Serotonergic Modulation of a Voltage-Gated Calcium Current in Parapodial Swim Muscle From *Aplysia brasiliana*

P. J. LAURIENTI AND J. E. BLANKENSHIP

*Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77555-1069*

Laurienti, P. J. and J. E. Blankenship. Serotonergic modulation of a voltage-gated calcium current in parapodial swim muscle from *Aplysia brasiliana.* J. Neurophysiol. 77: 1496–1502, 1997. Here we describe the effects of serotonin (5-HT) on dissociated parapodial muscle fibers from *Aplysia brasiliana.* 5-HT has previously been implicated as a modulatory transmitter at the parapodial neuromuscular junction. Exogenously applied or endogenously released 5-HT increases the amplitude of motoneuron-induced excitatory junctional potentials and contractions in parapodial muscle. Exogenously applied 5 μM 5-HT increases the amplitude of a voltage-gated inward calcium current in isolated muscle fibers by an average of 42% in response to a voltage step from −70 to −10 mV. The amplitude of the inward current was increased at all voltages tested, with the peak increase occurring between −30 and −20 mV. The dihydropyridine calcium channel antagonist nifedipine (10 μM) blocked this effect of 5-HT. The data indicate that 5-HT increases a previously identified calcium current in parapodial muscle fibers that is similar to the vertebrate L-type current. Although several types of K⁺ channels exist in these fibers, including Ca²⁺-dependent K⁺ channels, the results suggest that 5-HT has little effect on these currents. Parapodial muscle contractions during swimming behavior occur in response to bursts of motoneuron action potentials that produce graded muscle depolarizations that occur over a 1- to 2-s period rather than being instantaneous or rapid responses as might be produced by one or two action potentials or a brief voltage step. With the use of 1-s voltage ramps, we attempted to mimic physiological depolarization and demonstrate that 5-HT is able to increase the amplitude of the inward calcium current. The data presented in this paper provide evidence that 5-HT increases the Ca²⁺ current, which may be one mechanism by which 5-HT modulates muscle contractions during swim behavior.

**INTRODUCTION**

Aside from the established actions of amines as transmitters or modulators of heart muscle and vascular smooth muscle in vertebrates (Bean 1989; Benham and Tsien 1988; Deckert et al. 1994; Reuter 1983; Trautwein et al. 1986) and invertebrates (Liebeswar et al. 1975; Mayeri et al. 1974; Sawada et al. 1984), it is also well known that amines can serve as hormonal or heterosynaptic modulators at neuromuscular junctions (NMJs) involved in motor systems. Catecholamines and serotonin (5-HT) can modulate activity at vertebrate skeletal muscle NMJs (Arreola et al. 1987; Hirai and Koketsu 1980; Kuba 1970); but invertebrate preparations have offered an especially rich variety of examples of modulation of neuromuscular transmission by aminergic compounds, most commonly 5-HT and/or octopamine. In lobster and crayfish, 5-HT enhances neuromuscular transmission at postural muscles (Beltz and Kravitz 1986; Kravitz et al. 1985); in the locust, octopamine enhances transmission in the extensor tibiae muscle (Evans and Myers 1986; O’Shea and Evans 1979). In mollusks, 5-HT enhances neuromuscular transmission in a variety of buccal muscle preparations (Brezina et al. 1994d; Kobayashi and Muneko 1980; Lotshaw and Lloyd 1990; Weiss et al. 1978; Yoshida and Kobayashi 1995; Zoran et al. 1989) and in locomotor muscle (McPherson and Blankenship 1991c; Satterlie 1995). In most of these preparations the amine has a major effect on the postsynaptic element (muscle fiber) of the NMJ, but in some cases 5-HT acts presynaptically to enhance transmitter release. Until recently, rather few details were available concerning the mechanism of action of modulatory amines in muscle, but it is now clear that one effect of these compounds is to enhance L-type-like calcium currents in some muscle fibers (Arreola et al. 1987; Brezina et al. 1994d).

