Short communications

Action of botulinum toxin on transmission from sympathetic nerves to the vas deferens

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Botulinum toxin (Type A) depressed or abolished transmission from postganglionic nerves to smooth muscle of isolated preparations of guinea-pig and mouse vas deferens. The time course of blockade was 2 to 6 times slower than that observed with the same concentration of the same batch of toxin on the rat diaphragm. Spontaneous excitatory junction potentials were still observed after 7 h exposure, indicating that the smooth muscle membrane was still able to respond to noradrenaline. Depression of transmission is probably due to a presynaptic action of unknown nature.

The lethal effects of botulinum toxin are due to blockade of the release of acetylcholine at skeletal neuromuscular junctions. Although, according to Ambache (1949, 1951), peripheral noradrenergic synapses are resistant to botulinum toxin, there have been reports which suggest that its blocking action may not be limited to cholinergic nerve terminals. Westwood & Whaler (1968) and Bond (1969) have given evidence that botulinum toxin (Types D and A) blocks transmission in response to postganglionic nerve stimulation in the guinea-pig vas deferens. Rand & Whaler (1965) also observed depression of sympathetic transmission to the rabbit ileum and piloerector muscles of the cat's tail. These results led us to repeat some of these experiments and to extend them by recording intracellularly from smooth muscle cells of the guinea-pig and mouse vas deferens.

Methods.—Experiments were carried out on isolated preparations of vas deferens as described previously (Burnstock & Holman, 1961 ; Holman, 1970). Muscles were incubated in Type A toxin (Microbiological Research Establishment, Porton Down, Salisbury) at a dose of 10^5 MLD/ml, either before or during the experiment. In most experiments, one of the paired organs served as control and was incubated in Krebs solution for an equal period with toxin inactivated by boiling for 5 min, or in Krebs solution alone. Tension developed was measured with a strain gauge and pen recorder. For intracellular recordings with KCl filled microelectrodes, the organ was lightly stretched over a glass rod inserted in the lumen.

Transmural stimulation of postganglionic noradrenergic nerves was effected with pulses of 0·5 to 1·0 ms duration, at frequencies up to 30 Hz and amplitudes up to 35 V. For tension measurements the frequency of stimulation and the duration of the train of pulses were chosen so that the muscle developed maximal tension. For recording excitatory junction potentials, lower frequencies were used to avoid contractions.

Results.—Responses of both guinea-pig and mouse vas deferens to transmural nerve stimulation with brief trains of pulses were usually depressed after 1·5 h incubation in the toxin. In a typical experiment, tension developed was reduced by about 50% after 3 h but some tension (less than 5% of control) persisted for up to 6 hours. These results confirm previous work (Bond, 1969 ; Westwood & Whaler, 1968 ; Rand & Whaler, 1965) but stand in marked contrast to the block of skeletal neuromuscular transmission in mammalian preparations at 35–37° C, which is complete in 0·5–1·5 h (Brooks, 1956 ; Spitzer, 1972).

Spontaneous excitatory junction potentials, up to 10 mV in amplitude and roughly 1 Hz in frequency, were seen upon impaling smooth muscle cells in control preparations. Similar potentials were also observed in preparations that had been exposed to the toxin for periods up to 7 hours. Frequencies and amplitudes appeared to be within the normal range despite the fact that impalements were often difficult and resting potentials were frequently low. At skeletal neuromuscular junctions botulinum toxin does not completely block the spontaneous release of acetylcholine but reduces the frequency of miniature end plate potentials and alters their amplitude distribution from a normal to a skewed type (Harris & Miledi, 1971 ; Spitzer, 1972). Since smooth muscle cells are electrically coupled to each other, the distribution of spon-

taneous junction potential amplitudes is skewed in control preparations (Holman, 1970), and it is difficult to decide whether the toxin changes their amplitude.

Single nerve stimuli failed to evoke excitatory junction potentials in many preparations which had been exposed to the toxin for 5 to 7 hours. In some cases, where no response was detectable at 1 Hz, repetitive stimulation at 10 Hz caused facilitation and junction potentials reappeared. A few preparations did show small junction potentials in response to single stimuli. The records of Fig. 1 were taken after incubation for 5 hours. Although the initial response to the train of stimuli was small—approximately half that of the preceding spontaneous junction potential—successive responses at 10 Hz showed marked facilitation, reaching amplitudes of up to 15 mV. Control preparations showed little facilitation; junction potentials could be graded according to the strength of the stimulus and gave

