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Unexpected Consequences

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Abstract

Botulinum neurotoxin (BoNT) is a widely used therapeutic in part because its mechanism of action is much wider than initially expected. Since BoNT is taken up more avidly in active presynaptic terminals, there is some selectivity for weakening muscles involved in frequent involuntary movements. BoNT blocks gamma motoneurons as well as alpha motoneurons, hence reducing afferent spindle activity which appears to have a favorable effect. Some BoNT is retrogradely transported in the motor axons, leading at least to reduction in recurrent inhibition mediated by the Renshaw cell. There are also central nervous system changes after BoNT injections and these may be due to brain plasticity.

Keywords

Botulinum neurotoxin; dystonia; Renshaw cell; retrograde transport; brain plasticity

Botulinum neurotoxin (BoNT) is a fascinating molecule with an interesting history. At many steps along the way, the outcome was unexpected, and this was true from the beginning.

BoNT was first known as a cause of food poisoning. In southwestern Germany in the late 18th century, some outbreaks were related to sausages. The physician and poet Justinus Kerner in 1820 published the first clear description of what is now called botulism, the name coming from the Latin “*botulus*” meaning sausage (Meyler and Cooper 2007). He thought the cause was a biological toxin that interfered with motor and autonomic nerve conduction. Amazingly, he speculated that small amounts of this toxin might be used therapeutically in conditions of hyperexcitability. He expected the unexpected, but it turned out that others also developed similar ideas about the possible beneficial effects of toxins.

The therapeutic history traditionally begins with Alan B. Scott who was trying to find a treatment for strabismus (Truong et al. 2009). He had the idea that weakening the muscle

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Ethical statement

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opposite to the weak muscle would balance the eye position. Like Kerner, he proposed that a toxin might be therapeutic for this purpose. Multiple toxins failed until he came across botulinum toxin which he obtained from Edward J. Schantz. Scott was introduced to Schantz by Daniel Drachman who himself was using botulinum toxin to focally weaken muscle. Schantz credits Vernon Brooks with the idea of focal weakening, and Brooks credits Arnold Burgen, his PhD advisor, who suggested that this might work. Bergen showed that botulinum toxin blocks neuromuscular junction transmission, and Brooks showed that there was a specific block of acetylcholine release. After tests in animals, Scott obtained an IND (investigational new drug) from the Food and Drug Administration (FDA) to try botulinum toxin type A in humans in 1977. His first human results were published in 1980 (Scott 1980). Because of prior disclosure (making the information public), it was not possible to patent the therapy. Scott started a company, Oculinum, to produce the toxin, which enabled other researchers to test the agent for other conditions. Fahn and colleagues, in an abstract (Fahn et al. 1985), first showed the utility for blepharospasm, and Tsui and colleagues, in a pilot study (Tsui et al. 1985), first showed the utility in cervical dystonia. These were shortly followed by publications of double-blind studies both in blepharospasm (Jankovic and Orman 1987) and cervical dystonia (Tsui et al. 1986). Research expanded for treatment of other dystonias, hemifacial spasm, and spasticity, all before the end of the 1980s. Since then, the number of indications has expanded almost exponentially. Again, it was certainly unexpected that the medical indications of the BoNTs would be so many. In part, this has been due to the unexpected phenomenon that there can be block of other neurotransmitters at a variety of different types of nerves.

The original concept of the mode of action of BoNT was the simple block of acetylcholine release and the resultant weakness of muscle. The idea is that to treat unwanted muscle activity, all that is needed is to weaken the target muscle taking care not to weaken it so much as to cause excessive weakness and reduce function rather than improving it. There is no doubt that this is an important part of the efficacy of the toxin, but, unexpectedly, this is only part of the story. It is true that in some studies there does appear to be a good relationship between the amount of weakness and efficacy, perhaps best seen in the treatment of spasticity where there is little voluntary movement and reduction of tone is helpful in passive care of the patient. However, in many circumstances, particularly in dystonia, there was not such a relationship (Cohen et al. 1989). Benefit is often more than weakness. Indeed, for some patients with focal hand dystonia of mild degree, we were able to see benefit with such low doses of BoNT that there was virtually no weakness. Also, in some of the early studies of cervical dystonia, the benefit for the pain was more than the movement disorder, and this was one of the unexpected findings that led investigators to direct studies of pain (Tsui et al. 1986).

