Complementary Pre-Synaptic and Post-Synaptic Strategies to Fight Against Expression Wrinkles

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Introduction

Nowadays, looking youthful is considered a sign of beauty and success. The cosmetic world is constantly searching for new developments to prevent the appearance of the first signs of ageing on the skin, which is also a growing demand among the worldwide population.

Expression wrinkles are the first visible sign that reminds us that our body is ageing. Repeated facial muscle contraction is responsible for the appearance of these unpleasant lines on the top half of the face (crow's feet, frown wrinkles, etc), but also around the mouth, nose, etc, which can start showing as early as the age of 30. Both surgery and Botulinum Toxin injections are invasive and expensive methods compared to cosmetic alternatives to fight against them. However, if a cosmetic treatment targets the right mechanism entailed in muscle contraction, it can help to minimise these undesirable lines.

Muscle contraction is a process where both the nerve and the muscle are involved in a synapse named Neuromuscular Junction (NMJ). The motor neuron releases the neurotransmitter acetylcholine (ACh) which travels through the synapse to activate the ACh receptors (AChRs) on the fibre muscle surface, creating an action potential and leading to muscle contraction. However, contraction is a complex mechanism formed by pre-synaptic and post-synaptic mechanisms involving several proteins, channels and vesicles.

At the pre-synaptic pathway, a localised change in membrane potential, known as action potential, is transmitted along the neuron to the terminal where it triggers the entry of Ca^{2+} into the neuron. Natural enkephalins are endogenous opioids that modulate Ca^{2+} channels by binding to their receptors located on the outside of neurons. When calcium ions enter the pre-synaptic terminal, the fusion of vesicles containing ACh with the membrane is induced, releasing the neurotransmitter into the synapsis and leading to muscle contraction. On the other hand, the combination of SNARE (SNAp REceptor) proteins (VAMP, Syntaxin and SNAP-25) forms a complex which acts like a cellular hook capturing vesicles and fusing them with the membrane. This complex is essential for ACh release at the synapsis and mediates the final steps of the exocytosis.

Released acetylcholine diffuses along the synapse to reach the AChRs on the neuromuscular junction, thus starting the post-synaptic route. At the NMJ, release of the nerve-secreted proteoglycan agrin activates MuSK (Muscle-Specific Kinase) and induces the formation of AChR clusters. AChR clustering is essential for reaching the sufficient magnitude of potential to initiate an action potential which will propagate through the muscle and originate muscle contraction. If AChRs were not recruited by MuSK, the ACh signal would not be strong enough to trigger contraction. Thus, modulation of MuSK activation leads to attenuation of muscle contraction.

In order to target different parts of the formation of expression wrinkles, we have designed four peptides which act in pre-synaptic and post-synaptic mechanisms to relax the muscle. The pre-synaptic strategy involves different mechanisms that restrain or inhibit the release of the neurotransmitter (acetylcholine) from the motor neuron. The most well-known example of a pre-synaptic treatment is Botulinum Toxin, which cleaves the protein SNAP-25 irreversibly, preventing SNARE complex assembly and finally paralysing the muscle. However, we have developed safer cosmetic alternatives to Botulinum Toxin such as the well-known peptides Leuphasyl®, Argireline® and SNAP-8. The enkephalin-like peptide Leuphasyl® couples to the enkephalin receptor decreasing the neuron excitability and resulting in ACh release modulation. Argireline® and SNAP-8 peptides target the same protein complex as Botulinum Toxin A. Both actives are a mimic of the N-terminal end of SNAP-25