Intramuscular injections of Botulinum Neurotoxin A (BoNT A) are given children with spastic cerebral palsy (GMFCS I-III) with the aim of improving gait by reducing hypertonicity while preserving motor function. An important functional aspect of such locomotion is a fast force development at the beginning of contraction, which in human motor function often is achieved by firing double pulses with an inter-pulse interval as short as 1.4 ms. We hypothesize that BoNT A reduces the ability of the neuromuscular junction to transmit doublets and therefore also reduces their potentiating effect on force development.

**Introduction**

Intramuscular injections of Botulinum Neurotoxin A (BoNT A) are given children with spastic cerebral palsy (GMFCS I-III) with the aim of improving gait by reducing hypertonicity while preserving motor function. An important functional aspect of such locomotion is a fast force development at the beginning of contraction, which in human motor function often is achieved by firing double pulses with an inter-pulse interval as short as 1.4 ms. We hypothesize that BoNT A reduces the ability of the neuromuscular junction to transmit doublets and therefore also reduces their potentiating effect on force development.

**Methods**

Juvenile Wistar rats in general anesthesia (hypnorm/midazolam) were injected with BoNT A (4-6 U/kg bw) or a similar volume of 0.9% saline (sham) in the gastrocnemius muscle leaving the contralateral leg untreated. After one to four days, animals were sacrificed and soleus muscles isolated with intact motor nerve. The isolated muscle-nerve preparations were incubated in Krebs-Ringer buffer and stimulated electrically with 60 Hz train, single pulses or doublets with inter-pulse interval from 2 to 10 ms (~100-500 Hz) while isometric force was measured.

**Results**

BoNT A treatment caused a progressive decline in maximal tetanic force of the muscle-nerve preparations compared to force of untreated muscles and sham muscles (Fig. 2A). Thus, a BoNT A-induced reduction in nerve-stimulated tetanic force of <33% was defined as a mild treatment, a reduction of 34-66% degree as intermediate and a force reduction of >66% as a severe treatment. In accordance herewith, force of a single pulse and double pulses spaced by 2 and 4 ms was found to decrease with the degree of BoNT A treatment becoming more severe (Fig. 2B). Furthermore it was seen that a double pulse separated by 2 or 4 ms potentiates force of a single twitch in a manner that depended on the interval between the pulses and the degree of BoNT A treatment. Thus, double pulses separated by 2 ms increased force of a single twitch to ~200%, whereas a doublet with inter-pulse interval of 4 ms increased force to ~220% (Fig. 2C). This potentiating effect of doublets on force production is seen to decrease when muscles are treated with BoNT A. Panel C illustrates that this potentiation was attenuated by BoNT A in a manner that depended on the degree of BoNT A treatment and the time between the pulses. All data are expressed as mean±SD. * indicates P<0.001.

**Conclusion**

Intramuscular injections of BoNT A reduced the ability of the neuromuscular junction to transmit very closely spaced pulses in skeletal muscle. This indicates that if the treatment is severe, BoNT A may interfere with motor function during locomotion, which could compromise gait.

**Take home message**

Severe treatment with Botulinum Neurotoxin A in children with spastic cerebral palsy (GMFCS I-III) implies a risk of interfering with motor function which could compromise gait.

**Figure 1.** The experimental set-up for measurement of isometric force.

**Figure 2.** Effect of BoNT A on force potentiation. The figure shows that tetanic force of BoNT A-treated muscles decline rapidly compared to untreated controls and sham muscles (Panel A). Panel B shows that double pulses separated by 2 or 4 ms produce approximately 200% or 220% of force of a single twitch. This potentiating effect of doublets on force production is seen to decrease when muscles are treated with BoNT A. Panel C illustrates that this potentiation was attenuated by BoNT A in a manner that depended on the degree of BoNT A treatment and the time between the pulses. All data are expressed as mean±SD. * indicates P<0.001.

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