We have shown that 5-HT modulates transmission at the NMJ of parapodial muscle in the swimming gastropod mollusk *Aplysia brasiliana* (McPherson and Blankenship 1991c). The swim behavior is controlled by neural circuits located in the cerebral (Gamkrelidze et al. 1995a) and the two pedal (von der Porten et al. 1982) ganglia. Each pedal ganglion contains cholinergic motoneurons and a cluster of neurons designated parapodal opener phase (POP) cells that burst rhythmically in phase during parapodial opening (McPherson and Blankenship 1991a,b; Parsons and Pinsker 1988; von der Porten et al. 1980). McPherson and Blankenship (1991c) demonstrated that POP cells are serotonergic and play a facilitatory role at the motoneuron-activated parapodial NMJ by approximately doubling the amplitude of motoneuron-induced excitatory junctional potentials (EJPs) and increasing the amplitude of parapodial muscle contractions by an average of 300%; POP cell activity also causes a 40% increase in the rate of muscle relaxation. Exogenously applied 5-HT faithfully mimics the facilitatory effects of POP cell firing. We have preliminary evidence that 5-HT increases a Ca²⁺ component of motoneuron action potentials (Gamkrelidze et al. 1995b; personal observations), and this may account for a portion of the facilitation at the NMJ. It is also possible that POP cells modulate neuromuscular activity by a direct action on parapodial muscle fibers. The current study demonstrates the direct effect of 5-HT on isolated parapodial muscle fibers. Two distinct fiber populations have been identified in parapodial muscle (Laurienti and Blankenship 1996a). Type I fibers contain three voltage-gated membrane currents, including the delayed rectifier, the A current, and an inward Ca²⁺ current. Similar experiments on *Aplysia californica* (Brezina et al. 1994a–c) and on *Philine aperta* (Dorsett and Evans 1991) buccal muscle have revealed similar voltage-
activated currents. Type II parapodial muscle fibers contain these three voltage-gated currents as well as two Ca$^{2+}$-dependent K$^+$ currents (Laurienti and Blankenship 1996b).

The two Ca$^{2+}$-dependent K$^+$ currents can be characterized and differentiated, one being a rapid, transient current $[I_{K(Ca)}]$, the other a slower, nonactivating current $[I_{K(Ca/o)}]$. The Ca$^{2+}$-activated currents identified in parapodial muscle fibers appear to be different from those in Aplysia buccal muscle (Brezina and Weiss 1995). The experiments reported in this paper were all performed on type II fibers because they have more pronounced inward Ca$^{2+}$ currents (Laurienti and Blankenship 1996a).

METHODS

Specimens of A. brasiliana were collected from Laguna Madre near Port Isabel, TX. Specimens were housed in the aquarium facility of the Marine Biomedical Institute in large aquaria with recirculating artificial seawater (ASW) at room temperature and fed dried seaweed (laver) daily. All experiments were performed at room temperature. The methods described below for dissociating and recording from individual muscle fibers are the same as those used successfully by us for studies of voltage-gated ionic currents in these fibers (Laurienti and Blankenship 1996a). These methods are briefly reviewed here.

Muscle fiber preparation

Animals used for muscle dissociations ranged in size from 30 to 300 g. They were first anesthetized by injection of isotonic MgCl$_2$ (33 ml/100 g body wt) into the foot sinus. The muscle tissue was dissociated with the use of a modified version of the methods proposed by Ishii et al. (1986). Muscle fibers were dissociated in 0.2–0.4% type I collagenase in ASW. After 4–7 h of incubation, the enzyme solution was replaced with ASW. This muscle fiber suspension was supplemented with 100–250 U/ml penicillin and 100–250 µg/ml streptomycin and stored at 13°C until used. Under these conditions, muscle fibers were viable for up to 4 days. To immobilize muscle fibers for recordings, cells were embedded in low-melting-point agarose (Gibco-BRL; Brezina et al. 1994a), in which individual fibers could be easily visualized for impalement and recording on the stage of an inverted phase-contrast microscope. The bathing solution was normal ASW, unless otherwise noted, to which stock drug solutions could be added to yield desired final concentrations. The perfusion system allowed precise control over the concentration of drugs or ions to which the cells were exposed.

