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THE EFFECT OF RECEPTOR DESENSITIZATION ON THE ACTION OF α-BUNGAROTOXIN ON CULTURED SKELETAL MUSCLE

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The effect of receptor desensitization produced by carbachol on the antagonist action of α-bungarotoxin has been studied in cultured skeletal muscle from chick embryos. In control preparations, α-bungarotoxin irreversibly blocked depolarization produced by carbachol. However, after receptor desensitization, α-bungarotoxin was no longer effective in blocking the response to carbachol. It is concluded that desensitization protects cholinoceptors from the action of α-bungarotoxin.

Nicotinic receptors of a number of systems are blocked by some snake venom toxins, which appear to be highly specific and irreversible cholinoceptor antagonists (for review see Lee, 1972). Skeletal muscle fibres grown in culture respond to cholinomimetic drugs (Dryden, 1970; Fischbach, 1970) and the depolarization response to acetylcholine is abolished by cobra neurotoxin and α-bungarotoxin (Harris, Marshall & Wilson, 1973; Hartzell & Fambrough, 1973; Harvey, Dryden & Marshall, 1973). The binding of these toxins to cultured muscle fibres is reduced in the presence of a cholinomimetic (Patrick, Heinemann, Lindstrom, Schubert & Steinbach, 1972; Vogel, Sytkowski & Nirenberg, 1972). In the frog sartorius muscle preparation, desensitization protects the cholinoceptors from toxin (Miledi & Potter, 1971; Lester, 1972). Desensitization occurs in cultured skeletal muscle (Harvey & Dryden, 1974) and this study was carried out to determine whether desensitized receptors in cultured muscle are vulnerable to α-bungarotoxin.

Methods Monolayer cultures of skeletal muscle were obtained from the leg muscles of 10-11 day chick embryos as described by Harvey & Dryden (1974). α-Bungarotoxin was purified according to the method of Dryden, Harvey & Marshall (1974).

Standard electrophysiological techniques were used to measure intracellular membrane potentials. For each culture a series of control potential measurements was made. The control medium (Eagle's Minimum Essential Medium, MEM, Eagle, 1959) was removed and the test solution added. Carbachol and α-bungarotoxin solutions were made up in MEM. Measurements of membrane potentials in the presence of carbachol were made during a 5 min contact time, and the values obtained were averaged. Since the percentage depolarization of the membrane of a cultured muscle fibre to a given concentration of agonist is independent of the resting potential (Harvey & Dryden, 1974), depolarization responses are also given as per cent of control resting potential levels. Intracellular recordings were performed at room temperature (20-22° C) and incubation with toxin was at 37° C. Each culture was used once for one treatment only.

Results and Discussion The results of the experiments are shown in Table 1. To ensure a high degree of receptor desensitization, 5 min exposure to 10 mM carbachol was chosen as the desensitization treatment (Harvey & Dryden, 1974). The membrane potential returned to pre-drug level after about 15 min, and after 12 h 15 min had elapsed, the depolarization response to 1 mM carbachol was 38.9 ± 2.7% (mean ± s.e.). This is a considerably smaller response than the control response to 1 mM carbachol (71.5 ± 1.6%) measured after 12 h 15 min in MEM without initial carbachol treatment. Thus, after 12 h 15 min a degree of desensitization still existed in the carbachol pretreated preparations and this is in accordance with the previously reported slow rate of recovery from desensitization in this preparation (Harvey & Dryden, 1974).

A toxin concentration sufficient to eliminate the carbachol response was then established. Exposure for 15 min to 2 μg/ml α-bungarotoxin was found to abolish the response to 1 mM carbachol. After a 12 h recovery period from the toxin there was still no response to 1 mM carbachol, as could be anticipated from previous work (Hartzell & Fambrough, 1973; Harvey *et al.*, 1973).

To test for protection against toxin blockade, fibres were exposed to the desensitizing concentration of carbachol for 5 min, rinsed briefly in MEM and then immediately incubated for 15 min with α-bungarotoxin. The response to 1 mM carbachol was measured 12 h after removing

Table 1 The effect of desensitization and α-bungarotoxin on the depolarization response to carbachol

Desensitization treatment	*Toxin incubation*	*Recovery period*	*Response to 5 min in 1 mM carbachol*		
			Resting membrane potential before test response (−mV, mean ± s.e.)	*Membrane potential in presence of carbachol (−mV, mean ± s.e.)*	*Depolarization as % of control membrane potential (mean ± s.e.)*
–	–	12 h 15 min in MEM	31.47 ± 2.37 (n = 30)	8.97 ± 0.52	71.5 ± 1.6 (n = 30)
5 min in 10 mM carbachol	–	12 h 15 min in MEM	33.33 ± 0.92 (n = 36)	20.35 ± 0.89	38.9 ± 2.7 (n = 59)
–	15 min in 2 μg/ml α-bungarotoxin	5 min in MEM	34.56 ± 2.13 (n = 16)	34.65 ± 1.31	−0.3 ± 3.8 (n = 23)
–	15 min in 2 μg/ml α-bungarotoxin	12 h in MEM	35.06 ± 1.11 (n = 52)	34.35 ± 0.81	2.0 ± 2.3 (n = 54)
5 min in 10 mM carbachol	15 min in 2 μg/ml α-bungarotoxin	12 h in MEM	33.28 ± 0.88 (n = 56)	17.88 ± 0.74	44.6 ± 2.2 (n = 76)

n is the number of measurements made on individual fibres.
The average value of the resting potential of the cultures before addition of any substance was 34.00 ± 1.62 mV (inside negative).

the toxin. This was 44.6 ± 2.2%, which was not significantly different from the response to carbachol after desensitization treatment but without the toxin.

It is not established how complete was the removal of the conditioning carbachol but earlier studies (Miledi & Potter, 1971; Lester, 1972) which showed protection by desensitization suffer from the same problem, indeed these studies were performed, in part, with the continuous presence of the conditioning cholinomimetic. Rang & Ritter (1970) noted that recovery from blockade with tubocurarine was much more rapid than recovery after desensitization and suggested that the rate of recovery from desensitization is unlikely to have been limited by diffusion. Diffusion of drug from receptors should encounter fewer barriers in a monolayer cell culture. It is not considered likely that protection was due to residual carbachol.

It is therefore concluded that receptor desensitization prevents α-bungarotoxin from blocking acetylcholine receptors in cultured skeletal muscle. This is in agreement with results obtained with isolated frog sartorius muscle (Miledi & Potter, 1971; Lester, 1972) and provides further evidence that the nicotinic receptors present in cultured skeletal muscle are similar in properties to the receptors in normal muscle.

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