Congenital myasthenic syndromes: β-adrenergic receptor agonist treatment

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ABSTRACT

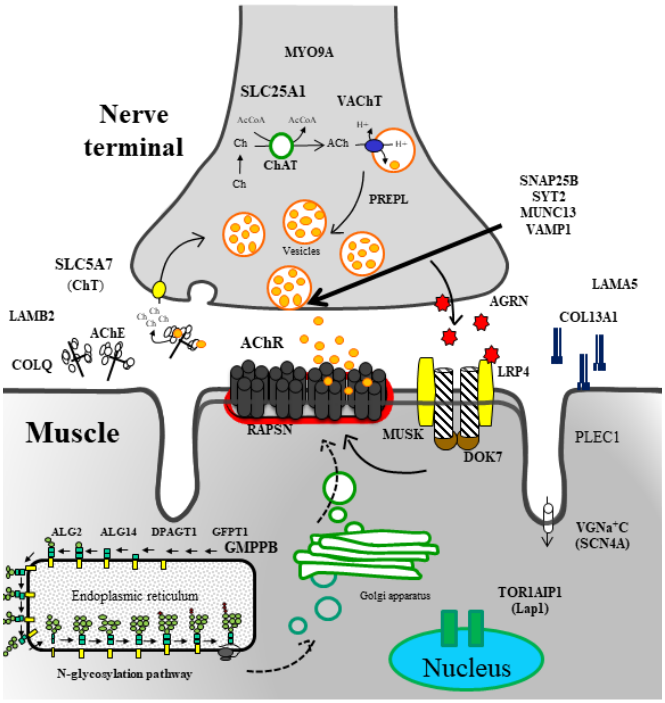
Acetylcholinesterase inhibitors, such as pyridostigmine, are the standard symptomatic treatment for myasthenia gravis (MG), and so have naturally been applied to the genetic forms of myasthenia, termed congenital myasthenic syndromes (CMS). Although effective for many CMS in others there is no clear response, and in some it is actually harmful. Now, with greater understanding of the mutations and molecular mechanisms underlying CMS, treatments can be tailored for the specific syndrome, and, depending on disease severity and patient response, this can include utilizing different combinations of drugs. In CMS, over the last 15-20 years β2-adrenergic receptor agonists have moved from occasional use to a mainstream medication. Many patients have life-transforming improvement both when the β2-adrenergic receptor agonists are used alone or in combination. Here we feature how the identification of DOK7-CMS first highlighted the consistent benefit of β2-adrenergic receptor agonists as medication and how its application to many different CMS subtypes evolved. The molecular pathogenic mechanisms for many CMS subtypes are now established, and this report will also discuss a hypothetical rationale for which forms of CMS are likely to benefit from the β2-adrenergic receptor agonists.

Key words: *Congenital myasthenic syndrome, β2-adrenergic receptor, ephedrine, salbutamol, albuterol, DOK7, COLQ, CHRNE*

Introduction

More than 30 genes have been identified in which mutations can underlie defective neuromuscular transmission (Figure1)[1,2]. The mutations can have their effect through a variety of molecular mechanisms, and even mutations within the same gene can lead to different phenotypes and very different clinical pictures. The congenital myasthenic syndromes (CMS) are hereditary disorders, and therefore there is no role for immunomodulatory agents. However, there are a number of drugs that can be used to provide symptomatic treatment

Figure 1. Diagrammatic representation of a motor endplate illustrating the potential location of the many genes/proteins in which mutations that underlie a congenital myasthenic syndrome are identified.



for the various different underlying molecular pathologies. The present repertoire includes acetylcholinesterase inhibitors (mainly pyridostigmine), 3,4-diaminopyridine (3,4-DAP), acetylcholine receptor (AChR) open-channel blockers (fluoxetine, quinidine), the β2-adrenergic receptor agonists ephedrine and salbutamol/albuterol, or different combinations of these agents [1,3]. It is important to recognize that drugs that benefit one form of CMS may be harmful in another, even when the mutations lie in the same gene.

