Effects of cholinesterase inhibitor therapy on the neuromuscular junction in a mouse model of anti-MuSK myasthenia gravis

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Myasthenia gravis (MG) is an autoimmune disease of fatiguing muscle weakness due to loss of postsynaptic acetylcholine receptors (AChR) from the neuromuscular junction (NMJ). Pyridostigmine, a reversible acetylcholinesterase (AChE) inhibitor, is the first line treatment for MG. It enhances the dwell time of acetylcholine in the synaptic cleft. The second most common form of MG involves autoantibodies against Muscle-Specific Kinase (MuSK). MuSK is the core of a receptor tyrosine kinase complex in the postsynaptic membrane that plays a coordinating role in differentiation of the embryonic NMJ. Clinical reports suggest anti-MuSK-positive MG patients can be non-responsive to and/or poorly tolerate pyridostigmine. Here we use a mouse model (Cole et al., 2008, 2010) to study the interaction of pyridostigmine with anti-MuSK MG.

Female C57Bl6J mice received daily intraperitoneal (ip) injections of IgG from high-titre MuSK-positive patients (anti-MuSK). Twelve days after the start of IgG injections the mice became weak, lost weight and were euthanized at 14 days (30mg sodium pentobarbital, ip). At the level of the NMJ, anti-MuSK-injected mice revealed a loss of tightly packed postsynaptic AChR and misalignment of nerve terminals from the postsynaptic AChR. To test the effect of pyridostigmine treatment the mouse was anaesthetized by 2-3% isofluorane/oxygen inhalation at day 7 of the injection series. An osmotic minipump was implanted subcutaneously. Infusion of pyridostigmine at therapeutically relevant levels (40% inhibition of blood cholinesterase activity), for a further 7 days did not prevent the onset of clinical weakness. Moreover, confocal imaging of NMJs from the diaphragm muscle of pyridostigmine-treated mice revealed greater loss of postsynaptic AChR cluster area and staining intensity compared to mice receiving the same anti-MuSK IgG injections but without pyridostigmine treatment. Intracellular recordings from fibres in the diaphragm muscle revealed significant reductions in the amplitudes of the endplate potential and spontaneous miniature endplate potentials in pyridostigmine-treated mice. Nerve terminal area and quantal content was not altered by pyridostigmine treatment, suggesting that the primary effect of pyridostigmine was to exacerbate the loss of postsynaptic AChRs. The same pyridostigmine dosage delivered to mice injected with control human IgG (no MuSK antibodies) did not cause these changes to the NMJ. Thus, pyridostigmine appears to potentiate the disassembly of postsynaptic AChR clusters that is initiated by MuSK autoantibodies.

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