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Effects of Neuromuscular Blocking Agents and Acetylcholinesterase Inhibitors on the Response of Pectoral Fin Muscle of the Sculpin (*Enophrys bison*) to Indirect Stimulation (41925)

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Abstract. The neuromuscular junction of the buffalo sculpin (*Enophrys bison*) was characterized *in situ* by examining the effects of various neuromuscular blocking agents and acetylcholinesterase inhibitors (ACHE-I) on pectoral muscle response to indirect stimulation. The injection of either *d*-tubocurarine (350 μg/kg) or α-bungarotoxin (α-Butx) (1 mg/kg) resulted in a flaccid paralysis. The depolarizing agents, succinylcholine (11 μg/kg) and decamethonium (42 μg/kg), produced a spontaneous contraction. The administration of the ACHE-I, diisopropyl fluorophosphate (DFP), and eserine resulted in responses which were contrary to those expected based on similar experiments using mammalian skeletal muscle. Twitch potentiation did not occur and the ability to maintain a tetanic response was not abolished even after the administration of clearly lethal concentrations of ACHE-I.

Investigations into the pharmacological aspects of neuromuscular transmission in fish have been limited. The effects of phenol on transmission in the red muscle of the goldfish (*Carassius auratus*) were examined by Kuba (1). Phenol was found to increase the quantity of transmitter released. Hidaka and Kuriyama (2) investigated the effects of catecholamines on cholinergic transmission in the pectoral fin of *C. auratus*. Their results indicated that norepinephrine acted on the prejunctional membrane and increased the amount of transmitter released, and epinephrine acted on the postjunctional membrane and increased the sensitivity of the membrane to the transmitter. The effects of tetanus toxin were investigated in *C. auratus* pectoral muscles where it was found to block the nerve action potential and the spontaneous release of acetylcholine from presynaptic terminals (3, 4).

Schneider and Weber (5, 6) examined the significance of acetylcholinesterase and its inhibition to neuromuscular transmission in the pectoral abductor muscle of the black bass (*Micropterus salmoides*). They evaluated the effects of diisopropyl fluorophosphate (DFP) on acetylcholinesterase activity in the muscle, and correlated the attendant acetylcholinesterase inhibition with response to nerve stimulation. Doses of DFP producing inhibition in excess of 97% were always accompanied by a well-maintained tetanic response, which is not typical of mammalian cholinergic systems.

The general objective of this study was to characterize the neuromuscular pharmacology of a marine teleost *in situ*, and to compare the system with that of the freshwater teleost and the classical mammalian neuromuscular model.

Materials and Methods. Buffalo sculpin weighing between 400 and 1000 g were collected from Yaquina Bay, Oregon, by otter trawl and held in outside tanks with aerated seawater at 6–14°C.

The fish were anesthetized with benzocaine (70 mg/ml) and the gills were irrigated with seawater containing the anesthetic. Each fish was then spinally transected, and the efferent branchial artery of the third gill arch was cannulated with PE 50 tubing, containing a heparinized (500 units) marine teleost saline solution. An incision was made posterior to the pectoral abductor muscle leaving a small cavity between the anterior extremity of the trunk musculature and the abductor. Sensory nerves were then dissected away from supporting tissue and cut. The pectoral abductor and adductor nerve bundle was decentralized by ligation and the adductor nerve was separated from the bundle and severed. A suture was attached to a muscle bundle of the dorsal section of the abductor and connected to a

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linear motion transducer, interfaced with a Clevite Brush Mark 260 recorder. The abductor was held under a constant tension of 30 g. An electrode was attached to the abductor nerve bundle distal to the ligation. The sculpin was placed on anesthetic-free seawater and allowed to recover for approximately 30 min.

The effects of various drugs on indirectly stimulated twitch and tetanus were studied. Twitch was produced by stimulating (Grass SD9 stimulator) the abductor nerve bundle at a frequency of 0.2 Hz. Tetanus was induced by stimulation at a frequency of 15 Hz. The optimal voltage for stimulation was determined for each preparation with a mean of 3.5 V.