Reversible, competitive acetylcholinesterase inhibitors, such as pyridostigmine have been the mainstay of treatment for myasthenia gravis (MG) for many years. By blocking the action of acetylcholinesterase, the presence of ACh within the synapse is prolonged, thus giving a greater probability of reaching the depolarization threshold for generation of a muscle action potential. Although effective for many CMS, in others there is no clear response, and in some it is harmful. Pyridostigmine is quite clearly contraindicated for endplate AChE deficiency due to mutations in COLQ, as there is already a deficit of acetylcholinesterase function [4]. Similarly, in the dominantly inherited slow channel syndrome, increasing the level and duration of ACh within the synaptic cleft is only likely to exacerbate this excitotoxic disorder [5]. The use of AChR open channel blockers, fluoxetine or quinidine, can be remarkably effective for

some slow channel mutations [6], but the response is less marked for others. Ephedrine, a β2-adrenergic receptor agonist that was originally derived from the *ephedra* plant family in China, was reported to produce some benefit for patients suffering from MG in the 1930s [7,8], but it was largely replaced once acetylcholinesterase inhibitors were found to give consistent and effective symptomatic treatment for the disease [9]. In CMS, clearly an alternative to cholinesterase inhibitors was required for endplate AChE deficiencies, and in these patients a beneficial response to ephedrine was reported [10]. In addition, anecdotally, in other CMS, patients would sometimes report having benefit from ephedrine or other adrenergic agonists. However, the identification of DOK7 mutations as a major cause of CMS [11] and their slow but remarkable improvement with β2-agonist medication [12] provided the impetus for their more widespread adoption and thus re-emerging as a mainstream option in treatment.

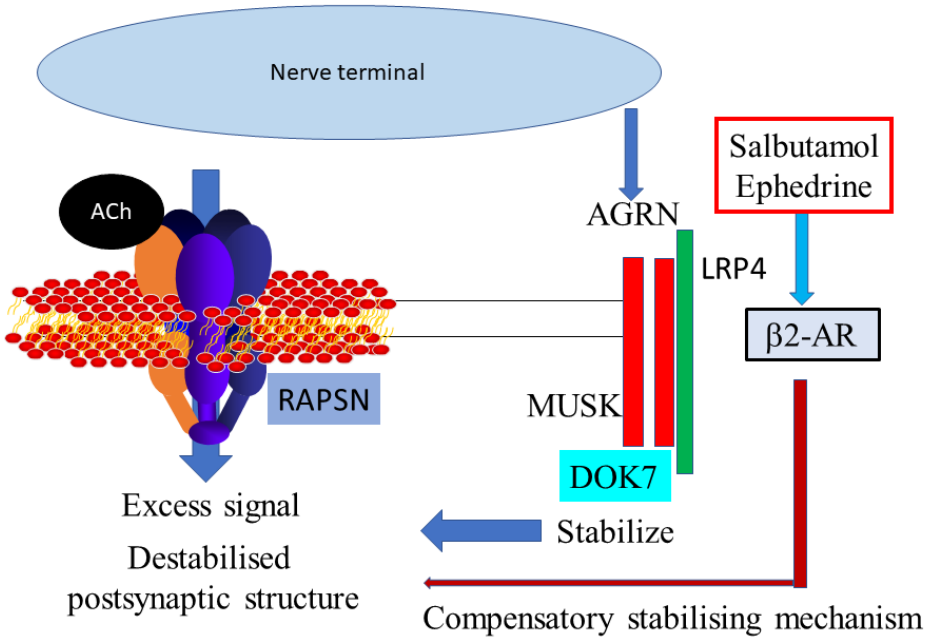
β2-adrenergic receptor agonists in the treatment of DOK7-CMS

