Actions of Cholinergic Drugs in the Nematode *Ascaris suum*

Complex Pharmacology of Muscle and Motorneurons

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ABSTRACT The cholinergic agonists acetylcholine (ACh), nicotine, and pilocarpine produced depolarizations and contractions of muscle of the nematode *Ascaris suum*. Dose-dependent depolarization and contraction by ACh were suppressed by about two orders of magnitude by 100 μM *d*-tubocurarine (dTC), a nicotinic antagonist, but only about fivefold by 100 μM *N*-methyl-scopolamine (NMS), a muscarinic antagonist. NMS itself depolarized both normal and synaptically isolated muscle cells. The muscle depolarizing action of pilocarpine was not consistently antagonized by either NMS or dTC. ACh receptors were detected on motorneuron classes DE1, DE2, DI, and VI as ACh-induced reductions in input resistance. These input resistance changes were reversed by washing in drug-free saline or by application of dTC. NMS applied alone lowered input resistance in DE1, but not in DE2, DI, or VI motorneurons. In contrast to the effect of ACh, the action of NMS in DE1 was not reversed by dTC, suggesting that NMS-sensitive sites may not respond to ACh. Excitatory synaptic responses in muscle evoked by depolarizing current injections into DE1 and DE2 motorneurons were antagonized by dTC; however, NMS antagonized the synaptic output of only the DE1 and DE3 classes of motorneurons, an effect that was more likely to have been produced by motorneuron conduction failure than by pharmacological blockade of receptor. The concentration of NMS required to produce these changes in muscle polarization and contraction, ACh antagonism, input resistance reduction, and synaptic antagonism was 100 μM, or more than five orders of magnitude higher than the binding affinity for [^{3}H]NMS in larval *Ascaris* homogenates and adult *Caenorhabditis elegans* (Segerberg, M. A. 1989. Ph.D. thesis. University of Wisconsin-Madison, Madison, WI). These results describe a nicotinic-like pharmacology, but muscle and motorneurons also have unusual responses to muscarinic agents.

INTRODUCTION

In nematodes, the evidence that implicates acetylcholine (ACh) as an excitatory neurotransmitter is strong. Much of this evidence has been collected in the large

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parasitic nematode *Ascaris suum*. Initially, a muscle strip preparation was used to measure muscle contraction in response to applied drugs, and, more recently, biochemical and electrophysiological techniques have been used to study identified neurons. Specifically, it has been shown that: (*a*) muscle cells depolarize and contract in response to exogenously applied ACh (Baldwin and Moyle, 1949; del Castillo, de Mello, and Morales, 1963; Aceves, Erlij, and Martinez-Maranon, 1970; Harrow and Gration, 1985); (*b*) *d*-tubocurarine (dTC) blocks these effects (Norton and de Beer, 1957; Martin, 1982) and also hyperpolarizes muscle cells (del Castillo et al., 1963); (*c*) acetylcholinesterase (AChE), the enzyme that degrades ACh, is present at many sites, including the ends of the muscle projections (muscle arms) that receive synapses from neurons in the nerve cords (Lee, 1961); inhibitors of AChE produce tonic muscle contractions and potentiate the action of exogenous ACh (del Castillo et al., 1963; Knowles and Casida, 1966); (*d*) choline acetyltransferase, the synthetic enzyme for ACh, is present in excitatory motorneurons and in nerve cords, but not in inhibitory motorneurons (Johnson and Stretton, 1985). There is also evidence suggesting that ACh is a transmitter at certain neuron–neuron synapses, such as excitatory motorneuron to inhibitory motorneuron, and excitatory interneuron to excitatory motorneuron synapses (Kass, Stretton, and Wang, 1984).

In both vertebrates and invertebrates, it is well known that there is a variety of ACh receptors (AChRs) that are pharmacologically distinct and are often specifically localized on different classes of cells. AChR classes have been further subdivided as new pharmacological distinctions, differences in second messenger systems, or families of related genes have been discovered. In nematodes, previous work has suggested that the muscle AChR is more related to classical nicotinic receptors than to muscarinic receptors (Natoff, 1969; Rozhkova, Malyutina, and Shishov, 1980; Colquhoun, Holden-Dye, and Walker, 1991). In *Caenorhabditis elegans*, it has been shown that there are binding sites for muscarinic antagonists (Culotti and Klein, 1983; Segerberg, 1989). Similar binding sites also occur in larval *Ascaris* (Segerberg, 1989). These putative muscarinic receptors were detected in crude homogenates, and therefore their cellular localization is unknown. Since we are interested in the role played by ACh in the control of locomotory behavior of *Ascaris*, we undertook a series of experiments to determine whether muscarinic receptors are present on muscle cells and/or motorneurons.

The motornervous system of *Ascaris* contains a set of seven classes of identified motorneurons with neuromuscular output in either the dorsal or ventral nerve cord, together with interneurons, almost exclusively in the ventral cord, which synapse onto the motorneurons (Stretton, Fishpool, Southgate, Donmoyer, Walrond, Moses, and Kass, 1978; Walrond, Kass, Stretton, and Donmoyer, 1985). Three classes of dorsal motorneurons (DE1, DE2, and DE3) excite dorsal muscle; DI motorneurons inhibit dorsal muscle; VI motorneurons inhibit ventral muscle; and V-1 and V-2 motorneurons are putative ventral excitors. DE1, DE2, DE3, DI, and VI motorneurons have processes in both dorsal and ventral nerve cords. The processes in each of these cell types are linked by a lateral dorsoventral process called a commissure. The ventral processes of DE1, DE2, and DE3 motorneurons receive synapses from interneurons. DI and VI neurons receive input from excitatory (or putative excitatory) motorneurons in the ventral and dorsal nerve cords, respectively.

Muscle cells receive synaptic input from motorneurons on the ends of muscle arms

that project from the cell body (sometimes called the belly) to the nerve cord (Rosenbluth, 1965). The cell body sits on top of the longitudinal contractile part of the muscle cell (the spindle). Although microelectrode penetrations of muscle cells are made in the cell body, which is located at some distance from the synapses, the depolarizing or hyperpolarizing responses to stimulation of excitatory or inhibitory motorneurons, respectively, can be recorded readily at these recording sites (Walrond et al., 1985).

Within the motornervous system, AChRs might be located at a multitude of sites with functional relevance for locomotory behavior. These include both synaptic (neuron–muscle and neuron–neuron) and extrasynaptic (muscle, motorneuron, and interneuron) locations. Exogenous applications of agonists and antagonists are likely to produce complex effects mediated by receptors located at more than one site and perhaps having different pharmacological characteristics. The picture is further complicated by the fact that there is tonic release of both excitatory and inhibitory neurotransmitters in *Ascaris* (del Castillo et al., 1963; Davis and Stretton, 1989*b*). However, techniques have been developed for recording intracellularly from the commissural processes of single, identified motorneurons (Davis and Stretton, 1989*a*). We can, therefore, make a preparation in which muscle can be affected by controlled changes in input from one or more identified commissural motorneurons; furthermore, muscle and motorneurons can be synaptically isolated and studied individually by removing calcium ions from the bathing medium or by replacing calcium with cobalt.

In the experiments described in this article, we have focused primarily on dorsal motorneurons and on dorsal muscle, since it is innervated almost exclusively by commissural motorneurons (Stretton et al., 1978) that are accessible for electrophysiological experiments (Davis and Stretton, 1989*a*). We report the changes in input resistance induced by a series of cholinergic agents in synaptically isolated DE1, DE2, and DI motorneurons. We also report the changes in membrane potential and contraction that are produced by the same agents in dorsal muscle having intact synaptic inputs from these three classes of motorneurons. Similar experiments were carried out in VI motorneurons and ventral muscle. We show that DE1, DE2, DI, and VI motorneurons have curare-sensitive AChRs. DE1 neurons, but not DE2, DI, and VI neurons, have receptors that are activated by the classical muscarinic antagonist *N*-methyl-scopolamine (NMS); this activation is not blocked by dTC. Finally, we show that the cholinergic pharmacology is mostly nicotinic-like, but that muscle and motorneurons have some unusual responses to muscarinic agents. Our findings provide evidence that these cells are not identical in their cholinergic pharmacology and that the muscle strip preparation, since it contains both muscle and motorneurons, is, therefore, more complex than can be revealed by studies of muscle cell pharmacology alone.

METHODS

Drugs

NMS, atropine, dTC, ACh, propionylcholine bromide (PCh), nicotine, oxotremorine, pilocarpine hydrochloride, hexamethonium bromide, decamethonium bromide, 3-(*N*-morpholino)propanesulfonic acid (MOPS), and neostigmine were obtained from Sigma Chemical Co.

(St. Louis, MO). Dexetimide hydrochloride and levetimide hydrochloride were obtained from Research Biochemicals Inc. (Natick, MA).

