Modulation of Radula Opener Muscles in *Aplysia*

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[1]*Department of Physiology and Biophysics and* [2]*The Fishberg Center for Research in Neurobiology, The Mt. Sinai Medical Center, New York City 10029; and* [3]*Center for Neurobiology and Behavior, Columbia University, New York City, New York 10032*

Evans, Colin G., Ferdinand S. Vilim, Orna Harnish, Irving Kupfermann, Klaudiusz R. Weiss, and Elizabeth C. Cropper. Modulation of radula opener muscles in *Aplysia*. *J. Neurophysiol.* 82: 1339–1351, 1999. We observed fibers immunoreactive (IR) to serotonin (5-HT), the myomodulins (MMs), and FMRFamide on the I7-I10 complex in the marine mollusk *Aplysia californica*. The I7–I10 muscle complex, which produces radula opening, is innervated primarily by one motor neuron, B48. B48 is MM-IR and synthesizes authentic MM_A. When B48 is stimulated in a physiological manner, cAMP levels are increased in opener muscles. cAMP increases also are seen when the MMs are applied to opener muscles but are not seen with application of the B48 primary neurotransmitter acetylcholine (ACh). Possible physiological sources of 5-HT and FMRFamide are discussed. When modulators are applied to resting opener muscles, changes in membrane potential are observed. Specifically, 5-HT, MM_B, and low concentrations of MM_A all depolarize muscle fibers. This depolarization is generally not sufficient to elicit myogenic activity in the absence of neural activity under "rest" conditions. However, if opener muscles are stretched beyond rest length, stretch- and modulator-induced depolarizations can summate and elicit contractions. This only occurs, however, if "depolarizing" modulators are applied alone. Thus other modulators (i.e., FMRFamide and high concentrations of MM_A) hyperpolarize opener muscle fibers and can prevent depolarizing modulators from eliciting myogenic activity. All modulators tested affected parameters of motor neuron-elicited contractions of opener muscles. MM_B and 5-HT increased contraction size over the range of concentrations tested, whereas MM_A potentiated contractions when it was applied at lower concentrations but decreased contraction size at higher concentrations. FMRFamide decreased contraction size at all concentrations and did not affect relaxation rate. Additionally, the MMs and 5-HT increased muscle relaxation rate, decreased contraction latency, and decreased the rate at which tension was developed during motor neuron-elicited muscle contractions. Thus these modulators dramatically affect the ability of opener muscles to follow activity in the opener motor neuron B48. The possible physiological significance of these findings is discussed.

INTRODUCTION

Many investigators that have sought to characterize the neural mechanisms important for plasticity in rhythmic behaviors have studied the neural circuits that generate these behaviors. Changes in the firing patterns of the circuits that mediate behavior are presumed to be indicative of changes in the behavior itself. Although this assumption is likely to be valid under some circumstances, it may not be valid under others. For example, it is not likely to be valid when muscle response dynamics are slow, i.e., when muscle tension cannot accurately follow changes in neural activity (Hooper et al. 1999; Morris and Hooper 1998). Systems with slow response dynamics that have been extensively investigated include the accessory radula closer (ARC) neuromuscular system in the marine mollusk *Aplysia californica* (e.g., Brezina et al. 1997) and stomatogastric muscles of the lobster *Panulirus interruptus* (e.g., Morris and Hooper 1997, 1998).

It has been shown that the relationship between motor neuron activity and the magnitude of the resulting muscle contraction can be quite complex even under steady-state conditions. For example, the peak or mean contraction amplitude may not be solely determined by the mean firing frequency of the motor neuron in that the particular firing pattern of the motor neuron also may be important (e.g., Brezina et al. 1997; Morris and Hooper 1997). Pattern dependence can be predicted from quantitative modeling (Brezina et al. 1997; Morris and Hooper 1997) but it is not always intuitively obvious. The term neuromuscular transform (NMT) has been introduced to refer to the complex nonlinear filter through which motor commands must pass before they are translated into muscle contractions (Brezina et al. 1999). Physiologically, the NMT comprises multiple steps including presynaptic Ca^{+2} elevation, neurotransmitter release, postsynaptic Ca^{+2} elevation, and activation of the contractile machinery. Thus the muscle contractions that will result from a particular pattern of neuronal activity often cannot be predicted unless the relevant NMT is understood.

To further complicate matters, it has become apparent that the NMT does not have to be a fixed filter that always operates in the same manner (Brezina et al. 1999). Instead it can be dynamic and can be modified. This plasticity is likely to be important because models have suggested that when the NMT is fixed, a system may not be able to generate behaviors with certain parameters (Brezina et al. 1999). When the NMT is "tuned," however, these behaviors become possible. To fully appreciate how neuronal activity is translated into a functional movement, therefore it has become apparent that it may be important to describe how the NMT can be altered. Modulatory neurotransmitters clearly can be important in this context. These modulators can be *intrinsic*, i.e., present as cotransmitters in the behavior-generating motor neurons themselves, and/or *extrinsic*, i.e., released as hormones or present in specialized modulatory neurons (Cropper et al. 1987a). Effects of modulatory neurotransmitters on neuromuscular function have been investigated in a number of preparations (Calabrese 1989) including the ARC neuromuscular system. Experiments in the ARC system have concentrated on effects of modulatory neu-

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rotransmitters that are the most striking in this preparation, i.e., effects of modulators on contraction amplitude and muscle relaxation rate. Work in the ARC neuromuscular system therefore has provided insights into how effects of modulators on certain aspects of the NMT are likely to be important for behavioral plasticity.

In the research described in this paper, we examined effects of modulatory neurotransmitters on the muscles that are antagonistic to the ARC muscles (the I7–I10 (radula opener) muscles in *Aplysia* (Evans et al. 1996). Modulators that are present in the I7–I10 complex were identified and their effects studied in whole muscle preparations. In a previous paper (Scott et al. 1997a), experiments were conducted on dissociated opener muscle fibers and the specific ion currents that were modulated were identified. Because I7–I10 muscles display different contraction characteristics than the ARC muscle, the studies described in this paper are designed to characterize additional aspects of modulation that will be incorporated into models of the experimentally advantageous radula closer-opener complex.

An abstract of this work has appeared (Evans et al. 1993).

METHODS

Animals

Aplysia californica (200–400 g) were maintained at 14–16°C in 150-gallon holding tanks containing aerated, artificial sea water (ASW). In all experiments animals were anaesthetized with isotonic magnesium chloride (50% wt/vol).

Methods used in physiological experiments

Physiological experiments were conducted in reduced preparations that have been described in detail (Evans et al. 1996). Briefly, one side of the buccal mass was cut away and the buccal ganglia were left attached to the remaining half of the buccal mass through buccal nerve 3. Preparations were placed in silicone elastomer (Sylgard)-lined dishes, and a small (5 ml) Lucite chamber was placed over the opener muscles to pharmacologically isolate them from the buccal ganglion. Preparations were grounded routinely using a chlorided silver wire.

When contractions of the I7 muscle were recorded, an isotonic transducer was used to detect muscle movements (Harvard Apparatus, MA) (Evans et al. 1996). Briefly, to connect I7 muscles to the transducer, a wooden beam was attached, approximately at its midpoint, to the rotating arm of the transducer. One end of the beam had a metal hook to which the odontophoral end of the I7 muscle was tied using a silk suture. The other half of the beam was marked with a centimeter scale, along which a known weight could be moved to vary the load on the muscle. When contractions of the I7 muscle were elicited by intracellular stimulation of motor neuron B48, we used intracellular double-barreled electrodes that were filled with a solution of 3 M potassium acetate containing 10 mM KCl. Electrodes had resistances of ~10 MΩ. When muscle contractions were elicited by stretching the I7 muscle, different loads were used for different muscles because stretch was elicited more readily in some cases than others. In general loads on muscle ranged from 36 to 410 mg. A "stop" was placed under the wooden beam to control muscle length (Evans et al. 1996). Experiments began with the stop at its highest point. The stop then was lowered to stretch muscles. Changes in relaxation rate were quantified by measuring the time it took for contractions to relax to two-thirds of their original size.

To obtain intracellular recordings from I7 muscle fibers, about one-third of the length of the I7 muscle was immobilized, with pins, on a raised piece of Sylgard within the Lucite chamber that separated the buccal ganglion from the I7 muscle. Electrodes used to record from muscle fibers were single barreled glass pipettes with resistances of 10–25 MΩ. In ion substitution experiments, preparations were grounded with a seawater agar bridge connected to a reservoir containing 3 M KCl and an Ag/AgCl pellet.

Methods used in immunocytochemical experiments

In most experiments, immunocytochemical experiments were performed using standard whole-mount methods (Longley and Longley 1986; Miller et al. 1991; Vilim et al. 1996b). Primary antisera were as follows: serotonin (5-HT)-rabbit host (kind gift from Dr. Hadassah Tamir, Columbia University), FMRFamide-rabbit host (Dia Sorin, Stillwater, MN), buccalin-rabbit host (Miller et al. 1992), SCP-rabbit host (kind gift from Dr. H. R. Morris Empire College), and myomodulin (MM)-rat host (raised against a peptide conjugated to bovine thyroglobulin with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (Vilim et al. 1996b). Primary antisera were applied for 48 h at room temperature at a dilution of 1:250. The secondary antibodies [lissamine rhodamine donkey anti-rat, and fluorescein donkey anti-rabbit (Jackson ImmunoResearch, West Grove, PA)] were applied for 24 h (1:500 dilution; room temperature).

