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THE PHARMACOLOGICAL PROFILE OF THE ACETYLCHOLINE RESPONSE OF A CRUSTACEAN MUSCLE

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SUMMARY

A pharmacological analysis was made of the depolarizing acetylcholine (ACh) response found on the gastric mill 1 muscles of the crabs *Cancer pagurus*, *Cancer irroratus* and *Cancer borealis*.

Acetylcholine, carbamylcholine, trimethylammonium, nicotine, and dimethyl-4-phenyl-piperazinium were effective in producing contractures and depolarizations in these muscles. No response to decamethonium, suberyldicholine, acetyl-β-methylcholine, carbamyl-β-methylcholine, pilocarpine and oxotremorine could be detected.

High concentrations of muscarinic agonists (10^{-4} to 10^{-3} M) potentiated and prolonged the ACh iontophoretic response. When the acetylcholinesterase activity was inhibited with neostigmine, or when the response was elicited with carbamylcholine, muscarinic agonists partially inhibited the response.

ACh responses were most effectively blocked by vertebrate nicotinic ganglionic antagonists, including dihydro-β-erythroidine, pempidine, and mecamylamine.

α-Bungarotoxin was without effect on the ACh response.

INTRODUCTION

Acetylcholine (ACh) is the neurotransmitter at vertebrate skeletal neuromuscular junctions and mimics the effects of the natural transmitter at neuromuscular junctions in many invertebrates (Gerschenfeld, 1973). These include animals as diverse as nematodes (Baldwin & Moyle, 1949; Del Castillo, DeMello & Morales, 1963), leech (Kuffler, 1978; Flacke & Yeoh, 1968), the polychaete worm *Syllis spongiphila* (Anderson & Mrose, 1978), and various molluscs (Twarog, 1960; Liebeswar *et al.* 1975; Taraskevich *et al.* 1977; Elliott, 1979).

Until recently there were no known cases of arthropod muscles with postsynaptic ACh receptors and cholinergic neuromuscular junctions. Futamachi (1972) first suggested the presence of ACh receptors on an arthropod muscle. This was followed by the discovery that some of the muscles of the stomatogastric system in decapod Crustacea receive cholinergic excitatory neuromuscular innervation (Marder, 1974*a*, *b*, 1976).

Although the pharmacology of the vertebrate skeletal muscle ACh receptor is well described (Koelle, 1975*a*), much less detailed information about the pharmacology of the many invertebrate muscle ACh receptors is available. Many earlier studies relied solely on inferences from bath applications of agonists and tension recordings, and in many preparations the muscle fibres are small, difficult to record from, electrically coupled, and diffusion barriers are present.

This paper describes the pharmacological profile of the nicotinic cholinergic response of the gastric mill 1 (gm1) muscles of the crabs *Cancer pagurus*, *Cancer irroratus*, and *Cancer borealis*. These muscles are typical crustacean striated muscles which receive excitatory innervation from four gastric mill (GM) motor neurones situated in the stomatogastric ganglion (Maynard & Dando, 1974). Previous work (Marder, 1974*b*, 1976; Lingle, 1980) showed that ACh mimicked the effects of the excitatory synaptic transmitter on these muscles. There is at present no evidence for any peripheral inhibitory innervation (Govind, Atwood & Maynard, 1975), nor do these muscles show any responses to γ-amino-butyric acid (GABA) (Marder & Paupardin-Tritsch, 1980).

These muscles are a convenient preparation for intracellular recording, iontophoretic agonist applications, and rapid changes of the bulk solution. Morever there appears to be only one type of cholinergic response on these muscles, thus facilitating its characterization.

METHODS

Most of the experiments were carried out with *Cancer pagurus*, obtained from fish markets in Paris, France. The remaining experiments were carried out with *Cancer irroratus* and *Cancer borealis*, bought from fishermen in Boston, Massachusetts. All animals were maintained in artificial-sea-water aquaria until used.

Experiments were performed on a preparation consisting of the isolated and intact pair of gastric mill 1 (gm1) muscles of the stomach (Maynard & Dando, 1974).