Saline Solutions

Normal *Ascaris* saline used in electrophysiological experiments had the following composition (modified from del Castillo and Morales, 1967): 125.1 mM sodium acetate, 3.9 mM sodium chloride, 24.5 mM potassium chloride, 9.8 mM magnesium chloride, 11.8 mM calcium chloride, and 5 mM MOPS buffer, adjusted to pH 6.8 with sodium hydroxide. Calcium-free saline had the same composition except for the omission of calcium chloride. Some experiments were carried out in saline with 40% reduced sodium acetate. Results obtained in reduced sodium acetate saline were indistinguishable from those in normal saline; therefore, they were pooled. In cobalt saline, calcium chloride was replaced by cobalt chloride. Phosphate-buffered saline (PBS) was composed of 150 mM NaCl and 50 mM sodium phosphate, pH 7.4.

Animals

Adult *Ascaris suum* worms were collected two or three times weekly at a local slaughterhouse from the small intestines of newly slaughtered pigs, transported to the laboratory in PBS at 37°C, rinsed, and maintained in PBS at 37°C. Female worms, 28–38 cm in length, were selected for electrophysiological experiments. Almost all experiments were conducted on worms within 2 d of collection.

Electrophysiological Recordings

Glass microelectrodes filled with 3 M KCl were used to impale muscle and nerve cells. The microelectrodes were mounted onto the head stages of model M-707 (World Precision Instruments, Sarasota, FL) or model 5A (Getting Microelectrode Amplifier, Iowa City, IA) preamplifiers which were equipped with current injection bridge circuits. Total current used for stimulation was measured through the preamplifier current monitor circuit, previously calibrated using a virtual ground current monitor and periodically tested for linearity in the range of injected currents used in these experiments. The dissected preparation was pinned to a Sylgard-lined chamber (volume ~1 ml) continuously perfused with saline preheated and maintained at 37°C. The perfusion was gravity-fed, and the flow rate of 2.5–3.0 ml/min was measured and controlled with a valve connected to a flowmeter (No. 11; Gilmont Instruments Inc., Barrington, IL). Test solutions were switched by means of a system of stopcocks connected to up to five test solution reservoirs.

Resting Potential Surveys

A cylindrical section of worm 2.5–3 cm in length, containing the commissures of the DE2-DI pair of motorneurons in the second segment and the first DE1-VI motorneuron pair of the third segment (Stretton et al., 1978), was removed from the intact animal under a dissecting microscope. The section was cut along the left lateral line, the intestine removed with fine forceps, and the body wall pinned flat, cuticle side down, in the Sylgard chamber. The right lateral line was cut both anteriorly and posteriorly such as to leave intact only the selected two pairs of motorneurons, of which three (DE2, DI, and DE1) innervate the dorsal muscle (Fig. 1). The bellies of dorsal muscle cells were impaled with 10–30 MΩ microelectrodes. 10 cells were impaled sequentially for each test saline in groups that included cells from both right and left dorsal quadrants. Resting potentials were determined by disimpalement and averaged. The perfusate was then switched to the next test solution, and, after the preparation had achieved a

steady state of contraction or relaxation, a new group of 10 muscle cells was impaled, each group posterior to the preceding group, so that no cell was impaled more than once.

Tension Measurements

A section of body wall was dissected and pinned as described above. A UC3 force transducer (Statham Instruments, Oxnard, CA) was attached to the posterior end of the dorsal body wall with silk suture thread stitched directly through the end of the muscle strip and then taken over a frictionless pulley to the transducer. The transducer, previously calibrated with analytical weights, was mounted on a micromanipulator and connected to a 100× d.c. amplifier in series

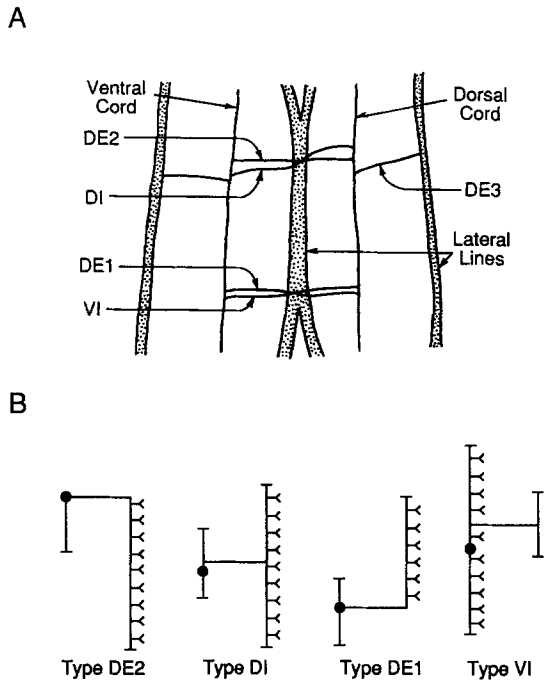

FIGURE 1. (*A*) Diagram of innervated muscle strip preparation in which simultaneous measurements of contractions and depolarizations were made. Intact motorneurons: DE1, DE2 (dorsal excitatory motorneurons), DI, and VI (dorsal and ventral inhibitory motorneurons, respectively); severed motorneuron: DE3 (a dorsal excitatory motorneuron); left lateral line was cut; right lateral line was partially cut, leaving intact the four dorsal and one ventral motorneurons. The dorsal muscle field (muscle bellies omitted from diagram) from which membrane potentials were sampled included those cells lying between the pairs of intact motorneurons and between the right and left lateral lines. Arrows indicate commissural processes of motorneurons. (*B*) Diagram of morphology of the four intact motorneurons. *Filled circles,* cell bodies in ventral nerve cord; *horizontal lines,* commissures; *Y,* neuromuscular output, which is in the dorsal nerve cord for neuron types DE2, DI, and DE1, and in the ventral nerve cord for type VI.

with a chart recorder. Baseline tension was adjusted by raising or lowering the micromanipulator. Test solutions were perfused through the chamber until the tension reached a steady state (usually within 10–15 min), and then the next test solution was applied. Before each dose–response series was begun, the preparation was exposed to test applications of 1 μM ACh with washout in drug-free saline between each exposure. This was done to ensure that the preparation had stabilized and was capable of achieving reproducible contractions. Three applications of ACh were usually sufficient. In agreement with the findings of Harrow and Gration (1985) and Colquhoun et al. (1991), we found no evidence for ACh-induced desensitization. Moreover, the complete reversibility of depolarization and contraction caused

by agonists and agonist–antagonist combinations demonstrated that antagonism was not due to fatigue of the preparation.

Pharmacology of Neuromuscular Junction

A 2.0–2.5-cm section of worm containing the commissures of a single pair of identified motorneurons was dissected in normal *Ascaris* physiological saline as described above. Muscle overlying the commissural processes on the ventral side was removed with fine forceps, and the desired commissure was impaled near the right lateral line with a 60–100-MΩ microelectrode (Davis and Stretton, 1989*a*). A muscle cell in the output zone (Walrond and Stretton, 1985) of the selected motorneuron was impaled with a 10–30-MΩ microelectrode. Although the sites of muscle and motorneuron impalement were somewhat distant from the neuromuscular synapses, which are located at the ends of the muscle arms at points of neuromuscular contact at the nerve cord (cf. Rosenbluth, 1965), excitatory or inhibitory responses could be recorded in muscle by stimulating the presynaptic motorneuron with injected current (Davis and Stretton, 1989*a*). A 100–200-ms square pulse of depolarizing current was passed into the motorneuron every 20–30 s and the response in muscle was recorded. Injected current was adjusted to give a submaximal response. The response of the muscle was recorded over a period of at least 3 min. While continuing the periodic stimulation, the perfusate was switched to drug-containing saline. Either after a change in muscle response to stimulation had reached a steady state, or after 10–30 min had elapsed, the perfusate was changed to the next test solution and the muscle response was allowed to reach a new steady-state level. Varying doses of NMS, dTC, and other cholinergic and noncholinergic agents were tested in this way.

Input Resistance Measurements

A cylindrical section of worm, 2.0–2.5 cm in length, containing the commissures of a selected motorneuron pair (Stretton et al., 1978; Walrond et al., 1985) was prepared for microelectrode penetration of a motorneuron commissure. Because of the difficulty of recording from the DE3 motorneuron class, chiefly because of its very fine commissural process, this cell was not investigated. The identity of the impaled motorneuron was confirmed by stimulating the cell with a 100-ms 0.1–0.3-nA square pulse of intracellular current and recording the appropriate response in a muscle cell in the synaptic output zone of the motorneuron. This verification was done in all cases except for the DE2 type motorneuron, which has a characteristic pattern of spontaneous excitatory postsynaptic potentials (EPSPs) peculiar to its class (Davis and Stretton, 1989*b*). The perfusate was then switched to cobalt-substituted saline, and, after synaptic transmission block was verified, the motorneuron was impaled with a second 60–100-MΩ microelectrode as close as possible to the first site of impalement. The input resistance of the motorneuron was determined by injecting depolarizing and hyperpolarizing square pulses of current (5-s duration, 0.1–2.5 nA) into the motorneuron through one microelectrode and measuring the voltage change at the second microelectrode. Then the preparation was perfused with drug-containing cobalt saline and, after 10–15 min of exposure, a second input resistance was determined. After these measurements, the preparation was returned to cobalt saline or to the next test solution and input resistance was measured again. An experiment was considered successful if drug-induced changes in input resistance were reversed to original or nearly original values by washing in drug-free cobalt saline. Those preparations in which an irreversible decline in input resistance was observed were assumed to be damaged by the electrode impalements and were not used in the interpretation of drug effects or in data summaries except as indicated in the text.