In all cases, tissues were viewed with a Nikon microscope equipped with epifluorescence and photographed with Tri-X (ASA 400) film. In experiments where immunocytochemistry was performed on B48 neurons, cells were identified by their position in the buccal ganglion and their ability to produce contractions of the I7 muscle (using physiological methods described in the preceding section). They then were injected with Lucifer yellow dye.

Methods used to radiolabel B48 neurons

In situ radiolabeling was done as in previous studies (see e.g., Cropper et al. 1987a;Lloyd et al. 1987). Briefly, 7 B48 neurons were physiologically identified (using physiological methods described in the preceding text). Neurons were marked by iontophoretic injection of Fast Green dye. Buccal ganglia were incubated for 24 h in 1 ml of 50% ASW, 50% *Aplysia* hemolymph containing 0.5 mCi of [^{35}S]methionine, 2.5 μl of 1 M colchicine (dissolved in DMSO), and 100 μl antibiotics (penicillin and streptomycin each at 50 units/ml). B48 neurons were dissected individually from the labeled ganglia (Ono and McCaman 1980), and radioactive MM was extracted in the presence of synthetic MM.

Coelution of radiolabeled and synthetic material was tested through two sequential reverse phase high-performance liquid chromatography (RP-HPLC) passes. In the first pass, an Aquapore RP-300 column was developed at 1 ml/min with a linear gradient of 5–50% solvent B in 45 min. Solvent A was 100% H_20, 0.01 M trifluoroacetic acid (TFA) and solvent B was 100% CH_3CN, 0.01 M TFA. In the second RP-HPLC pass, the same column was developed with a linear gradient of 15–45% solvent B in 30 min. Solvent A was 100% H_2O, 0.01 M heptafluoroacetic acid (HFBA) and solvent B was 100% CH_3CN, 0.01 M HFBA. In both passes, synthetic peptides were detected by absorbence measurements using a V-4 flow spectrophotometer (ISCO) at 215 nm. In the first pass, radiolabeled peptides were detected by scintillation counting of 10% of each fraction. After the second pass, whole fractions were counted.

Methods used for measuring cAMP levels in I7 muscles

In experiments with exogenous modulators, I7 muscles were removed and placed in ASW for 2 h to stabilize preparations. Muscles then were exposed to modulators at different concentrations for different periods of time (see RESULTS for descriptions of specific experiments). In experiments in which cAMP elevations were induced by the release of endogenous modulators, buccal ganglia were desheathed, and preparations were rested for 2 h. B48 neurons then

were stimulated in a pattern that mimicked physiological activity, i.e., B48 was fired at 3 Hz for 2 s followed by a pause of 4 s (Evans et al. 1996). B48 then was fired at 14 Hz for 1.2 s followed by a pause of 1.5 s. The 3-Hz stimulation then was repeated. Neurons were stimulated for a total of 5 min at 15°C. During the last burst of stimulation I7 muscles were frozen, i.e., the ASW bathing muscles was replaced with liquid nitrogen.

In all experiments, we extracted cAMP from I7 muscles by homogenizing them in 65% ethanol, 35% H_2O, heating them to 90°C for 5 min, and then spinning them in a clinical centrifuge for 2 min. We removed the resulting supernatant and stored samples at −20°C until we made cAMP measurements. cAMP levels were quantified using a commercially available RIA (Amersham). Proteins were measured using a BCA protein assay reagent (Pierce, Rockford, IL).

Reagents

The ASW used in these experiments had the following composition (in mM): 460 NaCl, 10 KCl, 11 $CaCl_2$, 55 $MgCl_2$, and 5 $NaHCO_3$. The pH was adjusted to 7.6. All salts, Fast Green dye, Lucifer yellow CH, and the *N*-methyl-D-glucamine were obtained from Sigma. Forskolin was obtained from Calbiochem and was dissolved in DMSO. The final concentration of DMSO during experiments with forskolin was 0.01%. Control experiments established that this concentration was not bioactive at the neuromuscular junction.

RESULTS

Modulators are present in the opener neuromuscular system

To identify potential modulators in the I7–I10 neuromuscular system, we used immunocytochemical techniques. Specifically, we sought to determine whether the I7–I10 muscles contain fibers that are immunoreactive to 5-HT and peptides that modulate neuromuscular activity in *Aplysia*, i.e., MM (Cropper et al. 1987b), buccalin (Cropper et al. 1988), FMRFamide (Weiss et al. 1986), and SCP (Lloyd et al. 1984). Fibers were 5-HT immunoreactive (IR) and MM-IR. Specifically, 5-HT and MM antisera stained dense networks of finely dividing processes that had numerous varicosities that extended over the surface of muscles (Fig. 1, *A* and *B*). Muscles were also immunoreactive to FMRFamide, but staining was confined to a much more sparsely distributed network of fibers (Fig. 1*C*). There was no detectable SCP-like or buccalin-like immunoreactivity (not shown).

In a previous study, we demonstrated that the I7–I10 muscle complex is innervated primarily by one motor neuron (Evans et al. 1996). This neuron was similar in size and location to B48, a neuron described by Church and Lloyd (1994). Additionally, Church and Lloyd found that stimulation of neuron B48 elicited radula opening/protraction as our neuron did and that B48 innervated the I8 muscle. If our motor neuron and B48 are in fact the same cell, we would expect our motor neuron to contain MM as B48 does (Church and Lloyd 1994). To determine whether this was the case, we initially performed immunocytochemical experiments on buccal ganglia. We found that our motor neuron was MM-IR but was not 5-HT or FMRFamide IR (not shown).

To determine whether our motor neuron synthesizes authentic MM, buccal ganglia were incubated in a radiolabeled form of an amino acid precursor of MM, namely [^{35}S]methionine. Motor neurons were dissected individually from ganglia and supplemented with quantities of synthetic MM, which are detected easily by optical measurements. Mixtures of native and synthetic material were subjected to sequential RP-HPLC. Native radioactivity did in fact precisely coelute with synthetic material through both stages of chromatography (Fig. 2).

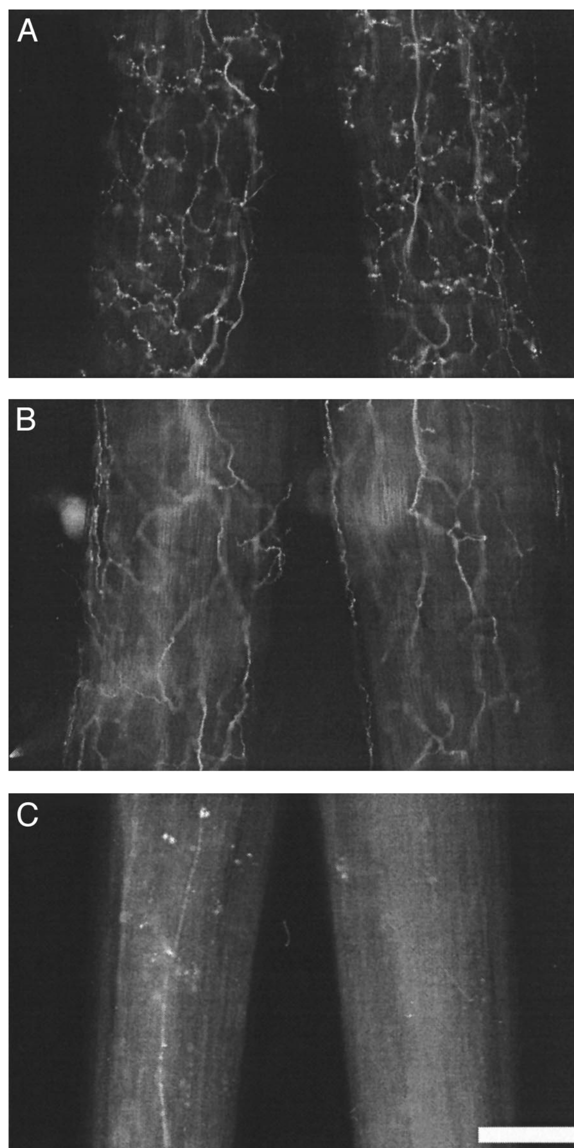

FIG. 1. Whole-mount immunocytochemistry of I7 muscles. Serotonin (5-HT)-like (*A*), myomodulin (MM)-like (*B*), and FMRFamide-like (*C*) immunoreactive fibers on the surface of the I7 muscle. Note that 5-HT and MM stain dense networks of finely dividing fibers, whereas FMRFamide stains a much more sparsely distributed network of fibers. *A* and *B* are from the same preparation and were visualized with different secondary antibodies (i.e., fluorescein in *A* and lissamine rhodamine in *B*). *C* is from a different preparation. Scale bar 200 μm.