All drugs were obtained from the Sigma Chemical Company, St. Louis, Missouri, except for atropine sulfate which was from the J. L. Baker Chemical Company, Phillipsburg, New Jersey. Drugs were administered intravenously (iv) in a marine teleost saline (KCl, 0.38 g/liter; NaCl, 7.25 g/liter; $CaCl \cdot 2H_2O$, 0.23 g/liter; $NaHCO_3$, 1.0 g/liter; $MgSO_4 \cdot 7H_2O$, 0.23 g/liter; Trizma Base, 0.80 g/liter; Trizma HCl, 6.85 g/liter; glucose, 2.0 g/liter) having an osmolality of 350 mosm. DFP administered both iv and ip was prepared in propylene glycol.

Results. *d-Tubocurarine.* The administration of 100 μg/kg (iv) *d*-tubocurarine resulted in an initial decline in twitch (Fig. 1a). Sculpin were injected with either 200, 250, or 350 μg/kg *d*-tubocurarine (Table I). In the three fish injected with 200 μg/kg *d*-tubocurarine, twitch decreased 23 ± 4.0% after 5 min. Twitch returned to its control level in one fish after 15 min and gradually declined in the other two. In the three sculpin injected with 250 μg/kg *d*-tubocurarine, twitch decreased 39 ± 11% with a recovery of twitch in one fish and a gradual decline in the other two. Twitch decreased 42.0 ± 6.0% after 5 min in three fish injected with 350 μg/kg *d*-tubocurarine and gradually declined in all three.

α-Bungarotoxin. The effects of α-bungarotoxin (α-Butx) on indirectly elicited twitch was studied in three fish (Table I). The

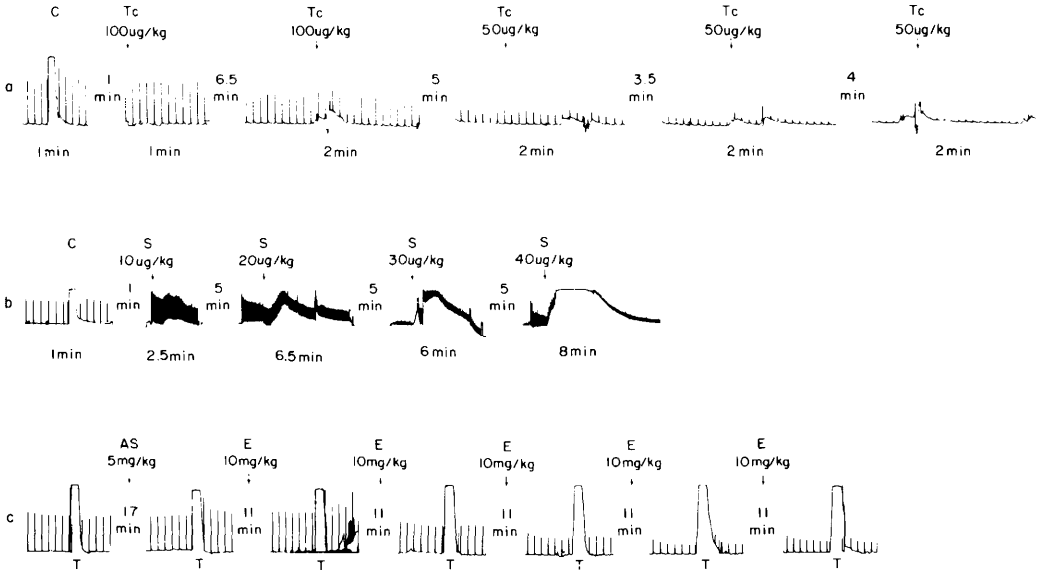

FIG. 1. Representative recordings illustrating the effects of drugs on indirectly stimulated contractions of the pectoral abductor muscle of the buffalo sculpin. Time between recordings refers to elapsed time between nerve stimulation. Time period below recordings refers to time period of nerve stimulation. C, control; T, tetanus. (a) The effects of *d*-tubocurarine (Tc), to a total dose of 350 μg/kg, on muscle twitch. Total time = 31 min. (b) The effects of succinylcholine (S) on muscle twitch. Total time = 41 min. (c) The effects of eserine, to a total dose of 50 mg/kg, on muscle twitch and tetanus. Preparation pretreated with 5 mg/kg atropine sulfate (AS). Stimulation sequence: 0.2 Hz for 30 sec, 15 Hz for 5 sec, 0.2 Hz for 25 sec. Time between recording refers to elapsed time between drug injection and nerve stimulation.

