme, INPP5a, hydrolyses IP\textsubscript{3}, and abolishes IP\textsubscript{3}R\textsubscript{1} signaling.\textsuperscript{6}\textsuperscript{5} We selectively reduced muscle IP\textsubscript{3} levels by overexpressing a plasmid encoding INPP5a in mouse TA muscle, and...
In comparison to siRNA-mediated IP3 R1-a-syntrophin, rapsyn, MUSK, and ErBb2, increased expression of the AChR subunit logic blockade of IP3 R with 2-APB on with IP3 R1 knockdown and pharmaco-

a similar profile to that of IP3 R1 silencing (to be involved in NMJ gene expression genes, and the transcription factors known to be involved in NMJ gene expression (Fig. 1). Results for IP3 reduction showed a similar profile to that of IP3R silencing in its effects on gene expression (Fig. 1A and B). Prevention of IP3 production increased expression of the AChR subunit genes, and the genes expressing AChE, a-syntrophin, rapsyn, MyoK, and E1B2. In comparison to siRNA-mediated IP3R silencing, IP3 reduction also produced qualitatively similar effects on the a and b subunits of the AChR, the observed increases in the expression of E1B2, myogenin, HDAC4 and GABPRx, as well as reduced expression of ColQ2 and Dach2 (Fig. 1C). These data suggest that both treatments act through the same pathways to affect muscle gene expression (Fig. 1).

We previously showed that IP3R silencing in muscle amplified quantal content, as well as spontaneous and evoked transmitter release, presumably by reducing a Ca2+-mediated retrograde signal that mediates homeostatic plasticity. Here, we provide evidence that the effect of IP3R silencing on homeostatic plasticity occurs through a change in vesicle dynamics. Since changes in transmitter vesicle release may be tightly coupled to changes in vesicle trafficking, we tested whether a reduction in IP3R-dependent Ca2+ release caused by IP3R silencing would mimic the effect on vesicle trafficking induced by cholinoergic blockade in NMJ. We used nBT as a blocking agent to affect a gross reduction of cholinoergic input in TA muscle, a treatment previously shown to lead to increased MECF frequency and quantal content. To demonstrate the effect on vesicle trafficking we used the retrograde marker, the B subunit of cholera toxin (CTB), which, unlike the styryl dyes such as FM-143, allows characterization of vesicle dynamics during prolonged treatments in living animals in vivo. When directly injected into TA muscle, CTB is transported trans-synaptically as a result of binding to GM1-gangliosides present on the nerve terminals and transported retrograde to motor neuron soma. We find that 12 h after CTB injection this tracer was readily detected in the synaptic nerves of mice treated with nBT (1 µg/day; 6 d) intraperitoneally, but not in saline-injected mice. CTB protein levels remain higher in nBT-treated mice than in control mice in the sciatic nerve at 18 h, but are at comparable levels as control at 24 h (Fig. 2A and B). Similarly, nBT causes a significant increase in the number of motor neurons labeled with CTB at 12 and 18 h (Fig. 2C) when compared with saline-treated control mice. The difference is transient, and when compared at 24 h, there is no difference in motor neuron labeling between saline-treated and nBT-treated mice, suggesting that the effect of nBT is to accelerate the initial rate of vesicle recycling. We then tested whether the effect of cholinoergic blockade by nBT on vesicle trafficking could be reproduced by IP3R knockdown in TA muscle. Nine days after siRNA or control (cRNA) treatment of TA muscles the same muscles were injected directly with CTB, and the expression level of tracer was followed in the sciatic nerve and lumbar motor neurons examined 12, 18 and 24 h after injection. We found that CTB protein was readily detected in the sciatic nerve innervating TA muscles treated with siRNA compared with those treated with cRNA at 12 and 18 h. After 24 h, there was no difference in CTB levels in the sciatic nerve between siRNA and cRNA (Fig. 2A and B). In the spinal cord, CTB-labeled motor neurons were first detected on the side innervating the siRNA-treated TA, 12 h after injection of CTB, compared with the cRNA-treated TA that had no CTB labeling of motor neurons at this time (Fig. 2C). At 18 h, the proportion of CTB-labeled motor neurons on the siRNA-treated side was nearly double the control-treated side. By 24 h, CTB labeling of siRNA- and control-treated sides were nearly identical (Fig. 2A and B) when compared with either treatment alone. By 24 h, there was no difference in CTB levels in the sciatic nerve between siRNA and cRNA (Fig. 2A and B).

