Neuroscience Letters 711 (2019) 134434

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Review article

Nicotinic acetylcholine receptor at vertebrate motor endplates: Endocytosis, recycling, and degradation

Rüdiger Rudolf[a,b,c,*], Tatjana Straka[a,b,c]

[a] Institute of Molecular and Cell Biology, Mannheim University of Applied Sciences, Mannheim, Germany
[b] Interdisciplinary Center for Neurosciences, Heidelberg University, Heidelberg, Germany
[c] Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany

ARTICLE INFO

Keywords:
Acetylcholine receptor
Autophagy
Endocytosis
Endplate
Neuromuscular junction
Recycling

ABSTRACT

At vertebrate motor endplates, the conversion of nerve impulses into muscle contraction is initiated by binding of acetylcholine to its nicotinic receptor (nAChR) at the postsynapse. Efficiency and safety of this process are dependent on proper localization, density, and molecular composition of the receptors. To warrant this, intricate machineries regulating the turnover of nAChR are in place. They control and execute the processes of i) expression, ii) delivery to the postsynaptic membrane, iii) clustering at the plasma membrane, iv) endocytic retrieval, v) activity-dependent recycling, and vi) degradation of nAChR. Concentrating on aspects iv-vi, this review addresses the current status of techniques, concepts, and open questions on endocytosis, recycling, and degradation of nAChR. A picture is emerging, that shows connections between executing machineries and their regulators. The first group includes the actin cytoskeleton, myosin motor proteins, Rab G-proteins, and the autophagic cascade. The second group features protein kinases A and C, Cdk5, and CaMKII as well as other components like the E3-ligase MuRF1 and the membrane shaping regulator, SH3GLB1. Recent studies have started to shed light onto nerve inputs that appear to master the tuning of the postsynaptic protein trafficking apparatus and the expression of critical components for nAChR turnover.

1. Introduction

The nAChR is the major ligand-gated ion channel at vertebrate neuromuscular junctions (NMJ). It mediates muscle contraction via Na^+ influx upon binding of acetylcholine, released from α-motoneurons [1]. nAChR accumulate at specific sites of the postsynaptic membrane and normally, nAChR clusters are organized in characteristic patterns, that have been defined as plaque-, pretzel-, or grape-like. In neuromuscular disorders or upon aging, nAChR levels or distribution at the NMJ are often altered [2–4]. NMJs are formed in the unborn with a nAChR subunit composition $\alpha_2\beta\gamma\delta$. During a perinatal period, γ-subunits are replaced by ε-subunits with different electrophysiological properties. This replacement is well studied in mouse where it occurs during the early postnatal phase [5]. A transcriptional switch from ε to γ and vice versa is observed upon denervation and re-innervation of NMJs, respectively [6–8]. In all these occasions, i.e. during development and de/reinnervation cycles, also changes in nAChR lifetime occur. While in normal adult NMJs, nAChR shows a half-life of several days, denervation dramatically shortens this to only 1–2 days [9–14]. Such fast turnover is also typical for the early postnatal phase [15,16]. Thus, regulation of nAChR turnover appears to be of principal importance in physiological and pathological processes, justifying a closer look onto it. Over the past decades, components, machineries, and signaling pathways involved in nAChR endocytosis, recycling, and degradation have been unraveled, for which the major available literature was summarized in this review. Agrin-induced clustering of nAChR and factors enhancing or reducing it, were topics of recent excellent reviews [17,18] and are not covered here. We apologize for studies that could not be mentioned due to space restrictions.

2. Framework for nAChR turnover studies

nAChR turnover has been extensively studied in the last decades. However, this would have been impossible without knowledge from neighboring fields, particularly, on cell biology and technological advancements. Indeed, the work of Claude, de Duve, and Palade massively contributed to both. Their studies laid the foundations of the secretory pathway, which is used by nAChR, and led to several principal methods, including electron microscopy, radioautography, pulse/chase labeling, and cell fractionation (reviewed in [19–21]). Together with

* Corresponding author at: Institute of Molecular and Cell Biology, Mannheim University of Applied Sciences, Mannheim, Germany.
E-mail address: r.rudolf@hs-mannheim.de (R. Rudolf).

https://doi.org/10.1016/j.neulet.2019.134434
Received 8 July 2019; Received in revised form 8 August 2019; Accepted 14 August 2019
Available online 14 August 2019

the introduction of α-bungarotoxin [22,23] and its derivatives (reviewed in [24,25]), which have served as efficient tools to identify or isolate nAChR, the scene was set in the mid-1970s to address nAChR trafficking. Live animal imaging [26,27] combined with radioactive or fluorescence pulse/chase analysis and genetic or pharmacological interventions were instrumental for the understanding of kinetic and molecular aspects of nAChR turnover [10,11,28–34]. Finally, genetically encoded molecular biosensors further refined the insights into the molecular machineries for nAChR trafficking [35–42].

