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Muscle specific kinase autoantibodies cause synaptic failure through progressive wastage of postsynaptic acetylcholine receptors

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ABSTRACT

In myasthenia gravis muscle weakness is caused by autoantibodies against components of the neuromuscular junction. Patient autoantibodies against muscle specific kinase (MuSK) deplete MuSK from the postsynaptic membrane and reproduce signs of myasthenia gravis when injected into mice. Here we have examined the time-course of structural and functional changes that lead up to synaptic failure. C57Bl6J mice received daily injections of anti-MuSK patient IgG for 15 days. Mice began to lose weight from day 12 and demonstrated whole-body weakness by day 14. Electromyography indicated synaptic impairment from day 6 in the gastrocnemius muscle and from day 10 in the diaphragm muscle. Confocal microscopy revealed linear declines in the area and density of postsynaptic acetylcholine receptors (3–5% per day) from day 1 through day 15 of the injection series in all five muscles examined. Intracellular recordings from the diaphragm muscle revealed comparable progressive declines in the amplitude of the endplate potential and miniature endplate potential of 3–4% per day. Neither quantal content nor the postsynaptic action potential threshold changed significantly over the injection series. The inverse relationship between the quantal amplitude of a synapse and its quantal content disappeared only late in the injection series (day 10). Our results suggest that the primary myasthenogenic action of anti-MuSK IgG is to cause wastage of postsynaptic acetylcholine receptor density. Consequent reductions in endplate potential amplitudes culminated in failure of neuromuscular transmission.

Introduction

While most cases of myasthenia gravis (MG) are caused by acetylcholine receptor (AChR) autoantibodies, a subset of MG patients instead display autoantibodies against the muscle specific kinase (MuSK) (Hoch et al., 2001; McConville et al., 2004) or an associated protein, low-density lipoprotein receptor-related protein-4 (LRP4) (Higuchi et al., 2011; Pevzner et al., 2011). Patient autoantibodies against AChRs are dominated by IgG subclass 1 (IgG1) and IgG3 and cause synaptic failure by accelerating AChR catabolism, activating complement or by blocking AChR channels (Conti-Fine et al., 2006; Kao and Drachman, 1977; Rodgaard et al., 1987; Toyka et al., 1975, 1977; Vincent and Newsom-Davis, 1982). Autoantibodies against MuSK are predominantly of the IgG4 subclass and disease severity correlates with the level of IgG4 autoantibodies (Klooster et al., 2012; Niks et al., 2008; Shiraishi et al., 2005). In animal models, anti-MuSK IgG4 caused failure of the neuromuscular junction (NMJ) (McConville et al., 2004; Niks et al., 2008) and myasthenic weakness independently of the complement system (Klooster et al., 2012; Mori et al., 2012). Instead, MuSK autoantibodies appear to exert their pathogenic actions by specifically interfering with the function of MuSK. When applied to heterologous cells, MuSK autoantibodies caused internalisation of recombinant MuSK, and when injected into mice they depleted MuSK from the endplate (Cole et al., 2010; Kawakami et al., 2011).

MuSK is a receptor tyrosine kinase that plays a central role in stabilising the NMJ. During development the nerve terminal secretes neural agrin, a proteoglycan that forms a complex with LRP4 and MuSK, activating MuSK and its associated transduction complex (reviewed in Ghazanfari et al., 2011; Wu et al., 2010). Pre- and postsynaptic differentiation was severely disrupted at NMJs of mouse embryos lacking MuSK (DeChiara et al., 1996). Conditional knockdown of the MuSK gene postnatally led to the break-up of postsynaptic AChR clusters and withdrawal of the nerve terminal

Abbreviations: AP, action potential; AChR, acetylcholine receptor; CMAP, compound muscle action potential; EPP, endplate potential; LRP4, low-density lipoprotein receptor-related protein-4; mEPP, miniature endplate potential; MG, myasthenia gravis; MuSK, muscle specific kinase; NMJ, neuromuscular junction; RNS, repetitive nerve stimulation; RMP, resting membrane potential.

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from the endplate (Hesser et al., 2006; Kong et al., 2004). Thus, MuSK is necessary to support pre- and postsynaptic differentiation of the NMJ during development.

MuSK autoantibodies have been reported to have diverse effects upon the NMJ. These effects have been assessed by actively immunising animals with MuSK or by injecting patient autoantibodies. Several studies reported impairment of postsynaptic AChR clusters, associated with evidence of reduced synaptic function (Cole et al., 2010; Jha et al., 2006; Shigemoto et al., 2006; ter Beek et al., 2009). Other studies reported evidence that MuSK autoantibodies caused a combination of pre- and postsynaptic abnormalities (Cole et al., 2008; Klooster et al., 2012; Punga et al., 2011; Richman et al., 2012 ; Viegas et al., 2012). Still other studies reported myopathic changes (Benveniste et al., 2005; Boneva et al., 2006; Martignago et al., 2009). Endplate potential (EPP) recordings from patient biopsies have produced contradictory results (Niks et al., 2010; Selcen et al., 2004; Shiraishi et al., 2005). Two groups have recently reported that the compensatory (homeostatic) upregulation of presynaptic quantal release was absent in mouse models of anti-MuSK myasthenia gravis (Klooster et al., 2012; Viegas et al., 2012). Here for the first time, we have tracked the time course of the pre- and postsynaptic changes that lead up to weakness using an established passive transfer mouse model of anti-MuSK myasthenia gravis. We show that anti-MuSK patient IgG produced parallel declines in postsynaptic AChR packing and quantal amplitude resulting in subthreshold EPPs, failure of neuromuscular transmission and weakness.