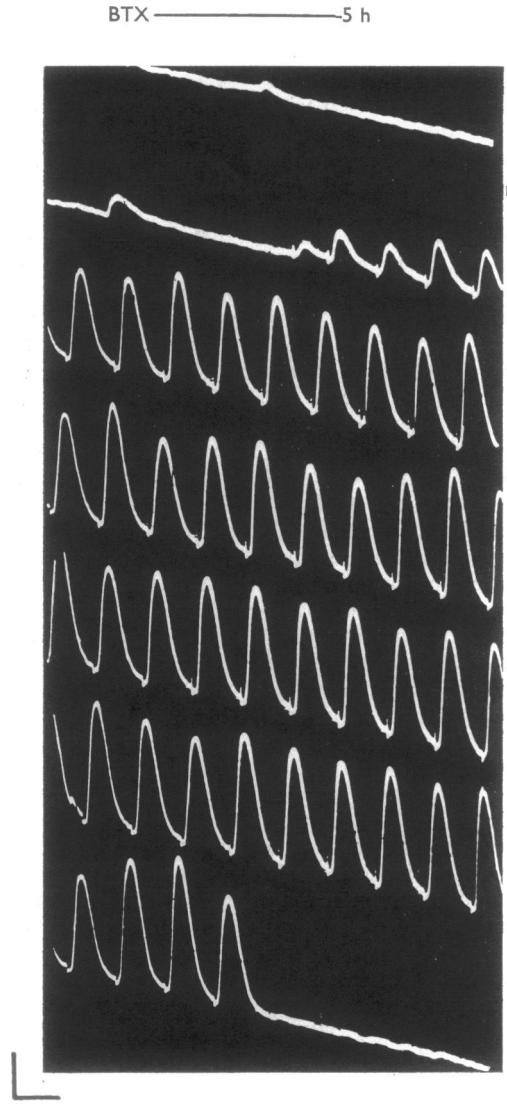

FIG. 1. Facilitation of excitatory junction potentials in a partially blocked preparation: transmural stimulation of mouse vas deferens incubated in toxin for 5 hours. Resting potential 50 mV. Scale 5 mV and 100 milliseconds. Stimulus parameters: pulses 0·5 ms duration, 37 V amplitude, 10 Hz.

rise to action potentials if they exceeded 30 to 35 mV; this usually happened at stimulus intensities less than 1/10 of those used in blocked preparations.

The possibility that there might be some change in the electrical properties of the smooth muscle membrane was investigated by direct stimulation of the muscle using the method of Tomita (1966), with pulses of 0·5 ms duration and from 0·5 to 60 V/cm. For periods of incubation up to 2·5 h electrotonus and action potentials arising from depolarizing pulses were indistinguishable from those in control preparations. Attempts to evoke action potentials by direct stimulation after periods of incubation of more than 3 h have been unsuccessful, although an injury discharge of action potentials could still be observed during some impalements.

Discussion.—The vas deferens of small laboratory animals (guinea-pig, rat and mouse) is frequently used as a model for studies on sympathetic transmission because of its rich noradrenergic innervation and lack of spontaneous contractile activity. We studied the mouse vas deferens in particular because contractions in response to nerve stimulation are not affected by atropine and there is evidence that such contractions are due to the release of noradrenaline (Farnebo & Malmfors, 1971). Further, the frequency of spontaneous junction potentials is relatively high in this preparation (Holman, 1970).

In agreement with Bond (1969), Rand & Whaler (1965) and Westwood & Whaler (1968), we conclude that botulinum toxin can depress noradrenergic transmission, although junctions in the vas deferens are very resistant to blockade compared with the skeletal neuromuscular junction. Since spontaneous junction potentials of normal size and frequency were seen when evoked release was blocked, the muscle membrane remained normally excitable by the transmitter and the effect of the toxin at noradrenergic junctions is, therefore, unlikely to be postsynaptic. The presence of spontaneous potentials at normal frequencies when evoked potentials are depressed or appear to be absent suggests that the mode of action of the toxin at sympathetic neuromuscular junctions may be different from its action on the skeletal neuromuscular junction. Recent work on skeletal nerve-muscle junctions (Harris & Miledi, 1971 ; Spitzer, 1972) has shown that the toxin does not prevent the nerve impulse from reaching the nerve terminal and that the site of blockade is the coupling process between action potential and transmitter release. The decline in size of the excitatory junction potentials seen in our experiments could be due to a similar blockade of transmitter release, or to a failure of the action potential to invade the nerve terminals. Further work is needed to resolve this issue.

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