Figure 1. IP3R activity controls synaptic gene expression in muscle. Expression levels (k = normalized to 0 time expression) of NMJ genes i.e., AChR (alpha) and gamma subunits (A); AChR (delta), ADR (e subunit (epison), acetylcholinesterase, the AChR collagen tail (ColQ), a-syntrophin, utrophin, EDB2, MUSK and rapsyn (B) and transcription-related proteins (C)) in TA muscle were measured after treatment for 9 d with IP3R knockdown (siRNA-9d), INPP5a-expressing plasmid (INPP5a-9d), or 2-APB intraperitoneally (2-APB-9d). These data show that these three modes of IP3R antagonism have comparable effects on expression of NMJ genes, although IP3R1 knockdown is the most effective. n = 5; *p < 0.01; **p < 0.001; 2-APB, IP3R blocker, 2-aminoethoxydiphenyl borate.
These results extend our previous studies and further demonstrate that the presence of the postsynaptic muscle IP$_3$R channel plays a central role in amplifying Ca$^{2+}$ signals that regulate both expression of subcellular genes and transcription factors, as well as the postsynaptic component of neuromuscular transmission. Increased quantal content and frequency results from an effect on postsynaptic vesicle trafficking at NMJ, presumably elicited via retrograde signaling.1-4,16

Materials and Methods

Mouse INPP5a cDNA, purchased from Open Biosystems (Thermo Fisher Scientific), was cloned into pEGRF-N1 (Clontech). tBT (1 mg/ml; Tocris Bioscience), in saline, was injected intra-peritoneally at a dose of 0.5 μg/day for 6 d.19 CTB (1 μg/μl; List Laboratory) diluted in saline, was injected into the left TA. The right TA was treated with DMSO or saline (0.9% w/v; USP, Lake Forest, US) as control. Antibodies recognizing CTB (1:500 dilution) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:5000 dilution) were obtained from Abcam and Ambion, respectively. Methods for quantitative PCR, intramuscular electroporation-mediated gene delivery, western blotting, tissue staining and imaging, quantitative studies in tissue sections and statistics were described in our previous publication.1