It turns out that there are many unexpected phenomena that lead to benefit being greater than the amount of weakness (Table 1). One has to do with the fact that toxin is taken up more avidly into nerves that are more active. This phenomenon was first demonstrated by Hughes and Whaler (Hughes and Whaler 1962) who studied a rat diaphragm preparation. The faster the nerve was stimulated, the faster the paralysis. We can understand this now to be due to more opportunity for toxin uptake due to more vesicle recycling with more transmitter release. This was subsequently demonstrated by Eleopra et al. (Eleopra et al. 1997)

comparing the amount of weakness of the extensor digitorum brevis (EDB) muscle after toxin injection, with and without stimulation of the fibular (peroneal) nerve. Chen et al. (Chen et al. 1999) then demonstrated this in a more clinical situation. Patients with writer's cramp were treated with BoNT and then in random order for successive injection cycles, either rested after the injection or wrote for 30 minutes (Fig. 1). The write condition resulted in greater strength reduction. This phenomenon can explain some of the selectivity of the toxin for just those muscles participating in the involuntary movements. For example, in hemifacial spasm, the toxin will be taken up more by the nerve endings in those muscles that are involved in the spasms and less in the muscles that are relatively quiescent (Hallett et al. 1994).

Another unexpected phenomenon has to do with the fact that a muscle contains intrafusal muscle fibers as well as the larger extrafusal fibers that lead to the muscle's contractile force. The intrafusal fibers contain the muscle spindles. The sensory afferents innervating the spindles convey information about muscle stretch. Intrafusal fibers are contractile and are innervated by the gamma motoneurons (as opposed to the larger alpha motoneurons that innervate the extrafusal fibers). When BoNT weakens the intrafusal fibers, the spindle becomes lax, and the spindle afferent discharge is reduced (Filippi et al. 1993). In some circumstances, it might be possible to block the gamma motoneurons without much block of the alpha motoneurons, and hence get reduced afferent activity without a change in muscle strength. Histological studies after BoNT injection has shown atrophy of the intrafusal muscle fibers, directly demonstrating this phenomenon (Rosales et al. 1996).

Another step in this mechanism relates to a reduction in spasm due to reduction in spindle afferent activity. It is the case that muscle vibration is a strong way to activate spindle afferents and lead to the tonic stretch reflex. The tonic stretch reflex is a spinal cord reflex that leads to muscle contraction in opposition to stretch of that muscle. It acts to stabilize the position of a limb in space. The sensory afferent activity with vibration mimics what would happen with stretch. In patients with dystonia, vibration leads to an augmentation of the dystonic movement (Kaji et al. 1995). Injection of low dose lidocaine into a motor nerve will lead to block of conduction in small fibers before large fibers, specifically gamma axons before alpha axons. This could lead to reduction of spindle afferent activity without weakness, and this can ameliorate dystonia. In the clinical situation it has been demonstrated that the tonic vibration reflex is suppressed more than maximal muscle strength (Trompetto et al. 2006).

While there has been suspicion that BoNT is retrogradely transported into the spinal cord, the central nervous system, there was a feeling that this might well just be non-functional fragments of the toxin molecule. Recently it has been demonstrated that the toxin can be retrogradely transported in its active state and even transcytosed to other neurons (Restani et al. 2011). The amount of toxin that is retrogradely transported must be small, but it might well have some effects. The most likely target for retrograde toxin is the synapse onto the Renshaw cell. The alpha motoneuron sends its axon to the periphery for the extrafusal muscle fibers, but there are collaterals of the axon in the spinal cord. One type of collateral innervates the Renshaw cell, which then in turn inhibits the alpha motoneuron which is the source of its innervation and other alpha motoneurons in the vicinity. This is called recurrent

inhibition and acts to restrain excessive activity. The neurotransmitter onto the Renshaw cell is also acetylcholine, so retrograde toxin could enter the collateral and block activity there, leading to a reduction in Renshaw cell activity. Functionally this would lead to reduced inhibition in the spinal cord. Such an effect would not support a reduction of net activity, of course, so would not be therapeutically beneficial, but if an effect could be demonstrated, it would provide evidence for functional retrograde transport.

While a study in the cat failed to find strong evidence of a change of recurrent inhibition (Hagenah et al. 1977, Wiegand and Wellhoner 1977), subsequently this has been demonstrated in the human by Marchand-Pauvert et al (Marchand-Pauvert et al. 2013). The method is complicated and will not be detailed here, but has been well validated. In the human study, the authors compared before and after BoNT injection and found a significant reduction in recurrent inhibition.

Another spinal reflex that has been studied is reciprocal inhibition. Reciprocal inhibition is an important general mechanism of motor control that works to dampen the power of a muscle when its antagonist is involved in a motor task. Loss of reciprocal inhibition would lead to excessive co-contraction, which is seen in dystonia. Notably, the process of reciprocal inhibition maybe impaired in dystonia (Hallett 2011). There are multiple processes of reciprocal inhibition in the spinal cord circuitry. The afferent activity from a muscle will lead to inhibition of its antagonist. If a single stimulus is given these processes can be studied separately by virtue of their latencies. In humans this has been studied by giving a stimulus to the radial nerve and looking for effects on the H reflex in the flexor carpi radialis (FCR) muscle. The first phase at about 10 ms interval between radial and median nerve stimuli appears due to disynaptic postsynaptic inhibition and the second phase appears due to oligosynaptic presynaptic inhibition. The second phase of reciprocal inhibition was reduced, that is, brought closer to normal, after BoNT injection (Priori et al. 1995). By increasing spinal inhibition, that might have a favorable therapeutic effect.