Microelectrodes for intracellular recording and current passing were either pulled on a vertical puller, a horizontal puller, or hand-fashioned with a de Fonbrune microforge. Electrodes for intracellular recording and current passing were filled with either 2·5 M-KCl or 0·5 M-K_2SO_4 and had resistances of 10–20 MΩ when filled with 2·5 M-KCl. No differences were detected between the results obtained with 2·5 M-KCl-filled electrodes and those obtained with 0·5 M-K_2SO_4-filled electrodes. Most experiments were done with two intracellular electrodes to monitor changes in membrane conductance. Electrodes for iontophoresis were filled with either ACh (0·1, 0·5 or 1 M) or carbamylcholine (Carb) (0·1 or 0·5 M). Iontophoretic electrodes were finer-tipped (40–60 MΩ when filled with 2·5 M-KCl) and more dilute agonist solutions were usually used to avoid the use of braking currents. If desensitization was still apparent braking currents (1–5 nA) were used.

All electrophysiological equipment was conventional; intracellular recordings were displayed on Gould pen-writers at high and low gain. Iontophoresis was accomplished with a stimulator through a 100 MΩ resistor monitored with a virtual ground current-voltage transducer, or with a WP Instruments microiontophoresis programmer. All experiments were done with a continuously running perfusion system (5–10

min^{-1} for antagonist application, 10–15 ml min^{-1} for agonist applications). Bath volume was 1–2 ml. All pharmacological agents were applied only after a stable iontophoretic response was found, and unless otherwise stated, all data in this paper were derived from experiments in which the iontophoretic response returned to within 5% of the control level after wash with the control solution.

The tension experiments were performed by pinning one of the muscle insertions into a wax-filled dish, and tying the other insertion to a Grass FT03 force-displacement transducer.

Cancer pagurus saline (Marder & Paupardin-Tritsch, 1980) was used for all experiments. All pharmacological solutions were made up directly before use in *Cancer pagurus* saline. Drugs were obtained from the following sources: acetyl-β-methyl choline bromide, acetylcholine chloride, arecoline hydrogen bromide, atropine sulphate, carbamylcholine chloride, carbamyl-β-methyl choline, decamethonium bromide, hexamethonium bromide, mecamylamine hydrogen chloride, neostigmine bromide, nicotine, picrotoxin, pilocarpine hydrogen chloride, and tubocurarine chloride (Sigma); nicotine bihydrogen tartrate and tetramethylammonium (BDH); dimethyl-4-phenyl piperazinium (Fluka); oxotremorine fumarate (Lab. Auclair Montrouge); pempidine tartrate (May and Baker); α-bungarotoxin, (batch BMA8-7Z) (Miami Serpentarium). Chlorisondamine was a gift from Ciba-Geigy; Gallamine (Flaxedil) was a gift from Rhone-Poulenc; benzoquinonium was a gift from Sterling Winthrop; dihydro-β-erthroidine was a gift from Merck & Co.; edrophonium and trimethaphan camsylat were gifts from Hoffman La Roche; and suberyldicholine was courtesy by B. Sakmann.

RESULTS

To determine which of a number of different cholinergic agonists were effective on muscle gml, two kinds of experiments were performed with agents known to activate different classes of ACh receptors in vertebrate and invertebrate tissues. Firstly, we performed tension experiments using the intact, paired gml muscles to determine which cholinergic agonists induced muscle contractures. Secondly, we bath-applied cholinergic agonists and recorded the depolarizations produced by relatively low concentrations of various agonists.

Cholinergic agonist-induced contractures. The paired gml muscles were attached with a thread to a force-displacement transducer and continuously superfused by a system which allowed the rapid changing of the bath solution.

Fig. 1A is a dose–response plot for the ACh and Carb induced gml contractures. The threshold for contracture was about 2×10^{-6} M ACh (in the presence of 10^{-5} M edrophonium or neostigmine to inhibit the cholinesterase) and about 2×10^{-5} M Carb; maximum contracture was produced by 2×10^{-5} M ACh and 5×10^{-4} M Carb (see also Marder, 1974*b*, for similar data on the cholinergic dorsal dilator muscle of *Panulirus interruptus*, and Lingle, 1980).

Fig. 1B shows typical records from experiments to determine which cholinergic agonists were effective in mimicking the ACh-contractures. Nicotine, trimethylammonium (TMA) and dimethyl-4-phenyl-piperazinium (DMPP) [the last two are agonists at vertebrate nicotinic ganglionic sites (Volle & Koelle, 1975)] were also