Electrophysiological equipment

Intracellular recordings were amplified with the use of an Axoclamp 2A amplifier (Axon Instruments). Signals were recorded permanently on a Gould chart recorder and converted to digital signals on a Digidata 1200 and stored on a computer with the use of pClamp software. A dual-beam oscilloscope was used for continuous monitoring of neural activity. An additional oscilloscope was employed to monitor the state of the voltage-clamp of Ca$^{2+}$ currents. The two Ca$^{2+}$-dependent K$^+$ currents can be characterized and differentiated, one being a rapid, transient current $[I_{K(Ca)}]$, the other a slower, nonactivating current $[I_{K(Ca/o)}]$. The Ca$^{2+}$-activated currents identified in parapodial muscle fibers appear to be different from those in Aplysia buccal muscle (Brezina and Weiss 1995). The experiments reported in this paper were all performed on type II fibers because they have more pronounced inward Ca$^{2+}$ currents (Laurienti and Blankenship 1996a).

RESULTS

Having recently characterized several voltage-gated membrane currents in isolated parapodial muscle fibers (Laurienti and Blankenship 1996 a,b), we sought to determine whether 5-HT was able to influence the amplitude of Ca$^{2+}$ currents. Muscle contractions are dependent on extracellular Ca$^{2+}$, because they are blocked in the absence of Ca$^{2+}$ or in the presence of Ni$^{2+}$ or Co$^{2+}$. By increasing Ca$^{2+}$ currents, 5-HT should increase the magnitude of the muscle contraction induced by a voltage step, a voltage ramp, or a constant amount of acetylcholine, which is the endogenous transmitter at the NMJ (McPherson and Blankenship 1991a). The present studies measured the effects of 5-HT on muscle Ca$^{2+}$ currents but did not directly measure muscle contractions.

Ca$^{2+}$ currents were recorded from muscle fibers with the...
large outward currents blocked with 50 mM TEA and 10 mM 4-AP. Ca\(^{2+}\) currents could be elicited by passing either depolarizing voltage steps or voltage ramps through the recording electrode. Fig. 1A shows unsubtracted current records from a typical cell when stepped from −70 to −10 mV under control conditions in the presence of 50 mM TEA and 10 mM 4-AP (trace labeled Ca), after the addition of 5 µM 5-HT (trace labeled Ca+5-HT) and in 0Ca(Co)SW (trace labeled Co). This figure demonstrates a previously identified inward Ca\(^{2+}\) current in type II fibers recorded in the presence of TEA and 4-AP (Laurienti and Blankenship 1996a). This inward current is enhanced by 5 µM 5-HT and blocked by 0Ca(Co)SW. After the addition of Co\(^{2+}\), a residual outward current was usually observed. The enhancement of the inward Ca\(^{2+}\) current observed in the presence of 5-HT may be due to a decrease in the amplitude of this residual outward current. However, addition of 5-HT to cells in 0Ca(Co)SW containing 50 mM TEA and 10 mM 4-AP had no effect on this residual outward current (n = 3, data not shown). In a few cases, Ca\(^{2+}\)-activated currents, especially $I_{K(Ca)}$, appeared to contribute to TEA- and 4-AP-insensitive outward current. In these cases, no attempt was made to analyze the effect of 5-HT on the Ca\(^{2+}\) current because the effect of 5-HT on the voltage-sensitive Ca\(^{2+}\) current could be contaminated by any change in the Ca\(^{2+}\)-activated potassium current.

$I$-$V$ relationships reveal that the Ca\(^{2+}\) current amplitude was increased at all voltages without a change in the activation threshold and with the maximal increase occurring just before peak (Fig. 1, B and C). In 7 cells tested, 5-HT increased the peak amplitude of the Co\(^{2+}\)-subtracted Ca\(^{2+}\) current by an average of 42 ± 9.6% (mean ± SE). Ca\(^{2+}\) current $I$-$V$ curves were constructed with the use of Co\(^{2+}\)-leak-subtracted currents so that all leak current and any Co\(^{2+}\)-insensitive voltage-gated currents were subtracted from the records before the data were plotted. Although TEA and 4-AP have been shown to block both voltage-sensitive and Ca\(^{2+}\)-dependent K$^+$ currents (Laurienti and Blankenship 1996a,b), it remained possible that 5-HT was actually decreasing an unblocked portion of the Ca\(^{2+}\)-dependent K$^+$ currents. Although it was not possible to determine conclusively the effect of 5-HT on the Ca\(^{2+}\)-dependent K$^+$ currents (see DISCUSSION), we were able to measure the effect of 5-HT on inward Ba\(^{2+}\) currents (Fig. 2), a condition in which the Ca\(^{2+}\)-activated K$^+$ currents are not active (Laurienti and Blankenship 1996b). As was reported for the Ca\(^{2+}\) current, 5-HT increased the amplitude of the Ba\(^{2+}\) current at all potentials tested. Furthermore, 5-HT increased the am-