Effects on the brain have been seen after BoNT injection. The phenomena will be discussed first and then possible explanations will be considered. The first is transcranial magnetic stimulation (TMS) mapping of muscles that have been injected (Byrnes et al. 1998). Homologous muscles were mapped on both sides of the body, and then mapping was done at baseline, at peak toxin effect and then at 3 months, after the effect had worn off. The centroid of the map of the injected muscle moved laterally transiently during the peak effect.

Another effect on the brain was also demonstrated with TMS. Using a paired TMS technique, it is possible to assess intracortical inhibition. This is thought to represent inhibition within a cortical column due to GABA-A effects. In patients with dystonia short intracortical inhibition (SICI) was reduced. At the peak of BoNT effect, one month, there was a normalization of SICI, which then returned to abnormal at 3 months (Gilio et al. 2000). In another study, however, this result was not reproduced (Boroojerdi et al. 2003).

How can such cortical effects be explained? There are at least two possible mechanisms. One would be transport of toxin to the cortex. As noted, there is good evidence for toxin being transported into the spinal cord and even jumping cells. However, the amount of such

toxin must be very small, and the amount that might reach the brain would have to be infinitesimal and likely not clinically meaningful.

It is more likely that the effects in the brain are due to a plastic change secondary to events in the periphery. The brain is a very plastic organ, and it is constantly changing. The brain changes with development; learning is accomplished by plasticity; the brain allows adaptation of environmental change; and plasticity is relevant for disease in many ways including recovery from brain lesions (Hallett 2001). Importantly with respect to a possible BoNT effect, disuse of a body part will lead to a decrease of the representation of that body part in the brain and an augmentation of the representations of nearby body parts. Even temporary block of afferent nerves will lead to rapid changes. Hence, BoNT should reduce the cortical representation of the injected muscles, since there will be less afferent activity from them. The physiological consequence of such a change is not clear, but could be advantageous. At least in the sensory cortex, body representations in dystonia appear to be more widespread than normal and also more intermixed with adjacent representations (Bara-Jimenez et al. 1998). Perhaps shrinkage of a representation could help normalize its function.

BoNT is a remarkable molecule. Although superficially simple, it has many direct and indirect effects. It already has widespread therapeutic use, but as we learn more about its unexpected consequences, we might find even more situations where it could be beneficial.

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Full Conflict of interest

Dr. Hallett serves as Chair of the Medical Advisory Board for and receives honoraria and funding for travel from the Neurotoxin Institute. He may accrue revenue on US Patent #6,780,413 B2 (Issued: August 24, 2004): Immunotoxin (MAB-Ricin) for the treatment of focal movement disorders, and US Patent #7,407,478 (Issued: August 5, 2008): Coil for Magnetic Stimulation and methods for using the same (H-coil); in relation to the latter, he has received license fee payments from the NIH (from Brainsway) for licensing of this patent. He is on the Editorial Board of 20 journals, and received royalties and/or honoraria from publishing from Cambridge University Press, Oxford University Press, Springer, and Elsevier. Dr. Hallett's research at the NIH is largely supported by the NIH Intramural Program. Supplemental research funds have been granted by Medtronic, Inc., for studies of deep brain stimulation, UniQure for a clinical trial of AAV2-GDNF for Parkinson Disease, Merz for treatment studies of focal hand dystonia, and Allergan for studies of methods to inject botulinum toxins.

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Highlights

The mechanism of action of BoNT is more than a simple decrease of neuromuscular transmission.

BoNT injection leads to reduced spindle afferent activity, changing spinal reflexes

There is some functional consequence to retrograde transport of BoNT

Cortical changes after BoNT injection may be due to brain plasticity

Fig. 1. Strength of muscle after botulinum neurotoxin injection. After the injection patients either rested or wrote continuously for 30 minutes. Strength was tested at 2 weeks, 6 weeks and 3 months after injection and was always less following the writing. From Chen et al. (Chen et al. 1999) with permission.

Table 1

Consequences of Botulinum toxin injection

Expected
Weakening of muscle due to block of neuromuscular transmission
Unexpected
Block of neurotransmission other than cholinergic synapses
Selective weakening depending on muscle activity
Reduction in spindle afferent activity reducing excitability of motoneurons
Retrograde transport into the spinal cord with effects at least on the Renshaw cell
Plasticity of the brain from peripheral denervation

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