SCIENTIFIC REPORTS

OPEN

Specific inhibition of acetylcholinesterase as an approach to decrease muscarinic side effects during *myasthenia gravis* treatment

Received: 18 May 2017
Accepted: 14 November 2017
Published online: 10 January 2018

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Non-selective inhibitors of cholinesterases (ChEs) are clinically used for treatment of *myasthenia gravis* (*MG*). While being generally safe, they cause numerous adverse effects including induction of hyperactivity of urinary bladder and intestines affecting quality of patients life. In this study we have compared two ChEs inhibitors, a newly synthesized compound C547 and clinically used pyridostigmine bromide, by their efficiency to reduce muscle weakness symptoms and ability to activate contractions of urinary bladder in a rat model of autoimmune *MG*. We found that at dose effectively reducing *MG* symptoms, C547 did not affect activity of rat urinary bladder. In contrast, at equipotent dose, pyridostigmine caused a significant increase in tonus and force of spontaneous contractions of bladder wall. We also found that this profile of ChEs inhibitors translates into the preparation of human urinary bladder. The difference in action observed for C547 and pyridostigmine we attribute to a high level of pharmacological selectivity of C547 in inhibiting acetylcholinesterase as compared to butyrylcholinesterase. These results raise reasonable hope that selective acetylcholinesterase inhibitors should show efficacy in treating *MG* in human patients with a significant reduction in adverse effects related to hyperactivation of smooth muscles.

Synaptic transmission at the skeletal neuromuscular junction (NMJ) is indispensable for survival of living organisms by transducing complexity of cerebral commands to muscular twitches. In the vertebrate NMJ presynaptic electrical signal is transmitted by acetylcholine (ACh) which is released from motor nerve ending, and then diffuses through synaptic cleft to activate postsynaptic nicotinic acetylcholine receptors (nAChRs) of muscle type $((\alpha 1)2\beta 1\delta\varepsilon)$[1]. This, in turn, leads to membrane depolarization (postsynaptic excitatory potential), triggering action potential (AP) and muscle twitch. Impairment of neuromuscular synaptic transmission results in muscle weakness and even death if synapses of respiratory muscles are affected.

The most common form of pathological muscle weakness is *myasthenia gravis* (*MG*). This chronic autoimmune neuromuscular disorder is characterized by fluctuating weakness of voluntary muscle groups. Weakness in *MG* is caused by autoantibodies directed specifically and primarily towards muscle type nAChRs. Antibodies reduce the number of functional nAChRs in the NMJs by a combination of complement-mediated membrane lysis and acceleration of receptor catabolism[2,3]. The cause of autoimmune response is unknown and only symptomatic therapies for *MG* are currently available. Clinically relevant treatments of *MG* include immunosuppressive drugs, plasmapheresis, thymectomy and inhibitors of cholinesterases (ChEs)[4]. All treatments suffer from a variety of side effects. For daily pharmacological correction of muscle weakness, the most frequently used drugs cause partial inhibition of AChE and butyrylcholinesterase (BChE). These enzymes catalyze hydrolysis of ACh, thus

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