Methods

Ethical approval

All mouse experiments described in this paper were conducted with the approval of The University of Sydney Animal Ethics Committee in compliance with the NSW Animal Research Act 1985 and the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 7th Edition NHMRC 2004. Patient consent was obtained in accordance with the Declaration of Helsinki. The project was approved by Human Research Ethics Committee of the Sydney South West Area Health Service.

Anti-MuSK IgG injections

Passive transfer experiments and IgG purifications were conducted as previously described (Cole et al., 2008). Female 6-week-old C57Bl6J mice (Animal Resources Centre, Western Australia) received daily intraperitoneal (i.p.) injections of 45 mg IgG from patient #4 (in sterile phosphate-buffered saline), as described in Cole et al. (2008),

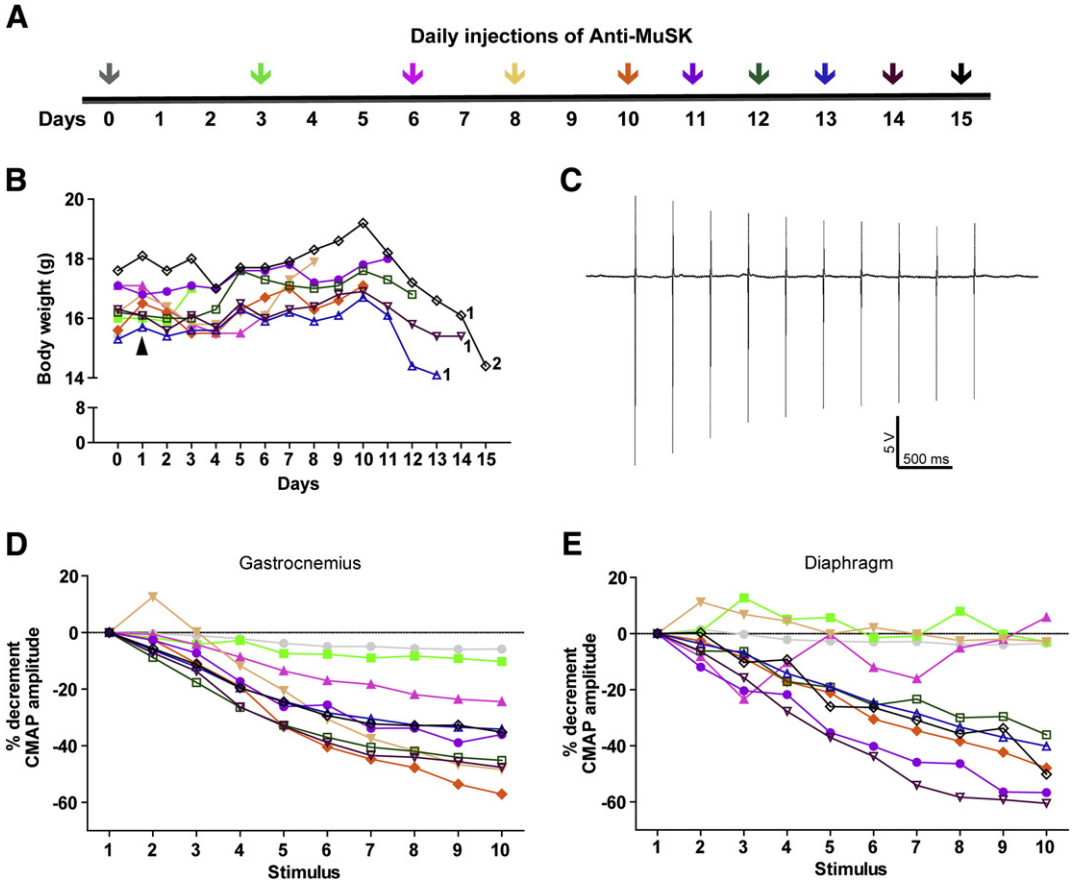

Fig. 1. Neuromuscular impairment in C57BL6J mice injected with anti–muscle-specific kinase (MuSK) IgG. (A) Experimental timeline: mice received daily injections of anti-MuSK-positive patient IgG (45 mg). One mouse each was taken out of the cohort and investigated for combined structure and function analysis at the time-points indicated by the coloured arrows. Three naive control mice were assessed on day 0. The colour code applies to panels B, D and E. (B) Body weight changes of individual mice during the course of anti-MuSK injections. Numbers beside symbols indicate whole-body weakness grades as described in *Methods*. The arrowhead indicates a single cyclophosphamide injection to suppress an active immune response to the human IgG. (C) A sample of serial compound muscle action potentials (CMAP) recorded from the gastrocnemius muscle showing a decremental response upon repetitive stimulation of the sciatic nerve at 3 per second, 13 days after the start of anti-MuSK IgG injections. (D) Decline (percent decrement) in the mean amplitude of the CMAP from the gastrocnemius muscle in response to 10 serial stimuli of the sciatic nerve at 3 per second. Each data point shows the mean amplitude of three recordings at a given time-point (see colour code in A). (E) Decline (percent decrement) in the mean amplitude of the CMAP from the hemidiaphragm muscle in response to 10 stimuli of the phrenic nerve at 3 per second (see colour code in A).