TABLE I. EFFECT OF DRUGS ON THE PECTORAL MUSCLE OF THE BUFFALO SCULPIN

Drug	Dose	Percentage decrease in twitch ($\bar{x}$ ± SE)	Percentage decrease in tetanus
d-Tubocurarine[a]	200 μg/kg	24 ± 4	NS[b]
	250 μg/kg	39 ± 11	NS
	350 μg/kg	42 ± 6	NS
α-Bungarotoxin[c]	450 ± 104 μg/kg	39 ± 3	NS
Eserine[d]	8 ± 3 mg/kg	28 ± 9	0
Atropine sulfate[e]	5 mg/kg	12 ± 5	0
Eserine	20 mg/kg	36 ± 6	0
	50 mg/kg	84 ± 6	0
	Dose (μg/kg, $\bar{x}$ ± SE)		Percentage of maximum tonic contraction
Succinylcholine[f]	11 ± 4		15 ± 5
	68 ± 16		100
Decamethonium[f]	42 ± 7		28 ± 9
	78 ± 12		100

[a] Twitch measurement 5 min after injection, $N = 3$ for each dose.
[b] not studied.
[c] Mean total dose of α-bungarotoxin resulting in initial decrease in twitch, $N = 3$.
[d] Mean total dose of eserine resulting in initial decrease in twitch, $N = 4$.
[e] Fish pretreated with atropine sulfate prior to eserine administration. Eserine dose represents total dose administered, $N = 5$.
[f] Mean dose of succinylcholine ($N = 6$) or decamethonium ($N = 3$) resulting in increases in tonic contraction represented as percentage of maximal tonic contraction.

dosage of α-Butx needed for a decline in twitch was from 250 to 600 μg/kg and complete blockade of twitch occurred after cumulative doses of 800 to 1000 μg/kg.

Succinylcholine. The dosage of succinylcholine required to cause an increase in muscle tension was inconsistent for the seven fish studied (Table I). Initial increases in muscle tension required doses of 5 to 60 μg/kg and complete contraction was achieved at doses ranging from 20 to 130 μg/kg (Fig. 1b).

Decamethonium. The effect of decamethonium on muscle tension was studied on four fish (Table I). Injections of 20 to 50 μg/kg decamethonium resulted in an initial increase in muscle tension, which was relatively consistent for the fish studied. Twitch declined as tension increased and tetanus was not affected when the muscle was not completely contracted.

Diisopropyl fluorophosphate. In preliminary experiments with DFP, an injection of 100 mg/kg DFP resulted in a 50% decrease in twitch with a slowing of the onset of tetanus, but the height of tetanic contractions remained unaltered. Tetanus did decline 9% after a cumulative dosage of 200 mg/kg. Opercular movements stopped approximately 10 min after the initial injection of 100 mg/kg. In two fish injected with 200 mg/kg DFP, intraperitoneally, (ip) both twitch and tetanus were abolished 40 min after injection. In an experiment in which 5 mg/kg atropine sulfate was injected iv prior to the ip injection of 200 mg/kg DFP, both twitch and tetanus declined 84 and 39%, respectively, but they were not completely abolished until a second injection of DFP was administered.

Eserine. A total dose of 8 ± 3 mg/kg eserine resulted in a decline in twitch, but there were no pronounced effects on tetanus in the four fish studied (Table I). Opercular movements became labored at approximately 10 mg/kg eserine, and ceased after a total dose of 12.5 mg/kg. The onset of tetanic contraction slowed at approximately 12.5 mg/kg eserine.

Atropine sulfate (5 mg/kg), injected prior to eserine to protect the animal against the systemic effects of eserine, elicited a slight decline in twitch of approximately 12 ± 4% (Table I). The five sculpin pretreated with atropine sulfate did not show a significant decline in twitch until after they received 20 mg/kg eserine (Table I). Tetanus was not

affected with a total dose of 50 mg/kg eserine (Fig. 1c). Opercular movements became labored at approximately 20 mg/kg eserine but were still present after a total dose of 50 mg/kg.

Controls. Injections of marine teleost saline (iv) and propylene glycol (iv and ip) had no effect on muscle response.

