Comparison of the Presynaptic Actions of Botulinum Toxin and β-Bungarotoxin on Neuromuscular Transmission

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Summary. Comparison was made between the presynaptic actions of type A botulinum toxin (BoTX) and β-bungarotoxin (β-BuTX) on isolated nerve-muscle preparations. On the mouse and rat diaphragms, BoTX is about 100 and 10 times more potent than β-BuTX, respectively, whereas on the chick biventer cervicis muscle, β-BuTX is 3–10 times more potent. The paralytic actions of both toxins are preceded by latency, antagonized by high concentrations of calcium or magnesium and by deficiency of calcium, accelerated by high frequencies of nerve stimulation and retarded by decrease of temperature. The paralytic actions of BoTX as well as β-BuTX appear to take place in two processes: first, binding with their respective target sites and second, the inhibitory changes of the target macromolecule of the nerve terminals leading to failure of transmitter release. The latter process is not reversed by washing but is retarded greatly by low calcium, high magnesium or low temperature. Binding of β-BuTX is faster than that of BoTX.

Miniature end-plate potentials of unreduced amplitude could be recorded in junctions blocked by either toxin. End-plate potentials were depressed and the successive decline of their amplitude during train of pulses was abolished by both toxins.

In contrast to the initial facilitatory actions after β-BuTX, BoTX has no sign of facilitation such as increase of the frequency of miniature end-plate potential, restoration of neuromuscular transmission, increase of quantal content of end-plate potential and occurrence of spontaneous fasciculations in low calcium media. Another difference between the two toxins is the typical Wedensky inhibition on repetitive stimulation and post-tetanic potentiation in β-BuTX paralysed muscles. By contrast, after BoTX, sustained contraction without post-tetanic potentiation was observed.

The two toxins show a mutual antagonism especially when β-BuTX is added before or simultaneously with BoTX. The action of the latter was completely antagonized in the presence of β-BuTX. Once it is bound to the target site, however, BoTX seems not to be antagonized by β-BuTX. On the other hand, BoTX appears to be able to retard the effect of bound β-BuTX.

Key words: Botulinum Toxin — β-Bungarotoxin — Presynaptic Actions — Low Calcium — High Magnesium — Effect of Temperature.
Both botulinum toxin (BoTX) isolated from *Clostridium botulinum* and \( \beta \)-bungarotoxin (\( \beta \)-BuTX) from *Bungarus multicinctus* venom have been shown to cause neuromuscular blockade by an inhibitory action on the release mechanism of the neurotransmitter (Burgen et al., 1949; Brooks, 1956; Thesleff, 1960; Chang and Lee, 1963; Chang et al., 1973a). The mutual antagonism between these two toxins (Chang et al., 1973b) suggests that they might be acting at different receptors and by different mechanisms. In fact, the muscle responded to repetitive stimulation of motor nerve with well sustained contraction after partial blockade by BoTX (Burgen et al., 1949) whereas the opposite, i.e., Wedensky inhibition, was observed after \( \beta \)-bungarotoxin (Chang and Lee, 1963). Moreover, \( \beta \)-BuTX causes an initial facilitation of transmitter release (Chang et al., 1973a) whereas no such phenomenon has been reported for BoTX. It was felt of importance, therefore, to compare the actions of the two toxins in order to understand further the modes of their presynaptic actions.

**Materials and Methods**

**Toxins.** Purified botulinum toxin, Type A, kindly supplied by Dr. Edward J. Schantz, was dissolved in 0.01 M phosphate buffer, pH 6.8, containing 1 mg/ml gelatin, to make 200 \( \mu \)g/ml as stock solution and stored at 4 °C. \( \beta \)-Bungarotoxin was isolated from the venom of *Bungarus multicinctus* as described by Lee et al. (1972).

**Neuromuscular Preparations**

The phrenic nerve-diaphragm preparation (Büllbring, 1946) was isolated from Long Evans rats of either sex weighing about 200 g or from mice (NIH strain) weighing 25–30 g. Chick biventer cervicis nerve-muscle preparation (Ginsborg and Warriner, 1960) was isolated from 4–9 days old male leghorn. The organ-bath contained 30 ml Tyrode solution (composition in mM: NaCl, 137; KCl, 2.8; CaCl\(_2\), 1.8; MgCl\(_2\), 1.1; NaH\(_2\)PO\(_4\), 0.33; NaHCO\(_3\), 11.9; Dextrose, 11.2) at 36 °C for BoTX or 37 °C for \( \beta \)-BuTX unless otherwise indicated and was gassed with 95% O\(_2\) + 5% CO\(_2\). Except in the experiment studying the effect of stimulus frequency, the nerve was stimulated with supramaximal rectangular pulses of 0.1 msec width every 10 sec for the diaphragms and 5 sec for the cervicis biventer muscle. The contraction was recorded isometrically with a force transducer for the diaphragms and isotonically on a kymograph for the chick muscle. Paralysis time was taken from the addition of toxins to complete paralysis of muscles to single stimulation.

**Intracellular Recordings**

End-plate potentials (e.p.p.s) and spontaneous miniature e.p.p.s (m.e.p.p.s) were recorded intracellularly with glass microelectrode filled with 3 M KCl. The organ-bath contained 25 ml oxygenated Tyrode solution and was kept at 35 °C. Effects on the e.p.p. were studied in rat diaphragm preparations equilibrated with 0.8 \( \mu \)g/ml d-tubocurarine in normal Tyrode solution. E.p.p.s were elicited by nerve stimulation at a rate of 0.5 Hz and the average of eight e.p.p.s was obtained for each end-plate by an averaging data processor (ATAC-201, Nihon Kohden, Japan). E.p.p.s as small as 0.06 mV could be distinguished from the noise by this procedure. For the study of initial facilitatory effect on transmitter release, quantal content of