After detecting mutations in DOK7 in a cohort of CMS patients with unknown genetics [13] it quickly became apparent from the clinical notes that there was a lack of response to cholinesterase inhibitors, but many patients insisted they felt better when taking β2-agonist medication, either ephedrine or salbutamol/albuterol [14]. Following this observation, a prospective study was set up to record the long-term response to ephedrine of patients with newly identified DOK7 mutations who were not previously on β2-agonist medication [12]. Ephedrine given at doses between 15 and 90 mg/ day improved muscle strength as measured by the quantitative myasthenia gravis (QMG) severity score and mobility scores [15]. Unlike treatments such as pyridostigmine or 3,4-DAP which in other forms of CMS take effect quickly, ephedrine was found to lead to delayed and progressive improvement in muscle strength taking place over months. Indeed, patients would often be found to be continuing to improve over a year from first starting treatment. The QMG score, designed for MG, is not an ideal method for severity scores in DOK7-CMS, and those scores that reflected the pattern of proximal muscle weakness seen in DOK7-CMS (such as times of arm raise, leg raise, or neck raise) were those that showed the most improvement. Moreover, the patients themselves reported profound benefit in their everyday living activities. What is also of note is that, while patients take a long time to improve, if they stop taking their medication they weaken rapidly back to baseline, usually within three or four days. Although ephedrine was used in this initial study, in a number of countries ephedrine is not easily available, in which case salbutamol/albuterol has been found to be an equally good alternative medication [16]. Salbutamol/albuterol is well tolerated in children. Many child neurologists have experience with using it in other neuromuscular disorders, and so it is frequently the drug of choice. Our initial observation and results of the prospective study are supported by numerous reports of the beneficial use of β2-agonists for DOK7-CMS where it is seen to be effective from early childhood through old age [17-21]

Treatment of CMS due to mutations in the AGRN-LRP4-MuSK-DOK7 pathway governing neuromuscular junction formation and stability

MuSK, plays a critical role in the formation of neuromuscular synapses and in maintenance of the synaptic structure [22]. MuSK is activated following the interaction of nerve-derived AGRN with LRP4, which in turn interacts with MuSK through its third β-propeller domain, leading to MuSK dimerization and phosphorylation [23]. Neuronal AGRN contains specific short RNA splicing inserts (of 4, 8, and 11 amino acids) that give it effective AChR clustering activity and that are not present in other AGRN forms, such as muscle-derived AGRN. Neuronal AGRN is secreted from the motor nerve terminal to perform its function within the synaptic cleft. DOK7, an intracellular protein, interacts with MuSK at the juxtamembrane phosphotyrosine binding site to amplify phosphorylation of both MuSK and DOK7[24]. This initiates what is still a poorly understood signalling pathway; it is thought to include the recruitment of Crk and Crk-L by DOK7 [25] that is crucial both for efficient clustering of the AChR on the postsynaptic membrane and development and maintenance of the synaptic structure. Mutations in DOK7 impair AChR cluster formation and cluster complexity in myotube cell cultures [26]. In muscle biopsies from patients with DOK7 mutations the neuromuscular junctions are found to be smaller than normal, and there is evidence for unstable or reforming synaptic structures [13,24]. It would appear that β2-agonists are able to partially compensate for the impaired DOK7 function, presumably through affecting the pathway responsible for maintaining synaptic structure somewhere downstream of DOK7. Therefore, it was not surprising to find that patients with mutations in MuSK, in the β-propeller domain of LRP4, or in AGRN also have a marked beneficial response to β2-adrenergic receptor agonists. However, the precise molecular mechanism has yet to be elucidated. It is likely to be through the increase in intracellular cAMP and activation of various protein kinases in the vicinity of the motor endplate. Although many protein kinases have been shown to activate or enhance AChR cluster formation in cell culture models, a definitive understanding of their

Figure 2. Representation of the destabilizing effect of neurotransmission which can lead to dispersal of AChR clusters and deconstruction of synaptic structure with the balancing signal from the AGRN-LRP4-MuSK-DOK7 signaling pathway that stabilizes endplate structure. It is hypothesized the β2-adrenergic receptor activation can provide additional input into this pathway downstream from DOK7.



