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Explanation of Timing of Botulinum Neurotoxin Effects, Onset and Duration, and Clinical Ways of Influencing Them

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Abstract

While the steps in the action of botulinum neurotoxin (BoNT) are well known, the factors underlying the timing of these steps are not fully understood. After toxin is injected into a muscle, it resides in the extracellular space and must be taken up into the nerve terminals. More toxin will be taken up if near the endplate. Toxin is distributed mainly by convection and there is likely little diffusion. Toxin that is not taken up will go into the general circulation where it may have a slight systemic effect. The uptake is activity and temperature dependent. Encouraging the unwanted muscle contractions after injection should be helpful. Cooling will decrease the uptake. The times for washout from the extracellular space and uptake of the toxin are not well established, but are likely measured in minutes. Toxin in the general circulation has a long half time. The time from injection to weakness is determined by how long it takes to get sufficient damage of the SNARE proteins to interfere with synaptic release. Toxins are zinc dependent proteases, and supplemental zinc may produce a greater effect. There will be weakness as long as there is residual toxin in the nerve ending.

Keywords

Endplate; Uptake; Duration of action; Cooling; Muscle activity; Zinc

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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, John Wiley & Sons, Wolters Kluwer, Springer, and Elsevier. He has received honoraria for lecturing from Columbia University. Dr. Hallett's research at the NIH is largely supported by the NIH Intramural Program. Supplemental research funds have been granted by the Kinetics Foundation for studies of instrumental methods to monitor Parkinson's disease, BCN Peptides, S.A. for treatment studies of blepharospasm, Medtronics, Inc., for studies of deep brain stimulation, Parkinson Alliance for studies of eye movements in Parkinson's disease, 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.

The botulinum neurotoxins (BoNTs) are commonly used for many different indications. The clinical effects are certainly well known, but a detailed explanation of all the phenomena is still lacking. In particular, while many of the steps in the action of the BoNTs are well understood, the factors underlying the timing of these steps are less so. In relation to effects at the neuromuscular junction, initial weakening does not occur for several days and the peak occurs in the order of several weeks. Effects subside at 2 months and strength generally returns to normal by 3 months. Improved knowledge of the physiology should have clinical implications to improve efficacy in the use of toxins. This article will review the sequential steps in toxin action, pointing out what is known and what is not, and will try to indicate clinical implications of these issues.

After toxin is injected into a muscle, it resides in the extracellular space and must be taken up into the nerve terminals. As this process occurs only at the endplate, clearly more toxin will be taken up if the injection is near the endplate. Endplates are generally located in the middle of muscle fibers, but as fibers are organized differently in different muscles, anatomical knowledge must guide the injector. Physiologically, the motor point is the place on the muscle where the stimulation intensity is least to evoke a muscle response. Sometimes this is the endplate zone, but sometimes not, so this is not reliable (Guzman-Venegas *et al.*, 2014). By EMG criteria, endplate spikes certainly are indicative of being in the right place, but these cannot always be found. If the motor unit configurations show initial negative phases, this means that the muscle action potentials are arising near the recording needle, and the endplate must be nearby. High density surface EMG can also define the endplate zone (Lapatki *et al.*, 2011). If attention is paid to injecting at the endplate, the effect will be greater (Delnooz *et al.*, 2014, Gracies *et al.*, 2009).

Toxin will be distributed in the muscle belly by means of convection, that is, the bulk movement of the fluid determined by the fluid volume and the force of the injection. Subsequently there might be diffusion; that is, spread from the initial site by Brownian motion determined by the concentration gradient and molecular size. Fascial boundaries between muscles do not appear to be significant barriers (Shaari *et al.*, 1991), so nearby muscles can be affected depending on the accuracy of needle placement and the volume of the injection. Diffusion takes time, and there may not be much time because there is continuous wash out from the extracellular space.