![Diagram](http://jn.physiology.org/doi/fig/10.220.33.5/55919690e/mr4709-02-9713:56:28)
FIG. 2. Effect of 5-HT on Ba\(^{2+}\) currents in dissociated muscle cells. **A**: superimposed Ba\(^{2+}\) currents recorded under control conditions in the presence of TEA and 4-AP (trace labeled Ba\(^{2+}\)) , after the addition of 5 \(\mu\)M 5-HT (trace labeled Ba\(^{2+}\)+5-HT) and in 0 Ba\(^{2+}\)/10 mM Co\(^{2+}\) artificial seawater (ASW) (trace labeled Co\(^{2+}\)). **B**: I-V curve for control Ba\(^{2+}\) current (●) and after the addition of 5-HT (○). As with inward Ca\(^{2+}\) currents, 5-HT enhanced the amplitude of the inward Ba\(^{2+}\) current at all voltages tested.

The Ca\(^{2+}\) current in parapodial muscle fibers has previously been identified as L-like because it has kinetic properties similar to the vertebrate L-type current and is blocked by 10 \(\mu\)M nifedipine (Laurienti and Blankenship 1996a). In the presence of 10 \(\mu\)M nifedipine, 5-HT no longer enhanced the inward Ca\(^{2+}\) current (Fig. 3, \(n = 4\)). This figure demonstrates an experiment in which the Ca\(^{2+}\) current was blocked before the addition of 5-HT. After the addition of 5-HT, the current was not enhanced and is superimposed over the nifedipine trace. The I-V curves shown in Fig. 3B are Ca\(^{2+}\) currents obtained under control conditions, in the presence of 10 \(\mu\)M nifedipine, and after the addition of 5-HT. This figure demonstrates that nifedipine blocks the Ca\(^{2+}\) current and that this prevents any effect of 5-HT.

All of the data reported above were from experiments in which voltage steps 80 ms in duration were used to elicit the inward Ca\(^{2+}\) currents. However, in the intact animal, muscle contractions during swimming are induced by summing EJPs from bursts of motoneuron action potentials. The responses are relatively slow and summate to produce ramplike depolarizations (McPherson and Blankenship 1991a). To determine whether 5-HT has effects at all voltages throughout a gradual depolarization, we attempted to mimic the natural time course of muscle depolarization with the use of 1-s voltage ramps to elicit inward Ca\(^{2+}\) currents (Fig. 4). In normal ASW, the voltage ramp induced an outward current similar to that reported previously (Laurienti and Blankenship 1996a). After the blockade of the outward K\(^+\) currents with 50 mM TEA and 10 mM 4-AP, an inward Ca\(^{2+}\) current was observed. In the presence of 5-HT, the inward Ca\(^{2+}\) current elicited by the voltage ramp was increased over all potentials in a manner similar to that shown in the voltage step experiments (see Fig. 3). In the presence of 0Ca(Co)SW, the 5-HT-sensitive inward current was blocked.