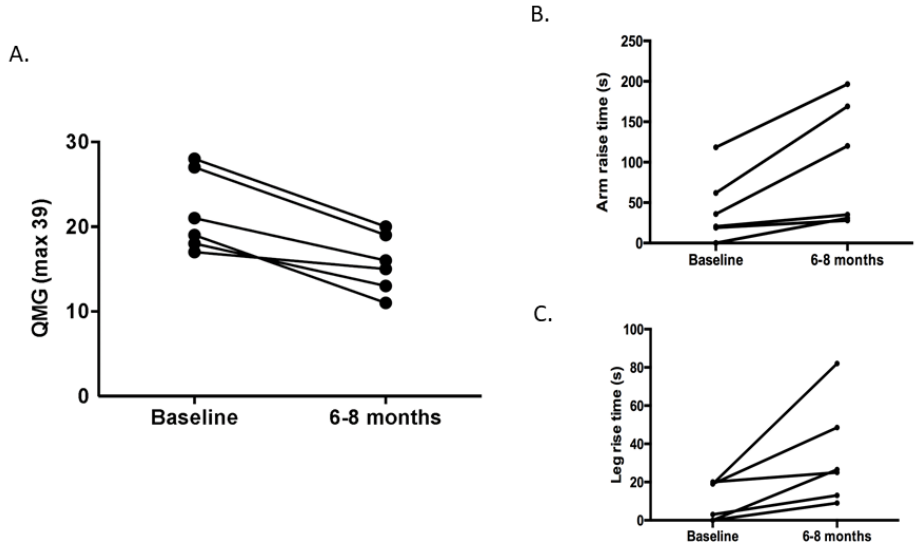
role at the neuromuscular junction *in vivo* is lacking. The slow and gradual response to β2-adrenergic receptor agonists treatment would argue against a direct effect on components of the AChR clustering pathway but rather for enhancement of the environment favoring stabilization of the synaptic structures [27]. Patients with MuSK or LRP4 mutations tend to respond equally as well as DOK7-CMS patients, but with AGRN mutations the response tends to be far less marked. This may be because AGRN is also synthesized by muscle (though not the neuronal RNA-spliced isoforms required for interaction with LRP4), and thus patients with mutations that also affect muscle AGRN often have a myopathic component to their weakness as well as impaired neuromuscular junction function. Though the β2-adrenergic receptor agonists may improve neuromuscular junction function they do not have a similar effect on the myopathic damage.

Patients with endplate acetylcholinesterase deficiency due to mutations in COLQ were identified well before DOK7-CMS was characterized and were reported to have a beneficial response to ephedrine [28], and this response has been confirmed in many subsequent reports [29,30]. The prolonged presence of acetylcholine in the synaptic cleft resulting from impaired breakdown of acetylcholine is thought to lead to excess calcium entry through the AChR and results in an endplate myopathy [31]. β2-adrenergic receptor agonists may help in repair of the disrupted neuromuscular junctions. Alternatively, there is some evidence suggesting that COLQ can interact with MuSK and contribute to the MuSK signalling pathway [32]. In which case the medication would be exacting a similar effect as seen in other cases with mutations in the AGRN-LRP4-MuSK-DOK7 pathway.

β2-adrenergic receptor agonists in treatment of severe AChR deficiency syndrome

Our current understanding of the maintenance of neuromuscular junction synaptic structure is largely based on a series of experiments in mice in which elements of the neuromuscular synaptic apparatus were 'knocked out' [22]. In the model derived from these experiments it has been proposed that the neurotransmitter ACh itself acts to destabilize both the neuromuscular junction structure and the aggregation of AChR on the postsynaptic membrane, but that the AGRN-LRP4-MuSK-DOK7 pathway works to counter this (Figure 2) [33,34]. COLQ mutations or anticholinesterases, by increasing the effective concentration and duration of ACh in the synaptic cleft, are likely to exacerbate destabilization of synaptic structures. Some evidence for the effect of anticholinesterases on the neuromuscular junction was obtained in the early 1970s, where in long-term usage they were found to affect the neuromuscular junction fine structure [35]. If β2-adrenergic receptor agonists can somehow enhance the AGRN-LRP4-MuSK-DOK7 pathway, they should be able to nullify this detrimental destabilizing effect of the cholinesterase

