Properties of Cholinergic Responses in Isolated Parapodial Muscle Fibers of Aplysia

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INTRODUCTION

The parapodia of opisthobranch molluscs comprise a bilateral pair of flaps, or wing-like extensions, of the dorsolateral body wall that fold over the back of the animal to cover the mantle cavity. In a small number of aplysids, the parapodia have become modified to serve also as well-muscularized swimming appendages that are flapped up and down rhythmically to propel the animal through the water. We have been studying the motor control of the parapodia in the swimming species Aplysia brasiliana (McPherson and Blankenship 1991a,b). Identified excitatory motoneurons in the pedal ganglia produce 1:1 excitatory junctional potentials (EJPs) in parapodal muscle fibers, and the muscle contracts in a graded fashion in proportion to the amount of summation and facilitation of EJPs; parapodal muscle fibers do not express action potentials. The EJPs are blocked by hexamethonium, a well-known specific blocker of cation-dependent cholinergic responses in Aplysia (e.g., Kehoe 1979), and acetylcholine-esterase has been identified histochemically in the muscle beds of the parapodia, suggesting that these motoneurons use acetylcholine as their transmitter (McPherson and Blankenship 1991a). Inhibitory junctional potentials (IJPs) occasionally are observed during in situ recordings from parapodal muscle fibers, but only excitatory motoneurons have been identified (McPherson and Blankenship 1991a,b).

Parapodal muscle fibers also are innervated by serotonergic modulatory neurons known as parapodal opener-phase (POP) cells (McPherson and Blankenship 1991c; Parson and Pinsker 1988). The amplitude of the cholinergic EJPs and the magnitude of the subsequent muscle contraction are both increased in the presence of exogenously applied serotonin or with concurrent activity in the modulatory POP cells (McPherson and Blankenship 1991c). Neither POP cell activity nor serotonin has any direct effect on muscle fiber resting potential or conductance; the amine works instead to modulate cholinergic transmission and muscle fiber calcium current (Laurienti and Blankenship 1996a,b, 1997).

We have studied the physiology of this neuromuscular synaptic complex as a model for aminergic modulation of synaptic transmission. However, the pharmacological properties of the cholinergic response have not been reported in detail. This paper considers this issue and develops evidence for a dual role of acetylcholine (ACh) at the neuromuscular junction (NMJ) in the parapodia of intact, swimming animals. Muscle fiber ACh receptors associated with excitatory motoneurons open cation-selective channels that lead to muscle depolarization and contraction. Other ACh receptors that are chloride selective and associated with putative inhibitory motoneurons could lead to fiber hyperpolarization and relaxation. Exogenous application of ACh to isolated muscle fibers activates both receptor types simultaneously and produces a voltage response that is relative small and variable and never produces muscle contractions.

Physiological, pharmacological, biochemical, and histochemical evidence clearly indicates that ACh is a common neurotransmitter in aplysidal molluscs, acting at a variety of neural-neural (Blankenship et al. 1971; Giller and Schwartz...
The inhibitory component is carried by chloride, activated simultaneously by any single motoneuron (Kozak et al. 1996). The excitatory cholinergic response. [A slow, K+-dependent ''fast'' inhibitory response and a cation-dependent hyperpolarizing, chloride-dependent response. In general, the properties of these two responses resemble those reported by others in Aplysia neurons and muscle. However, some significant differences occur that continue to obscure a clear pattern of ACh responses among molluscan excitable cells and their relationships to nicotinic ACh responses in vertebrate neurons and muscle. We further discuss the implication of the physiological properties of each response in the context of a behaving animal. An abstract containing preliminary data from some of these experiments has been reported previously (Laurienti and Blankenship 1994).

Methods

Specimens of A. brasiliana were collected from Laguna Madre near Port Isabel, Texas. They were housed in our Institute’s aquarium facility in large aquaria with recirculating artificial seawater (ASW) at room temperature and fed dried seaweed daily.

Dissociated parapodial muscle fibers

Animals used for these studies ranged in size from 30 to 300 g. Animals were anesthetized by injecting isotonic MgCl₂, into the foot sinus (dose, 33 ml/100 g body wt). The muscle tissue was dissociated using a modified version (Laurienti and Blankenship 1996a) of previously proposed methods (Brezina et al. 1994; Ishii et al. 1986). Briefly, the skin covering a parapodium was removed, and small pieces of muscle tissue were dissected out and placed into a vial containing 0.2–0.4% Type I collagenase. The vials were incubated at 30–33°C in a shaking water bath for 4–7 h. The enzyme solution then was removed with pipettes and replaced with ASW containing penicillin and streptomycin. Dissociated muscle fibers were stored ≤4 days in a chilled water bath at 13°C.