BoNT in the muscle has been imaged by MRI (Elwischger *et al.*, 2014). Toxin distributes along the long axis of muscle fibers and does not change much in a 10 minute period. The volume of distribution is similar, but might be slightly less, in spastic versus normal muscle. In fact, as modelled in mouse muscle, the volume of distribution becomes rather quickly less (Figure 1) (Tang-Liu *et al.*, 2003). By two hours it is appreciably smaller and by 12 hours, there is hardly any to be seen. The different marketed brands of BoNT exist in different forms and complexed with different amounts of protein, but the region of effect does not seem to differ if similar volumes are used for injection (Carli *et al.*, 2009).

Taken together, the evidence seems fairly clear that the distribution is determined primarily by convection, which in practical terms is the volume of the injection.

As noted already, there is continuous washout from the extracellular space. The half-life may only be a few minutes, but is at most a few hours as seen the mouse model (Tang-Liu *et al.*, 2003). It is difficult to find any studies about residual drug in the muscle after intramuscular injection as most studies report the opposite – how fast drug gets into the general circulation. One study of digoxin showed a washout halftime of 4 minutes (Hess and Muller, 1982). Hence, it is clear that any toxin taken up will have to be in a very short time. This time differs considerably from the time to effect.

Toxin that is not taken up into the nerve endings will be washed out and will go into the general circulation where it may have a slight systemic effect. There is a long half time in the circulation, perhaps several days (Simpson, 2013). It will have the opportunity to be taken up in other muscles or other presynaptic terminals elsewhere in the body. That there is only slight systemic effect is because there is so little toxin compared with the size of the body. It is not certain what happens to toxin not taken up, but it might be removed by the liver (Simpson, 2013).

Since the toxin is in the muscle for only a short time, it is fortunate that the uptake is rapid. In the rat hemidiaphagm, the halftime for binding is about 12 minutes and for subsequent internalization 5 minutes (Simpson, 1980).

The uptake on BoNT is activity dependent. The more activity, the more uptake. Activity dependence appears to occur because activity will give rise to more opportunities for binding of the toxin to the synaptic membrane. This phenomenon was first demonstrated in the rat diaphragm (Hughes and Whaler, 1962), but then subsequently in human extensor digitorum brevis muscle (Figure 2) (Eleopra *et al.*, 1997). Clinically, it has been demonstrated that activity after injection will increase the amount of weakness produced, but it was not possible to show an increased therapeutic effect (Chen *et al.*, 1999). Activity dependent uptake is likely partly responsible for the often seen clinical situation of a greater clinical effect than weakness effect. Uptake will be greater in the nerve terminals of those muscle fibers involved in the unwanted muscle spasms. Clinically, it makes sense to encourage the involuntary movements in the few minutes following the injection. This should produce more uptake and uptake more selectively in the desired nerve terminals.

Uptake is also temperature dependent (Poulain *et al.*, 1992). Muscle temperature is generally homeostatically controlled. Sometimes skin cooling is used to reduce the superficial pain of the injection (Irkoren *et al.*, 2014). To the extent that this will reduce muscle temperature, it should reduce uptake and reduce the effect of the injection.

In the presynaptic terminal, released light chains of BoNT enzymatically destroy one of the SNARE proteins. The SNARE proteins are being continuously made. Hence the time from injection to weakness must depend on how long it takes to get sufficient damage to interfere with synaptic release. This time must be days.

Toxins are zinc dependent proteases, and the question has arisen as to whether the intracellular zinc concentration might be limiting toxin action (Koshy *et al.*, 2012). It is difficult to find clear information on this point. Somewhat old data suggest that roughly half of persons in North America over 50 years old consume less zinc than federally

recommended, and nearly 30% of these individuals may show overt signs of zinc deficiency (Briefel *et al.*, 2000, Prasad *et al.*, 1993). If this is the case, then supplemental zinc may produce a greater effect. There is some evidence that supplemental zinc can produce a greater effect in patients not assessed for zinc deficiency (Koshy *et al.*, 2012). In a double blind, placebo controlled, cross over study of 77 patients with multiple disorders treated with three different toxin preparations, there was a subjective benefit of supplemental zinc given together with phytase. Phytase is given with the zinc to increase its bioavailability and absorption (Rimbach *et al.*, 1998). This study has been called into question, however, because of scientific rigor and the matter remains controversial (Cohen, 2014). The distribution of body zinc is not clear. Logically, supplemental zinc should be of value only if a muscle is zinc deficient, and it might be that muscle zinc is maintained even in the face of slight serum deficiency. Or, of course, it could be the other way around.