3. Endocytosis

Reduced nAChR lifetime and binding of ^{125}I-bungarotoxin at NMJs from myasthenic patients delivered first hints on the regulation of nAChR density at NMJs [43,44]. Ultrastructural analysis of muscles labeled with ^{125}I-bungarotoxin [45] or peroxidase-conjugated α-bungarotoxin [46–48] confirmed the localization of nAChR in endo/lysosomal vesicles. It has remained unclear, which type of endocytosis is used by nAChR. Work from C2C12 cells suggested that it occurs via lipid raft-mediated processes [49,50]. Conversely, crosslinking experiments in frog muscle cell cultures pointed to clathrin- and caveolin-dependent pathways [51]. Anyhow, live-imaging of mouse muscles upon injection with fluorescent α-bungarotoxin yielded small steady-state numbers of nAChR-positive endocytic vesicles, typically below 10 per NMJ [41,42]. Overexpression of Rab5 or its hyperactive mutants increased this number, suggesting that Rab5 is involved in nAChR endocytosis. This assumption was corroborated by the finding of puncta double-positive for Rab5-GFP and α-bungarotoxin as well as co-precipitation of Rab5 with α-bungarotoxin-biotin labeled preparations [41].

4. Recycling

Starting in 2005, a series of reports on nAChR trafficking used an elegant fluorescence-based pulse/chase-like labeling assay [28,29,31–33,52]. These combined injection of α-bungarotoxin-biotin at one time point (here referred to as “pulse”) with a sequence of immediate and later injections (“chase”) of streptavidin-species marked with different fluorophores. Subsequent microscopy revealed that surface-exposed nAChR labeled by injection of α-bungarotoxin-biotin plus streptavidin-dye would (i) remain α-bungarotoxin-biotin positive unless degraded, (ii) lose their fluorescence tag upon endocytosis, and (iii) expose a free streptavidin-binding site upon recycling. One insight from these studies was that ~25% of nAChR undergo recycling within four days [28]. Notably, this was subject to extensive regulation. In particular, antagonistic functions of PKC on the one hand and PKA and CaMKII on the other hand were described [29,33,53]. While activation of PKC or inhibition of PKA or CaMKII led to enhanced removal of recycled nAChR [29,33], the opposite was true upon inhibition of PKC and activation of PKA [33] (Fig. 1). A modified pulse/chase-like protocol with two different fluorescent α-bungarotoxin species injected at ten days interval combined with imaging of genetically encoded biosensors and pharmacological modulation revealed further details on the underlying recycling machinery and the regulatory mechanisms of PKA [35,36,54]. These reports suggested that nAChR-containing recycling vesicles profit from PKA activation only in close vicinity to the postsynapse, likely, because exclusively there the necessary cAMP microdomain for activating PKA was present [35]. Rapsyn was described as an anchoring protein (AKAP) linking PKA to nAChR-recycling vesicles [36]. Capturing of recycling vesicles in the postsynaptic cAMP microdomain needed a dense subsynaptic actin meshwork and the motor protein, myosin Va [35,54] (Fig. 1). In mouse, subsynaptic enrichment of myosin Va and PKA-dependent nAChR recycling were found to develop during the early postnatal phase [16], which is characterized by synaptic pruning [55] and maturation [56,57], and the γ-to-ε switch [5]. A sequential recruitment of main recycling apparatus components was found during recovery from Notexin treatment and their enrichment was reduced in dystrophic mdx mice [37], which are known to exhibit increased nAChR turnover and reduced receptor lifetime [37,58]. Imaging of muscles injected with fluorescent α-bungarotoxin and transfected with GFP-fusion proteins showed extensive colocalization of nAChR-positive vesicles with Rab5-GFP, Rab4-GFP, and Rab11-GFP [42]. Notably, the distribution of these components was not uniform. While colocalization of α-bungarotoxin puncta with the recycling markers Rab4-GFP and Rab11-GFP occurred mostly in close vicinity to the NMJ, Rab5-GFP and α-bungarotoxin double-positive puncta were also found at larger distance from the NMJ [42]. This suggests that nAChR endocytosis and recycling might occur at different sites. Preferential insertion of novel nAChR at rims of synaptic gutters was demonstrated by sequential labeling with differently colored α-bungarotoxin species [13,54] and with knock-in mice expressing nAChRγ-GFP [15,59]. Of note, nAChR recycling could not be observed in cultured myotubes made of C2C12 cells or primary myoblasts [60].

5. Degradation

As aforementioned, nAChR lifetime is affected by different patho/physiological conditions and muscle activity. Upon denervation, muscular dystrophy, myasthenia, or other atrophic situations, nAChR half-life can decrease from several days to one day or less [10,14,44,58]. At these occasions, nAChR subunit composition often switches from ε to γ, and nAChR gene expression is upregulated and less restricted to NMJ [6–8,43,61–63]. Thus, upon atrophy, comparably large amounts of nAChR are turned over. Likely, under such circumstances, recycling is being suppressed while receptor degradation is enhanced. A fasting-atrophy model in combination with live-imaging of muscles injected with fluorescent α-bungarotoxin and GFP-fusion proteins showed extensive colocalization of endocytosed nAChR with the autophagosomal components, LC3 and p62/SQSTM1, as well as with the E3-ligase, MuRF1 [39]. This suggested an involvement of ubiquitylation and selective autophagy in nAChR degradation (Fig. 1). Aberrant NMJ morphology, nAChR turnover, and increased nAChR vesicle numbers in autophagy-defective ATG7-knock out mice [64] and another study that used a mouse model of myasthenia gravis [50] corroborated the hypothesis of the crucial role of autophagy in nAChR turnover. In muscles from wildtype mice injected with fluorescent α-bungarotoxin and transfected with GFP-LC3 and RFP-labeled proteins, the autophagosomal and membrane shaping regulator, SH3GLB1 (aka Bif-1 or endophilin B1), colocalized with endocytosed nAChR and LC3, suggesting its role in nAChR degradation [38,39]. Upon denervation, the ratio of non-phosphorylated to phosphorylated SH3GLB1 increased together with the amounts of Rab5 and LC3 [41]. Furthermore, the number of endocytic nAChR was modulated by overexpression of phosphomutants of SH3GLB1. Since these also altered the expression of RN-tre and Rabex-1, inhibitors and activators, respectively, of Rab5, SH3GLB1 was proposed to control progression of nAChR-containing endosomes to autophagic degradation [41]. A dominant-negative mutant of Cdk5 abutted denervation-induced increase in nAChR vesicles and phosphorylation of SH3GLB1 [41]. Since Cdk5 is upregulated upon denervation [65] and generally affects nAChR stability [66–68], this could link NMJ activity status to a recycling / degradation switch for incoming nAChR vesicles (Fig. 1).