Modulators are bioactive in whole muscle preparations

Physiological experiments were conducted on the I7 muscles, which are the longest and most experimentally advantageous of the opener complex. Modulators tested were those that are physiologically relevant. Thus 5-HT and FMRFamide were tested because fibers on opener muscles are 5-HT-IR and FMRFamide-IR. Two MMs were tested (MM_A and MM_B) because we specifically localized MM_A to B48 in biochemical experiments, and we have found that MM_A is cleaved from a

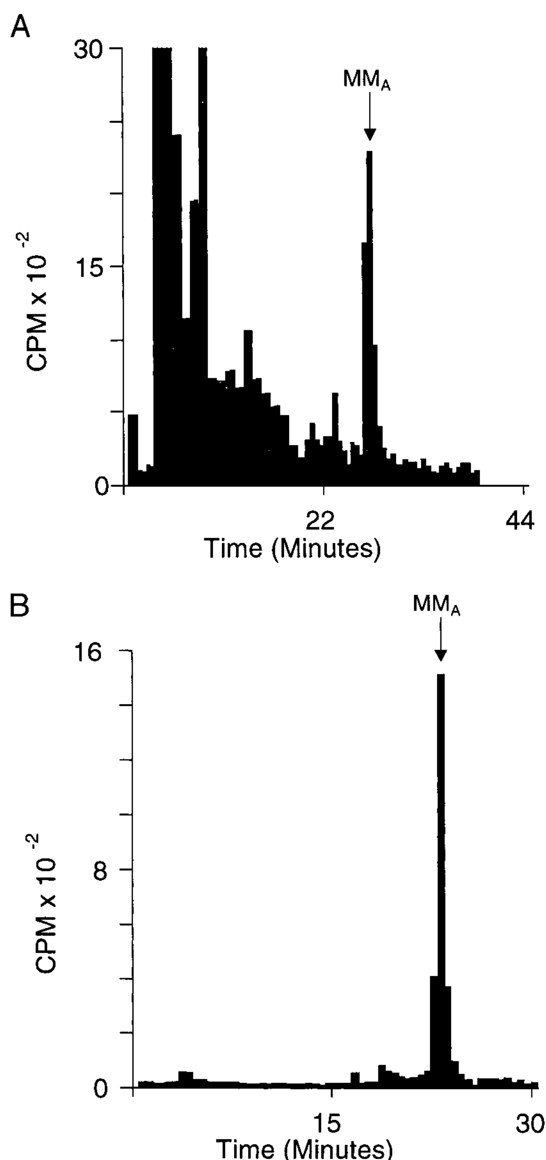

FIG. 2. Comparison of chromatographic properties of [^{35}S]methionine-labeled B48 peptides and synthetic MM_A. Methionine-containing peptides in 7 B48 neurons were radiolabeled in vivo and extracted in the presence of nanomolar quantities of synthetic MM_A. Extracted material was sequentially chromatographed through 2 high-performance liquid chromatography (RP-HPLC) passes. In both passes synthetic MM_A was detected by absorbance measurements at 215 nm. ↓, elution time of the MM_A. B48 radioactivity was detected by counting 10% of the fractions resulting from the first RP-HPLC pass and 100% of the last pass. Counts per whole fraction are plotted in *A* and *B*. *A*: 1st RP-HPLC pass, performed in the presence of 0.01 M trifluoroacetic acid (TFA). Fractions were collected every minute for the first 20 min, then were collected every 0.5 min. *B*: rechromatography of the MM_A peak shown in *A* in the presence of 0.01 M heptafluoroacetic acid (HFBA). Fractions were collected every minute for the first 16 min, then were collected every 0.5 min.

precursor protein that also encodes MM_B (Miller et al. 1993). Actually, the MM precursor encodes five other MMs in addition to MM_A and MM_B (Miller et al. 1993). We chose MM_A and MM_B because experiments in the ARC neuromuscular system have suggested that differences in the bioactivity of the MMs are most striking if these two peptides are compared (Brezina et al. 1995).

Previous experiments have shown that vigorous contractions of opener muscles are elicited if the motor neuron B48 is stimulated (Evans et al. 1996). In this study therefore, we sought to characterize effects of modulators on parameters of motor neuron-elicited muscle contractions. Additionally, previous experiments have shown that opener contractions can be elicited in the absence of neural activity if muscles are counterweighted so that they are stretched beyond their resting length (Evans et al. 1996). In unmodulated muscles, this does not, however, appear to occur unless muscles are stretched in an unphysiological manner (Evans et al. 1996). A second goal of these experiments, however, was to determine whether contractions of opener muscles are induced more readily by stretch in the presence of modulators. Finally, in some neuromuscular systems modulators themselves induce myogenic activity (e.g., Meyrand and Marder 1991). We, therefore also conducted experiments to determine whether similar effects would occur in the opener complex.

EFFECTS OF MODULATORS ON THE MEMBRANE POTENTIAL OF I7. When modulators were applied to I7 muscles, changes in membrane potential were observed. These effects were concentration dependent and reversible (Fig. 3). 5-HT and MM_B both induced a concentration-dependent depolarization of muscle fibers (Fig. 3, *A* and *B;* Table 1), whereas FMRFamide induced a concentration-dependent hyperpolarization (Fig. 3*D;* Table 1).

The effects of MM_A on membrane potential were more complex. At low concentrations, MM_A depolarized muscle fibers (Fig. 3*C;* Table 1). However, we found that the effects of MM_A at higher concentrations (i.e., 10^{-6} M) were biphasic. Specifically, MM_A produced a depolarization followed by a hyperpolarization (Fig. 4*A1*) when recordings were maintained for several minutes (n = 3). This could indicate that the MM_A-induced hyperpolarization had a delayed onset. Alternatively, MM_A could have simultaneously induced both the depolarizing and hyperpolarizing responses, but initially the hyperpolarizing response may have been masked by the depolarizing effect. To distinguish between these possibilities, we performed experiments in which we initially applied 10^{-6} M MM_B or 5-HT to saturate the depolarizing response, and then applied 10^{-6} M MM_A. We found that under these conditions, MM_A did in fact exert a pure hyperpolarizing action without a long delay (n = 3; Fig. 4*A, 3* and *4*). In other experiments, the normal ASW bathing the preparation was replaced with sodium-free ASW that contained *N*-methyl–D-glucamine. Under these conditions, modulator-induced depolarizations were not observed (n = 3; Fig. 4*B2*). When MM_A was applied to I7 muscles at 10^{-6} M in Na-free ASW, only hyperpolarizations were observed. These hyperpolarizations did not appear with a delayed latency.

In some systems, modulators that depolarize muscle fibers can exert striking effects on neuromuscular function in that they can cause muscles to contract in the absence of neural activity (e.g., Meyrand and Marder 1991). These muscle contractions can be as vigorous as those that are elicited by motor neuron activity and can have similar dynamics. We found that depolarization evoked by 5-HT did not itself lead to spontaneous muscle contractions or spiking (n = 10; Fig. 5*A*).

EFFECTS OF MODULATORS ON STRETCHED OPENER MUSCLES. In a previous study we demonstrated that opener muscles were depolarized in the absence of neural activity if they were stretched beyond resting length (Evans et al. 1996). When muscles were stretched in a physiological range, slight tension

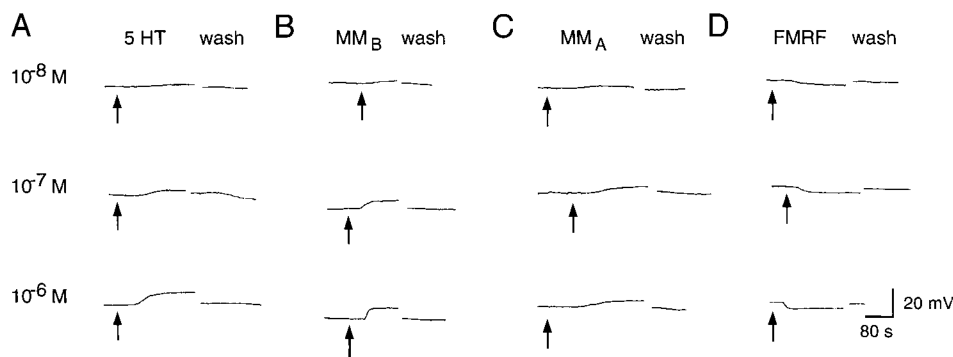

FIG. 3. Concentration-dependent actions of modulators on the membrane potential of I7 muscle fibers. See Table 1 for specific membrane potentials of muscle fibers and modulator-induced depolarizations. Note that 5-HT and MM_B produced concentration-dependent depolarizations (*A* and *B*), whereas FMRFamide elicited a concentration-dependent hyperpolarization (*D*). Effects of MM_A were more complex (*C*). At 10^{-7} M, it produced a depolarization (*middle*). At 10^{-6} M, this depolarization was actually less pronounced (*top*). Effects of MM_A therefore were considered in more detail in experiments such as the one shown in Fig. 4. *A–D* were all taken from different preparations.

increases were observed but vigorous contractions were not elicited (Evans et al. 1996). Thus modulators and stretch can both depolarize opener muscle fibers but neither manipulation alone elicits myogenic activity. What if the two manipulations are interacted? To answer this question, I7 muscles were attached to a movement transducer and stretched in a physiological range (Evans et al. 1996) so that a contraction was not elicited. 5-HT (10^{-6} M) then was applied to stretched muscles and muscle contractions were elicited ($n = 8$ of 10 preparations; Fig. 5*B*). These contractions were rhythmic, and each contraction was preceded by what appeared to be a "spike." These spikes were not large in amplitude, therefore they were presumably generated in regions of the muscle that were stretched and were only electrotonically conducted to fibers from which recordings were made.

