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Cellular Mechanisms Underlying Swim Acceleration in the Pteropod Mollusk *Clione limacina*[1]

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SYNOPSIS. The pteropod mollusk *Clione limacina* swims by dorsal-ventral flapping movements of its wing-like parapodia. Two basic swim speeds are observed—slow and fast. Serotonin enhances swimming speed by increasing the frequency of wing movements. It does this by modulating intrinsic properties of swim interneurons comprising the swim central pattern generator (CPG). Here we examine some of the ionic currents that mediate changes in the intrinsic properties of swim interneurons to increase swimming speed in *Clione*. Serotonin influences three intrinsic properties of swim interneurons during the transition from slow to fast swimming: baseline depolarization, postinhibitory rebound (PIR), and spike narrowing. Current clamp experiments suggest that neither I_h nor I_A exclusively accounts for the serotonin-induced baseline depolarization. However, I_h and I_A both have a strong influence on the timing of PIR—blocking I_h increases the latency to PIR while blocking I_A decreases the latency to PIR. Finally, apamin a blocker of $I_{K(Ca)}$ reverses serotonin-induced spike narrowing. These results suggest that serotonin may simultaneously enhance I_h and $I_{K(Ca)}$ and suppress I_A to contribute to increases in locomotor speed.

INTRODUCTION

Typically, *Clione* swim nearly continuously to maintain a stable position in the water column and avoid sinking due to negative buoyancy. Satterlie and Norekian (2001) described the general mechanisms of locomotor speed change in *Clione limacina*. *Clione* swim by dorsal-ventral flapping movements of their wing-like parapodia. Two groups of antagonistic interneurons (six interneurons in the dorsal group and seven interneurons in the ventral group) comprise the *Clione* swim central pattern generator (CPG) that produces the basic slow swim rhythm (Arshavsky *et al.*, 1985*b*). The two groups of interneurons reciprocally inhibit each other and exhibit postinhibitory rebound (PIR; Arshavsky *et al.*, 1985*c*; Satterlie, 1985).

Initiation of swim acceleration occurs in response to prey during feeding and as an escape response to tactile stimuli applied to the wings, body, or tail (Arshavsky *et al.*, 1985*a*). The *Clione* swim system uses many of the general mechanisms underlying locomotor speed changes including: 1) increases in limb cycle frequency, 2) increases in force of limb muscle contraction, and 3) changes in biomechanical properties of the locomotor system (Sillar *et al.*, 1997). In *Clione*, serotonin mediates many of these changes in swim speed, both in whole animals and reduced preparations. Bath application of serotonin or endogenous release of serotonin through identified serotonergic modulatory neurons causes increases in swim speed by means of enhancement of both cycle frequency and contractile force (Satterlie and Norekian, 1995; Satterlie, 1995). A compartmental serotonergic modulatory system alters swim speed through the activity of subsets of serotonergic neurons and is potentially capable of modifying cycle frequency and contractile force independently (Satterlie and Norekian, 1996).

Neuromodulation involves several changes at the network, synaptic and cellular levels including enhanced synaptic efficacy, recruitment of previously inactive neurons, and modification of the voltage-dependent and time-dependent properties of ion channels (Kaczmarek and Levitan, 1987; Levitan, 1988; Getting, 1989; Harris-Warrick and Marder, 1991; Calabrese, 1998; Grillner, 1999; El Manira and Wallén, 2000). Two groups of serotonergic cells in the cerebral ganglia of *Clione* (Cr-SA and Cr-SP neurons) modify swimming speed by modulating CPG interneurons and by recruiting additional interneurons and motoneurons into activity (Satterlie and Norekian, 1995).

Cr-S neurons recruit two groups of neurons that affect *Clione* locomotor speed. First, Cr-S neurons recruit two pairs of large motoneurons (GEMNs) that innervate both slow-twitch and fast-twitch swim muscles throughout their ipsilateral wings. Recruitment of the GEMNs during fast swimming in turn activates the fast-twitch musculature to enhance the intensity of wing contractions. Second, a pair of pleural interneurons (type-12 interneurons) are also recruited during fast swimming by the Cr-S neurons (Satterlie and Norekian, 1996). The type-12 interneurons reconfigure the swim CPG by providing early excitation of the dorsal-phase swim interneurons and early inhibition of ventral-phase swim interneurons to shorten the cycle period of the swim rhythm (Arshavsky *et al.*, 1985*d*).