6. Regulatory signaling pathways

Muscle denervation by severing peripheral nerves, saturating doses of α-bungarotoxin, or botulinus toxins, increases nAChR turnover and reduces receptor lifetime [11,13,14,58,69–72]. Since electrical muscle stimulation could largely rescue the kinetic rundown of nAChR stability [72], motor innervation appears to be crucial for receptor stabilization. Which neuronal molecules mediate modulation of nAChR turnover is still elusive, but they might act by metabotropic activity at the

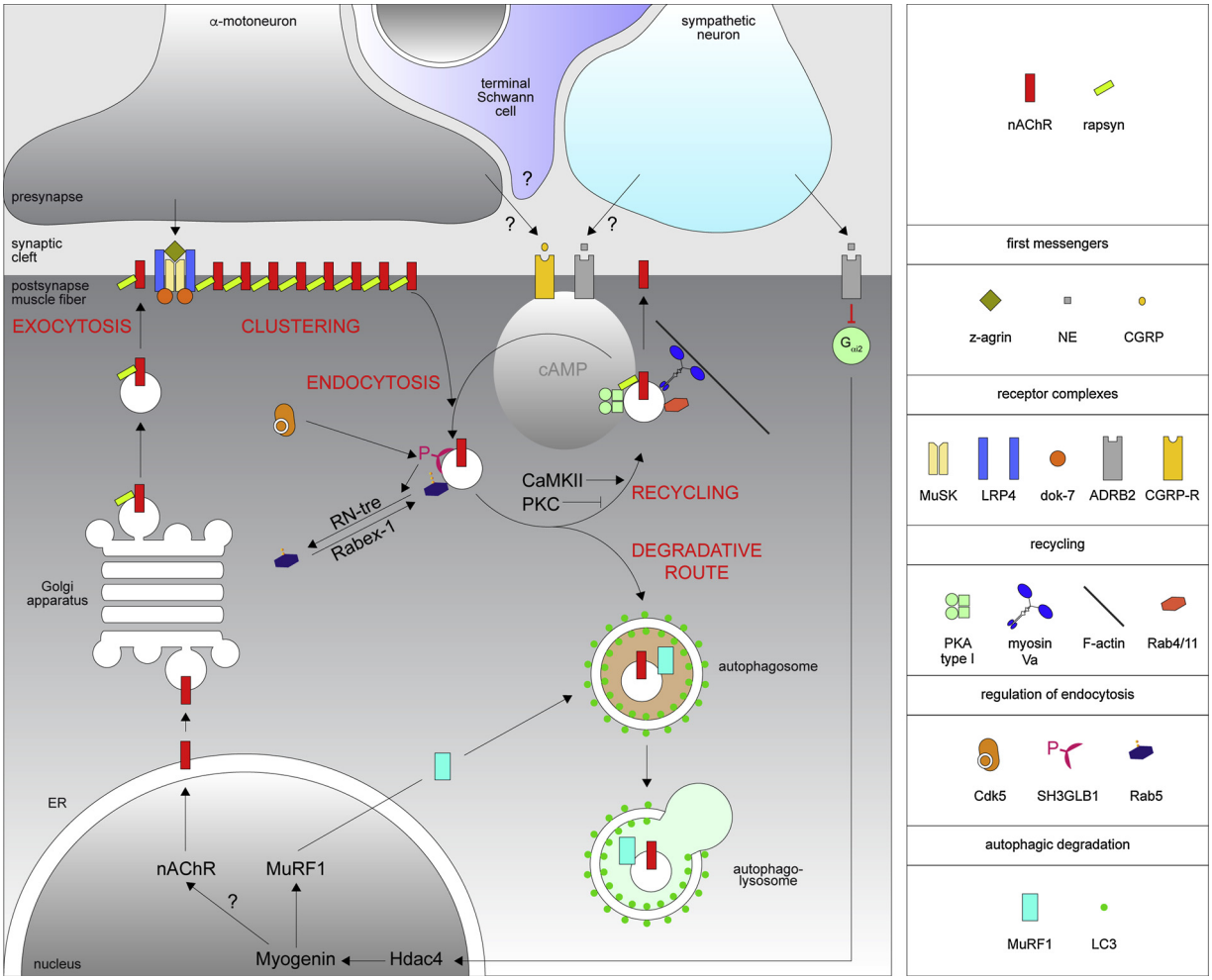

Fig. 1. Hypothetical schematic life cycle of nAChR at the vertebrate motor endplate.
Upon biosynthesis and glycosylation in ER and Golgi apparatus, nAChR gets escorted by rapsyn to the sarcolemma. Juxtaposed to the motoneuron terminal, nAChR clustering uses the agrin/MuSK/Lrp4 complex. Eventually, nAChR is endocytosed and recycled using cooperative function of myosin Va, PKA type I, and rapsyn, or degraded via MuRF1-dependent selective autophagy. Selection between these options involves CaMKII and PKC. SH3GLB1 might control progression of nAChR-containing vesicles towards autophagic degradation via Rab5 activation. Functional interaction between sympathetic neurons and the NMJ link between sympathetic signaling and nAChR turnover regulation via a $G\alpha_{i2}$-Hdac4-myogenin-MuRF1 axis. Whether other components of the NMJ like terminal Schwann cells (tSC) play a role in nAChR turnover, is still unclear.