There will be weakness as long as there is residual toxin in the nerve ending damaging the SNARE proteins. The SNARE proteins are constantly resynthesized, but this process apparently cannot keep up with the rate of lysis. Toxin appears to remain in the terminal for a matter of months, and this determines its duration of action (Rossetto *et al.*, 2014). The BoNTs are likely metabolized mainly by the proteasome pathway, but information on this is limited (Rossetto *et al.*, 2014).

To summarize the clinical implications:

1. Toxin should be injected near the motor endplate and there are electrophysiological methods for identifying the endplate
2. Accurate needle placement is needed to avoid affecting neighboring muscles
3. Avoid cooling the injection site
4. Encourage the unwanted muscle spasms in the minutes after the injection; probably after about an hour this is no longer of benefit
5. Consider zinc supplementation at the time of injection if a patient is zinc deficient

Clearly there is more to learn about the method of action of BoNT, and further insights may well lead to other ways to maximize clinical effects.

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Highlights

- After botulinum toxin is injected, it is taken up into nerve terminals, so injection into endplate zones is optimal.
- Botulinum toxin uptake is activity dependent.
- Injection of botulinum toxin into muscle distributes the toxin mainly by convection.
- Toxin not taken up into the nerve terminals is washed out of the muscle into the general circulation.
- Toxin is active in the presynaptic terminal until metabolized.