RESULTS

Muscle Pharmacology

Acetylcholine. A typical ACh dose–response curve is shown in Fig. 2, one of seven such experiments. Muscle depolarized and contracted in a dose-dependent fashion in response to ACh in normal saline. No inhibitors of AChE were used. In each of the experiments in which tension measurements are illustrated below, the maximum tension induced by the test agent, and, as a reference, the tension induced by 1 μM

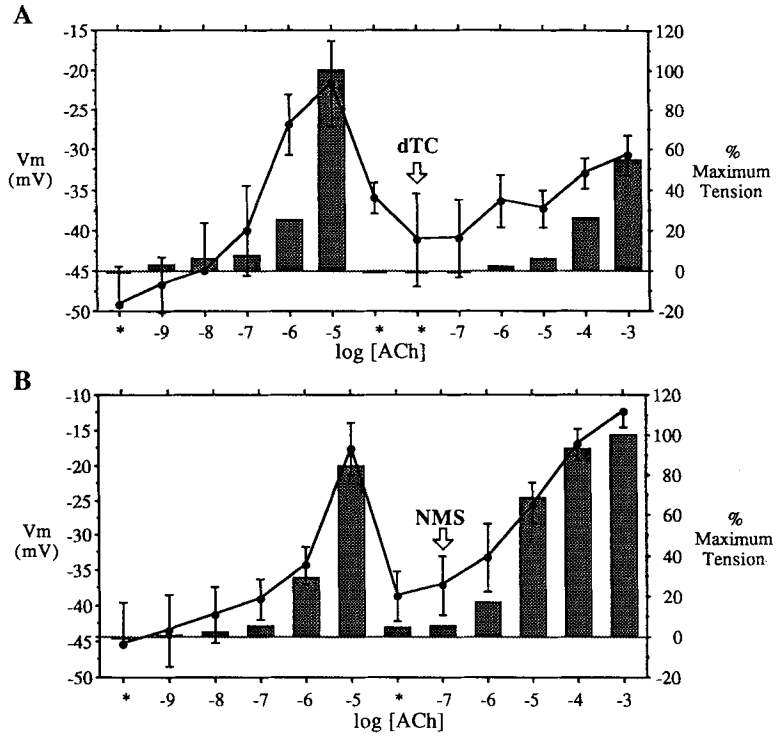

FIGURE 2. (*A*) dTC shift of the ACh dose–response curve in dorsal muscle. Histogram (ordinate label on right), dorsal muscle tension, expressed as percent of maximum tension, in response to increasing doses of ACh; superimposed line graph (ordinate label on left), dorsal muscle resting potential surveys done in the same preparation, with 95% confidence intervals indicated. Each dose was applied in order from left to right without washout between doses. Starred points on the abscissa represent ACh-free saline measurement. At the arrow, 100 μM dTC was applied, and successive doses of ACh contained 100 μM dTC. Maximum tension = 16.2 g; tension at 1 μM ACh = 4.1 g. (*B*) NMS shift of the ACh dose–response curve in dorsal muscle. Histogram (ordinate label on right), dorsal muscle tension in response to increasing doses of ACh; superimposed line graph (ordinate label on left), average dorsal muscle resting potential surveys done in the same preparation, with 95% confidence intervals indicated. Each dose was applied without washout between doses. Starred points on the abscissa represent ACh-free saline measurement. At the arrow, 100 μM NMS was applied, and successive doses of ACh contained 100 μM NMS. Maximum tension = 21.6 g; tension at 1 μM ACh = 6.3 g.

ACh were recorded. No further depolarization occurred at concentrations > 1 mM ACh, although the absolute level of depolarization varied among preparations, as did the initial baseline resting potential. In some preparations, muscle cells were synaptically isolated by perfusing with calcium-free saline until evoked synaptic responses failed to be elicited by stimulating the presynaptic motorneuron with injected current. Muscle also depolarized in a dose-dependent manner in response to ACh applied in calcium-free saline (data not shown), demonstrating that receptors for ACh are present on synaptically isolated muscle cells. Complete reversal of ACh-induced depolarization was not achieved by prolonged washing in either normal or calcium-free saline, although washout reversal was considerably more complete in normal saline.

NMS antagonized contractions induced by 1 μM ACh in a dose-dependent manner (Fig. 3). In this example, one of four such experiments, 100 μM NMS was the most effective concentration tested, blocking > 90% of the ACh-induced contraction, the

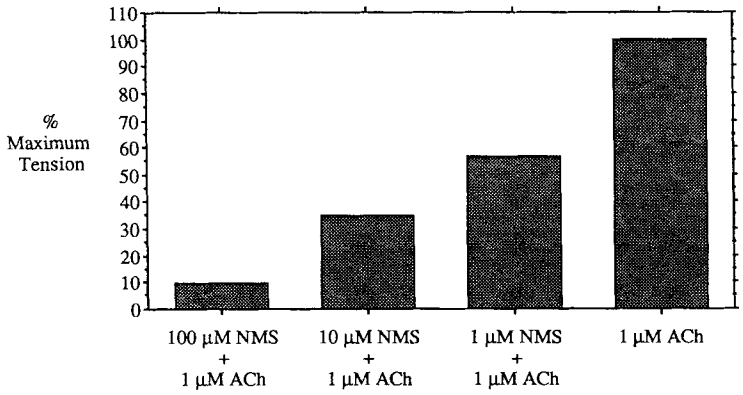

FIGURE 3. Decreasing doses of NMS applied simultaneously with 1 μM ACh. Each bar represents the tension produced by the drug-containing saline. Tension in this experiment at 1 μM ACh = 3.1 g; all other tension measurements expressed as a fraction of this tension.

absolute level of antagonism varying among preparations. A complete block was observed in another experiment (not shown) with 1 mM NMS.

Application of 100 μM NMS alone (the highest dose tested in these experiments) produced a 4–6-mV depolarization in dorsal and ventral muscle cells. NMS-induced depolarization persisted in calcium-free saline (Table I), demonstrating that the NMS-sensitive sites are present on muscle free of tonic inputs. Doses of < 100 μM NMS produced no significant depolarization. The nicotinic antagonist dTC hyperpolarized muscle cells, presumably due to blockage of the effects of ACh released tonically from excitatory motorneurons (del Castillo et al., 1963; Davis and Stretton, 1989*b*). The difference in effect between these two cholinergic antagonists suggests that they act on different targets.

The dose of NMS required to depolarize muscle, 100 μM, is more than five orders of magnitude larger than the binding affinity constant for [^{3}H]NMS measured in homogenates of the related nematode *Caenorhabditis elegans* (Culotti and Klein, 1983;

Segerberg, 1989) and the estimated affinity constant in *Ascaris larvae* (Segerberg, 1989). This discrepancy is so large that it casts doubt on any functional significance of the high-affinity [^{3}H]NMS binding sites in locomotory behavior, although they might, of course, be involved in other behaviors.

100 μM decamethonium was ineffective and 100 μM hexamethonium was partially effective in reversing a 1 μM ACh-induced contraction (data not shown). dTC was the most effective antagonist tested, reducing the sensitivity of ACh-induced depolarizations and contractions by more than two orders of magnitude, as shown in Fig. 2 *A*. In this experiment, the contraction produced by 10 μM ACh was effectively reversed by washing in saline; however, the resting membrane potential did not return to its original level, a frequent observation in these experiments. When the preparation was bathed in 100 μM dTC, the membrane repolarized nearly to baseline values. A second dose–response series was then performed, each dose of ACh containing 100 μM dTC.

TABLE I

Depolarization of Muscle Cells in the Presence of NMS

V_m in saline	V_m in NMS	Depolarization
mV	*mV*	*mV*
−36.3 ± 1.6	−32.7 ± 1.1	3.6
−37.4 ± 0.7	−33.5 ± 0.7	3.9
−41.3 ± 1.2	−35.3 ± 0.8	6.0
−35.3 ± 1.2	−29.2 ± 1.1	6.1
−39.8 ± 1.2	−33.7 ± 1.2	6.1

Depolarization of dorsal muscle cells, in five preparations, induced by 100 μM NMS applied in calcium-free saline. Each value is the average resting potential of 15 cells with indicated SEM. Except for the first figure (3.6 mV depolarization), all are significant within the 95% confidence limits.

NMS, by comparison, was much less effective than dTC in antagonizing ACh-induced contractions and depolarizations, reducing the sensitivity of ACh-induced depolarizations and contractions by less than an order of magnitude (Fig. 2 *B*). In these experiments, 100 μM NMS reduced a contraction produced by 1 μM ACh by $<50\%$.

Nicotine. This agonist also depolarized dorsal muscle in a dose-dependent fashion. Fig. 4 illustrates a dose–response curve taken in which contraction was also measured; it is one of four such experiments showing similar results. Nicotine also depolarized muscle in calcium-free saline, indicating that nicotine receptors are present on muscle cells free of tonic inputs (data not shown). Nicotine contractions and depolarizations were very difficult to reverse by washing in drug-free saline, even after >30 min.

dTC antagonized nicotine-induced depolarizations and contractions, reducing the ACh sensitivity by more than two orders of magnitude, similar to the result seen for ACh (Fig. 4 *A*).