postsynapse, involving the formation of cAMP [58,73]. By activating PKA, cAMP might stimulate nAChR recycling [33,35]. A candidate metabotropic neuropeptide, that could be released from motoneurons is α-calcitonin gene related peptide (CGRP) [74,75]. This was found to stimulate cAMP production [76] in a postsynaptic PKA type-I specific microdomain [35], nAChR expression [77], nAChR phosphorylation [78], and NMJ strength [79]. A single injection of CGRP in mouse muscle corrected denervation-induced NMJ fragmentation [35]. Another potential activator of the postsynaptic cAMP-PKA axis might be sympathetic co-innervation of NMJs [40]. In contrast to motor terminals, which at the light microscopic level are matching the distribution of postsynaptic nAChR, sympathetic endings were mostly found at the rims of nAChR cluster bands, at least in mouse hindlimb muscles [40,80]. These would be close to the zone of preferential integration of new nAChR (Fig. 1). As demonstrated by live-imaging of hindleg muscles injected with fluorescent α-bungarotoxin and transfected with fluorescent biosensors, stimulation of sympathetic ganglia led to immediate rise of postsynaptic cAMP levels, activation of β2-adrenergic receptors (ADRB2), and nuclear import of the transcriptional coactivator, PGC1α [40]. Local chemical sympathectomy led to massive muscle atrophy as well as functional rundown, simplification, and shrinkage of NMJ [40]. Simultaneous systemic treatment of mice with the sympathicomimetic, clenbuterol, reversed most of these effects [40]. Recently, the findings of a functional sympathetic innervation of NMJ were further supported by other studies [81–84]. In particular, Rodrigues and colleagues addressed effects of surgical sympathectomy on nAChR turnover [83]. There, membrane-bound nAChR was reduced while MuRF1 levels were increased. Furthermore, sympathectomy activated a Hdac4 – myogenin – MuRF1 regulation axis, likely mediated by reduced ADRB2 and $G\alpha_{i2}$ activity [83] (Fig. 1).

7. Conclusions

A picture emerges that can integrate most findings into a common regulatory scheme (Fig. 1). In some aspects, our understanding of the processes underlying nAChR turnover appears to be rather complete and interactions between the different executing and regulatory molecules are worked out in some detail. But still, large gaps of knowledge can be noted. For example, it has remained elusive, which are the targets of PKA for nAChR recycling and of the ubiquitination machinery for nAChR degradation. Although it is likely, that this is one or more of the nAChR subunits, formal proof is missing. With respect to the primary signals modulating the decision between endocytosis, recycling, and degradation, it is uncertain, if different triggers mediate a

simultaneous regulation, if they are all used throughout the lifespan or just under certain circumstances, and how much also other cell types than motoneuron, sympathetic neuron, and muscle cell are part of the regulatory scheme.

Acknowledgement

The work of RR was funded by DFG grants RU923/8-1 and RU923/8-2.