To determine whether 5-HT was inducing the release of ACh from motor neuron terminals, experiments were performed in the presence of 10^{-4} M hexamethonium [which blocks the cholinergic input from B48 (Evans et al. 1996)]. 5-HT was still effective at eliciting contractions ($n = 3$; Fig. 6*A*). 5-HT did not have to be added immediately after muscles were stretched to induce contractions; we were able to delay 5-HT application for as long as 16 min (times longer than 16 min were not tested.) Like 5-HT, MM_B also was able to induce contractions in stretched muscles ($n = 3$; Fig. 6*B*).

Scott et al. (1997a) have shown that modulators activate or enhance two types of inward currents in opener muscle fibers; I_{Ca} and $I_{Mod(cat)}$. We hypothesized that the effects of modulators on $I_{Mod(cat)}$ were likely to be the most important for inducing contractions in stretched muscles. I_{Ca} is a high-voltage-activated current; it does not appear until −40 mV and is maximal at ~0 (±10) mV (Scott et al. 1997a). As previously discussed, the resting membrane potential of opener fibers is approximately −70 mV. Stretches therefore would have to depolarize muscle fibers ~30 mV for I_{Ca} to become apparent. Previous experiments in which we measured depolarizations induced by typical stretches suggest that this is not likely to be the case (Evans et al. 1996).

Because the effects of modulators on I_{Ca} are cAMP dependent whereas effects of modulators on $I_{Mod(cat)}$ are not, we sought to determine whether the cAMP analogue 8-CPT-cAMP could induce contractions in stretched muscle fibers. We found that 8-CPT-cAMP was ineffective at eliciting contractions of stretched muscles ($n = 3$; Fig. 6*C*). Thus these data are consistent with the idea that effects of modulators on $I_{Mod(cat)}$ are likely to be more important for inducing contractions in stretched muscles than effects of modulators on I_{Ca}.

Our data show that modulators that depolarize muscle fibers can elicit myogenic activity if muscles are stretched. As discussed in the preceding text, however, not all modulators depolarize muscle fibers. FMRFamide, and MM_A at high concentrations hyperpolarize muscle fibers. This would suggest therefore that the ability of modulators to initiate contractions in the presence of stretch would depend on the specific combination of substances present. To determine whether this is the case, we performed experiments in which muscles were stretched, 10^{-6} M 5-HT was applied, and contractions were elicited. We then applied 10^{-8} M FMRFamide. Rhythmic contractions and "spiking" in I7 muscles completely ceased ($n = 3$; Fig. 7). To ensure that muscles had not been damaged, we then exchanged the FMRFamide-containing ASW with ASW containing 10^{-6} M 5-HT. Contractions and spiking were again elicited and could again be blocked by FMRFamide application (Fig. 7). FMRFamide was effective at inhibiting contractions even when contractions were increasing in frequency (e.g., see second FMRFamide application in Fig. 7). This suggests that the 5-HT effect was not simply desensitizing. We also found that contractions elicited by unphysiological stretches of the muscle were abolished by modulators that hyperpolarize muscle fibers, i.e., 10^{-7} M MM_A (Fig. 8*A; n* = 3), and 10^{-9} M FMRFamide ($n = 3$; Fig. 8*B*).

TABLE 1. *Effects of modulators on the membrane potential of I7 muscles*

Modulator (at 10^{-6} M)	*n*	Average Unmodulated Membrane Potential, mV	Change in Membrane Potential, mV
MM_A	5	−74.8	+6.1 ± 0.6
MM_B	7	−74.7	+7.2 ± 0.8
5-HT	9	−73.4	+7.3 ± 0.9
FMRFamide	3	−74.7	−5.7 ± 0.7

Membrane-potential changes expressed as means ± SE. 5-HT, serotonin; MM, myomodulin.

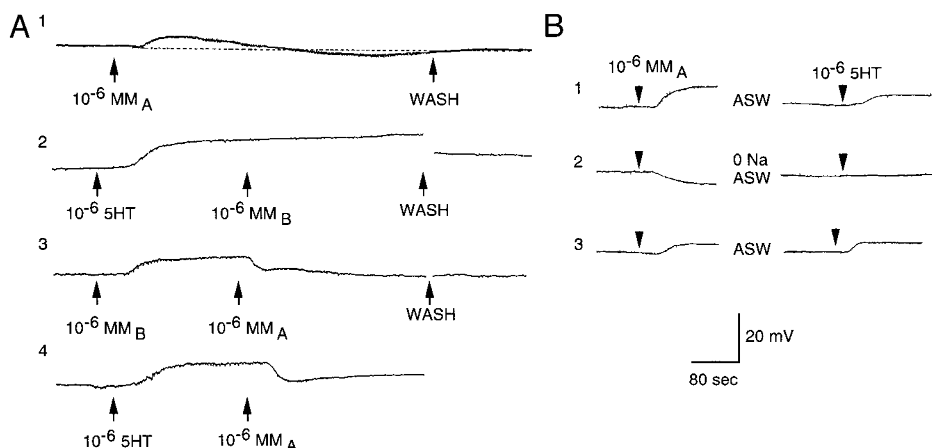

FIG. 4. *A1*: MM_A at high concentrations exerts both a depolarizing and a hyperpolarizing effect on opener muscle fibers (unmodulated fiber potential, −72 mV). *A2*: MM_B applied to muscles in the presence of 10^{-6} M 5-HT did not cause further depolarization of the fiber (unmodulated fiber potential, −76 mV). This suggests that the depolarizing response was saturated. In *A, 3* and *4,* 10^{-6} M MM_A was applied under the same conditions. A hyperpolarizing response was now apparent (unmodulated fiber potential −76 mV in *A3;* unmodulated fiber potential, −72 mV in *A4*). *B*: depolarizing effects of modulators are Na-dependent. In *B2* MM_A and 5-HT were applied in the presence of 0 Na artificial seawater (ASW). Under these conditions modulators did not depolarize muscle fibers. Depolarizing responses returned when the Na-free ASW was replaced with normal ASW (unmodulated fiber potential, −72 mV). Note that when MM_A is applied in Na-free ASW a hyperpolarization is observed.

EFFECTS OF MODULATORS ON B48-INDUCED CONTRACTIONS OF THE I7 MUSCLE. We next characterized the effects of various modulators on contractions induced by firing the opener motor neuron B48. We found that MM_B and 5-HT potentiated contractions over the range of concentrations tested, whereas MM_A potentiated contractions when it was applied at 10^{-9} to 10^{-7} M but decreased contraction size at 10^{-6} M (Fig. 9, *B* and *C*). FMRFamide decreased contraction size at all concentrations (Fig. 9, *B* and *C*).

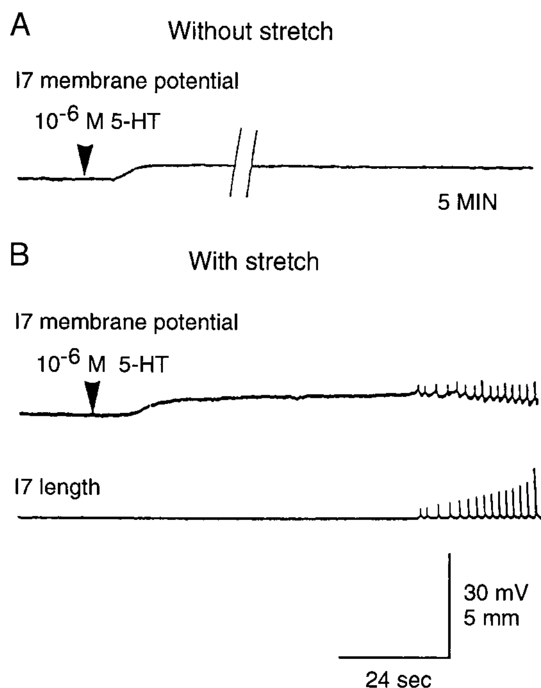

FIG. 5. 5-HT initiates spiking and muscle contractions, but only if muscles have been stretched. *A*: experiment in an unstretched I7 muscle. Application of 10^{-6} M 5-HT (▾) resulted in a depolarization of the muscle fiber. Recording was monitored for >5 min and "spiking" and contractions of the muscle were not observed. Depolarization persisted until the 5-HT was washed out. *B*: experiment in a stretched muscle. After the preparation used for the experiment in *A* was washed for 1 h, the I7 was stretched by 1 mm from its resting length. This stretch step did not elicit muscle contractions on its own. However, when 10^{-6} M 5-HT was added, simultaneous recordings of fiber membrane potential (*top*) and muscle length (*bottom*) show that what appeared to be spikes and contractions were induced ~1 min after 5-HT depolarized muscle fibers.

