Interactions of Neuromuscular Effects of Edrophonium, Alpha-Bungarotoxin and Beta-Bungarotoxin

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Interactions of neuromuscular effects of edrophonium, alpha-bungarotoxin, and beta-bungarotoxin were studied in 12 chickens using the sciatic-gastrocnemius nerve-muscle preparation to elucidate the mechanism of action of each drug. Modification by the toxins of neuromuscular effects of edrophonium depended on the level of block pre-established by the toxins. Edrophonium-induced augmentation of muscle twitch ("facilitation") was decreased by both toxins. As the block reached 50 per cent, the facilitation was nearly abolished. Edrophonium-induced contraction of the muscle was blocked by alpha-bungarotoxin only. At 25 per cent block, it was no longer observable in five of six preparations. Beta-bungarotoxin enhanced the contracture. At complete block, the contracture reached 156 (SE 11, n = 6) per cent of control. The authors conclude that edrophonium facilitates neuromuscular transmission by a prejuncional mechanism and causes contracture of the chicken muscle by a post-junctional activation. The beta-bungarotoxin-blocked nerve-muscle preparation of the chicken is a model of acute denervation potentially useful for the study of drug effects on the postjunctional membrane. (Key words: Neuromuscular transmission; Antagonism, Neuromuscular relaxants, edrophonium; Neuromuscular relaxants, alpha-bungarotoxin; Neuromuscular relaxants, beta-bungarotoxin.)

Edrophonium increases the mechanical response to motor nerve stimulation ("facilitation") and causes a sustained contraction ("contracture") in the chicken. The motor nerve terminal is thought to be the site of action for the facilitation.¹ ² Direct depolarization of the end-plate is probably responsible for the contracture.³ Alpha- and beta-bungarotoxins are polypeptides with molecular weights of 8,000 (alpha) and 28,500 (beta) that have been isolated from snake venoms. The purified toxins have been well studied and are widely employed as investigative tools in neuromuscular pharmacology and physiology.⁴ Alpha-bungarotoxin blocks neuromuscular transmission primarily by a postjunctional mechanism.⁴ Beta-bungarotoxin blocks prejunctionally.⁵ ⁶ In vivo, the purified toxins have no other measurable effects.⁴ ⁷

Both blocks are essentially "permanent," spontaneous recovery not being observed during periods of acute experimentation.⁵ ⁷ Interactions between edrophonium and the toxins may be valuable in confirming the mechanisms of neuromuscular actions of each drug. It can be predicted that alpha-bungarotoxin will block manifestations of both neuromuscular actions of edrophonium, the prejunctional facilitation and the postjunctional depolarization. Beta-bungarotoxin, on the other hand, will leave the depolarization unaltered. Besides quantitatively studying drug interactions, we attempted to create with beta-bungarotoxin an investigative model of pharmacology of the postjunctional membrane.

Methods

Twelve mature hens, averaging 2.3 (± 0.3 SD) kg in mass, were anesthetized with pentobarbital, 20–25 mg/kg, intravenously. The trachea, one carotid artery, and one subcutaneous vein were cannulated by cutdowns. The carotid arterial blood pressure was continuously recorded via a Statham pressure transducer. All chicken lungs were ventilated with air by use of a Palmer Pump delivering a stroke volume of 25 ml/kg, 18 times/min. The right sciatic nerve was exposed, crushed, and stimulated distally with square electric pulses of 0.2-msec duration and supramaximal voltage (approximately 10 volts) at 0.1 Hz. Stimuli were generated by a Grass S88 Stimulator and delivered to a pair of platinum electrodes in contact with the nerve via a Grass SIU 5 Stimulus Isolation Unit. The ipsilateral gastrocnemius muscle was detached from its insertion and attached to a Grass FT 10C force transducer by pre-stretched heavy silk. The knee and the ankle were immobilized by pins attached to a heavy metal frame. The force of contraction of the muscle was transduced, amplified, and recorded on a Beckman Type R Dynograph using a Strain Gauge Coupler. In addition, the electrical output of the preamplifier of this channel was tapped and used as the input to a second channel using a Beckman AC/DC coupler with a time constant set at 0.3 seconds. This arrangement allowed simultaneous selective recording on a separate channel of the twitch response without the contracture.

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The preload to (length of) the muscle necessary for maximal tension output of the twitch was individually determined, applied (approximately 200–300g) and maintained. The body temperature of each chicken was kept in its normal range (38–40 C), and heating applied as needed. After the preparation had stabilized, edrophonium chloride in doses of 0.5 and 3 mg/kg was tested for facilitation, contracture, and neuromuscular block. Each series of tests consisted of the injection of both doses. The larger dose was injected one minute after the smaller dose.

The control measurements were repeated to insure stability of response. After injection of either of the toxins, the series of tests was repeated at various levels of block, approximately 20–25 per cent, 50 per cent when time allowed, 75–80 per cent and complete block. Each chicken served as its own control. Six chickens received alpha-bungarotoxin; the remaining six received beta-bungarotoxin. Previous observations have shown that alpha-bungarotoxin, 0.2 mg/kg, or beta-bungarotoxin, 0.02 mg/kg, usually produces complete block of neuromuscular transmission with a conveniently slow time course in the chicken, 10–30 minutes from intravenous injection to beginning of block and an additional 30–180 minutes to total block, during which interacting effects of edrophonium can be studied. All drugs were injected intravenously.

Results

Preliminary observations in the chicken had shown that the intravenous injection of edrophonium, 0.5 mg/kg, normally produced within one minute an augmentation of the twitch response that was equivalent to 50–75 per cent of maximal augmentation. Addition of 3.0 mg/kg produced a contracture equivalent to 50–75 per cent of the maximal contracture produced by edrophonium. The total effect of these doses lasted less than 20 minutes, and there was no appreciable cumulative effect when the doses were repeated 20 minutes apart. These doses were well tolerated by the chicken.