References

[1] A. Karlin, Emerging structure of the nicotinic acetylcholine receptors, Nat. Rev. Neurosci. 3 (2002) 102–114.
[2] M. Gonzalez-Freire, R. de Cabo, S.A. Studenski, L. Ferrucci, The neuromuscular junction: aging at the crossroad between nerves and muscle, Front. Aging Neurosci. 6 (2014) 208, https://doi.org/10.3389/fnagi.2014.00208.
[3] R. Rudolf, M.M. Khan, S. Labeit, M.R. Deschenes, Degeneration of neuromuscular junction in age and dystrophy, Front. Aging Neurosci. 6 (2014) 99, https://doi.org/10.3389/fnagi.2014.00099.
[4] T. Taetzsch, G. Valdez, NMJ maintenance and repair in aging, Curr. Opin. Physiol. 4 (2018) 57–64, https://doi.org/10.1016/j.cophys.2018.05.007.
[5] V. Witzemann, B. Barg, Y. Nishikawa, B. Sakmann, S. Numa, Differential regulation of muscle acetylcholine receptor gamma- and epsilon-subunit mRNAs, FEBS Lett. 223 (1987) 104–112.
[6] Y. Gu, Z.W. Hall, Characterization of acetylcholine receptor subunits in developing and in denervated mammalian muscle, J. Biol. Chem. 263 (1988) 12878–12885.
[7] D. Goldman, H.R. Brenner, S. Heinemann, Acetylcholine receptor alpha-, beta-, gamma-, and delta-subunit mRNA levels are regulated by muscle activity, Neuron. 1 (1988) 329–333.
[8] J.C. Martinou, D.L. Falls, G.D. Fischbach, J.P. Merlie, Acetylcholine receptor-inducing activity stimulates expression of the epsilon-subunit gene of the muscle acetylcholine receptor, Proc Natl Acad Sci U S A 88 (1991) 7669–7673, https://doi.org/10.1073/pnas.88.17.7669.
[9] T.A. Levitt, R.H. Loring, M.M. Salpeter, Neuronal control of acetylcholine receptor turnover rate at a vertebrate neuromuscular junction, Science 210 (1980) 550–551.
[10] T.A. Levitt, M.M. Salpeter, Denervated endplates have a dual population of junctional acetylcholine receptors, Nature 291 (1981) 239–241.
[11] E.F. Stanley, D.B. Drachman, Denervation accelerates the degradation of junctional acetylcholine receptors, Exp. Neurol. 73 (1981) 390–396 doi:0014-4886(81)90274-0 [pii].
[12] E.F. Stanley, D.B. Drachman, Stabilization of acetylcholine receptors at neuromuscular junctions: analysis by specific antibodies, Ann. N. Y. Acad. Sci. 505 (1987) 121–132.
[13] S. Strack, Y. Petersen, A. Wagner, I.V. Röder, M. Albrizio, M. Reischl, I.U. Wacker, C. Wilhelm, R. Rudolf, A novel labeling approach identifies three stability levels of acetylcholine receptors in the mouse neuromuscular junction in vivo, PLoS One 6 (2011) e20524, , https://doi.org/10.1371/journal.pone.0020524.
[14] S. Strack, M.M. Khan, F. Wild, A. Rall, R. Rudolf, Turnover of acetylcholine receptors at the endplate revisited: novel insights into nerve-dependent behavior, J. Muscle Res. Cell. Motil. 36 (2015) 517–524, https://doi.org/10.1007/s10974-015-9418-0.
[15] P. Yampolsky, S. Gensler, J. McArdle, V. Witzemann, AChR channel conversion and AChR-adjusted neuronal survival during embryonic development, Mol. Cell. Neurosci. 37 (2008) 634–645.
[16] P. Yampolsky, P.G. Pacifici, L. Lomb, G. Giese, R. Rudolf, I.V. Röder, V. Witzemann, Time lapse in vivo visualization of developmental stabilization of synaptic receptors at neuromuscular junctions, J. Biol. Chem. 285 (2010) 34589–34596, https://doi.org/10.1074/jbc.M110.168880.
[17] L.A. Tintignac, H.-R. Brenner, M.A. Rüegg, Mechanisms regulating neuromuscular junction development and function and causes of muscle wasting, Physiol. Rev. 95 (2015) 809–852, https://doi.org/10.1152/physrev.00033.2014.
[18] L. Li, W.-C. Xiong, L. Mei, Neuromuscular junction formation, aging, and disorders, Annu. Rev. Physiol. 80 (2018), https://doi.org/10.1146/annurev-physiol-022516-034255 annurev-physiol-022516-034255.
[19] C. De Duve, Tissue fractionation. Past and present, J. Cell Biol. 50 (1971) 20d–55d.
[20] G.E. Palade, Intracellular aspects of the process of protein secretion, Science (80-.) 189 (1975) 347–358, https://doi.org/10.1126/science.189.4206.867-b.
[21] C. de Duve, Lysosomes revisited, Eur. J. Biochem. 137 (1983) 391–397.
[22] J.P. Changeux, M. Kasai, C.Y. Lee, Use of a snake venom toxin to characterize the cholinergic receptor protein, Proc. Natl. Acad. Sci. U. S. A. 67 (1970) 1241–1247.
[23] C.Y. Lee, C.C. Chang, Y.M. Chen, Reversibility of neuromuscular blockade by neurotoxins from elapid and sea snake venoms, Taiwan Yi Xue Hui Za Zhi 71 (1972) 344–349.
[24] C.Y. Lee, Chemistry and pharmacology of polypeptide toxins in snake venoms, Annu. Rev. Pharmacol. 12 (1972) 265–286, https://doi.org/10.1146/annurev.pa.12.040172.001405.
[25] D.M. Fambrough, Control of acetylcholine receptors in skeletal muscle, Physiol. Rev. 59 (1979) 165–227.
[26] R.J. Balice-Gordon, J.W. Lichtman, In vivo visualization of the growth of pre- and postsynaptic elements of neuromuscular junctions in the mouse, J. Neurosci. 10 (1990) 894–908.
[27] R.J. Balice-Gordon, S.M. Breedlove, S. Bernstein, J.W. Lichtman, Neuromuscular junctions shrink and expand as muscle fiber size is manipulated: in vivo observations in the androgen-sensitive bulbocavernosus muscle of mice, J. Neurosci. 10 (1990) 2660–2671.
[28] E. Bruneau, D. Sutter, R.I. Hume, M. Akaaboune, Identification of nicotinic acetylcholine receptor recycling and its role in maintaining receptor density at the neuromuscular junction in vivo, J. Neurosci. 25 (2005) 9949–9959.
[29] I. Martinez-Pena y Valenzuela, C. Mouslim, M. Akaaboune, Calcium/calmodulin kinase II-dependent acetylcholine receptor cycling at the mammalian neuromuscular junction in vivo, J. Neurosci. 30 (2010) 12455–12465, https://doi.org/10.1523/JNEUROSCI.3309-10.2010.
