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IS THE CONTRACTILE RESPONSE TO EXOGENOUS ACETYLCHOLINE DUE TO A PRESYNAPTIC EFFECT?

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1 Whether the contractile response induced by exogenous acetylcholine (ACh) chiefly involved the pre- or post-synaptic junctional site of the motor endplate was studied by using the cat gastrocnemius nerve muscle preparation poisoned with β-bungarotoxin (β-BuTX), a toxin isolated from the venom of *Bungarus multicinctus* which acts presynaptically.

2 After neuromuscular transmission was completely blocked by β-BuTX, the dose-response curve of the contractile response induced by close intra-arterial injection of ACh, was compared with that of the control. No appreciable difference was observed.

3 In contrast, the response to ACh was completely abolished when neuromuscular transmission was blocked by α-bungarotoxin, a toxin isolated from the same venom which acts postsynaptically.

4 It is concluded that postjunctional site of the motor end-plate is chiefly involved in the contractile response produced by exogenous ACh.

Introduction

Acetylcholine (ACh) has been considered as a neuromuscular transmitter which is synthesized in and released from the motor nerve terminal (for review, see Hubbard, 1973). However, Riker and his colleagues have claimed that intra-arterial ACh depolarizes the motor nerve endings, the *in vivo* contractile response of the innervated muscle to ACh being largely a result of this action and concluded that the functional integrity of the unmyelinated terminals is essential for the action of ACh (Riker, 1966; Okamoto & Riker, 1969; Riker & Okamoto, 1969). Recently, Okamoto, Longenecker, Riker & Song (1971) reported that the contractile response to exogenous ACh was severely impaired in the cat soleus and gastrocnemius muscles poisoned with the venom of black widow spider (*Latrodectus mactans tredecimguttatus*), which selectively destroys motor nerve endings (Clark, Mauro, Longenecker & Hurlbut, 1970; Okamoto *et al.*, 1971; Clark, Hurlbut & Mauro, 1972). As a result of these observations, Okamoto *et al.* (1971) concluded that the pre-junctional site of the motor endplate was chiefly involved in the contractile response produced by exogenous ACh.

α-Bungarotoxin (α-BuTX) and β-bungarotoxin (β-BuTX) were isolated from the venom of *Bungarus multicinctus*, the former producing neuromuscular block of the antidepolarizing type by acting on the postjunctional membrane of the motor endplate, and the latter acting exclusively on the presynaptic side of the neuromuscular junction and producing a marked reduction in ACh output from the rat diaphragm and a blockade of neuromuscular transmission which was not associated with any diminution of response to ACh (Chang & Lee, 1963; Lee & Chang, 1966; Chang, Chen & Lee, 1973).

Since both black widow spider venom (BWSV) and β-BuTX were considered to block neuromuscular transmission presynaptically, it was deemed to be of interest to see whether β-BuTX has the same effect as BWSV on the contractile response to exogenous ACh in the cat gastrocnemius nerve muscle preparation *in situ*. In addition the effect of α-BuTX was compared on the same preparation.

Methods

Purification of bungarotoxins

α-Bungarotoxin and β-BuTX were isolated from the venom of *Bungarus multicinctus* by means of column chromatography on CM-Sephadex C-50 using ammonium acetate buffer gradient according to the method of Lee, Chang, Kau & Luh (1972). The concentration of buffer ranged from 0.05 M, pH 5.0 to 1.0 M. pH 6.8. Fractions 2 and 5, which contained α-BuTX and β-BuTX respectively, were re-chromatographed on CM-cellulose with the same buffer system.

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Cat gastrocnemius-nerve muscle preparation

Cats of either sex (1.8–2.4 kg body weight) were anaesthetized with chloralose (80 mg per kg i.v.), the trachea was cannulated for artificial respiration (100 ml × 30 per min) and the left femoral vein for injection of drugs and toxins.

The gastrocnemius muscle preparation was prepared according to the method described by Brown, Dale & Feldberg (1936), except that the tension of the muscle was recorded with a Grass force displacement transducer FT 10C, which was connected with a direct writing Grass Model 7 Polygraph. ACh, dissolved in 0.1 ml 0.9% w/v NaCl solution (saline) was injected as rapidly as possible into the central end of the tibial artery for retrograde injection through the popliteal artery as described by Brown *et al.* (1936). The nerve to gastrocnemius muscle was placed on a pair of platinum electrodes for stimulation and all other branches of the sciatic nerve were sectioned. The stimuli were square wave pulses of 0.4 ms duration. Supramaximal single stimuli were delivered to the nerve at a frequency of 0.2 Hz. Atropine sulphate (1 mg per kg) was injected intravenously in order to prevent the lowering of blood pressure and other parasympathomimetic effects caused by ACh.

Results

An immediate muscle contraction was observed in response to intra-arterial ACh, exactly as described by Brown *et al.* (1936). Since the sensitivity of the gastrocnemius muscle to exogenous ACh might decrease after repeated intra-arterial injection of ACh, repeated test doses of ACh before administration of toxin were avoided. Instead, the response to ACh was tested only after neuromuscular transmission had been completely blocked by β-BuTX and the averaged ACh dose-response curve thus obtained on 8 cats was compared with that of a control group of 6 cats injected with saline.