Cr-S neuron activation also enhances the activity of a group of pedal serotonergic cells, the Pd-SW neurons. The axons of Pd-SW neurons extend from the pedal ganglia and innervate the slow-twitch swim musculature of the ipsilateral wing. Pd-SW neurons contribute to increased swim speed by enhancing con-

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tractility of the slow-twitch musculature, thus, modulating the force of muscle contraction. Pd-SW neurons can act independently of changes that occur centrally with regard to reconfiguration of the swim CPG and thereby can enhance locomotor performance within both slow and fast swimming modes (Satterlie, 1995; Satterlie and Norekian, 1996).

Bath application of serotonin or release of endogenous serotonin via direct Cr-S neuron stimulation induces three critical changes in swim interneurons of the basic CPG. First, serotonin depolarizes swim interneurons. Second, it enhances PIR amplitude and decreases in latency between the termination of hyperpolarization to peak PIR of swim interneurons. Third, serotonin decreases the spike duration of swim interneurons (Satterlie, 1991; Satterlie *et al.*, 2000). Tonic depolarization increases excitability of swim interneurons by bringing them closer to spike threshold. Modifying the trajectory of PIR decreases the time between subsequent swim interneuron spikes and provides an additional temporary increase in excitability. Finally, the decrease in spike duration acts permissively to allow high cycle frequencies by preventing the long duration spikes from exceeding their half cycle duration.

Here we provide data on the ionic mechanisms that may underlie many of the changes in CPG interneurons that occur at the cellular level during the transition between slow and fast swimming behavior in *Clione*. We have used both intracellular and single electrode voltage clamp (SEVC) recording techniques to describe ionic mechanisms of swim acceleration that result from serotonergic modulation.

METHODS

Clione limacina were collected from the breakwater at Friday Harbor Laboratories, Friday Harbor, WA. Specimens were maintained in gallon jars and either partially submerged in flowing seawater tables at 10°C or placed in a refrigerator at 4°C. Animals were pinned on SYLGARD®-lined Petri dishes (3 cm diameter) using cactus spines (*Opuntia* sp.) prior to dissection. The dissected preparation consisted of the central ganglia and wings. To facilitate penetration of intracellular microelectrodes through the ganglionic sheath, the ganglia were bathed in protease (Sigma type XIV) for 3 to 5 min. at 1 mg/ml concentration then thoroughly washed in natural seawater. A similar proteolytic treatment using collagenase (Sigma blend collagenase) for 3–5 min. at a concentration of 1 mg/ml was used to help physical removal of the ganglionic sheath with fine forceps. Removing the ganglionic sheath was necessary to isolate swim interneurons for single electrode voltage clamp experiments due to electrical coupling in *in situ* preparations. Swim interneuron isolation was done according to methods previously described by Arshavsky *et al.* (1986) and Satterlie *et al.* (2000), by pulling the cell out of the ganglia using the recording microelectrode.

During the entire dissection and throughout the ensuing experiments, the preparation was submerged in a natural seawater bath. The temperature of the bath was kept constant at 10–12°C using a peltier device (Dagan Instruments, Inc.). The bath volume was kept constant at 3 ml, and drugs were administered by pipetting the appropriate volume of an isotonic stock solution of the drug directly to the bath to give the desired end concentration. All the drugs were soluble in sea water. A gravity fed perfusion system in combination with a peristaltic pump was used to exchange larger solution volumes (*i.e.,* during high magnesium-high calcium saline administration and during wash in normal seawater). All drugs and chemicals used were obtained from SIGMA, Inc., (St. Louis MO) except apamin, which was purchased from Alomone Laboratories, Inc. (Israel). High magnesium-High calcium seawater (HI-DI) was made by adding 18.8 ml of 1 *M* $MgCl_2$, 3.8 ml $CaCl_2$, 0.8 ml 1 *M* KCl, and 51.6 ml of distilled water to 125 ml of natural seawater.