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References

1. Zhu H, Maricich CM. Skeletal muscular IP$_3$R receptors amplify physiological and pathological synaptic calcium signals. J Neurosci. 2011; 31(26):9935-45. PMID:21601873; http://dx.doi.org/10.1523/JNEUROSCI.3766-11.2011
2. van de Leemput J, Chandran J, Knight MA, Holtzclaw LA, Scholz S, Cookson MR, et al. Deletion at ITPR1 underlies ataxia in mice and spinocerebellar ataxia 15 in humans. PLoS Genet. 2007; 3:e108; PMID:17590087; http://dx.doi.org/10.1371/journal.pgen.0030108
3. Bezprozvanny I. Role of inositol 1,4,5-trisphosphate receptors in the neuromuscular junction in skeletal muscle. J Physiol 1992; 458:487-99; PMID:1302275
4. Powell JA, Molgó J, Adams DS, Colasante C, Williams LA, List Laboratory. Increased IP$_3$R1 receptors amplify physiological and pathological synaptic calcium signals in muscle fibers and Ca$^{2+}$ overload underlies ataxia in mice and spinocerebellar ataxia 15 in humans. PLoS Genet 2007; 3:e108; PMID:17590087; http://dx.doi.org/10.1371/journal.pgen.0030108
5. Zeleznik-Ray JJ, Gomez CM. Increased IP$_3$R1-trisphosphate receptor mediated activity in slow-channel syndrome. J CeReS. 2007; 43:343-52. PMID:17975294; http://dx.doi.org/10.1002/jcrs.2006.07.007
6. Laxminarayan KM, Chan BK, Tetaz T, Bird PI, Plomp JJ, van Kempen GT, Molenaar PC. The action of inositol 1,4,5-trisphosphate receptor in pathogenesis of Huntington’s disease and spinocerebellar ataxia. Neurotox Res. 2011; 36:1186-97; PMID:21210219; http://dx.doi.org/10.1007/s00011-010-0393-y
7. Pepple JJ, van Kempen GT, Molenaar PC. Adaptation of spinal cord to decreased postsynaptic sensitivity at single synapses in alpha-bungarotoxin-treated rats. J Neurophysiol. 1992; 68:467-95; PMID:13522775
8. Pepple JJ, van Kempen GT, Molenaar PC. The expression of actin-related protein 1a in alpha-bungarotoxin-treated rats. J Neurophysiol. 1992; 68:467-95; PMID:13522775
9. Bezprozvanny I. Role of inositol 1,4,5-trisphosphate receptor in pathogenesis of Huntington’s disease and spinocerebellar ataxia. Neurotox Res. 2011; 36:1186-97; PMID:21210219; http://dx.doi.org/10.1007/s00011-010-0393-y
10. Domet MA, Walsh CE, Wilson DF. Impact of alpha-bungarotoxin on transmitter release at the neuromuscular junction of the rat. Neurosci Lett 1995; 199: 49-52; PMID:7656206; http://dx.doi.org/10.1016/0304-3940(95)01254-1

11. Van der Kloot W. Loading and recycling of synaptic vesicles in the Torpedo electric organ and the vertebrate neuromuscular junction. Prog Neurobiol 2003; 73: 269-303; PMID:14698765; http://dx.doi.org/10.1016/S0301-0536(03)00089-3

12. Aman AT, Fraser S, Mestecky EA, Rodighiero C, Kennny M, Alto M, et al. A mutant cholera toxin B subunit that binds GM1 ganglioside but lacks immunomodulatory or toxic activity. Proc Natl Acad Sci U S A 2001; 98:8536-41; PMID:11447291; http://dx.doi.org/10.1073/pnas.161273098

13. Garfield MA, Bonf BJ. Imaging synaptic vesicle exocytosis and endocytosis with FM dyes. Nat Protoc 2004; 1:2916-21; PMID:17406552; http://dx.doi.org/10.1038/nprot.2006.476

14. Highlight AP, McGhee RD, Foster KD, Palmer JR, Hens S, Goldstein CS. Retrograde control of synaptic transmission by postsynaptic CAMKII at the Drosophila neuromuscular junction. Neuron 2003; 39:255-67; PMID:12873083; http://dx.doi.org/10.1016/S0896-6273(03)00627-4

15. Grozinger J, Spencer MJ, Bhattacharya BJ, Radziejewski E, Vohra BP, Zanin R, et al. Calcium activation impairs neurotransmitter transmission in a mouse model of the slow-channel myasthenic syndrome. J Clin Invest 2007; 117:2903-12; PMID:17653947; http://dx.doi.org/10.1126/jci283383

16. Micheva KD, Buchanan J, Holz RW, Smith SJ. Retrograde regulation of synaptic vesicle endocytosis and recycling. Nat Neuroscience 2003; 6:935-42; PMID:12891824; http://dx.doi.org/10.1038/nn1114

17. Holland RL, Brown MD. Postsynaptic transmission block can cause terminal sprouting of a motor nerve. Science 1980; 207:649-51; PMID:6243417; http://dx.doi.org/10.1126/science.6243417