The mechanism of EMFACE stimulation of muscle after the application of botulinum based neurotoxin

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***Abstract* — EMFACE is a new device that induces supramaximal muscle contractions of delicate facial muscles while heating the skin and underlying tissues. However, many EMFACE patients are users of botulinum based neurotoxins that are used to immobilize facial muscles in order to prevent repetitive skin folding. As such, facial muscles of these patients should not be contrated during EMFACE treatments. However, clinical studies have shown that it is possible to induce contractions of these muscles by external stimulation such as during EMAFCE. However, the mechanism of how it is possible is not entirely known.**

Keywords—Botulinum toxin, BOTOX, HIFES, EMFACE, Stimulation, Facial, Muscle

I. Introduction

Neuromodulators in aesthetic medicine, such as Botox, Dysport, Xeomin or Jeuveau have become the most frequently sought nonsurgical aesthetic procedure[1]. Type A botulinum-based neurotoxins have a myriad of clinical indications[2]. They are most frequently utilized to treat dynamic facial rhytides[3] involving the glabella, frontalis and periocular regions. Botulinum neurotoxins block neurotransmitter release in the synaptic neuromuscular junction to block voluntary muscle contraction. With blocked contractions, wrinkle formation is prevented as the overlying skin is not being repetitively folded during daily activities and thus aids in maintaining a more youthful skin appearance.[4,5] By decreasing the contraction strength of some facial depressor muscles, there is temporary increased tone of elevating facial muscles. There are also secondary effects such as reduction in erythema which may allow greater light reflection.

Recently, a novel device EMFACE was introduced to stimulate botulinum neurotoxin-blocked muscles to prevent muscle atrophy. EMFACE is able to stimulate the blocked facial muscle even though it is not possible voluntarily. In fact, the use of external stimulation on neurotoxin-blocked muscles has already been documented in multiple studies [6,7], however, the mechanism of how it is possible is not exactly known.

Although it is not entirely clear, all these suggest that external stimulation has the ability to bypass the effect of botulinum neurotoxins. The goal of this paper is to summarize existing knowledge on the topic and provide a viable hypothesis that could explain why such an effect is demonstrated during an EMFACE treatment.

II. The physiology of muscle contraction

To better understand the effect of botulinum neurotoxin, it is necessary to first explain the standard function of the neuromuscular junction and muscle contraction. The neuromuscular junction is responsible for the chemical transmission of an electrical impulse from the nerve to the muscle, to produce a muscle contraction. When a nerve impulse in the form of action potential reaches a nerve ending, voltage-gated calcium (Ca2+) channels are activated, which causes an influx of Ca2+ ions into the presynaptic neuron from the extracellular space. In the presynaptic neuron, the calcium cations interact with synaptic vesicles and enable their association with the presynaptic membrane. After the fusion of synaptic vesicles with presynaptic membrane, the content of the vesicles – neurotransmitter acetylcholine (Ach) is released into the synaptic cleft - quantal release.[8]

Under normal conditions, the released Ach transmits the electrical signal to the muscle fiber by depolarizing the muscle fiber membrane. Depolarized muscle membrane activates sarcoplasmic reticulum where Ca+ ions are stored and releases them.[8] The presence of the Ca+ ions in the muscle fiber results in a sliding process between actin and myosin filaments which slide alongside each other resulting in muscle contraction. The muscle contraction lasts as long as the Ca+ ions are present in the muscle fiber, however, Ca+ ions are quickly (fraction of second) returned into the sarcoplasmic reticulum unless there is another action potential that again increases the level of Ca+ ions or keeps it at the same level.[8] The collective shortening of the sarcomeres is the molecular mechanism behind a muscular contraction.[9]

III. The effect of botulinum neurotoxin

Botulinum based neurotoxin is affecting the process of muscular contraction at the level of neuromuscular junction. When applied, it works as a protease and prevents the fusion of the vesicles with the presynaptic membrane[10]. Without this fusion, the Ach cannot be released into the neuromuscular junction and trigger the muscle contraction as described above. It is a chemical denervation that causes partial paralysis of the innervated muscle. However, such

paralysis is not causing any damage to the nerve or the neuromuscular junction and is not permanent[11].