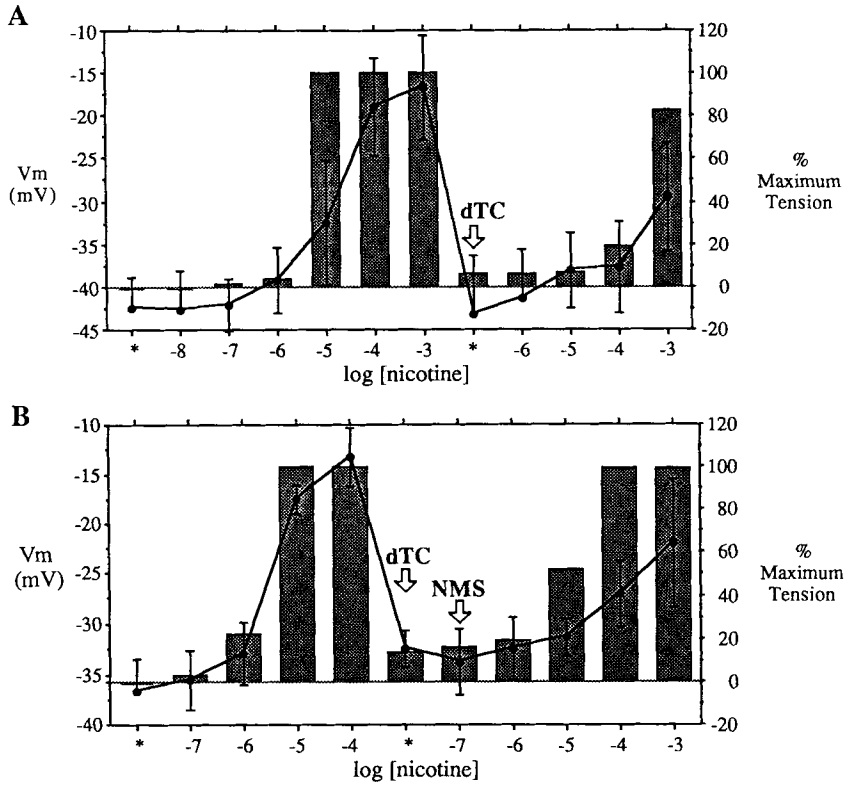

FIGURE 4. (*A*) dTC shift of the nicotine dose–response curve. Histogram (ordinate label on right), dorsal muscle tension expressed as percent of maximum tension; superimposed line graph (ordinate label on left), average dorsal muscle resting potential, with 95% confidence intervals indicated. Starred points on the abscissa indicate measurements taken in nicotine-free saline. At arrow, 100 μM dTC was added, and subsequent drug applications contained 100 μM dTC. Maximum tension = 13.1 g; tension at 1 μM ACh not measured. (*B*) NMS shift of the nicotine dose–response curve. Histogram (ordinate label on right), dorsal muscle tension expressed as percent of maximum tension; superimposed line graph (ordinate label on left), average dorsal muscle resting potential, with 95% confidence intervals indicated. Starred points on the abscissa indicate measurements taken in nicotine-free saline. The depolarization and contraction produced by 100 μM nicotine were reversed by applying 100 μM dTC (first arrow). 100 μM NMS was added (second arrow, no dTC present), and subsequent drug applications contained 100 μM NMS. Maximum tension = 4.2 g; tension at 1 μM ACh = 1.8 g.

NMS was a poor antagonist for nicotine-induced contractions, but was more effective in antagonizing depolarizations (Fig. 4 *B*). 100 μM nicotine induced a depolarization and contraction that was not reversible by washing in drug-free saline (not shown), but an application of 100 μM dTC repolarized and relaxed the preparation to nearly original levels.

Pilocarpine. The muscarinic agonist pilocarpine induced depolarizations and contractions in muscle in a dose-dependent manner in normal saline. Fig. 5 illustrates one of three such experiments in which a wide range of concentrations was

applied. Pilocarpine also depolarized muscle in calcium-free saline (not shown), suggesting that receptors for this drug are present on synaptically isolated muscle cells. There was great variation among preparations in their responses to pilocarpine: some preparations depolarized but only weakly contracted in response to increasing doses of pilocarpine, while for other preparations the reverse was true, suggesting that uncoupling of the depolarization–contraction relationship may have occurred with this agonist. Furthermore, contractions and depolarizations induced by pilocarpine were extremely difficult to reverse, even after prolonged washing in drug-free saline or by salines containing either muscarinic or nicotinic antagonists. Some preparations exhibited a pilocarpine-induced contraction that was enhanced rather than reversed by NMS (not shown). This contraction, uncoupled from a corresponding depolarization, could not be reversed by dTC or by prolonged washing in drug-free saline. Contraction that persisted after the drug was washed out was seen

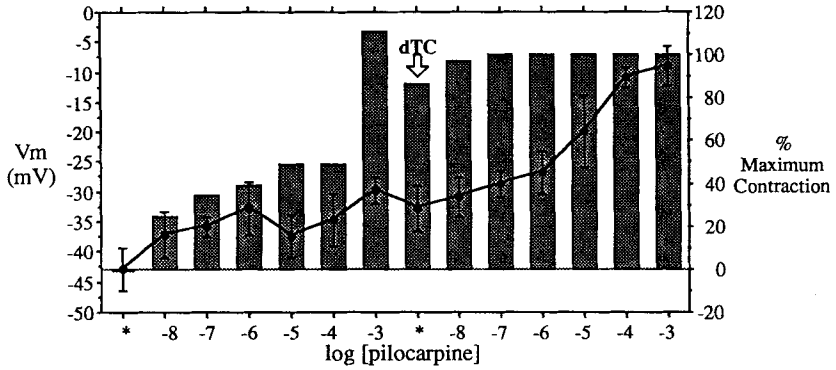

FIGURE 5. Histogram (ordinate label on right), dorsal muscle tension in response to increasing doses of pilocarpine in normal saline; superimposed line graph (ordinate label on left), average muscle cell resting potential measurements made in the same preparation, with 95% confidence intervals indicated. Starred points on the abscissa represent pilocarpine-free saline measurements. At arrow, 100 μM dTC was added and successive doses of pilocarpine contained 100 μM dTC. Maximum tension = 10.6 g; tension at 1 μM ACh = 1.1 g.

on several occasions in experiments with muscarinic agonist–antagonist combinations. These features of the pilocarpine response made actions of this drug difficult to evaluate. A pilocarpine dose–response curve with a second series containing dTC is also shown in Fig. 5. In this preparation, dTC only very slightly reversed the pilocarpine-induced contractions and also appeared, paradoxically, to enhance depolarization over the same range of pilocarpine concentrations that did not significantly depolarize muscle when applied without dTC.

Oxotremorine, another classical muscarinic agonist, also produced contractions in muscle (data not shown). This drug also depolarized muscle in a dose-dependent manner, but appeared to be somewhat less potent than pilocarpine, and its pharmacology was not investigated further.

Antagonism of Neuromuscular Transmission

In this series of experiments, the effects of various drugs on the muscle membrane potential responses induced by intracellular current injection into different classes of motorneurons were tested.

DE1. In Fig. 6, the predrug evoked response, 4.5–7.5 mV, of a dorsal muscle cell to intracellular stimulation of the DE1 motorneuron was reduced to almost zero after application of 100 μM NMS. This change was readily reversed by washing in drug-free saline. A second and third application likewise reversibly reduced muscle response to stimulation, although the drug appeared to be progressively less effective with successive applications. After the final washout of NMS, 100 μM dTC was added to the perfusate. After a brief period of enhanced responsiveness of muscle, the ability of the muscle cell to respond to DE1 stimulation was again reduced to almost zero. Applications of NMS in concentrations < 100 μM failed to antagonize transmission from DE1 to muscle.

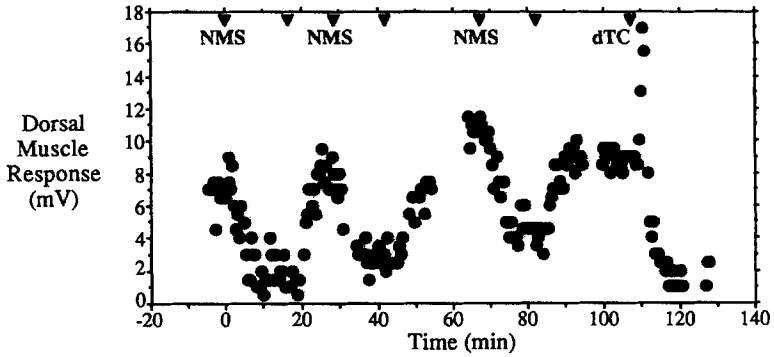

FIGURE 6. Effect of NMS and dTC on neuromuscular transmission of a DE1 motorneuron. Ordinate, depolarizing response (millivolts) of dorsal muscle cell to a 100-ms intracellular depolarizing current injected into DE1 every 30 s (see Methods). Abscissa, time (minutes). At arrowheads drug was added. Unlabeled arrowheads indicate beginnings of washout periods. [NMS] = 100 μM; [dTC] = 100 μM.