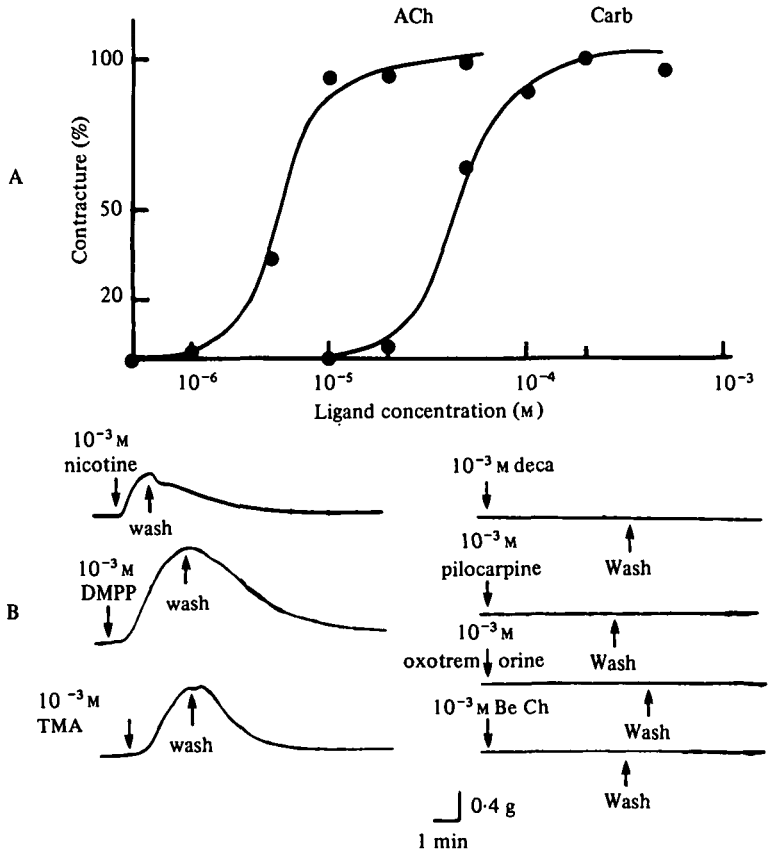

Fig. 1. Effects of cholinergic agonists on muscle contracture. (A) ACh and Carb dose–response curves for gml. Ordinate: percentage muscle contracture. Abscissa, concentration of agonist applied, log scale. (B) Tension records, gml. Downward arrows, perfusion changed to solution indicated. Upward arrows, wash. Bath volume, 2 ml. Perfusion rate 10 ml min^{-1}.

effective in producing gml contractures. Rough dose–response curves (not shown) for nicotine, TMA and DMPP showed thresholds for contracture at higher doses than for ACh and Carb, and even at 10^{-3} M the contractures produced by these three agents were smaller than the maximal contractures shown with ACh or Carb.

Decamethonium (deca) is a partial agonist at vertebrate skeletal muscle (Adams & Sakmann, 1978). As is shown in Fig. 1 B, 10^{-3} M deca was ineffective in producing contractures in this muscle. Carbamyl-β-methyl choline (BeCh), oxotremorine, pilocarpine, and arecoline, agonists at vertebrate muscarinic sites (Koelle, 1975*b*) were likewise without effect on muscle contracture at doses as high as 10^{-3} M (Fig. 1 B).

Cholinergic agonist-induced depolarizations. Since the threshold for contracture occurs at a dose that is usually higher than the dose for the threshold for depolarization, we performed a number of experiments to estimate the concentration necessary to give a just noticeable depolarization (2–5 mV), using rapid bath applications of agonists. ACh, Carb and nicotine were effective at doses of 10^{-6} M or less, whereas DMPP and TMA were effective at somewhat higher concentrations (5×10^{-6} M 2×10^{-5} M). (At low concentrations nicotine was an effective agonist, at the higher

concentrations used for the tension measurements it probably was a mixed agonist–antagonist (Marder & Paupardin-Tritsch, 1978).) At still higher concentrations (5×10^{-5} to 10^{-4} M), gallamine and arecoline depolarized the membrane slightly, although 10^{-3} M concentrations of these agents did not produce muscle contractures (above). Even at high concentrations (10^{-4} to 10^{-3} M), deca, pilocarpine, oxotremorine, acetyl-β-methyl choline (MeCh), and BeCh produced no change in membrane potential or membrane conductance (as monitored by current pulses passed through a second microelectrode).

Effects of agonists and antagonists on postsynaptic ACh responses. To determine which of many conventional cholinergic ligands were able to block the postsynaptic ACh response on these muscles, we performed a number of experiments in which ACh was iontophoretically applied to the muscle fibres. The agents tested were perfused over the muscle, and the resulting changes in membrane potential, membrane conductance and the amplitude of the ACh response recorded.