Isolated muscle fibers were embedded in agarose gel in a recording chamber before electrophysiological recordings according to the methods reported by Brezina et al. (1994). The chamber was placed on an inverted, phase-contrast microscope and attached to a perfusion system. The perfusion system allowed flow rates ≤10 ml/min; this provided rapid exchange of the chamber solution, which was ~200 µl. Previously published data have demonstrated rapid response times and adequate washout using this perfusion system (Brezina et al. 1994; Laurienti and Blankenship 1996a).

Electrophysiological methods

Conventional electronics were employed for intracellular recordings using an Axoclamp 2A amplifier (Axon Instruments, Foster City,
CA). Signals were recorded on a Gould (Valley View, OH) chart recorder and converted to digital signals on a Digidata 1200 (Axon Instruments) and stored on a PC computer using pClamp software (Axon Instruments). Dual-beam oscilloscopes (Tektronix, Beaverton, OR) were used for continuous monitoring of neural activity and to monitor the quality of the voltage clamp. Intracellular electrodes were pulsed from capillary glass on a horizontal puller. Electrodes were filled with KAc (3 M) and had resistances that ranged from 20 to 50 MΩ. The bath was grounded through an agar bridge.

Experimental data were collected under current- and voltage-clamp conditions using single electrodes. Current-clamp experiments were used to monitor membrane voltage responses to various pharmacological compounds. During all current-clamp experiments, 10-ms negative constant-current pulses were injected through the recording electrode to monitor changes in cell resistance. Muscle fiber current-voltage (I-V) relationships were obtained under voltage clamp using 1-s voltage ramps. Cells were clamped at a holding potential of −60 mV, and a voltage ramp was induced from −120 to 0 mV in the presence or absence of cholinergic compounds. These experiments allowed the determination of reversal potentials for specific cholinergic agonists. The physiological properties of the muscle membranes, including characterization of voltage-dependent and serotonin-induced currents, and the quality of the voltage clamp have been discussed previously in detail; these dissociated fibers are adequately spaced-clamped under our recording conditions (Laurienti and Blankenship 1996a,b, 1997). Because of the limited gain attainable with intracellular voltage-clamp recordings, the command voltage and the actual membrane voltage may slightly differ. However, the actual membrane voltage was recorded and was used in all data interpretation and figure preparation.

Solutions and drugs

Normal ASW was used to store isolated muscle fibers and to perform all experiments unless otherwise specified. ASW contained (in mM) 427 Na+, 499 Cl−, 10 K+, 10 Ca2+, 48 Mg2+, 3 HCO3−, and 26 SO4−. In some experiments Na propionate was used as a substitute for NaCl to produced 15% Cl− (propionate substituted)-SW. ACh, nicotine, hexamethonium, and Type I collagenase were obtained from Sigma; tubocurare, methylcarbamylcholine (MCC), and suberyldicholine were obtained from Research Biochemicals International (Natick, MS). All other chemicals used were obtained from either Sigma or Fisher. All drugs were applied by bath perfusion. Drugs typically were made in a stock solution in either ASW or distilled water and added to the bath chamber to yield the desired concentration.

RESULTS

Application of ACh (5–10 μM) to isolated muscle fibers results in an increase in membrane conductance that produces a voltage response that is quite variable in amplitude. ACh-induced responses, unlike motoneuron-induced EJPs, vary from hyperpolarizations in a few fibers to depolarizations in most fibers; an occasional fiber shows no voltage response to applied ACh but demonstrates a decrease in membrane resistance. This variability results from the simple fact that the resting potential and ACh reversal potential in these fibers are close to one another and vary independently such that in different fibers the ACh reversal level may be more negative or more positive than the resting level. On average, ACh produces a response with a reversal potential of −52 ± 2.1 (SE) mV (see following text and Fig. 8). The average resting membrane potential of isolated parapodial muscle fibers was −56 ± 1 mV (n = 40) (see Laurienti and Blankenship 1996a). Although the cholinergic response varies among muscle fibers, the response never depolarizes cells to contraction threshold. In fact, most responses result in membrane voltages between −50 and −60 mV. Such potentials are suggestive of a chloride component to the ACh-induced response.