Figure 3. The response of patients with severe AChR deficiency syndromes on optimized pyridostigmine to the introduction of salbutamol/ephedrine to their medication. **A**. Reduction of the QMG severity score at 6-8 months. **B, C.** Response of arm raise and leg raise times after 6-8 months, illustrating the marked and consistent improvement seen for two quantitative components of the QMG scoring system.



inhibitors. Using this model as a basis, a rational hypothesis can be put forward that many other forms of CMS that are treated with anticholinesterase medication might find additional benefit from β2-adrenergic receptor agonists.

Cholinesterase inhibitors such as pyridostigmine are the first line treatment for patients with a deficiency of endplate AChR due to mutations in the AChR ε-subunit (CHRNE). These patients respond well to cholinesterase inhibitors but also often have some structural changes in their endplates with the loss of postsynaptic folds and the area of the endplates that stain with α-bungarotoxin elongated along the muscle fiber [36]. A number of AChR deficiency patients seen in clinic were found to initially respond very well to pyridostigmine, but over time the response diminished. This cohort became severely affected despite many attempts at optimizing their treatment. It was therefore hypothesized that these patients might benefit from the addition of β2-agonists to their medication that would counter the long-term detrimental effect of the cholinesterase inhibitors on the synaptic structures in these patients. A prospective study was set up to quantify any potential improvement. Medication was given on an outpatient basis with incremental dosage dependent on body weight and tolerability; the final dose ranged between 0.5 and 1 mg/kg/d for ephedrine and 0.05 and 0.2 mg/kg/d for salbutamol. In all patients, baseline therapy with pyridostigmine and 3,4-DAP or pyridostigmine alone remained unchanged for at least a year before adding salbutamol or ephedrine and during the follow-up period. Blood pressure, heart rate, and ECG were performed before treatment and at each dosage increment. All patients showed unequivocal improvement in functional ability as measured using the QMG severity score (Figure 3). Four patients who had been non-ambulant for many years acquired the ability to walk independently. Whereas patients with DOK7-CMS tend to see the benefit from their medication with β2-agonists as a gradual improvement over a period of months, the CHRNE AChR deficiency patients were found to respond more quickly with the majority of the improvement felt within the first month after initiation. Follow up of the patients showed that in most cases the improvement was sustained for years [37]. In our experience less severe cases of AChR deficiency due to CHRNE mutations frequently also benefit from the addition of β2-agonists but the improvement may not be so dramatic due to starting from a less severe baseline score.

β2-adrenergic receptor agonists in in mouse models of CMS

While it is clear that CMS patients benefit from β2-adrenergic receptor agonists, it is important to establish that this is truly due to a function effect at the neuromuscular junction.

Since CMS are rare, the easiest way to investigate is through mouse models. One mouse model that accurately reflects the respective human condition is the model for AChR deficiency syndrome [38]. In humans the fetal form of the AChR that contains the γ-subunit is expressed at low levels in adult muscle throughout life, whereas in mice, expression of the γ-subunit is turned off by three weeks