[30] E.G. Bruneau, M. Akaaboune, Dynamics of the rapsyn scaffolding protein at the neuromuscular junction of live mice, J. Neurosci. 30 (2010) 614–619.
[31] I. Martinez-Pena y Valenzuela, C. Mouslim, M. Pires-Oliveira, M.E. Adams, S.C. Froehner, M. Akaaboune, Nicotinic acetylcholine receptor stability at the NMJ deficient in α-syntrophin in vivo, J. Neurosci. 31 (2011) 15586–15596, https://doi.org/10.1523/JNEUROSCI.4038-11.2011.
[32] C. Mouslim, M. Aittaleb, R.I. Hume, M. Akaaboune, A role for the calmodulin kinase II-related anchoring protein (alphakap) in maintaining the stability of nicotinic acetylcholine receptors, J. Neurosci. 32 (2012) 5177–5185, https://doi.org/10.1523/JNEUROSCI.6477-11.2012 32/15/5177 [pii].
[33] I. Martinez-Pena y Valenzuela, M. Pires-Oliveira, M. Akaaboune, PKC and PKA regulate AChR dynamics at the neuromuscular junction of living mice, PLoS One 8 (2013) e81311, , https://doi.org/10.1371/journal.pone.0081311.
[34] P.-J. Chen, I. Martinez-Pena y Valenzuela, M. Aittaleb, M. Akaaboune, AChRs are essential for the targeting of Rapsyn to the postsynaptic membrane of NMJs in living mice, J. Neurosci. 36 (2016) 5680–5685, https://doi.org/10.1523/JNEUROSCI.4580-15.2016.
[35] I.V. Röder, K.-R.R. Choi, M. Reischl, Y. Petersen, M.E. Diefenbacher, M. Zaccolo, T. Pozzan, R. Rudolf, Myosin Va cooperates with PKA RIalpha to mediate maintenance of the endplate in vivo, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 2031–2036, https://doi.org/10.1073/pnas.0914087107.
[36] K.-R.R. Choi, M. Berrera, M. Reischl, S. Strack, M. Albrizio, I.V. Roder, A. Wagner, Y. Petersen, M. Hafner, M. Zaccolo, R. Rudolf, I.V. Röder, A. Wagner, Y. Petersen, M. Hafner, M. Zaccolo, R. Rudolf, Rapsyn mediates subsynaptic anchoring of PKA type I and stabilisation of acetylcholine receptor in vivo, J. Cell. Sci. 125 (2012) 714–723, https://doi.org/10.1242/jcs.092361.
[37] I.V. Röder, S. Strack, M. Reischl, O. Dahley, M.M. Khan, O. Kassel, M. Zaccolo, R. Rudolf, Participation of myosin Va and Pka type I in the regeneration of neuromuscular junctions, PLoS One 7 (2012) e40860, , https://doi.org/10.1371/journal.pone.0040860.
[38] R. Rudolf, J. Bogomolovas, S. Strack, K.-R. Choi, M.M. Khan, A. Wagner, K. Brohm, A. Hanashima, A. Gasch, D. Labeit, S. Labeit, Regulation of nicotinic acetylcholine receptor turnover by MuRF1 connects muscle activity to endo/lysosomal and atrophy pathways, Age (Dordr) 35 (2013) 1663–1674, https://doi.org/10.1007/s11357-012-9468-9.
[39] M.M. Khan, S. Strack, F. Wild, A. Hanashima, A. Gasch, K. Brohm, M. Reischl, S. Carnio, D. Labeit, M. Sandri, S. Labeit, R. Rudolf, Role of autophagy, SQSTM1, SH3GLB1, and TRIM63 in the turnover of nicotinic acetylcholine receptors, Autophagy 10 (2014) 123–136, https://doi.org/10.4161/auto.26841.
[40] M.M. Khan, D. Lustrino, W.A. Silveira, F. Wild, T. Straka, Y. Issop, E. O'Connor, D. Cox, M. Reischl, T. Marquardt, D. Labeit, S. Labeit, E. Benoit, J. Molgó, H. Lochmüller, V. Witzemann, I.C. Kettelhut, L.C.C. Navegantes, T. Pozzan, R. Rudolf, Sympathetic innervation controls homeostasis of neuromuscular junctions in health and disease, Proc. Natl. Acad. Sci. 113 (2016) 746–750, https://doi.org/10.1073/pnas.1524272113.
[41] F. Wild, M.M. Khan, T. Straka, R. Rudolf, Progress of endocytic CHRN to autophagic degradation is regulated by RAB5-GTPase and T145 phosphorylation of SH3GLB1 at mouse neuromuscular junctions in vivo, Autophagy 12 (2016) 2300–2310, https://doi.org/10.1080/15548627.2016.1234564.
[42] F. Wild, M.M. Khan, R. Rudolf, Evidence for the subsynaptic zone as a preferential site for CHRN recycling at neuromuscular junctions, Small GTPases (2017) 1–8, https://doi.org/10.1080/21541248.2017.1324939.
[43] D.M. Fambrough, D.B. Drachman, S. Satyamurti, Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors, Science 182 (1973) 293–295.
[44] D.B. Drachman, R.N. Adams, E.F. Stanley, A. Pestronk, Mechanisms of acetylcholine receptor loss in myasthenia gravis, J. Neurol. Neurosurg. Psychiatry 43 (1980) 601–610.
[45] P.N. Devreotes, D.M. Fambrough, Turnover of acetylcholine receptors in skeletal muscle, Cold Spring Harb. Symp. Quant. Biol. 40 (1976) 237–251.
[46] G. Fumagalli, A.G. Engel, J. Lindstrom, Ultrastructural aspects of acetylcholine receptor turnover at the normal end-plate and in autoimmune myasthenia gravis, J. Neuropathol. Exp. Neurol. 41 (1982) 567–579.
[47] A.G. Engel, J.M. Lindstrom, E.H. Lambert, V.A. Lennon, Ultrastructural localization of the acetylcholine receptor in myasthenia gravis and in its experimental autoimmune model, Neurology 27 (1977) 307–315.
[48] A.G. Engel, G. Fumagalli, Mechanisms of acetylcholine receptor loss from the neuromuscular junction, Ciba Found. Symp. (1982) 197–224.
[49] S. Kumari, V. Borroni, A. Chaudhry, B. Chanda, R. Massol, S. Mayor, F.J. Barrantes, Nicotinic acetylcholine receptor is internalized via a Rac-dependent, dynamin-independent endocytic pathway, J. Cell Biol. 181 (2008) 1179–1193, https://doi.org/10.1083/jcb.200709086jcb.200709086.
[50] A. Du, S. Huang, X. Zhao, K. Feng, S. Zhang, J. Huang, X. Miao, F. Baggi, R.S. Ostrom, Y. Zhang, X. Chen, C. Xu, Suppression of CHRN endocytosis by carbonic anhydrase CAR3 in the pathogenesis of myasthenia gravis, Autophagy 13 (2017) 1981–1994, https://doi.org/10.1080/15548627.2017.1375633.