To determine if chloride contributes to the hyperpolarizing ACh response, we compared the reversal potential for large ACh-induced hyperpolarizations in normal ASW and in low-chloride ASW. Figure 1A demonstrates a typical current-clamp experiment where a muscle fiber is held at varying membrane potentials and ACh is applied. Under such conditions, an extrapolated reversal potential can be determined. In the example shown, the ACh reversal potential is −68 mV. In the presence of 15% Cl− (propionate substituted)-SW, the response is shifted such that the new reversal potential is extrapolated to −40 mV. Figure 1B shows data from three cells in which similar cholinergic responses were obtained in ASW and in low-chloride sea water. These data demonstrate that, on average, the reversal potential for such ACh responses in ASW is −69 mV, and in 15% Cl−-SW, the cholinergic reversal potential shifts to −41 mV, a displacement of 28 mV. The predicted Nernstian shift for a purely chloride-dependent response with a change in extracellular [Cl−] of this magnitude is 48 mV. The chloride reversal potential we have estimated is somewhat more negative than that measured in most other molluscan neurons (Gardner and Moreton 1985; Kehoe 1972a) and muscle fibers (Blankenship et al. 1977; Kozak et al. 1996 and references therein). This could indicate that parapodial fibers have a lower internal resting chloride concentration than that of most other neurons and muscle or that potassium ions as well as chloride are contributing to membrane current during the cholinergic response. The fact that the cholinergic response does not shift to the extent predicted by the Nernst equation also might suggest that chloride is not the sole ion contributing to the cholinergic response, but it also may reflect a passive reduction in [Cl−]i when [Cl−]o is reduced greatly or that propionate has some permeability through the chloride channel (see Blankenship et al. 1977; Kehoe 1972a; Kozak et al. 1996). Most importantly, we believe that those muscle fibers that produce a relatively large hyperpolarizing response to ACh represent fibers that are expressing a preponderance of chloride-sensitive receptors. ACh responses in most fibers were, however, small- to moderately sized depolarizing responses or small hyperpolarizations and could represent activation of additional receptors.

Several pharmacological compounds were identified that allowed us to distinguish two components to the cholinergic response. The pharmacological properties of the first component are illustrated in Fig. 2. This response, like that in Fig. 1, is another example of a relatively pure, uncontaminated version of a chloride-sensitive hyperpolarization. This component is activated by ACh and selectively mimicked by suberyldicholine (n = 42, Fig. 2A), nicotine (n = 36; Fig. 2B), and carbacol (n = 12; data not shown here; see following text). This hyperpolarizing response is blocked by curare (n = 30; Fig. 2C) but not by hexamethonium (n = 13; Fig. 2D). Nicotine consistently produces, like suberyldicholine, only hyperpolarizing responses in these muscle fibers and these responses are blocked by curare (data not shown, but see following text).

Many muscle fibers respond to ACh with a net depolarization, but the use of suberyldicholine reveals that this response is composed of both a depolarizing component and an occult
chloride-dependent hyperpolarization that apparently is masked by the simultaneously occurring large depolarizing response (Fig. 3A).

The second component, represented by the net depolarizing response to ACh, is blocked by hexamethonium (n = 13; Fig. 3B). Hexamethonium also blocks motoneuron-induced EJPs in reduced muscle preparations (McPherson and Blankenship 1991a). The depolarizing response also is mimicked selectively by MCC (n = 23; Fig. 4). As seen in Fig. 4A, increasing the dose of MCC produces a physiological dose response. All subsequent experiments were conducted using the 500 μM concentration of MCC as this is the lowest dose to induce a maximal depolarization. Neither nicotine nor carbachol was ever seen to activate this second, depolarizing component of the ACh response (see following text). The MCC-induced response is blocked partially by hexamethonium (Fig. 4B) and by curare (Fig. 4C).