Depolarizing potentials as large as 40–50 mV were recorded when ACh was applied iontophoretically to the gml muscle. These responses resulted from a direct action of ACh on the muscle membrane, as they could be elicited after synaptic transmission was blocked (Marder & Paupardin-Tritsch, 1980).

It was possible to study the ACh responses as a function of membrane potential when the muscle fibres were penetrated with two microelectrodes, and we found that these responses were depolarizing at all potential levels between -25 mV and -100 mV. The current–voltage relation of the muscle fibres was linear between about -45 and -95 mV. We were unable to depolarize the membrane effectively past -25 mV. The reversal potential was *estimated*, by extrapolation, to be about 0 mV.

It was possible to elicit ACh responses with an electrode placed almost anywhere on the muscle membrane if the iontophoretic current used was sufficiently large. Very rapid responses with very small currents were more difficult to elicit, suggesting that there is some patchiness in the distribution of ACh receptors in the muscle membrane. At present, however, we have no direct measure of the number and distribution of the ACh receptors.

The effects of muscarinic agonists. In the previous section, it was shown that high concentrations of bath-applied muscarinic agonists were ineffective in producing muscle contractures. Furthermore, in several experiments MeCh or pilocarpine were iontophoretically applied to a number of sites along the muscle, subsequently shown to respond to ACh, but no responses to the muscarinic agonists occurred. These same compounds, however, were found to have two effects on ACh responses. High concentrations of muscarinic agonists potentiated the ACh response (Fig. 2A). This potentiation, which developed slowly, outlasted the presence of the agonists in the bath, eventually reversing with extensive washing (Fig. 2A). Since it is known that many cholinergic ligands effective at ACh receptors also block the acetylcholinesterase activity when assayed biochemically (Changeux, 1966; Mooser & Sigman, 1975), we suspected that this potentiation was due to the partial block of the muscle cholinesterase by the muscarinic agonists. This was confirmed by experiments using Carb as agonist (Fig. 2B) or by pretreating the muscles with an anticholinesterase agent, neostigmine (Fig. 2C). Under these conditions, no potentiation of the ACh response

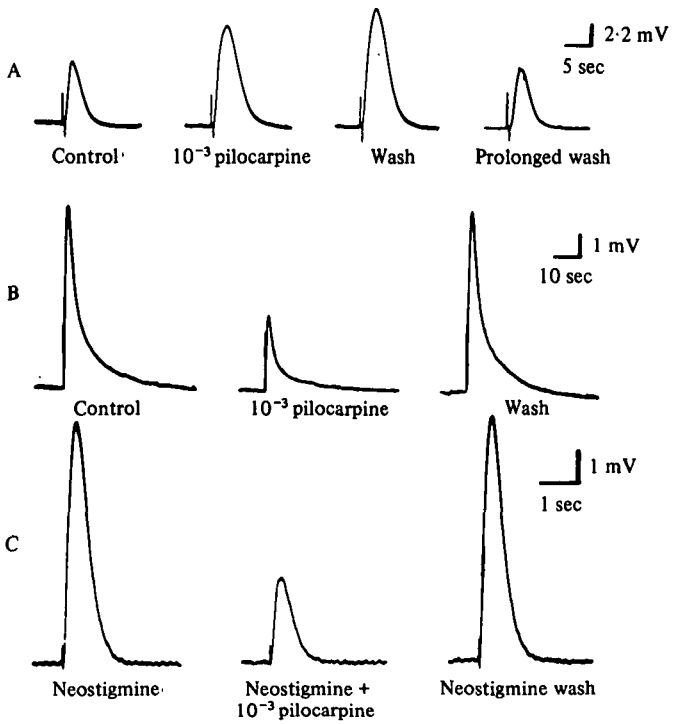

Fig. 2. (A) Effects of pilocarpine on ACh iontophoretic response. First record, ACh response, control saline. Second record, ACh response potentiated by bath application of 10^{-3} M pilocarpine. Third record, ACh response still potentiated after 10 min wash. Fourth record, ACh response returned to control level after 30 min wash. Resting potential, −70 mV all records.

(B) Effects of pilocarpine on Carb iontophoretic response. First record, Carb response, normal saline. Second record, Carb response partially blocked by bath application of 10^{-3} M pilocarpine. Third record, Carb response after wash. All records, −63 mV.

(C) Effects of pilocarpine on ACh response in neostigmine-treated muscle. Muscle was pretreated for 30 min with 10^{-5} M neostigmine. First record, ACh response, in 10^{-5} M neostigmine. Second record, ACh response in neostigmine (10^{-5} M) partially blocked by bath application of 10^{-3} M pilocarpine. Third record, wash in 10^{-5} M neostigmine. Resting potential, −65 mV all records.