after birth. To reflect the human condition, the human γ-subunit was introduced into the mice under the muscle actin promoter to induce continuous low-level expression of the γ-subunit along the muscle fiber. The mice generated are myasthenic with fatigable muscle weakness, reduced endplate receptor number, and electrophysiological evidence of impaired neuromuscular junction function [38]. These mice were subjected to different treatment regimens to mirror what might occur in clinic, and in particular two cohorts were compared where one was given pyridostigmine alone and a second had salbutamol/albuterol added six weeks after pyridostigmine was initiated. The results of the study showed that addition of salbutamol reduced fatigable muscle weakness, reduced amplitude decrement of the compound muscle action potential on repetitive stimulation, and increased postsynaptic area labelled by α-bungarotoxin. Whereas pyridostigmine treatment reduced postsynaptic folds, the addition of salbutamol restored postsynaptic folding [37]. Thus, there is direct confirmation of the beneficial effect of salbutamol on neuromuscular junction structure and function. Similar results have been seen in a mouse model of acetylcholinesterase deficiency [39] and DOK7-CMS [40], although the DOK7-CMS mouse model is so severely affected that it is difficult to make direct comparison with the human situation. However, salbutamol did increase survival and the number of detectable endplates in the DOK7 CMS mouse model, again demonstrating its effect at the neuromuscular junction.

Concluding remarks

Treatment of CMS is often challenging. The current repertoire of drugs is not specifically licensed for CMS largely due to rarity and consequent lack of randomized controlled trial evidence of efficacy. Nevertheless, the CMS are a group of genetic disorders that mostly respond well to the current symptomatic treatments, which are often life-transforming. As stated earlier, an agent that provides benefit in one CMS subtype can be harmful in another. Thus, it is important to obtain an early genetic diagnosis, and it may also be crucial to establish molecular pathology for a particular mutation to guide treatment. It should be noted that some syndromes such as DOK7-CMS or MuSK-CMS may give the impression of a good response to cholinesterase inhibitors at first dosing but may subsequently suffer severe deterioration in their condition, emphasizing the imperative of a molecular diagnosis. Moreover, because each patient's response may be different or vary over time, it is important to optimize treatment and treatment combinations, and to provide follow up.

Over the last 10–15 years, β2-agonists have re-emerged as a mainstream option in treatment. Clearly an alternative to cholinesterase inhibitors was required for endplate AChE deficiencies, and in these patients a beneficial response to ephedrine was reported [28]. However, it was following the identification of DOK7 mutations as a major cause of CMS and their slow but remarkable improvement with β2-agonist medication that provided the impetus for its more widespread adoption. The idea that acetylcholinesterase inhibitors can be detrimental to neuromuscular junction structure suggests that the β2-agonists are potentially beneficial as a counteracting agent wherever cholinesterase inhibitors are appropriately used. In simple terms, this is whenever increased synaptic duration and density of acetylcholine can enhance signal transmission at the neuromuscular junction, then β2-agonists could be used to alleviate long term detrimental effects. Ephedrine and salbutamol can be used interchangeably, although we currently use salbutamol more frequently, because there is more safety data for its use in children and it is easier to prescribe. However, ephedrine is a good alternative for those in whom salbutamol causes side effects. Higher doses appear to give a greater response, but this needs to always be weighed against the side effect profile. In general, we would recommend increasing salbutamol progressively up to 4 mg twice a day over the course of 6 months when side effects are not apparent. Medication can be increased further up to 8 mg twice a day in older children/adults if required. In a few patients we, and others, have found that the beneficial response can diminish over time, which in most of the cases we have observed is associated with an adolescent growth spurt. Some have tried a 'drug holiday' to restore efficacy, but in our experience patients suffer an often serious and rapid decline with the withdrawal of medication which can then take many weeks or months to regain the functional levels seen prior to drug withdrawal. The precise function of β2-adrenergic receptors at the neuromuscular junction is not known, but there are reports that they are present at high density and that neuromuscular junctions may receive direct sympathetic innervation [41,42]. It is also known that β2-adrenergic receptor blockers are detrimental for myasthenia gravis or CMS patients, which further suggests a direct role at the neuromuscular junction. With time the role of β2-adrenergic receptors at the neuromuscular junction will be elucidated, but until then it is useful to view treatment for the many phenotypically different CMS as a balancing act between functional enhancement of signal transmission by cholinesterase inhibitors, that long-term are detrimental to synaptic structure, with the counterbalancing enhancement of structure by β2-agonists.

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