Studies in which effects of 5-HT and neuropeptides have been modeled in the ARC neuromuscular system have suggested that effects of modulators on muscle relaxation rate may be of fundamental importance during normal feeding behavior (Deodhar 1999; Weiss et al. 1992). We therefore sought to determine whether relaxation rate also could be modulated in the I7–I10 complex. We found that relaxation rate was in fact increased by the MMs and 5-HT (Fig. 9*A*).

Previous experiments have shown that stretch of the opener muscles will decrease the latency of motor neuron elicited muscle contractions (Evans et al. 1996). We have suggested that this phenomenon at least partially may be accounted for by the fact that stretch depolarizes muscle fibers. If so, it might be expected that modulator-induced changes in membrane potential would produce similar effects. To determine whether this was the case, we applied 5-HT to nonstretched muscles and elicited contractions by stimulating B48. Decreases in contraction latency were clearly observed ($n = 3$; Fig. 10). In part this decrease appears to result from the fact that excitatory junction potentials (EJPs) that were previously subthreshold for eliciting a contraction of the muscle become threshold. For example, in the experiment shown in Fig. 10, the first EJP of the burst of action potentials only elicited a change in tension when the preparation was bathed in 10^{-6} M 5-HT. When this occurred, a significant decrease in contraction latency was observed because the muscle contraction was elicited by relatively low-frequency stimulation. Specifically, because the motor neuron was stimulated at ~3 Hz, an EJP was elicited every 333 ms. Consequently, when the previously subthreshold first EJP now elicited a contraction, this contraction was advanced by 333 ms (the total duration of the contraction is ~1 s).

Additionally, 5-HT increased the rate at which tension

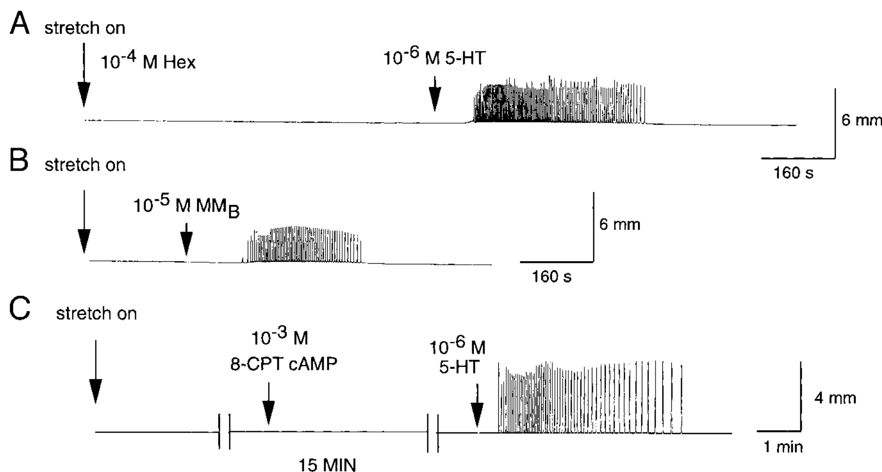

FIG. 6. *A*: 5-HT can initiate contractions in stretched I7 muscles in the presence of hexamethonium [which blocks effects of the opener motor neuron primary neurotransmitter, acetylcholine (ACh)]. In this experiment, muscles were placed in 10^{-4} M hexamethonium and stretch was applied (as indicated, ↓). 5-HT (10^{-6} M) then was added directly to muscles. *B*: a similar experiment showing that MM_B can initiate myogenic activity. *C*: membrane permeable cAMP analogue, 8-CPT-cAMP, did not elicit contractions of stretched I7 muscles. In this experiment, muscles were stretched and 10^{-3} M 8-CPT-cAMP was applied directly to muscles. Contractions were not elicited. After 15 min, muscles were tested with 10^{-6} M 5-HT to determine whether they were capable of contracting. *A–C* are from different preparations.

was developed in that the time it took to reach 50% of the maximum amplitude was decreased. For example, in the experiment shown in Fig. 10 contractions were at half-maximal amplitudes ~420 ms after tension began to develop. In contrast, in the presence of 10^{-6} M 5-HT half-maximal amplitudes were reached ~250 ms after tension began to develop. This increase in the rate of tension development did not result from an increase in EJP size. In fact, the EJP time constant was actually decreased. Modulated EJPs were, however, more effective at producing tension increases in muscles than unmodulated EJPs, at least during the time that the overall contraction amplitude was increasing (compare EJP #2 in Fig. 10*A* to EJP #1 in Fig. 10*B*). Because tension increases produced by modulated EJPs were larger, they summated more efficiently.

5-HT and MM increase muscle cAMP levels

We found that MM_A, MM_B, and 5-HT all increased cAMP levels in opener muscles in a concentration-dependent (Fig. 11*A*), and time-dependent (Fig. 11*B*) manner. FMRFamide (Fig. 11*A*) and the B48 primary neurotransmitter ACh (Fig. 11*B*) did not increase cAMP levels. Additionally, we found that forskolin and 8-CPT-cAMP mimicked effects of 5-HT and the MMs on motor neuron evoked muscle contractions ($n = 3$; Fig. 12). Thus they increased contraction size (Fig. 12*A*) and relaxation rate (Fig. 12*B*).

Indirect evidence for release of modulators under physiological conditions

To indirectly determine if MM might be released during motor neuron firing, we took advantage of the fact that MM increases cAMP levels in opener muscles. Previous experiments have shown that B48 fires at least twice during the opening/protraction phase of ingestive motor programs (Evans et al. 1996). One relatively high-frequency burst of activity occurs during visible opening/protractions, the second burst of low-frequency activity occurs at peak retraction. We mimicked this firing pattern, i.e., we stimulated B48 neurons at 14 Hz for 1.2 s followed by a pause of 1.5 s. We then stimulated B48 at 3 Hz for 2 s followed by a pause of 4 s. The burst of 14 Hz activity then was repeated. After periods of stimulation, we measured the resulting levels of cAMP in the I7 muscle. Other preparations were processed in a similar manner except that we did not stimulate B48 neurons. (B48 bilaterally innervates the I7–I10 complex (Evans et al. 1996) so we could not make within animal comparisons, i.e., use one I7 as a control (unstimulated) muscle and one as an experimental (stimulated) muscle.) We found that physiological stimulation

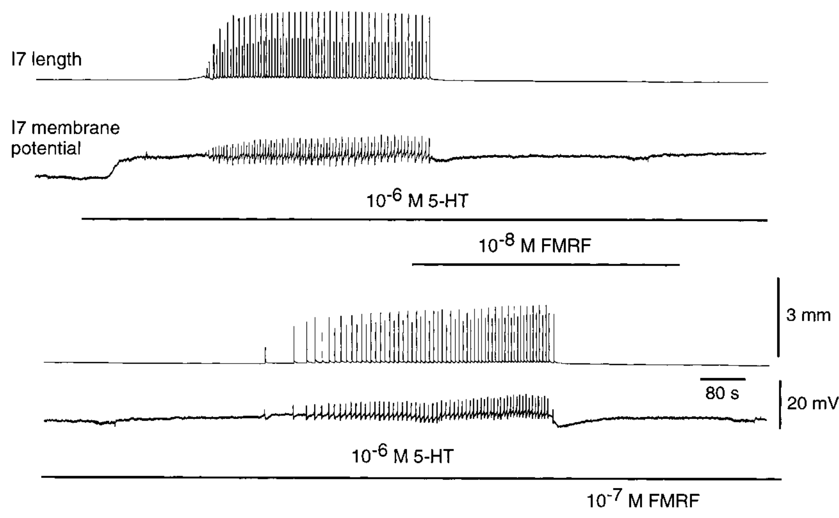

FIG. 7. Myogenic activity in stretched muscles is not seen when modulators that depolarize muscle fibers are applied with modulators that hyperpolarize muscle fibers. *Top*: continuous recording of I7 muscle length. *Bottom*: continuous recording of membrane potential. Unmodulated fiber potential was −74 mV. Before the record begins the muscle was stretched and then monitored for 5–6 min to ensure that stretch activated contractions had not been induced. 5-HT (10^{-6} M) was added to the muscle, which caused the muscle fiber to depolarize and eventually contract. FMRFamide (10^{-8} M) then was added, which hyperpolarized the fiber and inhibited spiking and muscle contractions. FMRFamide then was washed out with ASW containing only 10^{-6} M 5-HT until contractions returned. Subsequent addition of 10^{-7} M FMRFamide caused a bigger hyperpolarization and again inhibited contractions.