Discussion. *Neuromuscular blocking agents.* The dose of *d*-tubocurarine needed for a competitive neuromuscular blockade in the sculpin, approximately 350 μg/kg, is close to that dose reported by Schneider (7) for the black bass (*M. salmoides*), 275 μg/kg. The administration of *d*-tubocurarine into the sculpin resulted in a flaccid paralysis, which is characteristic of mammalian preparations. The dose of *d*-tubocurarine required to produce a 90% block in indirectly elicited twitch in rats and cats is 70 and 400 μg/kg, respectively (8), and 217 μg/kg is the mean concentration needed in man (9). Hence, the dose of *d*-tubocurarine required for blockade in the sculpin is higher than reported in rats, but is comparable to the dose needed in other mammalian species.

α-Butx is purified from the venom of the elapsid snake (*Bungarus multicinctus*) and results in a neuromuscular block similar to *d*-tubocurarine, but is irreversible (10). This peripheral blockade produces a flaccid paralysis, and results in death by respiratory failure in most animal species.

In the sculpin, approximately 500 μg/kg resulted in labored opercular movement, and the animals died after an accumulation of 1 mg/kg over 80 min. This dose is high when compared to other animals. α-Butx injected subcutaneously (sc) in mice has a LD_{50} of 140 μg/kg and a LD_{50} of 110 μg/kg when injected ip (11). Lee and Tseng (12) injected rabbits iv with 1 mg/kg α-Butx and saw respiratory arrest within 10 min.

Depolarizing agents. Succinylcholine and decamethonium interrupt neuromuscular transmission by producing a prolonged depolarization of the muscle endplate region (13). The administration of these agents into the sculpin resulted in a muscle contracture which was dose dependent in duration and magnitude.

Avian tonic fibers also react to the depolarizing drugs with a spontaneous contracture, and a distinct twitch (14). Ginsborg (15) found that the muscle fibers in the hen which respond with a contracture contain multiply innervated fibers, and those which respond with a flaccid paralysis contain mostly focally innervated fibers. The results of the sculpin experiments suggest that tonic fibers are present in the sculpin pectoral muscles.

Acetylcholinesterase agents. The effects of DFP and eserine on the neuromuscular system of the sculpin are similar to those reported by Schneider and Weber (6) for the black bass. They found that although twitch decreased, tetanic response was not usually altered. Schneider and Weber (6) also found similar results in black bass pretreated with atropine sulfate; tetanus was not affected with up to an accumulated dose of 500 mg/kg DFP (iv). The 100 mg/kg DFP (iv) required for tetanus to decline in the sculpin is greater than values reported in mammalian studies. Faff *et al.* (16) studied the effects of DFP in a sciatic nerve–anterior tibialis muscle preparation in rats and saw depressions in indirectly elicited tetanus within 16 min after an injection (iv) of 2.0–2.5 mg/kg DFP.

The effects of increasing doses of eserine on twitch and tetanus were similar to those of DFP. Tetanus did decline in some experiments, but the decrease occurred after opercular movements became labored. This may suggest that the toxic effects precede the neuromuscular effects in the sculpin. However, a decline in opercular movement may not indicate a cessation of the animals' ability to receive adequate oxygenation since the seawater perfusion of the gills may be providing adequate oxygenation.

These results are contrary to those of classical mammalian cholinergic systems. In mammals, ACHE-I result in two dose-dependent responses in indirectly stimulated phasic muscles: (i) potentiation of twitch, and (ii) depression in twitch and a rapid increase in tetanic tension followed immediately by partial or complete relaxation (17).

When the ACHE-I neuromuscular experiments of this study are examined with those of Schneider and Weber (6), it becomes apparent that the basic cholinergic characteristics of the skeletal muscles of these two teleosts are different from those of mammals. These discrepancies could be explained by

differences between teleost and mammalian acetylcholinesterase, particularly since fish are poikilotherms and the enzymes operate at different temperatures. However, Schneider and Weber (6) have shown that bass do have true acetylcholinesterase, and greater than 97% of it can be inhibited and tetanus is still not depressed. It is apparent from the studies on both the sculpin and the bass that acetylcholinesterase is not as important for termination of acetylcholine's effect in neuromuscular transmission in these species of fish as in other vertebrates. This implies that other mechanisms for termination of acetylcholine activity may exist.

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