All electrophysiological recordings were made using Axon Instruments, Inc. AxoClamp 2B amplifier, Digidata 1321, and pCLAMP software. Electrodes were filled with 2M potassium acetate, and electrode resistances used for single electrode voltage clamp ranged from 5–10 MΩ while electrode resistances used for intracellular recording ranged from 10–30 MΩ.

All averaged data are reported as mean ± SE. Paired sample *t*-tests were used to show significant differences between mean values measured under different conditions. An ANOVA with post hoc multiple comparison analysis was used to test for significant differences between multiple means.

RESULTS

Ionic currents of swim interneurons

Preliminary single electrode voltage clamp experiments on physically isolated *Clione* swim interneurons suggest the presence of at least six different ionic currents. These include a sodium current (I_{Na}), a low-voltage-activated calcium current (LVA-I_{Ca}), a delayed rectifier potassium current (I_K), an inactivating, transient potassium current (I_A), a calcium-activated potassium current ($I_{K(Ca)}$), and an hyperpolarization-activated cation current (I_h). Five of these are shown in Figure 1. The sixth, $I_{K(Ca)}$ is not directly shown, but is instead represented by the characteristic 'N-shaped' current-voltage relationship (Fig. 1F).

The immediate goal is to find ionic currents that are serotonin-sensitive since applied serotonin, or activation of serotonergic cerebral neurons, triggers a distinct acceleration of swimming speed in *Clione*. However, we have thus far not been able to demonstrate direct serotonergic modulation of the ionic currents mediating swim acceleration. Our initial experiments presented here focus on the three subthreshold currents I_h, I_A, and $I_{K(Ca)}$. By pharmacologically blocking these subtrheshold currents in separate current clamp experiments, we examine the function that they may play in the modulation of swimming speed with respect to the three characteristic changes that occur in CPG inter-