In (A)–(C) membrane conductance was unchanged by pilocarpine.

was seen, but another action of these muscarinic agents was observed. At high concentrations these same agents reduced the amplitude of the ACh response (when neostigmine treated) (Fig. 2C), even when no change in muscle membrane potential or conductance was seen. Table 1 shows that 10^{-3} M concentrations of these agents blocked about 50% of the ACh or Carb response under these conditions.

Effects of cholinergic antagonists. A wide variety of pharmacological agents, known for their action on cholinergic receptors in other preparations, were tested for effectiveness in blocking the ACh iontophoretic response in these muscles. For all the data presented below, the experimental paradigm was essentially the same. After stable iontophoretic responses were located, drug solutions were added to the bath. When three successive iontophoretic responses of equal amplitude were obtained in the drug-containing solution, the preparation was washed. All data reported here are from experiments in which the response amplitude recovered to within a few per cent

Table 1. *Muscarinic agonist block of the ACh response*

Muscarinic Agonist (10^{-3} M)	% block of carb response	% block of ACh response, neostigmine-treated
Acetyl-β-methyl choline	80 ± 3	82 ± 3
Carbamyl-β-methyl choline	58 ± 5	52 ± 4
Pilocarpine	60 ± 2	58 ± 12
Oxotremorine	58 ± 12	56 ± 6
Arecoline	62 ± 4	61 ± 7

Each value is the mean of five experiments, standard deviation indicated. For all agonists except arecoline there was no change in membrane potential or conductance; arecoline sometimes produced small depolarizations.

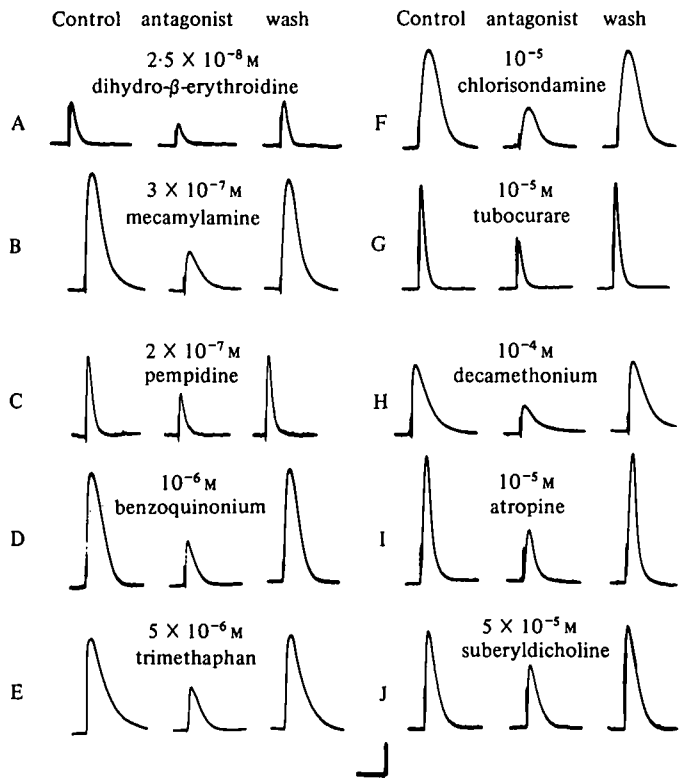

Fig. 3. Effects of cholinergic antagonists on iontophoretic ACh responses. For all records, first trace, normal saline; middle trace, drug concentration indicated; last trace, after wash. Resting potentials (constant during drug applications): A, −64 mV; B, −44 mV; C, −45 mV; D, −60 mV; E, −69 mV; F, −68 mV; G, −50 mV; H, −70 mV; I, −66 mV; J, −62 mV. Vertical bar: 1 mV, E, G, I; 2 mV, A, F, H, J; 4 mV, C, D; 5 mV, B. Horizontal bar; 2 s, A, B, C, D, F, J; 4 s, I; 8 s, E, G, H.

of the control after washing with normal saline. Fig. 3 shows typical records from some of these experiments; the most effective blocking agents are shown first. It can be seen that the most effective antagonist was dihydro-β-erythroidine, and that other nicotinic ganglionic blocking agents such as mecamylamine, pempidine, and trimethaphan (Ascher, Large & Rang, 1979) were also quite effective.