The muscle-fiber responses to ACh were only occasionally purely of either the depolarizing or hyperpolarizing type. Most responses to ACh were found by use of selective agonists (Fig. 4D) or antagonists (Fig. 4E) to comprise both a hyperpolarizing (chloride-dependent) and a depolarizing (caticonic-dependent) component, these occurring in different proportions from cell to cell. The depolarizing component is activated selectively by MCC and blocked selectively by hexamethonium. The hyperpolarizing, chloride-dependent response is activated selectively by suberyldicholine (and, unexpectedly, by nicotine, which in other molluscan preparations also activates the caticonic, depolarizing response). Curare blocks both responses. Others have reported that α-bungarotoxin selectively blocks the chloride-dependent fast hyperpolarizing response (Kehoe et al. 1976; Kozak et al. 1996). We have not yet tested whether α-bungarotoxin blocks the hyperpolarizing response.
Using voltage clamp it is possible to determine the reversal potential of the pharmacological agonists by inducing voltage ramps in the presence and absence of drug. Such data are plotted on an $I-V$ curve with the crossing points of the two curves being the reversal potential for the agonist. Figure 5 demonstrates $I-V$ curves for suberyldicholine, MCC, carbachol, and nicotine, with the lower portion of each panel representing difference currents. The difference currents were obtained mathematically by subtracting the current recorded in control ASW from that recorded in the presence of the cholinergic agonist. These difference currents represent the current that is activated by the specific agonists and are free of any leak currents or voltage-gated currents. As demonstrated in this figure, suberyldicholine induces a current that reverses near $-70 \text{ mV}$ (near the range of the chloride reversal potential). Carbachol and nicotine induce currents that reverse near $-60 \text{ mV}$, again indicating predominant activation of a chloride current. MCC, however, as an agonist more selective for the depolarizing component of ACh responses, induces a current

FIG. 3.  A: 5 μM Ach induces a depolarization in a cell held near the resting membrane potential. Application of 5 μM suberyldicholine to the same cell induces a hyperpolarization typical of the hyperpolarizing chloride response. B: in a cell with a resting potential near the reversal potential for Cl, 50 μM hexamethonium blocks most of the Ach-induced depolarization, leaving a small depolarizing response. (Remaining response is likely to be an inverted chloride-dependent response, but it could represent some cationic response not blocked by hexamethonium.) Effects of hexamethonium reversed after washout. Voltage and time calibrations for all traces are to the right of A.

FIG. 4. Pharmacology of the cationic Ach response. A: micromolar concentrations of methylcarbamylcholine (MCC) produce depolarizations in isolated muscle fibers in a dose-dependent fashion. B: 50 μM hexamethonium (50 Hex) largely blocks the depolarization caused by 500 μM MCC. C: although 50 μM curare effectively blocks the Ach hyperpolarization as shown in Fig. 2C, it also blocks a portion of the MCC (500 μM) depolarizing response. D: in a single fiber, 10 μM Ach, 500 μM MCC, and 10 μM suberyldicholine (Suberyl) all induce different responses. E: 50 μM hexamethonium selectively blocks the Ach (10 μM)-induced cationic response, revealing the underlying hyperpolarization. Voltage and time calibrations for all traces are shown to the right of C.

FIG. 5. Current-voltage ($I-V$) curves for suberyldicholine (Suberyl), MCC, carbachol, and nicotine. Voltage ramps of 1 s were applied 1st in control ASW, then in the presence of the various compounds at their respective concentrations. Beneath each pair of records a difference current, computer subtracted, is presented. Vertical dashed line represents the reversal level for the current in the presence of each compound. Suberyldicholine selectively activates $\alpha_\text{ACH}$ and reverses at $-68 \text{ mV}$. MCC activates both Ach receptors; however, this drug is more efficacious at the cationic receptor. In this cell, MCC had a reversal potential of $-32 \text{ mV}$. Carbachol and nicotine produce currents effectively indistinguishable from those produced by suberyldicholine, acting predominantly also on $\alpha_\text{ACH}$. None of the difference currents displays significant voltage dependence.
that reverses near $-30 \, \text{mV}$, which is below (more positive than) the contraction threshold for these cells. This current presumably is carried by a mixed cationic current, probably including sodium, potassium, and calcium.