The facilitatory effect of edrophonium normally preceded and outlasted the contracture, during which
it was diminished, but not eliminated. After the contracture, an apparently "recurrent" facilitation was observed in the control condition (fig. 1). During partial neuromuscular block pre-established by either toxin, the facilitation was not only diminished in amplitude but also shortened in duration. At the level of 50 per cent block of twitch it was eliminated (figs. 1–3). The recurrence of facilitation was likewise first diminished and then eliminated. In addition, during partial block pre-established by beta-bungarotoxin a superimposing neuromuscular block appeared during the contracture (fig. 1).

Alpha-bungarotoxin completely prevented edrophonium-induced contracture in five of six chickens at the level of 25 per cent block of the twitch, and in all chickens at higher levels of block. Beta-bungarotoxin, by contrast, enhanced the contracture (figs. 2 and 3). The contracture increased as the block increased, and reached 156 (SE 11) per cent of control at peak. The increase was not due to a cumulative effect of edrophonium because prolonged rest did not prevent it.

Discussion

Alpha- and beta-bungarotoxins both blocked the neuromuscular facilitatory action of edrophonium. This can be also viewed as failure of edrophonium to reverse the action of either toxin effectively. Both toxins are known to block irreversibly, their block proceeding unhalted in vivo except during very transient antagonism following injection of anticholinesterases.7

Contracture of chicken muscle has been a classic sign of depolarization of the postjuncional membrane.8,9 Our results indicated that with alpha-bungarotoxin, the edrophonium-induced contracture of the chicken gastrocnemius muscle was totally blocked in five of six preparations when the neurally elicited twitch had been blocked by 25 per cent. This observation is compatible with Waud and Waud's report10 that more than 70 per cent of receptors must be occluded before neuromuscular transmission fails.

Failure of beta-bungarotoxin to prevent edrophonium-induced contracture of the avian muscle indicates that this toxin has no appreciable postjuncional blocking effect in vivo. A neuromuscular preparation completely paralyzed by this toxin thus may be useful as a physiological model for investiga-

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**Fig. 2.** Blockade of neuromuscular effects of edrophonium by \( \alpha \)-BuTX. **Broken line:** Tension output of neurally elicited muscle twitch during the time course of neuromuscular block produced by \( \alpha \)-BuTX. **Open circle:** Tension output of neurally elicited twitch following injection of edrophonium, 0.5 mg/kg. **Closed circle:** Tension output of drug-induced unstimulated contracture following addition of edrophonium, 3 mg/kg, after 1 min. **Unit of ordinate:** Tension output of control twitch. **Abscissa:** Percentage depression of the twitch produced by \( \alpha \)-BuTX, with zero per cent referring to the control condition before injection of \( \alpha \)-BuTX. Bars indicate SD.

**Fig. 3.** Effect of \( \beta \)-BuTX on neuromuscular effects of edrophonium. **Broken line:** Tension output of neurally elicited muscle twitch during the time course of neuromuscular block produced by \( \beta \)-BuTX. **Open circle:** Tension output of edrophonium-facilitated twitch. **Closed circle:** Tension output of edrophonium-induced unstimulated contracture. **Unit of ordinate:** Tension output of control twitch. **Abscissa:** Percentage depression of the twitch by \( \beta \)-BuTX. Bars indicate SD. (See figure 2 for details.)
tions of the postjunctional mechanisms of drugs, where elimination of prejunctional activity is desired. It is, in essence, a preparation of acute and practically permanent denervation. It should be realized that few neuromuscular drugs are purely pre- or postjunctional, and that neither toxin is literally irreversible.

The advantage of the acutely denervated muscle preparation over the chronically denervated muscle preparation is that the muscle does not have time to develop supersensitivity or to suffer trophic changes. Chicken muscle fibers are normally multiply innervated and are already very sensitive to depolarizing drugs. With this model it is possible to compare the innervated and the denervated preparations within a short period. This increases the probability that comparative studies are done under comparable conditions, and that the cholinceptors on the end-plate have not undergone time-dependent pathologic changes. Additional advantages of this experimental model include the stability, the affinity, and the specificity of binding of the toxins to the acetylcholine receptors at the neuromuscular junction.\(^4\)

We cannot explain the enhancement of edrophonium-induced contracture by beta-bungarotoxin. If acetylcholinesterase were inhibited by beta-bungarotoxin, the additional contracture following injection of edrophonium could have been attributable to acetylcholine accumulation. It is not due to cumulative effect of edrophonium, either, because increased intervals between injections did not reverse the trend. Furthermore, it is known that edrophonium in the dose range used is not cumulative.\(^5\)

The dosage of edrophonium used in these experiments did not produce a neuromuscular block during control conditions, nor during partial neuromuscular block pre-established by alpha-bungarotoxin. However, it did produce a block during the partial block pre-established by beta-bungarotoxin (fig. 1). This finding was unexpected. Careful examination of the neuromuscular effect of edrophonium during the control stage reveals that shortly after the onset of facilitation, a neuromuscular block did seem to superimpose and diminish the facilitation (fig. 1, upper panel). In other words, the facilitation had more than compensated for the block. By blocking the prejunctionally originated facilitation, beta-bungarotoxin then revealed the postjunctional neuromuscular blocking effect of edrophonium.

In conclusion, edrophonium facilitates neuromuscular transmission by a prejunctional mechanism, and causes a contracture in chicken muscle at higher dosage by depolarization of the end-plate. In vivo, alpha-bungarotoxin blocks neuromuscular transmission postjunctonally. Beta-bungarotoxin blocks prejunctionally.

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