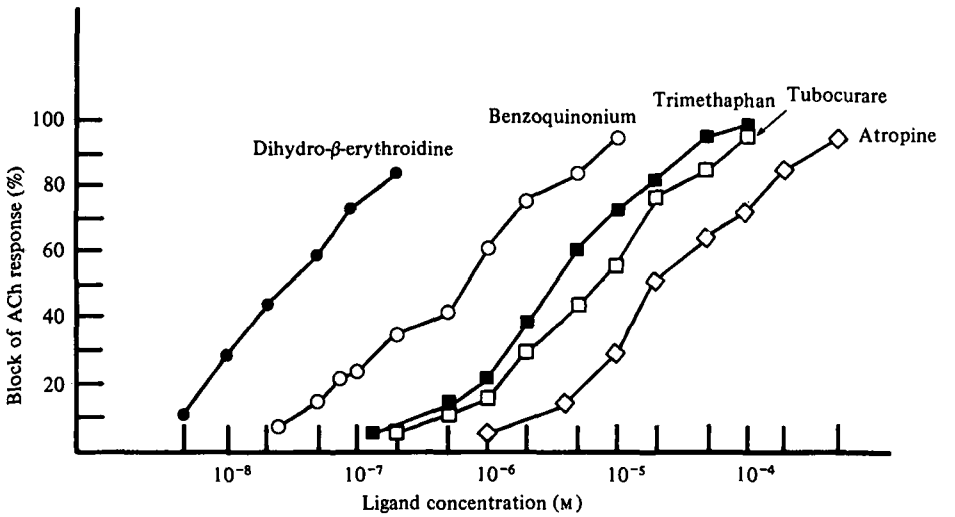

Fig. 4. Percentage block of the ACh response with respect to drug concentration. All experiments, drugs were applied at concentration indicated and then washed completely before a different concentration was applied. Each point on the graph is the mean of at least five experiments, which showed complete recuperation of the response after wash. Error bars not shown to avoid visual confusion, but standard errors of the mean were calculated for all points and were in all cases only a few per cent. Ordinate, percentage block of the iontophoretic response; abscissa, drug concentration on a log scale.

Fig. 4 shows the percentage block of the ACh response at different drug concentrations for some of the compounds tested. These plots (and others not shown) give an estimate of the drug concentration required to produce a 50% block of the physiological ACh response (Table 2). Table 2 shows that the most effective antagonists were indeed those which block vertebrate nicotinic ganglionic receptors (Ascher *et al.* 1979), while curare and decamethonium were less effective. Atropine, a highly potent muscarinic antagonist, was effective at concentrations at which it blocks the nicotinic receptor found at vertebrate skeletal muscle (Adler, Albuquerque & Lebeda, 1978; Feltz, Large & Trautmann, 1977).

Apparent insensitivity to α-bungarotoxin. Although α-bungarotoxin (α-Bgt) blocks the vertebrate skeletal muscle ACh receptor, the vertebrate nicotinic ganglionic ACh response is, under many experimental conditions, resistant to blockade by α-Bgt (Brown & Fumagalli, 1977; Carbonetto, Fambrough & Muller, 1978; Ravdin & Berg, 1979). Under ordinary physiological conditions the ACh response was not affected by α-Bgt (Fig. 5). These experiments were performed in two ways. For some experiments α-Bgt was mixed in *Cancer* saline to a final concentration of 10^{-7} to 10^{-6} M and superfused over the muscle, thus avoiding desensitization and ensuring complete mixing of the bulk solution. In the experiments shown in Fig. 5A no effect was seen after 47 min of superfusion with 2×10^{-7} M α-Bgt or 33 min in 10^{-6} M α-Bgt.

In order to study the effects of high α-Bgt concentrations for many hours a different paradigm was used, as illustrated by the experiment shown in Fig. 5B. The perfusion system was stopped, α-Bgt was added to the bath to a final concentration of 10^{-6} M

Table 2. *Effect of cholinergic ligands on ACh responses*

Drug	Concentration necessary to block 50% of the ACh response
Dihydro-β-erythroidine	3×10^{-8}
Mecamylamine	2×10^{-7}
Pempidine	2×10^{-7}
Benzoquinonium	6×10^{-7}
Trimethaphan	2×10^{-6}
Chlorisondamine	5×10^{-6}
Curare	1×10^{-5}
Decamethonium	$1{\cdot}5 \times 10^{-5}$
Atropine	$2{\cdot}5 \times 10^{-5}$
Picrotoxin	4×10^{-5}
Hexamethonium	5×10^{-5}
Suberyldicholine	9×10^{-5}
Acetyl-β-methyl choline	3×10^{-4}
Pilocarpine	$\sim 10^{-3}$
Arecoline	$\sim 10^{-3}$
Oxotremorine	$\sim 10^{-3}$
Carbamyl-β-methyl choline	$\sim 10^{-3}$

The values listed here were obtained from plots as in Fig. 4. The experiments with the muscarinic agonists (the last five) were made on preparations which were pretreated with 10^{-5} M neostigmine.