**DISCUSSION**

The experiments performed here on isolated muscle fibers provide evidence that a portion of the facilitatory effect of 5-HT on parapodial contractions may be achieved by increasing the amplitude of voltage-activated Ca\(^{2+}\) currents in muscle fibers. The effects of 5-HT on inward Ca\(^{2+}\) currents must be interpreted carefully because at least a small part of the enhancing effects could be due to a decrease in Ca\(^{2+}\)-activated K\(^+\) currents. However, Ba\(^{2+}\) can substitute for Ca\(^{2+}\) as the charge carrier for the inward current (Laurienti and Blankenship 1996a), and similar effects of 5-HT were observed when Ca\(^{2+}\) was replaced with Ba\(^{2+}\) (Fig. 2). Because it has previously been demonstrated that in the presence of Ba\(^{2+}\), Ca\(^{2+}\)-activated K\(^+\) currents are blocked (Laurienti and Blankenship 1996b), this shows a direct and major effect of 5-HT on the calcium channels. We attempted to detect any effect of 5-HT on the Ca\(^{2+}\)-activated K\(^+\) currents, but the results were inconclusive because it was not possible to separate these currents from the voltage-gated Ca\(^{2+}\) and K\(^+\) currents. However, it is not likely that the 5-HT effects observed here were due to decreasing Ca\(^{2+}\)-activated K\(^+\) currents, because 5-HT increased the amplitude of the Ba\(^{2+}\)
FIG. 3. Nifedipine (10 μM) prevents the enhancing effects of 5-HT on Ca$^{2+}$ currents in muscle fibers. A: superimposed currents recorded in response to a voltage step from −70 to −10 mV. Bottom trace: control Ca$^{2+}$ current. Top 2 traces: in the presence of nifedipine and nifedipine plus 5-HT. 5-HT does not increase the amplitude of the inward current. B: I-V curves for control Ca$^{2+}$ currents (●), in the presence of 10 μM nifedipine (■), and after the further addition of 5 μM 5-HT (▲). In this record, nifedipine blocked 88% of the inward current. The addition of 5-HT did not increase the amplitude of the residual Ca$^{2+}$ current. The records in A have been leak corrected after acquisition and these currents were used to produced the I-V curve after Co$^{2+}$ subtraction.

Current when the Ca$^{2+}$-activated K$^+$ currents were not active. This effect of 5-HT occurs at all voltages in which the Ca$^{2+}$ current is active and does not lower the threshold for activation of this current. 5-HT has been shown to induce a similar increase in the amplitude of Ca$^{2+}$ currents in the accessory radula closer (ARC) muscle of Aplysia (Brezina et al. 1994d). Similar L-type Ca$^{2+}$ currents are enhanced by noradrenalin in smooth muscle cells from rabbit ear artery (Benham and Tsien 1988) and by endothelin in porcine vascular smooth muscle (Goto et al. 1989). As with the above-mentioned preparations, dihydropyridine antagonists, nifedipine in this case, blocked the enhancing effects of 5-HT. These data suggest that 5-HT enhances an L-type-like calcium current. Although an exact characterization of the type of calcium channel was not made, there are several examples of the ability of 5-HT to enhance a voltage-gated calcium channel with little effect on potassium conductance in Aplysia (Pellmar 1984; Pellmar and Carpenter 1980) and Helix neurons (Cottrell 1981; Hill-Venning and Cottrell 1992; Paupardin-Tritsch et al. 1986). Additionally, 5-HT is able to enhance the inward Ca$^{2+}$ current in parapodial muscle fibers over prolonged depolarizations that more closely resemble the depolarizations that would be expected in the intact animal. These results demonstrate that the 5-HT-sensitive current does not rapidly inactivate and further support its classification as L-type like.

Although modulation of Ca$^{2+}$ currents appears to be a common mechanism for the enhancement of muscle contraction amplitude, it is not the only possible mechanism by which modulation occurs, and not all aminergic effects act to increase muscle contraction. In frog skeletal muscle, 5-HT decreases the sensitivity of the acetylcholine receptor and thus depresses neuromuscular transmission (Akasu et al. 1981). 5-HT inhibits or suppresses muscle contractions in certain Rapana (Muneoka and Kobayashi 1980) and Aplysia (Ram et al. 1981) buccal muscles; and in other Aplysia buccal muscles, contractions are diminished by peptides that increase a voltage-sensitive K$^+$ current (Brezina et al. 1994e).