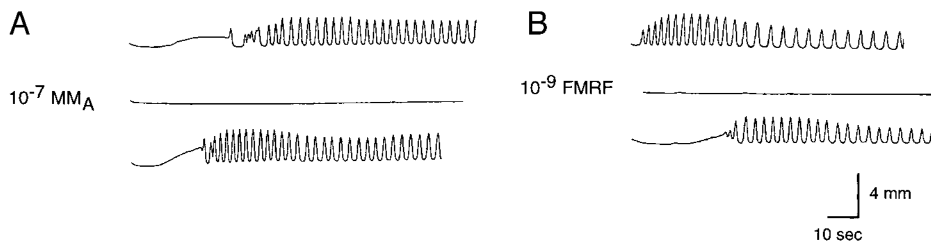

FIG. 8. Actions of the modulators on contractions of the I7 muscle elicited by stretch. *Top*: made before modulator application. *Middle*: made in the presence of modulators. *Bottom*: made after modulators were washed out. Stretch was applied at the onset of each recording and was removed after recordings were completed. Note that stretch-induced contractions of opener muscles were not observed in the presence of 10^{-7} M MM_A (*A*) or in the presence of 10^{-9} M FMRFamide (*B*).

of B48 produced a significant increase in cAMP levels in the I7 muscle (Fig. 13).

DISCUSSION

Source of modulatory input to the I7–I10 complex

We show that there are MM-IR neural processes and varicosities on opener muscles. The opener motor neuron B48 synthesizes authentic MM_A (Fig. 2) (Church and Lloyd 1994) and is therefore one physiological source of MM input to the I7–I10 complex. We also demonstrate that there are neuronal processes on opener muscles that are FMRFamide-IR. Our data and data of Church and Lloyd (1994) indicate that the FMRFamide-like peptide is not a cotransmitter in B48. We showed that FMRFamide-IR processes had an appearance that was clearly different from the appearance of MM-IR processes.

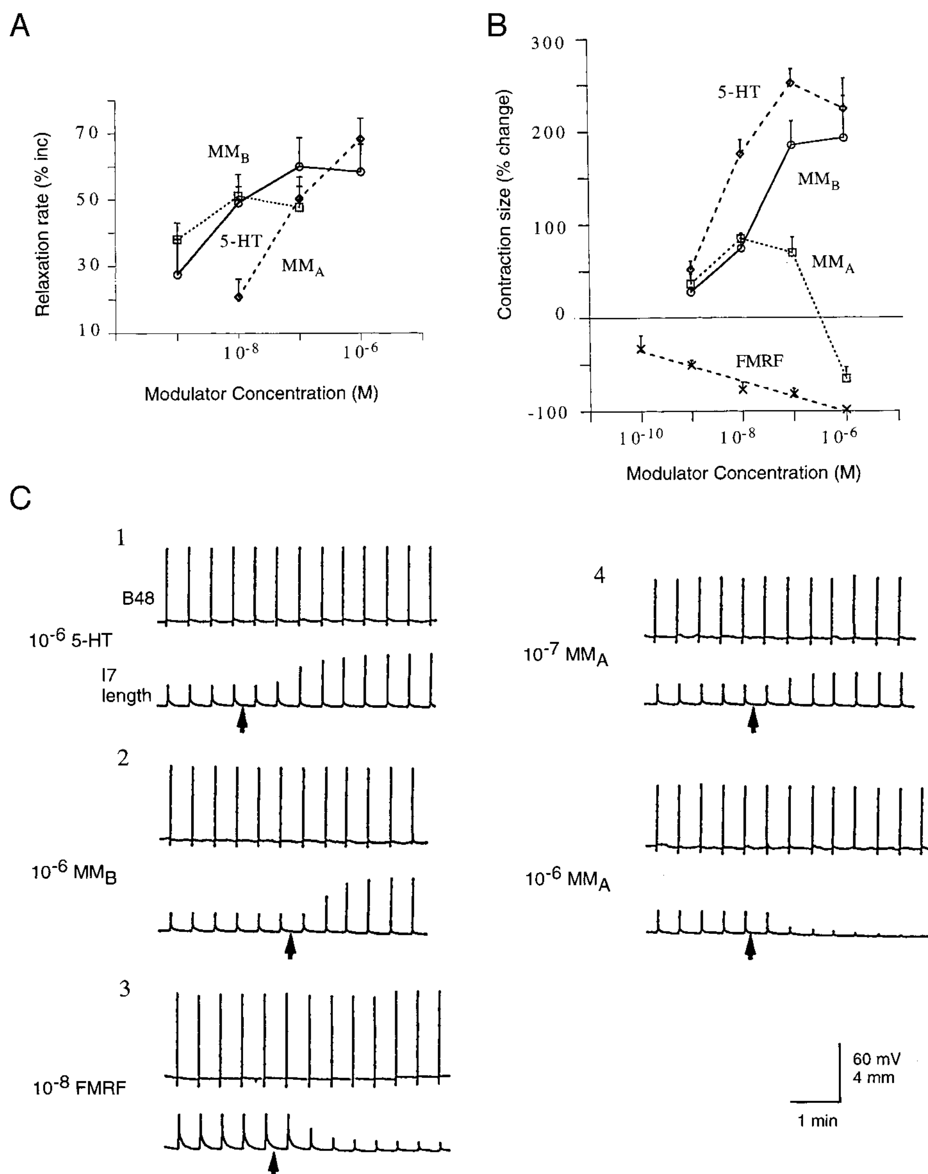

FIG. 9. Modulation of motor neuron elicited contractions of I7 muscles. *A*: effects of modulators on the relaxation rate of B48-induced muscle contractions ($n = 3$ for each point; SEs are indicated). *B*: effects of modulators on the amplitude of B48-induced contractions of I7 muscles ($n = 3$ for each point; SEs are indicated). *C*: examples of data used to generate the plots shown in *A* and *B*. *Top*: action potentials in the motor neuron B48; *bottom*: resulting muscle contractions. Modulators were applied in the concentrations indicated (↑). Note that 5-HT and MM_B produce concentration-dependent increases in contraction size (*B* and *C, 1* and *2*) and relaxation rate (*A*). MM_A increases contraction size (*B* and *C4, top*) and relaxation rate (*A*) at lower concentrations and decreases contraction size (*B* and *C4, bottom*) and increases relaxation rate (*A*) at higher concentrations. FMRF decreases contraction size at all concentrations (*B* and *C3*) and has no effect on relaxation rate.

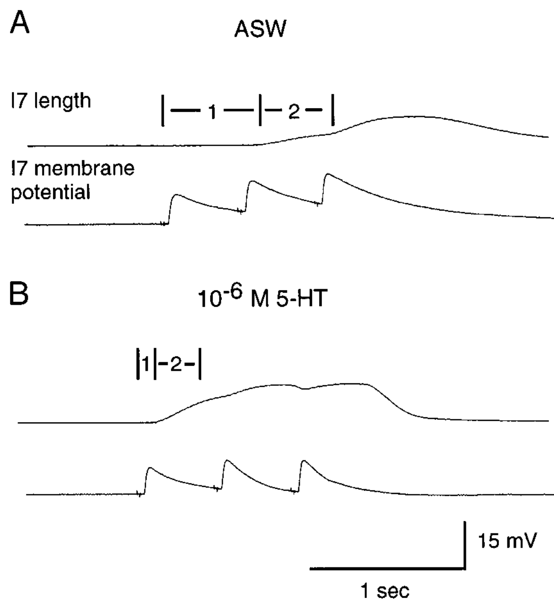

FIG. 10. High-speed record of the effects of 5-HT on contractions of the I7 muscle. Bracketed 1s indicate contraction latency (the 1st bracket indicates when motor neuron stimulation begins, the 2nd bracket indicates when a change in tension is first detected). The bracketed 2s indicate contraction rise time (the 1st bracket indicates when tension is first detected, the 2nd bracket indicates when contractions reach half-maximal amplitude). *A*: control record obtained in ASW. Contractions of the I7 muscle were elicited by stimulating motor neuron B48 (not shown). *Top*: resulting change in length in the I7 muscle. *Bottom*: intracellular recording of motor neuron-elicited excitatory junction potentials (EJPs). Artifacts in the EJP trace indicate when neuron B48 is stimulated. *B*: similar recordings obtained in the presence of 10^{-6} M 5-HT. Note that contractions in 5-HT are phase advanced with respect to contractions elicited under control conditions, i.e., there is less time between the point at which stimulation begins and the point at which tension is first detected (compare 1 in *A* vs. *B*). Also note that tension develops at a faster rate in the presence of 5-HT, i.e., there is less time between the point at which tension is first detected and the point at which contractions reach half-maximal amplitude (compare 2 in *A* vs. *B*).

Namely, the MM antibody stained a dense network of finely dividing processes that had numerous varicosities (Fig. 1*B*). In contrast, the FMRFamide antibody stained a much more sparsely distributed network of fibers (Fig. 1*C*). Church and Lloyd (1994) have shown that B48 does not synthesize FMRFamide, and we found that B48 is not FMRFamide-IR. One source of the FMRFamide-like input to the opener complex is likely to be the multifunction neurons B4/B5. These neurons innervate the opener complex (Evans et al. 1996) and synthesize FMRFamide (Church and Lloyd 1991). Additionally, FMRFamide-like peptides may originate from buccal S (sensory) cells, which are strongly FMRFamide-IR (Lloyd et al. 1987) and innervate many muscles of the buccal mass (Jahan-Parwar et al. 1983).