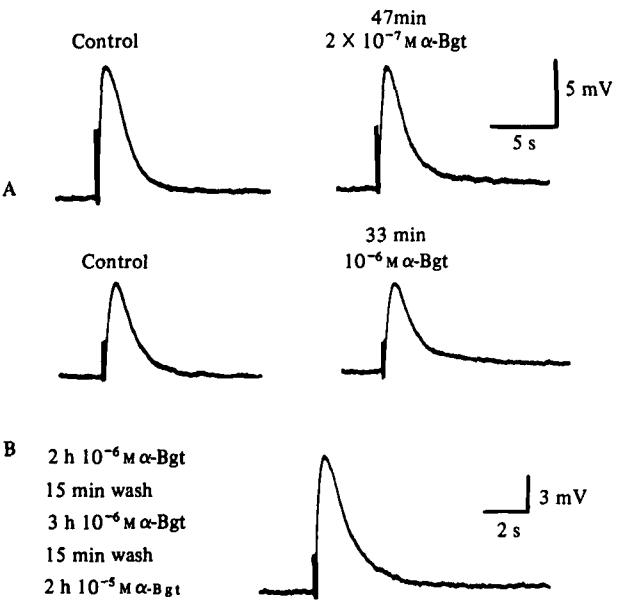

Fig. 5. Lack of effect of α-Bgt. All experiments, bath volume 2 ml. (A) α-Bgt made to concentration indicated and perfused at final concentration into bath. Perfusion with toxin was continued for 10 min to ensure complete exchange and mixing of bulk solution. Perfusion was stopped, and then restarted (still with toxin containing saline) 2 min before the records shown here were taken. (B) α-Bgt added to the bath from a 10^{-4} M stock solution to make the final concentrations indicated. Bath circulation and mixing were effected by pipetting the solution up and down a few times every 5 min. Response shown here was after the treatment indicated on the figure. This fibre was penetrated 2 h before the record shown was taken and the response was stable during that time.

and incubated for 2 h. Since desensitization was rapid and marked without perfusion even with braking currents, we washed with normal saline for 5–10 min before comparing ACh responses before and after toxin application and the ACh response after 10 min of wash was identical to the pretoxin control. After 15 min wash, 10^{-6} M toxin was again added and the preparation was incubated for 3 h, and once again when tested (after 10 min wash) the ACh responses were normal. Finally, in the experiment of Fig. 5B the muscle was incubated for 2 h in 10^{-5} M α-Bgt, and the response shown in Fig. 5B was recorded subsequent to the whole treatment, a total of 7 h in toxin containing saline (see Fig. 5B). At the end of this experiment ten different muscle fibres were penetrated and normal ACh responses were recorded in all of them, indicating that even if the ACh receptors underneath the iontophoretic electrode were protected from blockade by ACh leaking from the electrode, that other sites, remote from the electrode, were equally untouched.

DISCUSSION

This paper describes the pharmacological characteristics of the ACh response recorded from some of the stomach muscles of decapod crustaceans (Marder, 1974*a*, *b*, 1976; Lingle, 1980). These arthropod muscle ACh receptors resemble vertebrate nicotinic ganglionic receptors, and not vertebrate skeletal muscle receptors, in three ways.

(1) Vertebrate nicotinic ganglionic agonists (DMPP and TMA as well as nicotine) mimicked the effects of ACh. Skeletal muscle agonists (decamethonium and suberyldicholine) and muscarinic agonists (pilocarpine, acetyl-β-methyl choline, carbamyl-β-methyl choline, oxotremorine, and arecoline) did not mimic the effects of ACh. However, we did show that high concentrations of muscarinic agonists appear to inhibit acetylcholinesterase, thus producing a potentiation and prolongation of the ACh iontophoretic response.

(2) The most effective antagonists we tested were those best known for their effects on vertebrate nicotinic ganglionic sites, including mecamylamine, pempidine, and dihydro-β-erythroidine. Our experiments provide no information about the mechanism by which these drugs inhibit the physiological response to ACh. It is likely that some of these drugs competitively block the ACh recognition site, while others may affect the channel (see Ascher *et al.* 1979).