The ability of NMS and dTC to block DE1 stimulation of muscle was repeated in 21 preparations. Two preparations appeared to be NMS insensitive, but these were also unresponsive to dTC, although they were unremarkable in other respects.

This experiment was repeated with atropine, the classical muscarinic antagonist. 100 μM atropine reduced the muscle response to DE1 stimulation in a manner similar to NMS (not shown), except for the time of action: atropine was somewhat slower acting and took more than twice as long to reverse by washout than NMS. Although NMS and atropine are very similar in chemical structure, they differ in charge, and this may result in different on and off rates.

True NMS- or atropine-sensitive sites in vertebrate tissues are distinguishable from nonreceptors by their responses to stereoisomeric pairs of benzetimide: receptors are 100-fold more sensitive to (+)-dexetimide than to (−)-levetimide (Beld and Ariens,

1974). However, in *Ascaris,* neither drug, applied in the same concentration as NMS, affected the transmission from DE1 to muscle (not shown).

The NMS block of neuromuscular transmission from a DE1 motorneuron could be overcome by increasing the strength of the current injected into the neuron (Fig. 7). No such increase was seen in similar experiments carried out during dTC antagonism.

DE2. In contrast to DE1, the transmission from DE2 to muscle was not antagonized by NMS. Instead, the muscle cell responsiveness appeared to be enhanced in a manner similar to that seen when dTC was applied to DE1 or DE2 (Fig. 8). In some preparations, the period of enhancement was quite prolonged for both dTC and NMS. NMS failed to antagonize DE2 stimulation of muscle in each of the 24 preparations in which this experiment was performed.

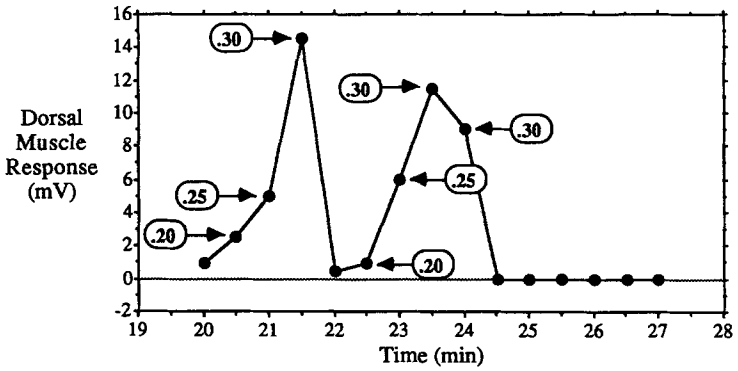

FIGURE 7. Effect of increasing stimulus strength during apparent NMS blockade of DE1-muscle synapse. Ordinate, depolarizing response (millivolts) of dorsal muscle to DE1 stimulation by 100-ms, 0.15-nA depolarizing current pulses injected at 30-s intervals. Abscissa, time (minutes). 100 μM NMS was added at t = 15 min. Muscle depolarization amplitude in response to DE1 stimulation dropped to almost zero at t = 20 min. Circled numbers are current injection values in nanoamperes. Unlabeled points are responses at 0.15 nA. Average muscle response to stimulus before addition of NMS was 13.8 mV (n = 13; range, 10.5–15.0 mV).

DE3. This motorneuron type was similar to DE1 in its NMS sensitivity (Fig. 9): 100 μM NMS reduced the dorsal muscle response to intracellular stimulation of DE3. Maintaining impalement of DE3 over the course of drug addition and washout is difficult because the commissure is small in diameter compared with DE1 and DE2; thus, no data were obtained regarding the effect of dTC on this cell type. Fig. 9 illustrates one of two experiments in which the recording was extensive enough to demonstrate the NMS sensitivity of DE3 output to muscle.

Input Resistance of Synaptically Isolated Motorneurons

The average input resistance of commissural motorneurons taken in cobalt-substituted saline were as follows (R_{in} ± SE; in megaohms): DE1, 9.4 ± 0.3 (n = 31); DE2, 12.4 ± 0.7 (n = 26); DI, 15.3 ± 0.8 (n = 20); VI, 12.1 ± 0.6 (n = 15). Each class of

motorneuron has several members arranged at reproducible positions along the worm, which have been arbitrarily assigned to different segments; each segment includes one each of the DE2, DE3, and DI motorneurons and two pairs of DE1 and VI motorneurons (Stretton et al., 1978). The majority of measurements were taken in the second DE1 and VI pair of the second segment; likewise, experiments on DE2 and DI were done primarily in the second segment. No discernable differences were observed among cells of different segmental position within a class, e.g., the first DE1 vs. the second DE1 in the second segment. Therefore, the measurements for each class were pooled.

These input resistance values are only slightly different from those reported by Davis and Stretton (1989*a*), for motorneurons in normal calcium-containing saline. DE1 appeared to have a somewhat higher (9 vs. 6 MΩ), and VI a somewhat lower (12 vs. 20 MΩ), input resistance in cobalt saline than in normal saline. However, these

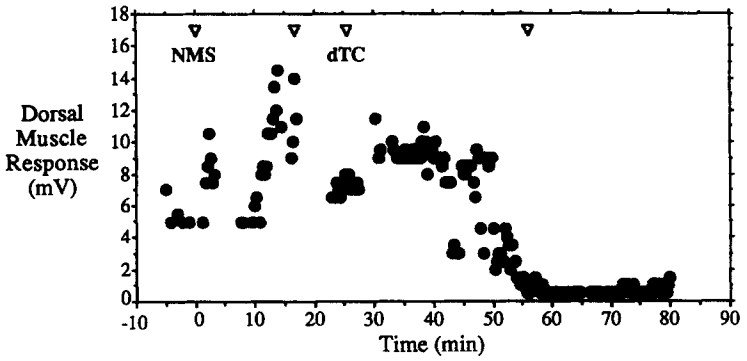

FIGURE 8. Effect of NMS and dTC on neuromuscular transmission of a DE2 motorneuron. Ordinate, depolarizing response (millivolts) of dorsal muscle cell to a 100-ms intracellular depolarizing current injected into DE2 every 20 s (see Methods). Abscissa, time (minutes). At arrowheads drug was added. Unlabeled arrowheads indicate beginnings of washout periods. Approximately 5 min after NMS was applied, the motorneuron became disimpaled and was then quickly reimpaled. Impalement was again lost and regained during NMS washout and dTC addition. [NMS] = 100 μM; [dTC] = 100 μM.

differences may not be significant since the number of observations taken in calcium-containing saline was relatively small. On the other hand, one would expect that input resistance would be different in cobalt- and in calcium-containing saline because tonic release of neurotransmitter, including ACh, was blocked by cobalt. Since ACh lowers the input resistance of all commissural motorneurons tested (see below), and since there is evidence that VI motorneurons receive tonic cholinergic input from dorsal excitatory motorneurons, and that DE1 motorneurons receive tonic cholinergic input from a large interneuron (Davis and Stretton, 1989*b*), an increase in input resistance of these motorneurons would be expected in cobalt-substituted saline; however, the expected effect was seen only in DE1. It is possible that input resistance differences would be small, however, and might only be detectable within 95% confidence limits in a very large sample size.

Pharmacology of Motorneurons

Acetylcholine. Acetylcholine reversibly reduced the input resistance of all motorneurons tested. Examples of these experiments are shown in Fig. 10. (Table II summarizes input resistance changes induced by cholinergic agents in all the motorneurons examined.) The reduction appeared to be dose dependent (see Table II, DE2 and DI), although no attempt was made to investigate the ACh dose dependence systematically. The AChE inhibitor neostigmine was required to observe input resistance changes in these motorneurons. In experiments in which neostigmine was omitted, no changes in input resistance were observed to occur when ACh was applied in doses ranging from 10 to 100 μM.

The inhibitory motorneurons DI and VI were ACh sensitive, although to a slightly lesser extent than the excitors DE1 and DE2. All ACh-induced resistance changes in inhibitors were reversible by washing in cobalt saline and were antagonized by 100 μM dTC, which, as in the excitors examined, was most effective when applied before exposure to ACh (not shown).

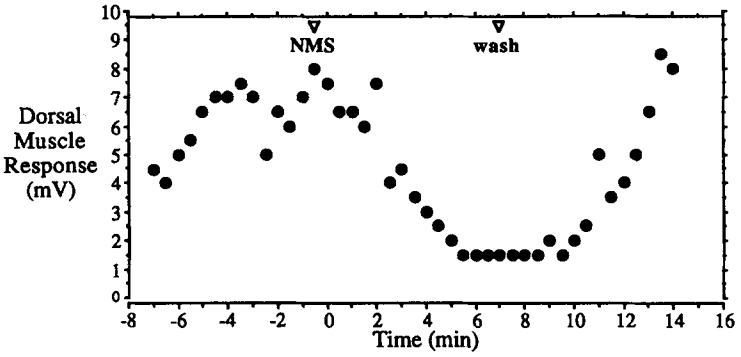

FIGURE 9. Effect of NMS on neuromuscular transmission of a DE3 motorneuron. Ordinate, depolarizing response (millivolts) of dorsal muscle cell to a 100-ms intracellular depolarizing current injected into DE3 every 30 s (see Methods). Abscissa, time (minutes). At arrowheads, 100 μM NMS was added or washout begun, as indicated.

dTC antagonized approximately half of the ACh-induced drop in input resistance in all motorneurons tested when applied after ACh. The effectiveness of dTC was almost doubled when the antagonist was applied before the agonist (data not shown). However, the reversal of ACh-induced resistance reductions by dTC was not complete even when dTC was applied before ACh.