[51] C.W. Lee, H. Zhang, L. Geng, H.B. Peng, Crosslinking-induced endocytosis of acetylcholine receptors by quantum dots, PLoS One 9 (2014) e90187, , https://doi.org/10.1371/journal.pone.0090187.
[52] E.G. Bruneau, M. Akaaboune, The dynamics of recycled acetylcholine receptors at the neuromuscular junction in vivo, Development 133 (2006) 4485–4493.
[53] P.G. Nelson, M.A. Lanuza, M. Jia, M.X. Li, J. Tomas, Phosphorylation reactions in activity-dependent synapse modification at the neuromuscular junction during development, J. Neurocytol. 32 (2003) 803–816.
[54] I.V. Röder, Y. Petersen, K.R. Choi, V. Witzemann, J.A. Hammer 3rd, R. Rudolf, Role of Myosin Va in the plasticity of the vertebrate neuromuscular junction in vivo, PLoS One 3 (2008) e3871, https://doi.org/10.1371/journal.pone.0003871.
[55] Y. il Lee, W.J. Thompson, M.L. Harlow, Schwann cells participate in synapse elimination at the developing neuromuscular junction, Curr. Opin. Neurobiol. 47 (2017) 176–181, https://doi.org/10.1016/j.conb.2017.10.010.
[56] C.R. Slater, Postnatal maturation of nerve-muscle junctions in hindlimb muscles of the mouse, Dev. Biol. 94 (1982) 11–22.
[57] M.J. Marques, J.A. Conchello, J.W. Lichtman, From plaque to pretzel: fold formation and acetylcholine receptor loss at the developing neuromuscular junction, J. Neurosci. 20 (2000) 3663–3675.
[58] R. Xu, M.M. Salpeter, Acetylcholine receptors in innervated muscles of dystrophic mdx mice degrade as after denervation, J. Neurosci. 17 (1997) 8194–8200.
[59] P. Yampolsky, P.G. Pacifici, V. Witzemann, Differential muscle-driven synaptic remodeling in the neuromuscular junction after denervation, Eur. J. Neurosci. 31 (2010) 646–658, https://doi.org/10.1111/j.1460-9568.2010.07096.x.
[60] H.R. Brenner, M. Akaaboune, Recycling of acetylcholine receptors at ectopic postsynaptic clusters induced by exogenous agrin in living rats, Dev. Biol. 394 (2014) 122–128, https://doi.org/10.1016/j.ydbio.2014.07.018.
[61] R. Miledi, L.T. Potter, Acetylcholine receptors in muscle fibres, Nature 233 (1971) 599–603.
[62] S.P. Ringel, A.N. Bender, W.K. Engel, Extrajunctional acetylcholine receptors. Alterations in human and experimental neuromuscular diseases, Arch. Neurol. 33 (1976) 751–758.
[63] R.R. Gomes, F.W. Booth, Expression of acetylcholine receptor mRNAs in atrophying and nonatrophying skeletal muscles of old rats, J. Appl. Physiol. 85 (1998) 1903–1908, https://doi.org/10.1152/jappl.1998.85.5.1903.
[64] S. Carnio, F. LoVerso, M.A. Baraibar, E. Longa, M.M. Khan, M. Maffei, M. Reischl, M. Canepari, S. Loefler, H. Kern, B. Blaauw, B. Friguet, R. Bottinelli, R. Rudolf, M. Sandri, Autophagy impairment in muscle induces neuromuscular junction degeneration and precocious aging, Cell Rep. 8 (2014) 1509–1521, https://doi.org/10.1016/j.celrep.2014.07.061.
[65] W.-Y. Fu, A.K.Y. Fu, K.-C. Lok, F.C.F. Ip, N.Y. Ip, Induction of Cdk5 activity in rat skeletal muscle after nerve injury, Neuroreport. 13 (2002) 243–247.
[66] A.K.Y. Fu, F.C.F. Ip, W.-Y. Fu, J. Cheung, J.H. Wang, W.-H. Yung, N.Y. Ip, Aberrant motor axon projection, acetylcholine receptor clustering, and neurotransmission in cyclin-dependent kinase 5 null mice, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 15224–15229, https://doi.org/10.1073/pnas.0507678102.
[67] W. Lin, B. Dominguez, J. Yang, P. Aryal, E.P. Brandon, F.H. Gage, K.-F. Lee, Neurotransmitter acetylcholine negatively regulates neuromuscular synapse formation by a Cdk5-Dependent mechanism, Neuron. 46 (2005) 569–579, https://doi.org/10.1016/j.neuron.2005.04.002.
[68] F. Chen, L. Qian, Z.-H.H. Yang, Y. Huang, S.T. Ngo, N.-J.J. Ruan, J. Wang, C. Schneider, P.G. Noakes, Y.-Q.Q. Ding, L. Mei, Z.-G.G. Luo, Rapsyn interaction with calpain stabilizes AChR clusters at the neuromuscular junction, Neuron 55 (2007) 247–260, https://doi.org/10.1016/j.neuron.2007.06.031 S0896-6273(07)00488-6 [pii].
