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Motor Nerve Filament Block Produced by Botulinum Toxin[1]

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Burgen, Dickens, and Zatman (*1*) have shown that during the neuromuscular paralysis produced by botulinum toxin (type A) excitation of a motor nerve releases no acetylcholine (ACh) from its muscular terminals. This finding could be explained by assuming either that the toxin blocks motor nerve terminals just proximal to the site of ACh-release, or that it interferes with the process of release itself. Experiments were carried out to decide between the two alternatives, using the cat's gracilis muscle *in situ* (*2*) and the guinea pig's excised serratus muscle (*3*), mounted in a bath of oxygenated Ringer-Locke solution.

Neuromuscular block was produced by intravenous injection of 10^8 mouse LD_{50}, or by addition of toxin to the muscle bath (10^3 to 5×10^4 mouse LD_{50}/ml bath fluid). Action potentials of either groups of muscle fibers or of single fibers were recorded from the surfaces of the muscles at an end plate region. The end plate region was located by applying decamethonium or curare and finding sites from which end plate potentials could be recorded. Toxin was administered after neuromuscular transmission had been restored.

Conduction in nerve trunks, or in muscle fibers that were stimulated directly, was not affected by the toxin. On the other hand, it could be shown that the constituent muscle fibers of a motor unit become inexcitable to stimulation through the nerve trunk one at a time, or in very small groups. It was found that the block produced by botulinum toxin in its early stages can be overcome by the second of two motor nerve volleys, separated by at least 0.8 msec. If botulinum toxin paralyzes by reducing the ACh-output at nerve endings, rather than by blocking conduction in motor terminals, then the second, successful volley should be preceded by an end plate potential in response to the first, unsuccessful volley. However, no end plate potentials could be recorded in response to the first volley when it failed to excite.

The above electrophysiological evidence suggested that action of the toxin is on the nerve filaments rather than on the mechanism of ACh-release. If that is true, stimulation of the nerve terminals resulting from the current that passes through the muscle during direct tetanization of the muscle should release the normal amount of ACh from a preparation that was paralyzed to nerve trunk stimulation by botulinum toxin. Measurements were therefore made of the ACh released by the guinea pig's excised diaphragm (*a*) during tetanization of the phrenic nerves, and (*b*) during direct stimulation of the muscle. Direct stimulation of the muscle released the same amount of ACh as indirect stimulation, of the same frequency and duration. Blocking doses of toxin prevented the release of ACh by nerve stimulation, but failed to alter the release by direct stimulation.

It is concluded that botulinum toxin (type A) produces neuromuscular paralysis by interfering with conduction in the terminal twigs of motor nerves, close to, or at, the points of final branching, but proximal to the site of ACh-release.

[1] This work will be reported in full at a later date. The project was supported by a grant from the Defence Research Board of Canada.

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