N-methyl-scopolamine. NMS, a muscarinic antagonist in other systems, had a surprising effect in *Ascaris:* it lowered the input resistance of one class of motorneuron, DE1, but not of DE2, DI, or VI motorneurons (Fig. 11). The resistance drop was rapid, occurring within 2–3 min after drug exposure in most preparations, and was readily reversed by washing in drug-free saline. The NMS-induced reduction in the input resistance of DE1 was reproducible among different preparations (see Table II), and was not antagonized by 100 μM dTC. The concentration of NMS used in these experiments was 100 μM, the dose at which blockade of transmission from the

DE1-to-muscle synapse has been observed. In one experiment in which three NMS concentrations (1, 10, and 100 μM) were applied to DE1, the input resistance drop was observed only at the highest dose.

Atropine (100 μM) also reduced the input resistance of DE1 (not shown). The effect of atropine was not easily reversed by washing in drug-free saline, however, even after prolonged periods.

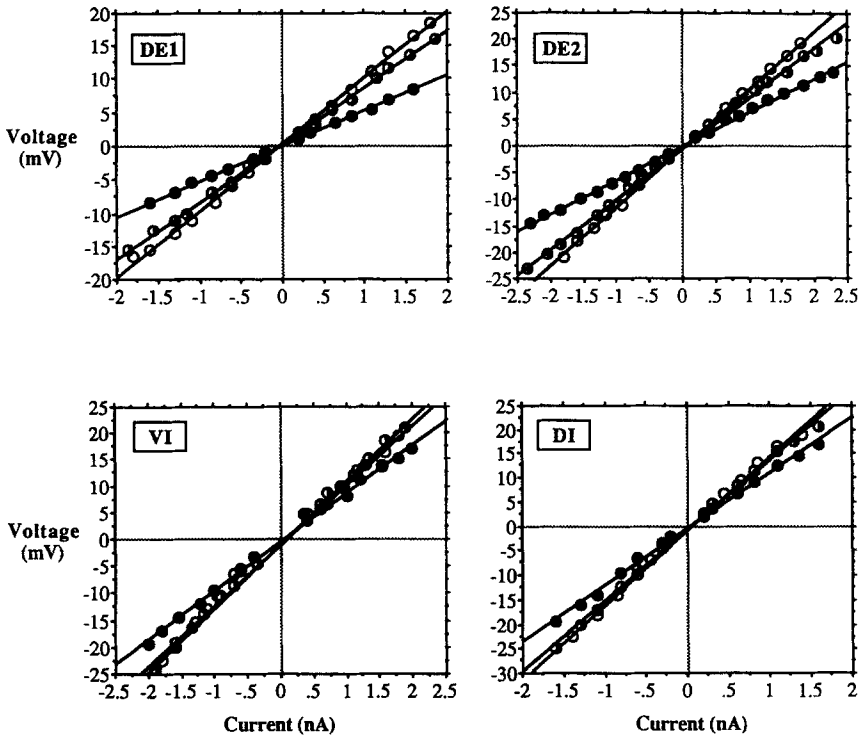

FIGURE 10. Examples of current–voltage plots for four commissural motorneurons in cobalt saline (*open circles*), 20 μM ACh + 20 μM neostigmine (*filled circles*), and after washout in drug-free cobalt saline (*half-filled circles*); clockwise from upper left: DE1, DE2, DI, and VI. Input resistance measurements obtained from the slopes of these plots (in megaohms) for each experiment are as follows: DE1, cobalt saline, 10.0, *open circles;* ACh + neostigmine, 5.3, *filled circles;* cobalt wash, 8.5, *half-filled circles;* DE2, cobalt saline, 11.1, *open circles;* ACh + neostigmine, 6.4, *filled circles;* cobalt wash, 9.5, *half-filled circles;* VI, cobalt saline, 11.2, *open circles;* ACh + neostigmine, 9.1, *filled circles;* cobalt wash, 11.8, *half-filled circles;* DI, cobalt saline, 15.3, *open circles;* ACh + neostigmine, 11.5, *filled circles;* cobalt wash, 14.5, *half-filled circles.*

Pilocarpine. The muscarinic agonist pilocarpine (100 μM) had no effect on DE1 or VI, but it lowered the input resistance of DE2 and, to a lesser degree, DI (Fig. 12). Pilocarpine-induced changes in input resistance did not wash out easily in any experiment in either DE2 or DI. Antagonism of pilocarpine in these cells by either dTC or NMS is less clear: dTC appeared to be more effective than NMS in DE2, while neither antagonist reliably blocked pilocarpine in DI and, in three of six

experiments, both dTC and NMS enhanced the drop in input resistance. The number of such experiments is small, however, and more are needed to further characterize pilocarpine as an agonist.

Propionylcholine. Another choline ester, PCh, was also quite effective in lowering the input resistance of DE1, DE2, and VI in a small number of experiments. Of five experiments, two with DE1, two with VI, and one with DE2, all demonstrated significant, easily reversible reductions of input resistance upon application of PCh

TABLE II

Effects of Cholinergic Agents on the Input Resistance of Synaptically Isolated Motorneurons

Cell	[Drug]	*N*	$\overline{\%\Delta R_{in}}$	Range
DE1	100 μM NMS	9	−26	−14.3 – −41.5
	100 μM atropine	3	−26	−23.0 – −28.4
	100 μM dTC	5	+3	0 – +9.3
	20 μM ACh/neo	2	−34	−21.9 – −47.0
	100 μM pilo	4	0	0 – −0.3
	30 μM PCh	1	−14	—
	30 μM PCh/neo	1	−55	—
DE2	100 μM NMS	5	0	0 – −0.6
	100 μM dTC	6	+8	0 – +17.3
	10 μM ACh/neo	4	−21	−6.4 – −43.9
	20 μM ACh/neo	3	−38	−16.1 – −53.7
	100 μM pilo	3	−28	−25.7 – −32.4
	40 μM PCh	1	−15	—
DI	100 μM NMS	5	0	0 – −0.4
	100 μM dTC	4	0	0 – −0.3
	10 μM ACh/neo	3	−10	0 – −24.8
	20 μM ACh/neo	1	−14	—
	100 μM pilo	7	−8	0 – −11.2
VI	100 μM NMS	6	0	0 – −1.0
	100 μM dTC	3	0	0 – −0.4
	20 μM ACh/neo	1	−23	—
	100 μM pilo	3	0	0 – −0.2
	20 μM PCh/neo	1	−21	—
	30 μM PCh/neo	1	−25	—

Summary of drug-induced input resistance changes, expressed as averages, in *Ascaris* motorneurons. Pilo, pilocarpine; neo, neostigmine. Neostigmine concentration was always 20 μM. $\%\Delta R_{in}$, average percent change in input resistance.

(Table II). In addition, the PCh response was antagonized by 100 μM dTC. DI has not yet been examined. Since the sample size was small, and antagonism by NMS was not investigated, more experiments are needed to describe the effects of PCh and its antagonists more completely.

Neostigmine was not required to produce measurable reductions in input resistance by PCh in DE1 and DE2 (not done for VI); however, when neostigmine was added to PCh-containing saline, the input resistance change was enhanced signifi-

cantly. This response contrasted with that of ACh, which required AChE inhibitor in order to observe significant ACh-induced reduction of input resistance in all motorneuron classes tested.

DISCUSSION

Muscle Pharmacology

Cholinergic agonists. The cholinergic agonists ACh, nicotine, and pilocarpine depolarized and contracted dorsal muscle at low doses and in a dose-dependent manner.