Figure 6 shows an experiment in which the effects of ACh, suberyldicholine, and MCC were all recorded in the same cell. The experiment then was repeated in the presence of hexamethonium which selectively antagonizes the depolarizing current. This figure shows that in the presence of hexamethonium all three compounds activate a current with a reversal potential near $-65 \, \text{mV}$; this is consistent with the chloride reversal potential. As previously demonstrated, suberyldicholine is selective for the hyperpolarizing response. ACh is nonselective and activates both the hyperpolarizing and depolarizing response with differing selectivities across different cells, and, finally, MCC predominantly activates the depolarizing response but does activate the chloride response to some degree. It can be seen from the Fig. 6, right, that in the presence of hexamethonium, the amplitude of the MCC current is smaller than that activated by ACh or by suberyldicholine.

To more clearly demonstrate the individual components of the ACh-induced current, we used computer-aided subtraction protocols. In Fig. 7 the chloride and cationic components of the ACh current have been isolated. The chloride component (ACh(Cl)) was derived by subtracting the control current in Fig. 6B from the current recorded in response to suberyldicholine in the presence of hexamethonium (Fig. 6B). This trace represents the hexamethonium-insensitive current free of leak and background currents, or the pure inhibitory response. The cationic current [ACh(cat)] was isolated by subtracting the MCC-induced current in the presence of hexamethonium (Fig. 6B) from the MCC current obtained in normal ASW (Fig. 6A). Therefore this trace represents only the hexamethonium-sensitive current and any chloride component has been subtracted out. As is evident from Fig. 7, the chloride current reverses at $-75 \, \text{mV}$, which is near the reversal potential for the chloride ion. The presumptive cationic current reverses near $-20 \, \text{mV}$, which is well below (more positive than) the contraction threshold for these cells.

We consistently observed muscle contractions when MCC was applied to the chamber bath (data not shown). However, no contractions were observed in response to either ACh or suberyldicholine. Figure 8 is a graph of the reversal potential of these three cholinergic agonists. The reversal potentials were determined using voltage-clamp experiments; each compound was tested in at least nine different cells. The graph clearly demonstrates that MCC has an average reversal potential below (more positive than) the contraction threshold ($-40 \, \text{mV}$) (Laurienti and Blankenship 1996a) for the muscle fibers, whereas ACh and suberyldicholine have reversal potentials above (more negative than) the contraction threshold.

**DISCUSSION**

We have presented the pharmacological properties of the cholinergic response of isolated parapodial muscle fibers. In the whole muscle, motoneurons induce 1:1 EJPs that are antagonized by hexamethonium, which also blocks motoneuron-induced muscle contractions. Of interest, however, is the fact that application of ACh to the isolated muscle cells resulted in mixed responses, relatively small hyperpolarizations or depolarizations but never contractions. The average reversal potential of ACh as determined in voltage-clamp experiments was $-52 \pm 2.1 \, \text{mV}$, which is above (more negative than) the contraction threshold for these cells (see Fig. 8) and near the normal resting potential for these fibers ($-56 \, \text{mV}$) (Laurienti and Blankenship 1996a).

The ACh-induced response has two pharmacologically distinct components: one component is carried predominantly by chloride and the other component is likely to be carried by mixed cations. The chloride-dependent response is activated by suberyldicholine and closely mimicked by carbachol and nic-
muscle fibers closely resemble those of vertebrate skeletal properties of the chloride-dependent receptor in parapodial and Kehoe 1975; Kehoe 1972b, 1979; Kozak et al. 1996), the (data not shown).

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partially by curare. This response has a reversal potential of 6 1.5 mV, which is close to the calculated Cl- reversal potential in these fibers. ACh had an average reversal potential of −52 ± 2.1 mV, which is more negative than contraction threshold for these fibers. This response is due to a co-activation of AChCl and AChCat. The MCC-induced responses reversed, on average, at −34.3 ± 1.3 mV, which is more positive than the contraction threshold. When applied alone, MCC was the only drug tested that was capable of inducing muscle contractions. Although MCC activates both ACh receptors, it is more effective on the cationic response.

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In keeping with the findings of other investigators (Ascher and Kehoe 1975; Kehoe 1972b, 1979; Kozak et al. 1996), the properties of the chloride-dependent receptor in parapodial muscle fibers closely resemble those of vertebrate skeletal nAChRs. However, the lack of sensitivity of the receptors controlling the cationic response to nicotine and carbachol in these fibers appears to set them apart from other molluscan neurons and muscle fibers and makes their relationship to vertebrate neuronal nAChRs unclear. Although there are some small differences in the pharmacological profiles of buccal and parapodial cation-related receptors, it is clear that each muscle type expresses both a cation- and chloride-dependent receptor in dissociated fibers. Despite this, the fibers respond differently to exogenously applied ACh that simultaneously activates both receptors, and the fibers appear to be innervated in different ways in the intact animal.

