Antibody to MyoD Decreases Myogenin Gene Expression and Agrin-induced Acetylcholine Receptor Clustering

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Objective: A family of myogenic regulatory factors, including MyoD and myogenin, guide myogenesis and neuromuscular synapse formation. Myogenin gene expression is activated by MyoD, and one of myogenin's functions is to activate gene expression of the acetylcholine receptor (AChR) at the neuromuscular synapse. Motor neurons release agrin as they near skeletal muscle fibers in development, which drives the clustering of existing AChRs to the site of neuromuscular synapse formation. We have previously demonstrated that continuous exposure to antibody to MyoD or myogenin decreases agrin-induced AChR clustering in C2C12 skeletal muscle cell culture. Our objective was to more specifically establish how MyoD and myogenin interact in development. Methods: C2C12 cell cultures were exposed to experimental manipulations including antibodies to MyoD and myogenin, and myogenin morpholino. Endo-Porter was used to enhance cell uptake of experimental manipulations. AChR clustering assays were performed to assess the effect of antibody or morpholino on agrin-induced AChR clustering. Western blots were performed to assess myogenin gene expression after antibody or morpholino exposure. Results: The results reported here demonstrate that exposure as short as eight hours for antibody to myogenin can decrease agrin-induced AChR clustering in myotubes. We have previously demonstrated that some experimental manipulations reduce myogenin gene expression concurrent with a decrease in agrin-induced AChR clustering. The current results establish more specifically how MyoD and myogenin interact in neuromuscular synapse formation by demonstrating that exposure to antibody to MyoD reduces myogenin gene expression concurrent with a decrease in agrin-induced AChR clustering. Conclusion: These results suggest that MyoD is essential for agrin-induced AChR clustering through a mechanism that includes activation of myogenin gene expression, leading to activation of AChR gene expression, and ultimately production of an appropriate level of AChR for agrin-induced AChR clustering and neuromuscular synapse formation.