In addition to peptide immunoreactivity, there is 5-HT immunoreactivity in neuronal processes on opener muscles. One source of this immunoreactivity is likely to be the serotonergic (Eisenstadt et al. 1973; Weinreich et al. 1973) metacerebral cells (MCCs). The MCCs have been studied extensively, and it has become apparent that these neurons act both centrally on neurons that generate feeding behavior and peripherally on the muscles of the buccal mass that execute feeding behavior (e.g., Fox and Lloyd 1998; Lotshaw and Lloyd 1990; Weiss et al. 1978). Neurons B48 and B4/B5 are not 5-HT-IR, and 5-HT is not present in buccal neurons.

Modulation of stretch-induced contractions of opener muscles

In this study we show that some of the modulatory neurotransmitters in the opener neuromuscular system (e.g., 5-HT and MM_B) can induce rhythmic muscle contractions if muscles are stretched. The induction of myogenic activity in the absence of neural activity has been described in other systems, e.g., in the pyloric dilator muscle of the shrimp *Palaemon* (Meyrand and Marder 1991; Meyrand and Moulins 1986), and in cardiac muscle of the leech *Hirudo* (Li and Calabrese 1987). In the shrimp there are times when the pyloric dilator motor neuron is silent and rhythmic contractions of the pyloric dilator muscle could occur naturally (Meyrand and Moulins 1988). In contrast, we expect that myogenic activity in the I7–I10 complex is not likely to occur in the absence of neural activity, at least during ingestive motor programs. During this type of activity, large presumably motor neuron-induced excitatory junctional currents (EJCs) are always recorded from I7 muscles during the radula opening/protraction phase of behavior (Evans et al. 1996). We also demonstrate that myogenic activity is only elicited in the I7–I10 complex when modulators that depolarize muscle fibers are applied to stretched muscles alone, i.e., when they are not applied with modulators that hyperpolarize muscle fibers.

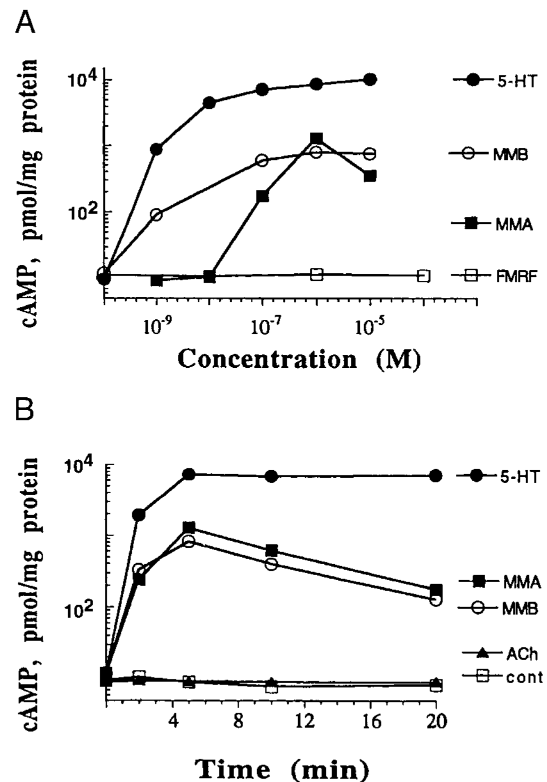

FIG. 11. MM_A, MM_B, and 5-HT increase levels of cAMP in opener muscles in a concentration-dependent (*A*) and time-dependent (*B*) manner. FMRFamide and ACh do not increase cAMP levels. In *A*, muscles were incubated in modulators for 5 min. In *B*, modulators were applied at 10^{-6} M. From 3 to 9 muscles were processed for each point.

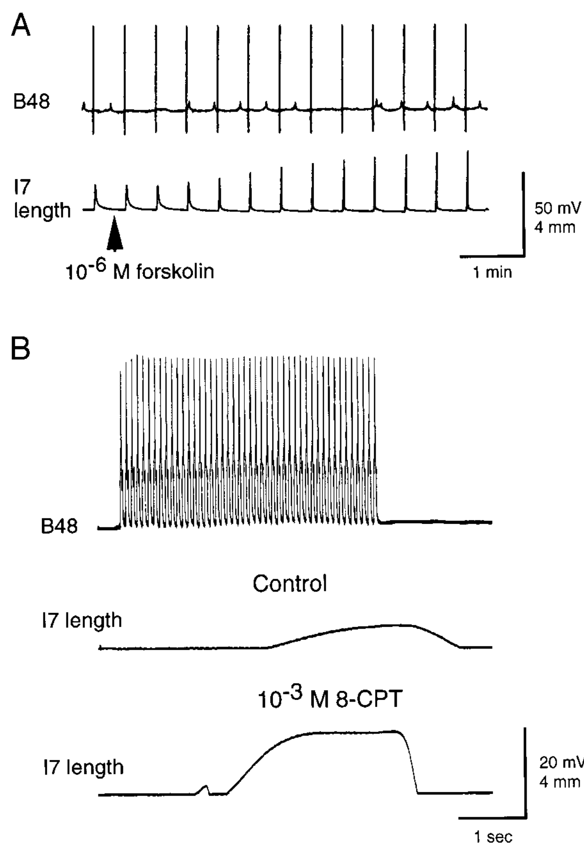

FIG. 12. Actions of membrane permeable cAMP drugs on B48 elicited contractions of the I7 muscle. *A*: 10^{-6} M forskolin was applied peripherally to a neuromuscular preparation (↑). Action potentials in motor neuron B48 are shown in the *top trace;* resulting muscle contractions are shown in the *bottom trace*. *B*: experiment conducted in the presence of 10^{-3} M 8-CPT cAMP. Action potentials in neuron B48 are shown in the *top trace.* A resulting contraction of the I7 muscle is shown in the middle trace under control conditions, i.e., before the cAMP analogue was added. The bottom trace shows a contraction of the I7 muscle after 10 min in 8-CPT-cAMP. For clarity, the action potentials generating the contraction shown in the *bottom trace* are not shown. *Middle* and *bottom*: appropriately aligned traces. Note that cAMP analogues increase the size (*A*) and relaxation rate (*B*) of motor neuron elicited muscle contractions, as do modulators that increase cAMP levels in opener muscles. *A* and *B* are taken from different preparations.

Our results taken together with those of Scott et al. (1997a) suggest that the induction of myogenic activity results, at least in part, from the activation of an $I_{Mod(cat)}$, which is primarily a Na current (Scott et al. 1997a). This inward current can be activated at resting membrane potentials and can summate with the inward current that results from stretch, which also appears to be primarily a Na current (Evans et al. 1996). Together these inward currents depolarize muscle fibers; this is likely to indirectly increase intracellular Ca levels. A direct effect of modulators on the characterized I_{Ca} is unlikely to occur in this context because I_{Ca} is activated at relatively depolarized membrane potentials (Scott et al. 1997a). It should be noted, however, that recent data suggest that there may be a second source of calcium in opener muscle fibers, i.e., muscle contractions appear to be activated at more negative voltages than the characterized I_{Ca} (Scott et al. 1997b). Because this source of Ca has not been specifically identified, it has not been possible to determine whether it is activated or enhanced by modulators.

Modulation of motor-neuron elicited contractions of the I7–I10 muscles: mechanisms of action of modulatory neurotransmitters

In this study, we show that the parameters of motor neuron elicited contractions of the opener muscles are altered by modulatory transmitters. Namely, we show that contraction size can be increased or decreased, muscle relaxation rate can be increased, contraction latency can be decreased, and the rate at which tension is developed can be increased. In the following text, we discuss likely mechanisms for these effects.

INCREASES AND DECREASES IN CONTRACTION SIZE. As described in the preceding text, Scott et al. (1997a) have shown that some modulators activate the inward Na current $I_{Mod(cat)}$ at resting membrane potentials. Although activation of this current is likely to play a role in producing increases in motor-neuron-elicited muscle contractions, a second inward current is also likely to be important in this context. Specifically, modulators that activate $I_{Mod(cat)}$ also enhance a dihydropyridine-sensitive "L"-type Ca current that is observed at relatively depolarized membrane potentials (Scott et al. 1997a). Scott et al. (1997a) suggested therefore that modulators that increase contraction size do so in part by their effect on I_{Ca}. They postulated that this effect is augmented by the activation of $I_{Mod(cat)}$.