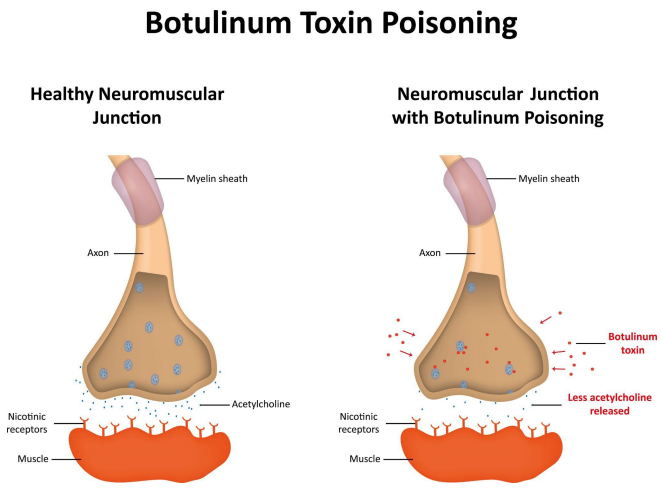

Figure 1: Botulinum toxin blocks the release of acetylcholine to neuromuscular junction, which does not allow the signal transmission into the muscle and thus blocks the muscle contraction.

IV. External stimulation of botulinum-paralysed muscle

There are two approaches to induce muscle contraction externally. First, muscles can be stimulated by applying an external electrical field to the muscle innervating neuron, where it induces an action potential, which is then carried to the neuromuscular junction.[12] Muscle can also be stimulated directly, as the muscle itself is an electrically excitable tissue.[13] If the muscle is stimulated through the neuron and, the whole muscle or all muscles innervated by this neuron are stimulated and contracted. With direct stimulation of the muscle tissue, only those muscle fibers in the electrical field are affected and a high stimulation intensity is needed to induce such contraction.[14] To contract the entire muscle by direct stimulation of the muscular tissue, a critical number of muscle fibers must be recruited.[13]

Multiple studies have shown it is possible to stimulate even the botulinum-paralysed muscles [7,15]. However, it is not entirely clear how such stimulation overcomes the barrier made by the botulinum neurotoxin. Upon the application of botulinum toxin, the membrane of the presynaptic neuron should be practically impermeable to Ach molecule due to its size as the the fusion of vesicles and presynaptic membrane ("quantal release") is blocked. Yet, the clinical trials are showing that externally it is possible to overcome this barrier and although the mechanism of how this happens is not entirely clear, several hypotheses were proposed to explain such mechanism:

Non-quantal Ach release

One of the possible explanations could be the non-quantal Ach release. It has been shown that aside from the above-described quantal release of Ach, also a non-quantal release occurs at the neuromuscular junction[16].

Non-quantal Ach release was proposed to be caused by the high-affinity choline transporter, which under normal physiological conditions returns the inactivated Ach (choline) from the synaptic cleft back to the nerve ending. Experimental findings indicate that this transporter may also transport the Ach to the neuromuscular junction. Its activity appears to be dependent on the concentration of calcium in the cytoplasm, which correlates with the firing rate of the neuron (number of induced action potentials)[16–18]. Considering that maximum firing rate of a neuron during voluntary muscle contractions reaches up to 25 Hz[19], while with external stimulation it is possible to induce firing rates in the order of hundreds of Hz, it could be assumed, that with an increased firing rate frequency, also the cytoplasmic calcium concentration increases, thus also increasing the level non-quantal Ach release.

Based on such findings, it could be hypothesized that such a non-quantal release of small amounts of Ach into the synapse still occurs, even in botulinum toxin denervated muscle. However, during voluntary contractions, the amount of the Ach is not sufficient to cause depolarization and the muscles thus remain relaxed. By applying an external high-frequency electrical field that surpasses the frequency of brain signals, the activity of the high-affinity choline transporter could be elevated, leading to exaggerated non-quantal release of Ach in amounts sufficient enough to cause muscle depolarization and contraction.