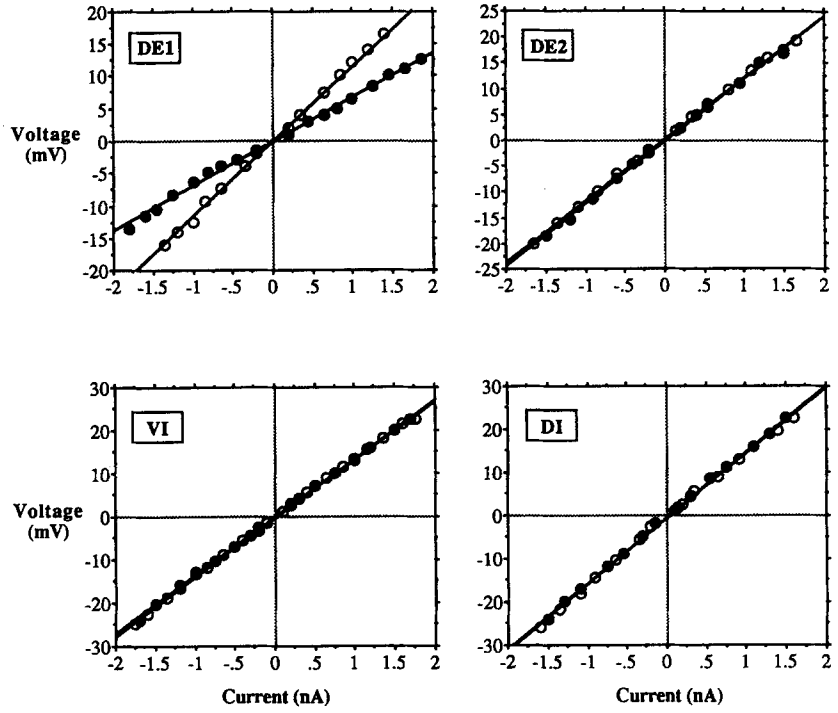

FIGURE 11. Current–voltage plots of four commissural motorneurons in cobalt saline (*open circles*) and in 100 μM NMS (*filled circles*): DE1 (*upper left panel*), DE2 (*upper right panel*), DI (*lower right panel*), and VI (*lower left panel*). Input resistance measurements obtained from the slopes of these plots (in megaohms) for each experiment are as follows: DE1, cobalt saline, 11.8, *open circles;* NMS, 6.9, *filled circles;* DE2, cobalt saline, 12.0, *open circles;* NMS, 12.1, *filled circles;* VI, cobalt saline, 13.7, *open circles;* NMS, 13.5, *filled circles;* DI, cobalt saline, 15.2, *open circles;* NMS, 15.3, *filled circles.*

The depolarizing action persisted when the cells were synaptically isolated by perfusion in calcium-free salines, indicating that receptors for these agents are present on muscle cells. Pennington and Martin (1990) reached a similar conclusion by studying the ACh response in single channel recordings from muscle vesicles.

Although the absolute levels of depolarization and contraction induced by ACh and nicotine differed among preparations, these agents produced typical sigmoidal dose–response curves and the examples used to illustrate these curves do not differ significantly from averaged values taken for each drug. The actions of pilocarpine were considerably more complicated, unpredictable, and variable: irreversibility, uncoupling of contraction and depolarization, nonsigmoidal dose–response curves, contraction during drug washout, and antagonist-induced contraction were all

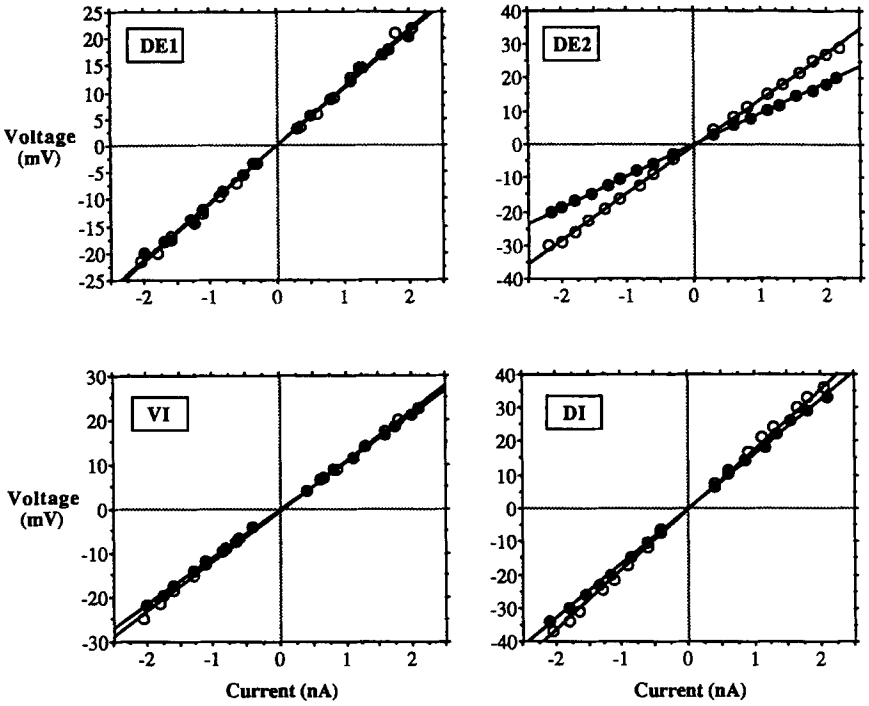

FIGURE 12. Current–voltage plots of four commissural motorneurons in cobalt saline (*open circles*), and in 100 μM pilocarpine (*filled circles*): DE1 (*upper left panel*), DE2 (*upper right panel*), DI (*lower right panel*), and VI (*lower left panel*). Input resistance measurements obtained from the slopes of these plots (in megaohms) for each experiment are as follows: DE1, cobalt saline, 10.9, *open circles;* pilocarpine, 10.6, *filled circles;* DE2, cobalt saline, 13.9, *open circles;* pilocarpine, 9.4, *filled circles;* VI, cobalt saline, 11.3, *open circles;* pilocarpine, 10.8, *filled circles;* DI, cobalt saline, 18.5, *open circles;* pilocarpine, 16.5, *filled circles.*

variously observed. This diversity of response could be a product of many factors, such as genetic variation, prior exposure of the worm to anthelmintic agents, slight differences in dissection procedures, or other unknown conditions. The physiological preparation used in these experiments is a complex one, with dorsal muscle cells receiving tonic synaptic input from three intact dorsal motorneurons, DE1, DE2, and DI. However, in the less complex preparation in which synaptically isolated motorneurons were tested for their responsiveness to cholinergic agonists, including

pilocarpine, we found that both DE2 and DI possess pilocarpine receptors. These receptors were not identical in pharmacology, and this difference may account for at least some of the variation in responsiveness seen in the innervated muscle strip preparation. Because of the wide variation in sensitivity to pilocarpine among preparations, it was difficult to rank these drugs in order of potency. In addition, since both nicotine and pilocarpine had very long washout times and were sometimes apparently irreversible, it was never possible to apply both drugs to the same preparation for comparison with regard to contraction. Furthermore, in some preparations, doses of pilocarpine as low as 10 nM were capable of causing muscle to contract and/or depolarize (Fig. 5). It was generally true, however, that nicotine-induced contractions were faster, stronger, more consistent, and more reproducible than contractions produced by pilocarpine. The long washout time required for nicotine may be a consequence of its lipid solubility. Pilocarpine, on the other hand, is a more polar molecule and may wash out more rapidly than nicotine. The observation that reversal is slow may indicate that something more complex may operate during pilocarpine agonism, such as the triggering of a second messenger cascade. For these reasons, the following order of potency is a tentative one: ACh > nicotine > pilocarpine > oxotremorine.

The apparent uncoupling of the depolarization–contraction link occurred most noticeably in the responses to pilocarpine, but also to some extent in response to nicotine, when various antagonists were present. These experiments do not shed any light on the possible mechanisms underlying the control of muscle tension. However, they suggest that muscle tension is controlled not only by muscle membrane potential but also by chemical signaling mechanisms not directly linked to the membrane potential (cf. Adams and O'Shea, 1983).

Cholinergic antagonists applied alone. del Castillo et al. (1963) first reported that bath-applied dTC hyperpolarized muscle, leading to the postulation that cholinergic excitation by motorneurons is tonic, and that muscle hyperpolarization arises from postsynaptic receptor blockade by dTC. This argument was strengthened by the finding of Davis and Stretton (1989*b*) that direct hyperpolarization of excitatory motorneurons by injected current caused a corresponding hyperpolarization in muscle resting membrane potential and that direct hyperpolarization of inhibitors produced depolarization in muscle. We have confirmed that dTC hyperpolarizes muscle (data not shown).

NMS, on the other hand, was found to depolarize muscle by 4–6 mV. Several possible explanations may account for this observation: (*a*) NMS directly or indirectly causes inhibitors to hyperpolarize, thereby reducing tonic inhibitory input to muscle; (*b*) NMS blocks presynaptic negative feedback receptors for tonically released ACh onto muscle, leading to increased ACh release; (*c*) NMS directly opens or closes ion channels on muscle; (*d*) NMS alters the action of a pump responsible for maintaining the electrochemical gradient of muscle cells (Brading and Caldwell, 1971; Caldwell, 1973); (*e*) NMS acts in a nonspecific manner on muscle and/or nerve cells. Although NMS may act at a multiplicity of sites controlling muscle membrane potential in this preparation, the observation that NMS depolarizes synaptically isolated muscle cells suggests that muscle cells carry NMS-sensitive sites and rules out the first two possibilities as the sole explanation for NMS-induced depolarization in muscle. The

question of whether NMS acts on ion channels or on membrane pump(s) remains unresolved by these data.

Cholinergic agonists vs. antagonists. Table III summarizes data obtained in resting membrane potential surveys and tension recordings in dorsal muscle.