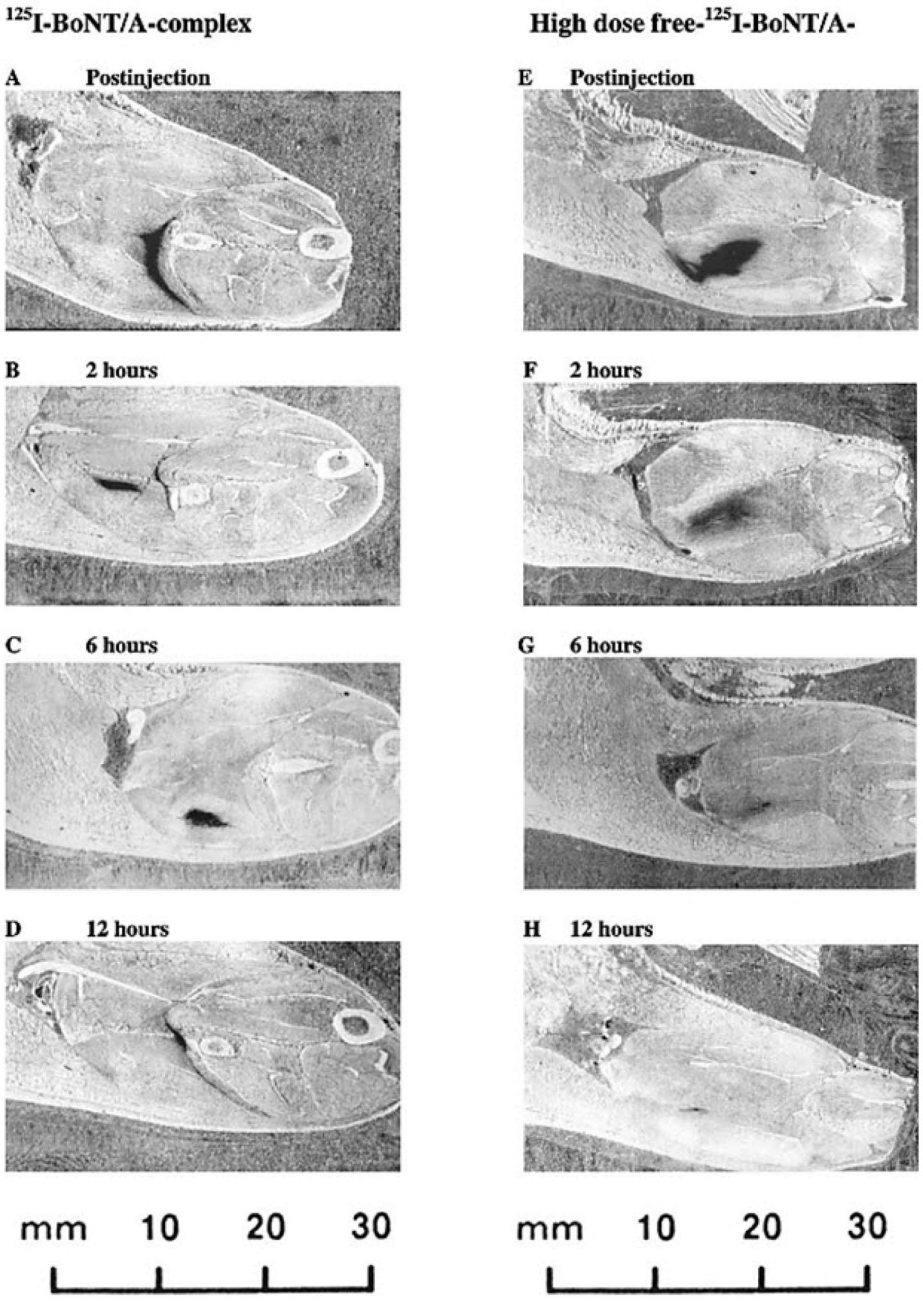

Figure 1.

Autoradiographs of sections through the gastrocnemius muscle injected with 125I-BoNT/A-complex, from four separate rats, (A) immediately after injection, (B) 2 h postinjection, (C) 6 h postinjection, and (D) 12 h postinjection. Sections correspond to the central plane of the injection and range from 5.6 to 8.3 mm below the surface of the preparation.

Autoradiographs of sections through the gastrocnemius muscle injected with high-dose free-125I-BoNT/A, from four separate rats, (E) immediately after injection, (F) 2 h postinjection, (G) 6 h postinjection, and (H) 12 h postinjection. Sections correspond to the

central plane of the injection and range from 3.6 to 4.9 mm below the surface of the preparation. From (Tang-Liu *et al.*, 2003) with permission.

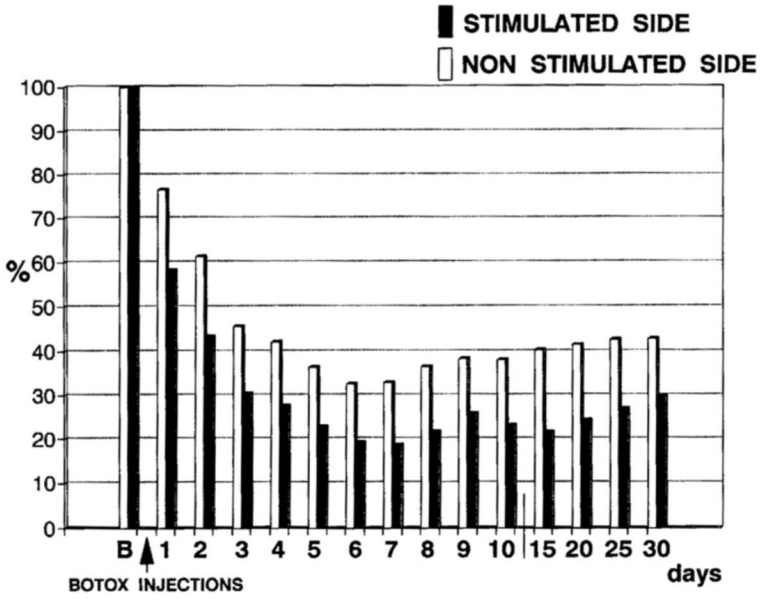

Figure 2.

The normalized compound muscle action potential (CMAP) from extensor digitorum brevis muscles on the two sides at various times after onabotulinumtoxinA injection. Injections were similar on the sides, but after injection the peroneal nerve was stimulated only on one side. Beginning at one day and lasting to 30 days, the CMAP amplitude was less on the stimulated side. From (Eleopra *et al.*, 1997) with permission.