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Neuregulins show their support for neuromuscular junctions

Proteins help establish a solid foundation for nerve–muscle connections.

Like teeth and fence posts, acetylcholine receptors (AChRs) need to be solidly anchored. Schmidt et al. reveal that proteins called neuregulins help ensure that the receptors are securely settled in the postsynaptic membrane of the neuromuscular junction (1).

Neuregulins are signaling molecules essential for everything from heart development to nerve cell differentiation. Another of their tasks is maintaining the neuromuscular junction where nerve and muscle meet, but how they do this is unclear. One hypothesis suggests that they alter the activity of genes that code for part of the AChR, the main receptor in the neuromuscular junction (2). But in a previous study, the researchers found that short-circuiting neuregulin signaling barely reduced the expression of these genes (3). Schmidt et al.'s new work indicates that, instead of changing gene activity, neuregulins modify the structure of the neuromuscular junction.

AChRs go through an involved "life cycle." Receptors in the postsynaptic membrane move into the neighboring perisynaptic membrane. Once there, the receptors are bundled up and returned to the cytoplasm. Although some of these internalized receptors are destroyed, many of them plug back into the postsynaptic membrane and start working again (4). The researchers followed AChRs in mice that were missing two neuregulin receptors, ErbB2 and ErbB4, and thus lacked neuregulin signaling in the muscle fiber. Schmidt et al. discovered that removal of AChRs from the postsynaptic membrane was faster in ErbB-deficient mice. However, the effect was much more pronounced for AChRs that had recycled to the postsynaptic membrane. The team found that curtailing neuregulin signaling sped up the recycled receptors' transfer to the perisynaptic membrane, suggesting that neuregulin usually helps these secondhand receptors become embedded at the postsynapse.

> "[Neuregulin induces a] post-translational modification of one of the building blocks of the synaptic anchoring scaffold."

High resolution confocal microscopy showed the impact on the postsynaptic membrane of blocking neuregulin signaling. Bald patches developed where AChRs were absent. Mice lacking ErbB2 and ErbB4 in muscle also showed subtle defects. The neuromuscular junction typically contains extra AChRs, allowing it to maintain impulse transmission, particularly during essential functions such as breathing. But interruption of neuregulin signaling cuts the number of spare AChRs, reducing this safety factor.

But how do neuregulins help hold AChRs in place? Loss of the ErbB receptors triggered several proteins to depart from the scaffold that undergirds the postsynaptic membrane, suggesting that neuregulins help strengthen this scaffold. The key link, Schmidt et al. discovered, is the protein α-dystrobrevin1 (α-DB1), part of the postsynaptic dystrophin-associated glycoprotein complex (DGC) that anchors AChRs at the synapse. Compared with mice deficient in ErbB receptors, rodents lacking α-DB1 show similar but more severe abnormalities in the neuromuscular junction.

Focal Point

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(Left to right) Mohammed Akaaboune, Hans Rudolf Brenner, Nadine Schmidt, Nadesan Gajendran, and colleagues (not pictured) dissected how neuregulins affect the stability of acetylcholine receptors in the postsynaptic membrane of the neuromuscular junction. Heat-map images (right) of the postsynaptic membrane show that recycled receptors thin out over the 48h after neuregulin signaling is blocked by depletion of the neuregulin receptors ErbB2 and ErbB4.

Schmidt et al. investigated α-DB1's role using muscle cell myotubes grown in culture. Adding an antibody that neutralizes neuregulin reduced the size of AChR clusters in wild-type myotubes but had no effect on muscle cells lacking α-DB1. When prodded by neuregulin, ErbB receptors phosphorylated α-DB1. A mutant form of this scaffold protein that can't be phosphorylated wasn't able to stabilize AChR clusters in response to neuregulin.

This study reveals a novel "post-translational modification of one of the building blocks of the synaptic anchoring scaffold," says senior author Hans Rudolf Brenner. It also highlights several unanswered questions to pursue. One mystery is why neuregulin only seems to affect the stability of recycled acetylcholine receptors. Even in normal muscles, the recycled receptors seem to be more loosely attached. Whether these reused receptors differ structurally from freshly made ones is unknown, Brenner says. The findings suggest that phosphorylation of α-DB1 spurs it to clamp the AChRs in place, but how α-DB1 solidifies the postsynaptic scaffold remains unknown.

1. Schmidt, N., et al. 2011. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201107083.
2. Schaeffer, L., et al. 2001. *Neuron*. 31:15–22.
3. Escher, P., et al. 2005. *Science*. 308:1920–1923.
4. Bruneau, E., et al. 2005. *J. Neurosci.* 25:9949–9959.