A quantification of acetylcholine receptors of the chick biventer cervicis muscle

The chick biventer cervicis nerve-muscle preparation introduced by Ginsborg & Warriner (1960) has been widely used in the study of neuromuscular blocking agents because it responds with contraction not only to nerve stimulation but also to exogenously applied acetylcholine and other nicotinic agonists. Recently, the extrinsic receptors which respond to low concentrations of exogenous acetylcholine in the absence of anticholinesterase were found to be different from those synaptic receptors which respond to intrinsic acetylcholine released by nerve impulse (Chang & Tang, 1974; Chang & Su, in preparation). Exogenous acetylcholine was found to act preferentially on extrajunctional receptors because its penetration into the narrow synaptic cleft is greatly hindered by enzymic hydrolysis during diffusion. The present study was undertaken to quantify the junctional and extrajunctional acetylcholine receptors by tagging these receptors with labelled α-bungarotoxin, a specific irreversible binding agent of nicotinic receptors (Chang & Lee, 1963; Miledi & Potter, 1971; Barnard, Wieckowski & Chiu, 1971; Berg, Kelly & others, 1972, Hartzell & Fambrough, 1972; Chang, Chen & Chuang, 1973). Attempts were also made to correlate the number of receptors and their function in neuromuscular transmission.

The biventer cervicis nerve-muscle preparation was isolated from 4–10 days old male white Leghorns and the experiments were performed in Tyrode solution at 37°. Tri [^{3}H]acetyl α-bungarotoxin (Chang & others, 1973), $4{\cdot}5 \times 10^8$ toxin molecules per d. min^{-1} was used. Its potency to block neuromuscular transmission was about one third of the unmodified toxin. At 1·0 μg ml^{-1} [^{3}H]α-bungarotoxin, the neuromuscular transmission, as tested by the contractile response to single nerve stimulation, was completely blocked after incubation for 62 ± 3 min (mean ± s.e., n = 9), whereas at 0·3 μg ml^{-1} of the toxin incubation for 117 ± 4 min (n = 4) was needed. The number of [^{3}H]toxin molecules remaining bound to each biventer cervicis after 2 h incubation and subsequent washings for 3 h is shown in Fig. 1. Non-specific bindings were mostly removed after washing. It is evident that the receptor sites on the muscle were saturated when the toxin concentration was 1 μg ml^{-1} or higher. Not more than half of the receptors were bound with the toxin at 0·3 μg ml^{-1} although the neuromuscular transmission was just completely blocked. This result is quite apart from that obtained in mouse diaphragms (Barnard & others, 1971) and rat diaphragms (Chang, Chuang & Huang, 1975). In the latter muscles, occupation of more than 80% of the receptors is needed for neuromuscular blockade. The safety factor for neuromuscular transmission in the chick biventer cervicis muscle is therefore less than 2 in contrast to 4–5 for rodent diaphragms.

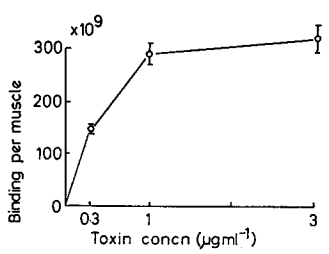

FIG. 1. The number of [^{3}H] acetyl α-bungarotoxin molecules bound to the chick biventer cervicis at various toxin concentrations. The muscles were treated with the toxin at 37° for 2 h and then washed for 3 h. Mean ± s.e., n = 4–8.

On saturation, there were about $3{\cdot}4 \times 10^{11}$ toxin molecules bound on each muscle. No difference was observed between muscles isolated from 4-day and 8-day old chicks. When the muscle was incubated with 1 μg ml^{-1} [^{3}H] toxin in the presence of 100 μg ml^{-1} acetylcholine, [^{3}H]α-bungarotoxin bound to the muscle was reduced by 53%. In this experiment, the contractile response to exogenous acetylcholine, which has been shown to act preferentially on extrajunctional receptors (Chang & Tang, 1974), was nearly completely protected from the blockade by the toxin whereas the response to nerve stimulation was not significantly protected. The number of [^{3}H] toxin molecules bound to the muscle under protection of acetylcholine may be therefore considered to represent the binding sites of junctional receptors. The fraction of [^{3}H]α-bungarotoxin-binding sites protected by acetylcholine may represent the extrajunctional receptor sites. The chick biventer cervicis muscle was found to be composed of approximately 8000 muscle fibres with average diameter 10 μm and 15 mm length. Since the majority of them are diffusely innervated at an interval of 340 μm (Ginsborg, 1960), it may be calculated that there are about $4{\cdot}6 \times 10^5$ receptor sites in one endplate. This is a much lower value than those ($2–4 \times 10^7$) found for the chick posterior latissimus dorsi muscle (Albuquerque, Barnard & others, 1974) and other mammalian focally innervated muscle fibres (Miledi & Potter, 1971; Barnard & others, 1971; Hartzell & Fambrough, 1972; Chang & others, 1973; Albuquerque & others, 1974). Interestingly, the maximal sensitivity to iontophoretic acetylcholine has been similarly found to be 15 times lower in anterior latissimus dorsi muscle (diffusely innervated) than in posterior latissimus dorsi muscle (focally innervated) (Fedde, 1969). The density of extrajunctional receptors, calculated by assuming that these receptors distribute evenly along the whole length of muscle fibre, is about 50 μm^{-2} whereas that in a mammalian focally innervated muscle is less than 5 μm^{-2} (Hartzell & Fambrough, 1972). This result is in agreement with the observation that the sensitivity to acetylcholine at the non-endplate zone in diffusely innervated muscle is higher than that in focally innervated muscle (Fedde, 1969).

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