FIG. 4. I-V curves produced with the use of 1-s voltage ramps from −120 to +10 mV. This figure demonstrates the total outward current (control; trace labeled ASW), the Ca$^{2+}$ current recorded when the K$^+$ currents are blocked with ASW containing 50 mM TEA and 10 mM 4-AP (trace labeled Ca), the enhanced Ca$^{2+}$ current in the presence of 5-HT (trace labeled Ca+5-HT), and after the blockade of the Ca$^{2+}$ current with 0Ca/Co/ASW (trace labeled Co). The figure provides evidence that the 5-HT-enhanced Ca$^{2+}$ current does not inactivate during prolonged depolarization. Current calibration is located above current records (— — — : 0 current level).
Although we have demonstrated that 5-HT increases the muscle Ca\(^{2+}\) currents, no direct evidence has been acquired demonstrating that this increase potentiates muscle contractions. Experiments designed to determine whether the effect of 5-HT on parapodial muscle directly influences contraction amplitude will require measurements of individual muscle contractions. Brezina et al. (1994d) were able to demonstrate convincingly that 5-HT is able to enhance contractions of individual ARC muscles by recording length changes in individual muscle fibers. Comparable results have been reported by Ram et al. (1984a,b, 1991) in various Aplysia buccal muscle preparations. A technique similar to that described by Brezina (1994) could be employed to measure contraction of isolated parapodial fibers.

**Functional role of 5-HT in the intact animal**

In an intact, behaving animal, POP cells fire in regular bursts in synchrony with the firing of opener phase parapodial motor neurons only during swimming behavior. When the animal is not swimming, POP cells are silent or fire irregularly (von der Porten et al. 1980). Although the frequency of opening of the parapodia is controlled by a central pattern generator (von der Porten et al. 1982), and the amplitude of muscle contractions can be increased by increasing the frequency of motoneuron firing (McPherson and Blankenship 1991a), 5-HT provides an independent mechanism by which the amplitude and the force of the muscle contractions can be further increased. We have demonstrated here that 5-HT increases Ca\(^{2+}\) currents in muscle cells; and we have evidence that 5-HT also increases Ca\(^{2+}\) current in the presynaptic motoneurons (Gamkrelidze et al. 1995b). By increasing Ca\(^{2+}\) in the presynaptic element, 5-HT may increase the amount of transmitter that is released and thus increase the amplitude of the motoneuron-induced EJPs. By increasing the muscle Ca\(^{2+}\) current, 5-HT may increase the amplitude of a muscle contraction to a given depolarization. 5-HT also increases the rate of relaxation, providing a mechanism to ensure that parapodial opening is complete and that these muscles are relaxed before the antiphasic parapodial closing movement is initiated.

In Aplysia buccal muscle, there is extensive evidence that muscle membrane currents and contractions are modulated not only by 5-HT but also by several families of peptidergic cotransmitters (small cardiovascular peptides, myomodulins, and buccalins) that are expressed in cholinergic motoneurons (see Weiss et al. 1992, 1993 for reviews). Except that McPherson and Blankenship (1992) showed by immunocytochemical staining of functionally identified cells that buccalin-like immunoreactivity did not appear to be present in parapodial motoneurons, we have not examined whether other modulatory peptides may be present, nor have we examined whether any of these peptides affect parapodial muscle fibers. It is possible that members of the myomodulin and small cardiovascular peptide families may be present in parapodial motoneurons and contribute further to the modulation produced by 5-HT.

This work demonstrates that 5-HT is able to increase Ca\(^{2+}\) currents in isolated parapodial muscle fibers. It will be critical to determine the effect of 5-HT on depolarization- or acetylcholine-induced muscle contractions in future experiments. Furthermore, the role of second-messenger systems awaits analysis. Once the molecular bases of this modulatory phenomenon are fully evaluated, manipulation of these systems in the intact animal or in cocultures of motoneurons and muscle fibers will help elucidate the importance of each (pre- and postsynaptic) modulatory event.

We thank Drs. V. Brezina and K. R. Weiss for helpful advice on this work, and Dr. G. N. Gamkrelidze for discussion of the experiments. This work was supported by the John Sealy Memorial Endowment Fund for Biomedical Research (J. E. Blankenship) and by National Institute of Neurological Disorders and Stroke Grant T32 NS-07185 to P. J. Laurienti. Address for reprint requests: J. E. Blankenship, Marine Biomedical Institute, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-1069.

Received 13 August 1996; accepted in final form 22 November 1996.

**REFERENCES**


Gamkrelidze, G. N., Laurienti, P. J., and Blankenship, J. E. Serotonin...