(3) We failed to observe a block of the physiological response to ACh by prolonged treatments with high concentrations of α-Bungarotoxin, similar to the results in autonomic ganglia (Brown & Fumagalli, 1977; Carbonetto *et al.* 1978). It is conceivable, of course, that the toxin did not reach the receptors. However, we think this an unlikely reason for the failure of the toxin to block, since ACh responses are easily located by positioning the electrode over the exposed surface of the muscle fibres, and other drugs act very rapidly. In a previous study (Marder, 1976) a block by high α-Bgt concentrations was reported, and it is likely that this was due to the presence of a contaminating toxin component (Ravdin & Berg, 1979).

The gml muscles are polyneuronally and multiterminally innervated. In these experiments we made no attempt to distinguish between junctional and ext

functional receptor sites. No heterogeneity of pharmacological sensitivites was observed that would have indicated more than one population of ACh receptors on these muscles, although it is unlikely that our techniques would have permitted us to distinguish subtle differences between receptor types.

It is somewhat difficult to ascertain how similar these Arthropod ACh receptors are to those found on other invertebrate muscles. Many of the early studies (cf. Gerschenfeld, 1973) were performed solely with tension recordings on preparations which may have contained more than one type of muscle fibre, but some relevant information is available.

The leech body wall ACh response is similar in pharmacological characteristics to the vertebrate skeletal muscle ACh receptor (Flacke & Yeoh, 1968; Walker, Woodruff & Kerkut, 1970). Other annelids may be different (Alvarez, del Castillo & Sánchez, 1969).

Molluscan muscle ACh receptors appear to fall outside the vertebrate classification system. The anterior byssal retractor muscle of *Mytilus* contracts after application of ACh. Cambridge, Holgate & Sharp (1959) showed that hexamethonium, curare, atropine, and benzoquinonium were equally effective in blocking the ACh response and that acetyl-β-methyl choline and carbamyl-β-methyl choline were weak agonists. Only fragmentary evidence is available on some *Aplysia* muscles excited by ACh (Taraskevich *et al.* 1977; Liebeswar *et al.* 1975). Elliott (1979) provided a detailed pharmacological analysis of the inhibitory effects of ACh on the clam heart and showed that this response resembles very closely the slow inhibitory response found on *Aplysia* neurones (Kehoe, 1972) which is unlike any vertebrate receptor.

At the present date, the ACh response on these arthropod muscles is the only known invertebrate muscle receptor with striking pharmacological similarity to the vertebrate ganglionic nicotinic receptor. Full comparisons of these ACh receptors with other invertebrate muscle ACh receptors require further detailed studies on many other preparations.

Finally, it is interesting to compare these muscle ACh responses with neuronal ACh responses in the same animals. Although the importance of cholinergic mechanisms in arthropods is unchallenged, the data, both physiological and biochemical, concerning the types and pharmacological properties of ACh receptors in arthropods have been confusing (Callec, 1974; Sattelle, 1977; Donnellan & Harris, 1977; Dudai, 1979). Recent binding studies have provided evidence for more than one type of ACh receptor in arthropod nervous systems. We recently demonstrated that neurones of the stomatogastric ganglion of the crab, *Cancer pagurus* showed several distinguishable classes of ACh responses (Marder & Paupardin-Tritsch, 1978*a*, *b*): (1) a nicotinic depolarizing excitatory response, (2) muscarinic depolarizations which may also activate 'burst' conductances, (3) an inhibitory response due to an increase in K^+ conductance. The nicotinic muscle response described in this paper is pharmacologically very similar to the nitotinic depolarizations which are the predominant cholinergic response found in these neurones.

Numerous investigators have employed α-Bgt binding techniques to describe and locate cholinergic receptors in arthropod systems (Schmidt-Nielsen *et al.* (1977); Marquis *et al.* 1977; Jones, Gallaso & O'Brien, 1977; Hildebrand, Hall & Osmond, 1979; Chester *et al.* 1979; Dudai, 1979). Although α-Bgt did not block the physio-

logical ACh response of this muscle, it may still be possible to demonstrate α-Bgt binding, as in autonomic systems (Carbonetto *et al.* 1978; Fumagalli & Brown, 1977). At present it is still unclear whether any of the arthropod α-Bgt binding sites correspond to physiologically active ACh receptors. The gml muscles of decapod crustacea may provide a preparation which will allow parallel biochemical and physiological studies on an arthropod ACh receptor.

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