Additionally, a long-term insufficient concentration of Ach in the synapse, due to the application of botulinum toxin can lead to an increased expression of n-acetylcholine receptor (nAChR) on the postsynaptic membrane and therefore also to increase in the sensitivity of the muscle to Ach [20,21]. A lower amount of Ach would thus be needed to induce such depolarization.

Direct stimulation of the muscle fiber membrane

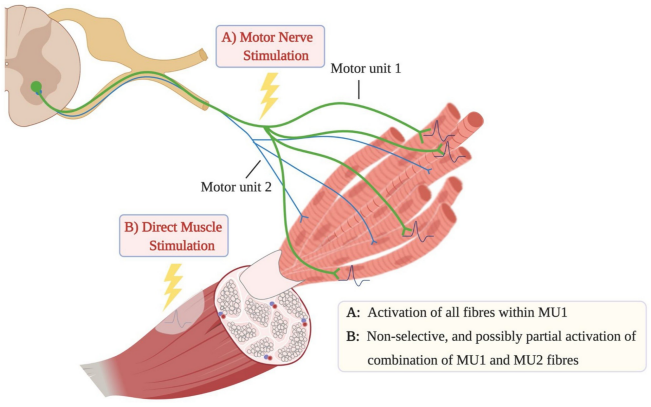

Figure 2: The principle of direct muscle stimulation vs. motor nerve stimulation. With nerve stimulation, the nerve is stimulated and signal propagates towards the muscle, while with direct stimulation the muscle fiber is stimulated directly. Image adopted from Guo et al.[22]

Another possible explanation for the externally induced contraction of botulinum-denervated muscles could be via the direct stimulation of the postsynaptic membrane. The

external electrical field could possibly induce action potential directly on the muscle fiber membrane and thus trigger the influx of Ca+ ions into the muscle fiber to contract the muscle.

Nevertheless, multiple studies contradict such hypothesis. Studies investigating the use of external stimulation of muscles fully denervated by physical transection showed that very high intensity (pulses in ms) is needed to depolarize the muscle fiber membrane and induce contractions of these muscles directly, while EMFACE uses lower energy pulses in μs.[23,24] Furthermore, with direct muscle fiber membrane stimulation it is difficult to stimulate the entire muscle, rather it has been shown to stimulate only a portion of the muscle, while with EMFACE the entire muscles are contracted.

However, all these conclusions regarding the direct muscle stimulation are based on studies performed on skeletal muscles only. Facial muscles are of significantly different proportion and are much more superficial located in low depths of 3-8 mm[25]. All this may influence the response. As the facial muscles are more delicate, lower intensity may suffice to irritate the muscle membrane. Since the thickness of some facial muscles may be as small as 0.5mm[26], it may be possible that such stimulation is able to recruit enough muscle fibers to induce contraction of the entire muscle. In addition, this could actually explain the non-stimulation of the masseter muscle during the EMFACE treatment. In the vast majority of patients the masseter is not stimulated and it could be explained by the fact that the neuronal branch innervating the muscle is not in the application field, but also by the fact that masseter muscle is one of the largest facial muscles with thickness of up to 3.5mm[27] and during the treatment not enough muscle fibers are recruited to induce full masseter activation.

V. Summary & Conclusion

It is clear that it's possible to externally stimulate botulinum-denervated muscles, yet, the underlying mechanism is not. Current paper proposes a possible explanation of this phenomenon based on the existing knowledge of the processes on the neuromuscular junction and muscle contraction. However, multiple different factors may play a role in the mechanism. More experimental studies are needed to fully understand why it is possible to externally stimulate the botulinum-paralysed muscles.

In regard to EMFACE specifically, a study by Chilukuri et al.[28] showed that during the EMFACE treatment the botulinum-denervated muscles are being contracted and what is most important, the EMFACE treatment does not interfere with the effect of botulinum toxin itself. No negative effects of the EMFACE stimulation on the efficacy of the botulinum toxin were found.

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