[69] J.S. Andreose, R. Xu, T. Lomo, M.M. Salpeter, G. Fumagalli, Degradation of two AChR populations at rat neuromuscular junctions: regulation in vivo by electrical stimulation, J. Neurosci. 13 (1993) 3433–3438.
[70] R.H. Loring, M.M. Salpeter, Denervation increases turnover rate of junctional acetylcholine receptors, Proc. Natl. Acad. Sci. U. S. A. 77 (1980) 2293–2297.
[71] D.A. Ramsay, D.B. Drachman, R.J. Drachman, E.F. Stanley, Stabilization of acetylcholine receptors at the neuromuscular synapse: the role of the nerve, Brain Res. 581 (1992) 198–207 doi:0006-8993(92)90709-I.
[72] M. Akaaboune, S.M. Culican, S.G. Turney, J.W. Lichtman, Rapid and reversible effects of activity on acetylcholine receptor density at the neuromuscular junction in vivo, Science 286 (1999) 503–507.
[73] S.L. Shyng, R. Xu, M.M. Salpeter, Cyclic AMP stabilizes the degradation of original junctional acetylcholine receptors in denervated muscle, Neuron 6 (1991) 469–475.
[74] D.R. Poyner, Calcitonin gene-related peptide: multiple actions, multiple receptors, Pharmacol. Ther. 56 (1992) 23–51.
[75] B. Fontaine, A. Klarsfeld, T. Hokfelt, J.P. Changeux, Calcitonin gene-related peptide, a peptide present in spinal cord motoneurons, increases the number of acetylcholine receptors in primary cultures of chick embryo myotubes, Neurosci. Lett. 71 (1986) 59–65.
[76] R. Laufer, J.P. Changeux, Calcitonin gene-related peptide elevates cyclic AMP levels in chick skeletal muscle: possible neurotrophic role for a coexisting neuronal messenger, EMBO J. 6 (1987) 901–906.
[77] B. Fontaine, A. Klarsfeld, J.P. Changeux, Calcitonin gene-related peptide and muscle activity regulate acetylcholine receptor alpha-subunit mRNA levels by distinct intracellular pathways, J. Cell Biol. 105 (1987) 1337–1342.
[78] K. Miles, P. Greengard, R.L. Huganir, Calcitonin gene-related peptide regulates phosphorylation of the nicotinic acetylcholine receptor in rat myotubes, Neuron 2 (1989) 1517–1524.
[79] B. Lu, W.M. Fu, P. Greengard, M.M. Poo, Calcitonin gene-related peptide potentiates synaptic responses at developing neuromuscular junction, Nature 363 (1993) 76–79.
[80] T. Straka, V. Vita, K. Prokshi, S.J. Hörner, M.M. Khan, M. Pirazzini, M.P.I. Williams, M. Hafner, T. Zaglia, R. Rudolf, Postnatal development and distribution of sympathetic innervation in mouse skeletal muscle, Int. J. Mol. Sci. 19 (2018) 1935, https://doi.org/10.3390/ijms19071935.
[81] A.K. Snyder-Warwick, A. Satoh, K.B. Santosa, S. Imai, A. Jablonka-Shariff, Hypothalamic Sirt1 protects terminal Schwann cells and neuromuscular junctions from age-related morphological changes, Aging Cell (2018) e12776, , https://doi.org/10.1111/acel.12776.
[82] L. Clausen, J. Cossins, D. Beeson, Beta-2 adrenergic receptor agonists enhance AChR clustering in C2C12 myotubes: implications for therapy of myasthenic disorders, J. Neuromuscul. Dis. 5 (2018) 231–240, https://doi.org/10.3233/JND-170293.
[83] A.C.Z. Rodrigues, M.L. Messi, Z.-M. Wang, M.C. Abba, A. Pereyra, A. Birbrair, T. Zhang, M. O'Meara, P. Kwan, E.I.S. Lopez, M.S. Willis, A. Mintz, D.C. Files, C. Furdui, R.W. Oppenheim, O. Delbono, The sympathetic nervous system regulates skeletal muscle motor innervation and acetylcholine receptor stability, Acta Physiol. (2018) e13195, , https://doi.org/10.1111/apha.13195.
[84] A.Z.C. Rodrigues, Z.-M. Wang, M.L. Messi, O. Delbono, Sympathomimetics regulate neuromuscular junction transmission through TRPV1, P/Q- and N-type Ca2+ channels, Mol. Cell. Neurosci. 95 (2019) 59–70, https://doi.org/10.1016/j.mcn.2019.01.007.