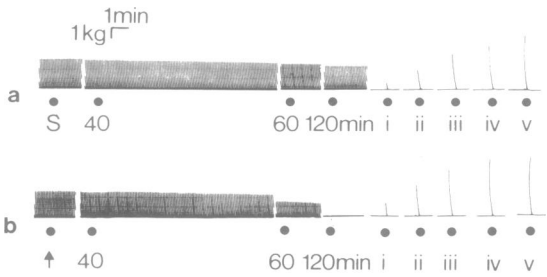

Figure 1 Contractions of the cat gastrocnemius muscle *in situ*. Supramaximal indirect stimulation of 0.4 ms duration was applied at a rate of 12 per minute. Electrical stimulation was interrupted during close intra-arterial injection of acetylcholine (ACh). (a) Control, At S, saline was injected intravenously. (b) β-Bungarotoxin-poisoned muscle. At arrow, β-bungarotoxin (0.5 mg per kg i.v.) was injected. At i, ii, iii, iv and v, indirect stimulation was stopped and ACh 3, 10, 30, 100 and 300 μg, each dose dissolved in 0.1 ml saline, was injected (i.a.) respectively.

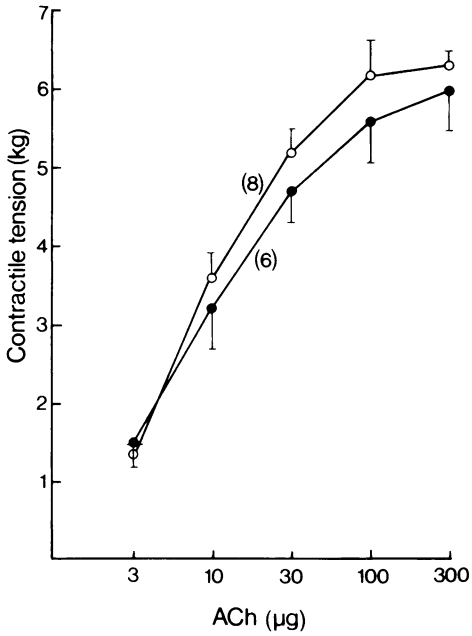

Figure 2 Dose-response curves to exogenous acetylcholine (ACh) in the cat gastrocnemius muscle. Vertical lines represent standard error of mean. Number of experiments is indicated on the figure. (●) Saline-injected control group; (○) β-bungarotoxin-injected group.

When β-BuTX was injected intravenously into the cat at a dose of 0.5 mg per kg, the twitch resulting from single indirect stimulation began to decline about 40 min after injection and complete block occurred in 120.2 ± 5.05 min (mean ± s.e., $n = 8$). After neuromuscular transmission was completely blocked, the responses to different doses of ACh were examined (Figure 1b). In the saline-injected cats (control group), a dose-response analysis of ACh was made similarly after the sciatic nerve was stimulated for about 120 min (Figure 1a). No appreciable difference was observed between the ACh dose-response curve in the β-BuTX-treated cats and that in the saline-injected group (Figure 2). On the other hand, in the α-BuTX (1 mg per kg, i.v.)-treated cats, the gastrocnemius muscle contraction produced by ACh was progressively depressed before the decline of the response to indirect stimulation and finally abolished within 60 min after the injection, whereas

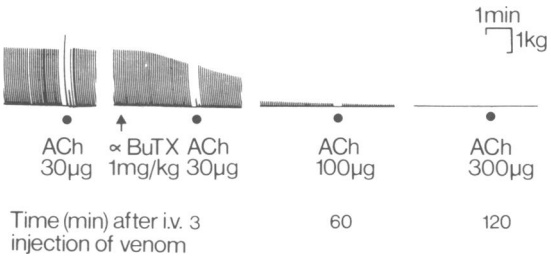

Figure 3 Effects of α-bungarotoxin (α-BuTX) on contractions of the cat gastrocnemius muscle *in situ*. Stimulation of the sciatic nerve (0.4 ms) once every 5 seconds. Electrical stimulation was interrupted during close intra-arterial injection of acetylcholine (ACh). At arrow, α-bungarotoxin was injected intravenously. At ACh, acetylcholine was injected intra-arterially.

complete neuromuscular block took place gradually in about 2 h (Figure 3).

Discussion

Although both BWSV and β-BuTX had been shown to block neuromuscular transmission presynaptically, their effects on the contractile response to exogenous ACh were quite different. According to Okamoto *et al.* (1971), the contractile response of both the cat gastrocnemius and the soleus muscle produced by exogenous ACh was severely impaired after complete poisoning with BWSV. The dose-response curve to intra-arterial ACh in the BWSV-treated cats was flattened and shifted to the right as compared to that of the control. As a result of these observations, they concluded that the prejunctional site of the motor endplate was chiefly involved in the contractile response produced by exogenous ACh. However, in the present experiments, the ACh dose-response curve in the β-BuTX-poisoned muscle was not appreciably different from that of the control, whereas the response to ACh was completely abolished by α-BuTX. Our results are in good accordance with the previous finding that β-BuTX does not affect the ACh receptor of the post-synaptic membrane (Chang & Lee, 1963; Lee & Chang, 1966; Chang *et al.*, 1973). Our results strongly indicate that the post-junctional site of the motor endplate is chiefly involved in the contractile response produced by exogenous ACh.

There are two possibilities for the discrepancy between our results and those of Okamoto *et al.* (1971): (1) BWSV might contain some component(s) that could affect post-synaptic membrane excitability to ACh, although Okamoto *et al.* (1971) claimed that BWSV was a toxin selectively poisoning the motor nerve ending. In this connection, Gruener (1973) has reported that BWSV can produce marked loss of excitability of the squid giant axon, whereas β-BuTX has no such action. (2) The sensitivity of the gastrocnemius muscle to exogenous ACh might have been reduced after repeated intra-arterial application of ACh in the experiments of Okamoto *et al.* (1971).

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