Since dTC, NMS, and hexamethonium had similar effects on both ACh and nicotine action, it can be tentatively concluded that ACh and nicotine act at the same receptors. Pilocarpine, although capable of depolarizing and contracting *Ascaris* muscle at low doses, was not reliably antagonized by either dTC or NMS (the antagonist commonly effective against this drug). In some cases dTC was weakly effective in reversing pilocarpine-induced contractions, but this observation was not consistent. It is possible that pilocarpine acts on a receptor that is activated in vivo by a ligand other than ACh.

Neuromuscular junction. The action of NMS and dTC at the neuromuscular junction is summarized in Table IV. DE1 and DE2 outputs to dorsal muscle from motorneurons were dTC sensitive. However, NMS antagonized DE1–muscle and DE3–muscle transmission, leaving DE2–muscle interactions intact. Enhancement of

TABLE III

Interaction between Agonists and Antagonists on Ascaris

Agonists	Antagonists			
	dTC	NMS	Hexa	Deca
ACh	Yes	Partial	Partial	No
Nicotine	Yes	Partial	Partial	Yes
Pilocarpine	No	No	ND	ND

Summary of resting potential survey and tension recording data. *Yes,* antagonist is effective in blocking agonist-induced depolarizations and contractions; *no,* antagonist is ineffective; *partial,* antagonist is only weakly effective; *ND,* not done. All antagonist concentrations were 100 μM. Hexa, hexamethonium; deca, decamethonium.

muscle responsiveness preceding blockade was often observed with both dTC and NMS. Such effects can often be explained by the presence of transmitter autoreceptors that diminish release from the presynaptic cell (cf. Fosbraey and Johnson, 1980). Since *Ascaris* motorneurons show tonic release of transmitter (Davis and Stretton, 1989*b*), blockade of such receptors would augment release.

The observation that NMS specifically affected neuromuscular transmission of certain classes of *Ascaris* excitatory motorneurons suggests that its effect is presynaptic. There are no discernable differences in synaptic morphology among these motorneuron types (Donmoyer, J. E., P. A. Desnoyers, and A. O. W. Stretton, unpublished observations); furthermore, contacts between all three types of motorneurons and muscle are often made within a few micrometers, making it unlikely that there could be effective separation of different subtypes of postsynaptic receptors between the synapses of each motorneuron (Walrond, J. P., J. E. Donmoyer, and A. O. W. Stretton, unpublished observations). Furthermore, the finding that NMS specifically alters the input resistance of the DE1 motorneuron, but not that of DE2, DI, or VI, directly implicates the motorneuron rather than the muscle as the site of

action of NMS. Thus, there seem to be two distinct responses to NMS, one producing an enhancement and the other a diminution of neuromuscular transmission. Furthermore, these NMS-sensitive sites are differently distributed among the motorneuronal types.

Motorneuron Pharmacology

Table V summarizes the drug responses observed in *Ascaris* in four classes of synaptically isolated motorneurons. ACh receptors were detected on all motorneuron classes examined. The effect of ACh was to lower input resistance. ACh receptors on motorneurons were dTC sensitive. However, dTC was more effective when applied before ACh, perhaps indicating a difference in speed of penetration to sensitive sites, or in association kinetics between agonist and antagonist, or suggesting drug-induced conformation changes in the receptor molecule. The actions of cholinergic antagonists were complex. dTC applied alone increased the input resistance of the excitors DE1 and DE2 slightly but significantly, but had no effect on the inhibitors DI and VI. This was surprising since input resistance measurements were carried out in cobalt-

TABLE IV

Action of NMS and dTC on Neuromuscular Transmission of Different Types of Motorneurons

Drug	Motorneuron				
	DE1	DE2	DE3	DI	VI
NMS	+	−	+	−	−
dTC	+	+	ND	−	−

Summary of neuromuscular junction antagonism experiments. +, Drug apparently blocked evoked muscle response to motorneuron stimulation; −, drug was ineffective; ND, experiment was not done.

substituted saline in order to block calcium-dependent tonic neurotransmitter release. It is possible (*a*) that there exists in *Ascaris* non–calcium-dependent, cobalt-insensitive release or leakage of neurotransmitter or neuromodulator (cf. Schwartz, 1987) capable of lowering the input resistance of excitors at dTC-sensitive sites; or (*b*), that dTC can directly or indirectly close ion channels present on DE1 and DE2 membranes. In either case, the question could be resolved by voltage- or patch-clamp experiments.

NMS reversibly lowered the input resistance of DE1 but not DE2, DI, or VI. This result is consistent with the effects on neuromuscular response described above: NMS antagonized DE1 and DE3 neuromuscular transmission but had no effect on the transmission from other commissural motorneurons. From this, we surmise that DE3 also possesses NMS-sensitive sites. Furthermore, it is probable that the blockage of neuromuscular transmission of the DE1 and DE3 neurons was not true pharmacological antagonism at the neuromuscular synapses, but rather the result of conduction failure in the motorneuron brought about by current leakage through channels opened by NMS. This interpretation is reinforced by experiments showing that

increasing the intracellularly injected stimulating current reversed the NMS effect on neuromuscular transmission. The paradoxical agonist-like effect of NMS is rare, but not unprecedented: in N1E-115 mouse neuroblastoma cells, Hedlund and coworkers (Hedlund, Arhem, Lorentz, and Sydbom, 1985) have described a tetrodotoxin-insensitive sodium conductance that is opened by muscarinic antagonists, leading to a reduction in input resistance in these cells.

Since the concentration of NMS required to produce such an effect is several orders of magnitude higher than the apparent affinity constant of [^{3}H]NMS for membrane-bound receptors in *Ascaris* and *Caenorhabditis elegans* homogenates, it is likely that these low-affinity NMS sites are not the same as those measured in the binding studies (Culotti and Klein, 1983; Segerberg, 1989). In addition, the low-affinity NMS-sensitive channels, at which NMS acts to reduce membrane input resistance in a manner similar to that of an agonist, appear to be dTC insensitive, unlike the ACh-activated sites. Although it was hard to prove that dTC completely blocked the effect of ACh (because of progressive deterioration of the responsiveness

TABLE V

Changes in Input Resistance of Motorneurons in Response to Drugs

Drug	Motorneuron			
	DE1	DE2	DI	VI
Acetylcholine	+	+	+	+
d-Tubocurarine	+	+	–	–
N-Methylscopolamine	+	–	–	–
Pilocarpine	–	+	+	–
Propionylcholine	+	+	ND	+

Summary of drug responses observed in commissural motorneurons of *Ascaris* based on changes in input resistance in response to the indicated agent. + means change in R_{in} was observed; – means no R_{in} change was observed; ND means experiment was not done.

of the preparation), no more than 10% of the input resistance change could be a dTC-resistant ACh response. Since NMS produces a change of >20% in input resistance in the presence of dTC, we conclude that the NMS response might include the activation of channels that may not be sensitive to ACh. Future studies that examine motorneuron pharmacology in more detail may reveal more effective cholinergic antagonists for ACh which also block this agonist-like action of NMS.

In contrast to the actions of NMS, pilocarpine lowered the input resistance of DE2 and DI, but not DE1 or VI. The receptors for pilocarpine were not identical in DE2 and DI, since dTC was an effective pilocarpine antagonist in DE2, and was even more effective than NMS, its conventional antagonist in other systems; however, in DI, both dTC and NMS enhanced the effect of pilocarpine in reducing input resistance. The complex actions of pilocarpine paired with cholinergic antagonists in DE2 and DI may explain the unusual effects these drugs had on dorsal muscle.

To further characterize these drug actions it would be useful to know how changes in input resistance correlate with changes in membrane potential in motorneurons.

This information is important in interpreting the combined effects of drugs suspected of stimulating multiple receptor subtypes in a preparation having tonic release of neurotransmitters. However, accurate determination of resting potential requires that the measurements be taken by disimpalement, an exceedingly difficult addition to the procedures described here. More direct determination of ion channel properties and sites of action will require techniques such as voltage-clamp and patch-clamp analysis.

Propionylcholine, an ester of choline related to ACh, was also capable of reducing input resistance in all motorneurons examined (DE1, DE2, and VI). PCh induced these changes without requiring neostigmine, although its effects were greatly enhanced in the presence of neostigmine. In *Caenorhabditis elegans*, kinetically different subforms of AChE can hydrolyze PCh (Johnson and Russell, 1983). It is not yet known if nematode choline acetyltransferase (ChAT) produces PCh in vivo, but there is evidence that homogenates of *C. elegans* make higher orders of choline esters such as butyryl- and valyrylcholine (Rand, J., personal communication). If *Ascaris* ChAT also synthesizes PCh, it is evident from the data presented here that there are receptors that can recognize this compound. Furthermore, if AChE hydrolyzes different choline esters at different rates, with differential localization of kinetically different forms of AChE (cf. Johnson and Stretton, 1980), the functional consequences of such an arrangement are complex.

The finding of NMS-sensitive sites in DE1, and pilocarpine-sensitive sites in DE2 and DI, provides a view of a more intricate system than had been anticipated. The functional significance of these sites is not clear. The ion channel opened by NMS in DE1 and by pilocarpine in DI may not be linked to cholinergic receptors, but may be activated by other endogenous neurotransmitters or neuromodulators.

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