Postsynaptic packing of acetylcholine receptors at the neuromuscular synapse and its regulation: a FRET study

O.L. Gervasio, P.F. Armson and W.D. Phillips , School of Medical Sciences (Physiology), Bosch Institute, University of Sydney, NSW 2006, Australia.

Fast, efficient synaptic transmission at the neuromuscular synapse depends upon the close packing of acetylcholine receptors (AChRs) in postsynaptic AChR aggregates, but not all postsynaptic AChRs are aggregated. Precisely how packing and retention of postsynaptic AChRs in aggregates is regulated during development remains to be defined. Here we describe a new confocal Fluorescence Resonance Energy Transfer (FRET) assay that allows semi-quantitative comparison of the degree to which AChRs are aggregated at synapses. During the first month of postnatal life the mouse tibialis anterior muscle showed increases both in the number of postsynaptic AChRs and in the degree to which they were packed together in aggregates (as defined by FRET). These changes were concurrent with a two-fold increase in immunofluorescent labeling for the cytoplasmic, AChR-associated protein, rapsyn. When one-month old muscle was denervated there was a further increase in the number of postsynaptic AChRs, but postsynaptic rapsyn immunostaining was reduced and so was the efficiency of AChR aggregation. *In vivo* electroporation of rapsyn-EGFP into muscle fibers increased the postsynaptic ratio of rapsyn-to-AChR. For muscle electroporation, 4-week old female FVB mice were anaesthetized with ketamine and xylazine (50mg/kg of each, I.P.) followed by buprenorphine (subcutaneous, 12 hourly; 0.03mg/kg) during recovery. Those synapses with higher ratios of rapsyn-EGFP to AChR displayed a slower turnover of AChR. Conversely, the reduction of postsynaptic rapsyn after denervation was accompanied by an acceleration of AChR turnover. Thus, changes in the amount of rapsyn targeted to the postsynaptic membrane may constitute a developmental mechanism controlling the extent to which postsynaptic AChRs are packed into aggregates and retained within the postsynaptic membrane.