Consistent with the general idea that modulation of inward currents will produce increases in contraction size, we found that the size of motor-neuron-elicited muscle contractions was increased by a modulator that primarily modulates inward currents at all concentrations (i.e., MM_B). In contrast, FMRFamide, which does not activate $I_{Mod(cat)}$ or enhance I_{Ca}, did not increase the size of muscle contractions. With respect to the specific roles of I_{Ca} and $I_{Mod(cat)}$, we found that increases in contraction size were observed when cAMP analogues were applied to neuromuscular preparations. Scott et al. (1997a)

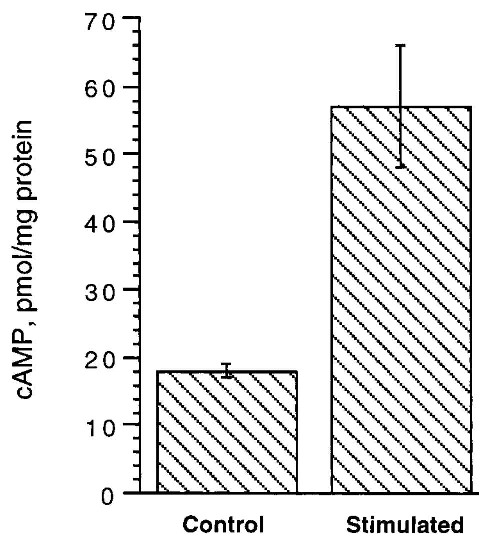

FIG. 13. Stimulation of the opener motor neuron B48 at physiological frequencies significantly increases cAMP levels in the I7 muscle [n = 7 for control (i.e., unstimulated) muscles; n = 6 for experimental (i.e., stimulated) muscles. SEs are indicated.]

have shown that effects of modulators on I_{Ca} are cAMP dependent, whereas effects of modulators on $I_{Mod(cat)}$ are not. Our data at least indicate therefore that increases in contraction size can be seen in the absence of effects on $I_{Mod(cat)}$. If effects of modulators on inward currents produce increases in contraction size, it might be expected that activation of outward currents would decrease contraction size. As discussed in the preceding text, Scott et al. (1997a) have characterized one such outward current, $I_{Mod(K)}$. FMRFamide specifically activates this current (Scott et al. 1997a) and does in fact decrease contraction size. Thus modulators that activate or enhance characterized inward currents do in fact increase contraction size, and a modulator that activates an outward current decreases contraction size.

We cannot, however, conclude that effects of modulators on the size of motor neuron-elicited muscle contractions are produced solely by effects on characterized currents. For example, MM_A increases contraction size at doses that do not produce significant increases in cAMP levels (i.e., at 10^{-9} M and 10^{-8} M; Figs. 9*B* vs. 11). Presumably, therefore I_{Ca} is not modulated. Although MM_A is likely to activate $I_{Mod(cat)}$ at these doses, it is possible that an additional current or currents are modulated. This is suggested by the fact that effects of MM_A and MM_B on contraction size are similar at 10^{-9} M and 10^{-8} M (Fig. 9*B*). Nevertheless, although both peptides similarly activate $I_{Mod(cat)}$ (Scott et al. 1997a), only MM_B increases cAMP levels at 10^{-9} M and 10^{-8} M (Fig. 11), and therefore presumably modulates I_{Ca}. Thus it is likely that there are two components to low dose effects of MM_B. Possibly the same is true for MM_A. If so, this current or currents are not cAMP dependent and have not been described.

INCREASES IN MUSCLE RELAXATION RATE. Biophysical correlates of modulator-induced increases in relaxation rate have not been observed in either opener muscles or ARC muscles. In the ARC neuromuscular system, data suggest that these types of effects result from a direct effect of modulators on the contractile machinery (Probst et al. 1994). More specifically, modulators appear to phosphorylate a large (i.e., >750 kDa) protein that is structurally related to the muscle protein twitchin (Heirerhorst et al. 1994; Probst et al. 1994). The phosphorylation state of this protein is well correlated with the relaxation rate of the muscle (Probst et al. 1994). Effects of modulators on twitchin are at least partially mediated through cAMP (Probst et al. 1994). A similar mechanism may be important in the opener neuromuscular system. We show that cAMP analogues do in fact produce increases in muscle relaxation rate.

EFFECTS ON CONTRACTION LATENCY AND THE RATE AT WHICH TENSION IS DEVELOPED. As is typical for molluscan muscle, a single motor neuron spike (Evans et al. 1996) generally does not elicit contractions of opener muscles. The opener motor neuron B48, however, fires in bursts during ingestive feeding behavior (Evans et al. 1996). Consequently, motor-neuron-elicited EJPs summate so that although a contraction is not elicited by the first EJP, it is elicited by subsequent EJPs. Contraction latency therefore can be decreased if EJPs that were previously subthreshold for eliciting contractions of muscle fibers become suprathreshold. This is one effect we observed (Fig. 10). In addition to decreases in contraction latency, modulators also increase the rate at which tension is developed in opener muscles. This appears to result from the fact that modulated EJPs continue to be more effective at producing tension increases in muscles than unmodulated EJPs. Because tension increases produced by modulated EJPs are larger, they summate more efficiently. This effect of 5-HT on contraction latency is different from the effect that is observed on the I3 muscle of *Aplysia* (Fox and Lloyd 1997, 1998). In the I3 muscle 5-HT increases EJP size, which results in greater EJP summation. In contrast, in the opener system, EJP size is not significantly increased and the EJP time constant actually is decreased. Consequently the summation of modulated EJPs in the opener muscle is actually reduced. Enhancement of tension occurs in spite of this because the effects of 5-HT on tension development are so powerful.

Although we cannot specifically assess postsynaptic and presynaptic contributions to the modulation seen in the opener system, we can at least conclude that postsynaptic effects of 5-HT application were observed, i.e., because the EJP time constant was decreased, a conductance increase has presumably occurred. As discussed in the preceding text, Scott et al. (1997a) have shown that 5-HT enhances or activates two inward currents in opener muscle fibers, an I_{Ca} and an $I_{Mod(cat)}$. Of these two currents, $I_{Mod(cat)}$ will be the most pronounced at resting membrane potentials or at slightly depolarized membrane potentials, such as those that will be reached during the course of one 10-mV EJP. Effects of 5-HT on $I_{Mod(cat)}$ therefore may be partially responsible for decreases in latency. If so the following may occur: 5-HT will activate $I_{Mod(cat)}$ and depolarize muscle fibers. Although motor-neuron-elicited EJPs will not be significantly increased in size, they will be occurring at more depolarized membrane potentials. Consequently, Ca influx will be enhanced. Obviously, however, biophysical changes in opener muscle fibers may not be completely responsible for decreases in contraction latency. Other as yet uncharacterized biochemical changes in muscle properties may occur in parallel.

Functional consequences of modulation of motor-neuron-elicited contractions of the I7–I10 muscles

Functional consequences of neuromuscular modulation have been modeled in the ARC system of *Aplysia*. The ARC muscles are radula closers and function as antagonists of the I7–I10 muscles. One important finding that has guided current conceptualizations of modulator function in the ARC neuromuscular system is that modulator release is enhanced as the rate at which feeding behavior is executed is increased, i.e., as animals are aroused (Cropper et al. 1990; Vilim et al. 1996a; Whim and Lloyd 1989). When animals are aroused, feeding behavior not only occurs more rapidly but bite strength also is increased (Weiss and Kupfermann 1977; Weiss et al. 1980). Consequently, muscle contractions must be increased in amplitude but must have limited durations because interbite intervals are decreased. If contraction duration is not limited, one muscle will not have relaxed before its antagonistic begins to contract (Weiss et al. 1992) and individual contractions will occur before the previous contraction has returned to baseline (Deodhar 1999). Modulators appear to be important in this context because they alter the NMT so that both contraction amplitude and muscle relaxation rate are increased (Cropper et al. 1988, 1990). Previous work in the ARC neuromuscular system therefore has suggested that modulators can be impor-

tant because they can affect muscle relaxation rate and determine when a contraction ends.

In this study, we show that modulators in the radula opener complex can additionally affect contraction duration by strongly affecting other parameters of motor neuron elicited muscle contractions. More specifically we show that modulators can decrease contraction latency and the rate at which tension is developed as a result of motor neuron activity. These effects actually are observed in conjunction with the modulatory effects that are striking in the ARC neuromuscular system, i.e., modulators affect contraction amplitude and muscle relaxation rate in the I7–I10 system as they do in the ARC neuromuscular system. Thus in the most general sense, modulators in the I7–I10 neuromuscular system presumably also act to alter the NMT so that contraction duration is decreased when behavior is executed more rapidly. In the opener complex, however, effects on both tension development and muscle relaxation presumably decrease contraction duration.

Increases in the rate at which tension is developed in the opener neuromuscular system are not, however, likely to exclusively result from the release of modulatory neurotransmitters. Posttetanic potentiation (PTP) is observed in this system. Consequently, even when intraburst stimulation parameters are kept constant and EJPs are larger and summate more efficiently, contractions are potentiated if the interburst interval is decreased. We therefore can speculate that modulators are released in the opener system when behavior is executed more rapidly, and it is important that contraction dynamics are adjusted so that contractions can be increased in size without corresponding increases in contraction duration. In considering effects on muscle relaxation rate, it is intuitively obvious why modulator release may be important. Current data indicate that increases in muscle relaxation rate cannot be produced by changes in primary transmitter release. In contrast, in considering effects of modulators on tension development, it is more difficult to appreciate why modulator release is necessary. When behavior is executed more rapidly interburst intervals obviously will decrease and tension development will be increased by the resulting PTP. Thus there appears to be convergence in the opener system in that as behavior is executed more rapidly the rate at which tension is developed will presumably increase both as a result of PTP and as a result of modulator release.

In summary, like modulators in the ARC neuromuscular system, modulators in the opener neuromuscular system are likely to be released when animals are aroused and behavior is executed relatively rapidly. Under these conditions, modulators are likely to alter the NMT so that muscles contract and relax more rapidly. These changes in muscle dynamics are likely to be important since they enable muscles to more faithfully "follow" neural activity.

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