In buccal muscle of A. californica, application of ACh always results in a depolarization and one sufficient to induce a muscle contraction (Kozak et al. 1996). These findings are in keeping with observations that ACh applied to whole buccal muscle causes contractions (Cohen at al. 1978). In parapodial muscle fibers, this is not the case. Furthermore we have observed in several experiments using intact parapodial muscle in reduced preparations of both A. californica and A. brasiliana that direct application of ACh, either by superfusion onto skinned muscle preparations or by perfusion through parapodial blood vessels, even in the presence of cholinesterase inhibitors, never produces contractions of parapodial muscle. Furthermore in buccal muscle, both intact and dissociated from both species, we have always found the muscle to respond to exogenous ACh with brisk contractions (unpublished observations). On the other hand, the application of MCC, which is more selective for the cationic response, does consistently induce contractions in dissociated parapodial muscle fibers. Furthermore the MCC response is antagonized by hexamethonium, the same antagonist that blocks motoneuron-induced EJPs and contractions in the whole muscle. These data pose an interesting contrast concerning the physiological roles of the two ACh receptors in the intact animal. To account for the discrepancy, we propose two models for the arrangement of the cholinergic receptors in the behaving animal (Fig. 9).

The first model, the one we believe most likely applies to intact parapodial muscle, involves receptor segregation where the excitatory receptors are clustered in the synaptic regions of excitatory motoneurons and the inhibitory receptors are clustered separately at the synaptic regions of inhibitory motoneurons. Although no inhibitory motoneurons have been identified, IJPs have been recorded in muscle fibers in intact parapodial preparations (McPherson and Blankenship 1991a).
This model also requires that the excitatory and inhibitory motoneurons be activated selectively and that contractions would occur in response to selective activation of excitatory motoneurons, which is in fact the case (McPherson and Blankenship 1991a,b).

The second model, one believed to obtain in buccal muscle (Kozak et al. 1996), does not require selective receptor clustering but instead suggests that the two receptor types are mingled close together and are activated by a single class of excitatory motoneuron. This model does require that the number or efficacy of functional excitatory receptors be greater than the number or efficacy of inhibitory receptors so that the net effect of Ach and of all motoneuron activity is to depolarize the muscle cell. The role of the chloride-dependent response in buccal muscle is to limit the amount of depolarization Ach can produce in a single fiber, and to do so by being colocalized and co-activated at a common synaptic site with the cationic receptor not separated from the excitatory site as a unique and independent locus for JPs (Kozak et al. 1996).

This model is less likely to obtain in parapodial muscle because the Ach reversal potential is near the resting potential, and exogenous Ach application never induces a contraction and often produces a net hyperpolarization of fibers. In other words, application of Ach to isolated parapodial fibers does not mimic the response of fibers to excitatory motoneuron input as is the case in buccal fiber responses. Furthermore, unlike the situation in buccal muscle (Cohen et al. 1978), JPs are observed in parapodial muscle and serve as candidates for the physiological response of a unique chloride-dependent input from cholinergic inhibitory motoneurons. However, it is possible that enzymatic dissociation procedures may alter either the excitatory cholinergic receptors or the inhibitory ones so as to confer different response properties on isolated fibers compared with those in situ. It is also possible that either of two types of clustered receptor could respond differently with repetitive activation due to desensitization or sensitization or either type, as has been observed in certain cholinergic neural synapses in Aplysia (Wachtel and Kandel 1971). In preliminary experiments where we have made repetitive applications of Ach to isolated fibers, we have seen no evidence of significant alterations in response amplitude with multiple stimulations. Thus it is not possible at this time to be certain whether two separate receptor populations are activated by specific motoneurons, but this seems the most likely case. The presence of separate excitatory and inhibitory inputs to parapodial muscle, using the cationic and chloride channels, respectively, could serve at least two roles: inhibitory inputs to one set of muscles could complement excitation of antagonists during the alternating up-and-down flaps of the parapodia during swimming, and direct inhibitory input may play a special role in muscle control when the parapodia are not utilized for swimming and instead are folded quietly over the back of the animal to enclose the mantle cavity.

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