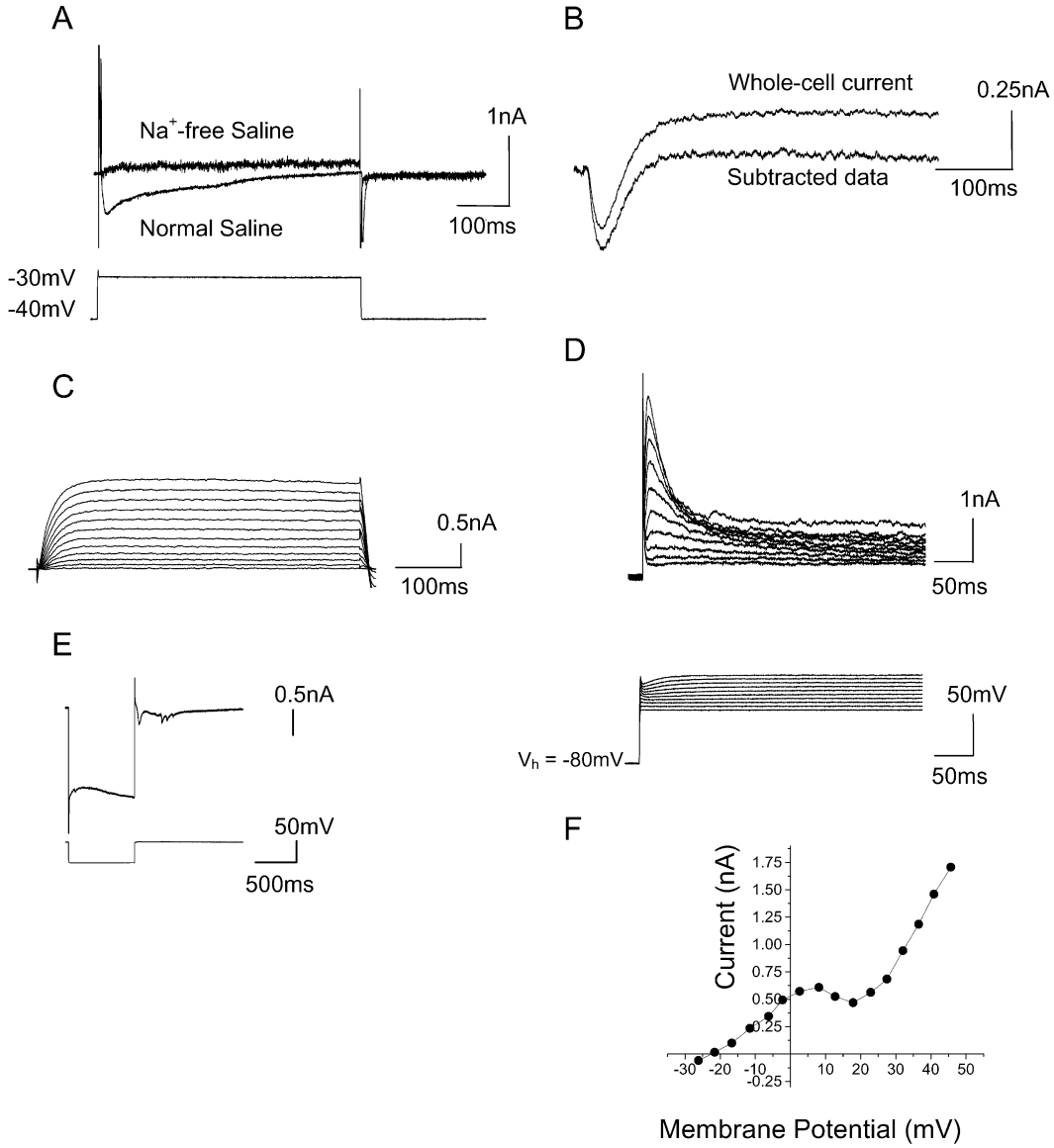

FIG. 1. Ionic currents characteristic of *Clione* swim interneurons. **A.** An inward current that closely resembles I_{Na} activated with depolarizing voltage steps from a holding potential of −40 mV. This current did not persist in Na^+ free (N-methyl-d-glucamine substitution for Na^+) saline. **B.** An inward current activated from a holding potential of −80 mV and insensitive to TTX (10 μM) closely resembles a low-voltage activated calcium current (LVA-calcium current or I_{CaT}). This inward current was blocked by Mg^{++} (166 mM) and Cd^{++} (2 mM). In this experiment, equivalent current traces elicited from a holding potential of −80mV are shown. The upper current trace in normal saline shows the whole cell current while the lower current trace shows the isolated calcium current obtained by subtracting the equivalent current trace in the presence of 166 mM $MgCl_2$ (not shown) from the upper current trace. **C.** Outward current traces evoked by depolarizing voltage steps from a holding potential of −40 mV in the presence of TTX (10 μM) closely resemble the delayed rectifier type potassium current (I_K). **D.** Outward current traces evoked by depolarizing voltage steps from a holding potential of −80 mV closely resemble the transient inactivating potassium current (I_A). **E.** Slow inward current evoked by hyperpolarizing voltage steps from a holding potential of −50 mV closely resembles the hyperpolarization-activated inward current (I_h). **F.** The current-voltage relationship of an isolated swim interneuron shows the distinctive ''N-shape'' indicative of the calcium-activated potassium current ($I_{K(Ca)}$).

neurons during swim accelerations—baseline depolarization, changes in PIR latency, and spike narrowing.

Role of I_h and I_A in mediating baseline depolarization

Antagonists to I_h and I_A, ZD7288 and 4-aminopyridine respectively, were used to test the hypothesis that I_h and I_A mediate changes in baseline depolarization. In other systems, I_h is activated at hyperpolarizing voltages below resting membrane potential, and does not exhibit inactivation. I_h conducts both Na^+ and K^+ and has a reversal potential around −30 mV (Pape, 1996; Lüthi and McCormick, 1998). Furthermore, I_h is sensitive to selective block by two antagonists—CsCl and ZD7288 (Pape, 1996; Satoh and Yamada, 2000).

Both CsCl and ZD7288 were observed to hyperpolarize the membrane potential of *Clione* swim interneurons (Fig. 2). In this experiment, a 1:1 mixture of

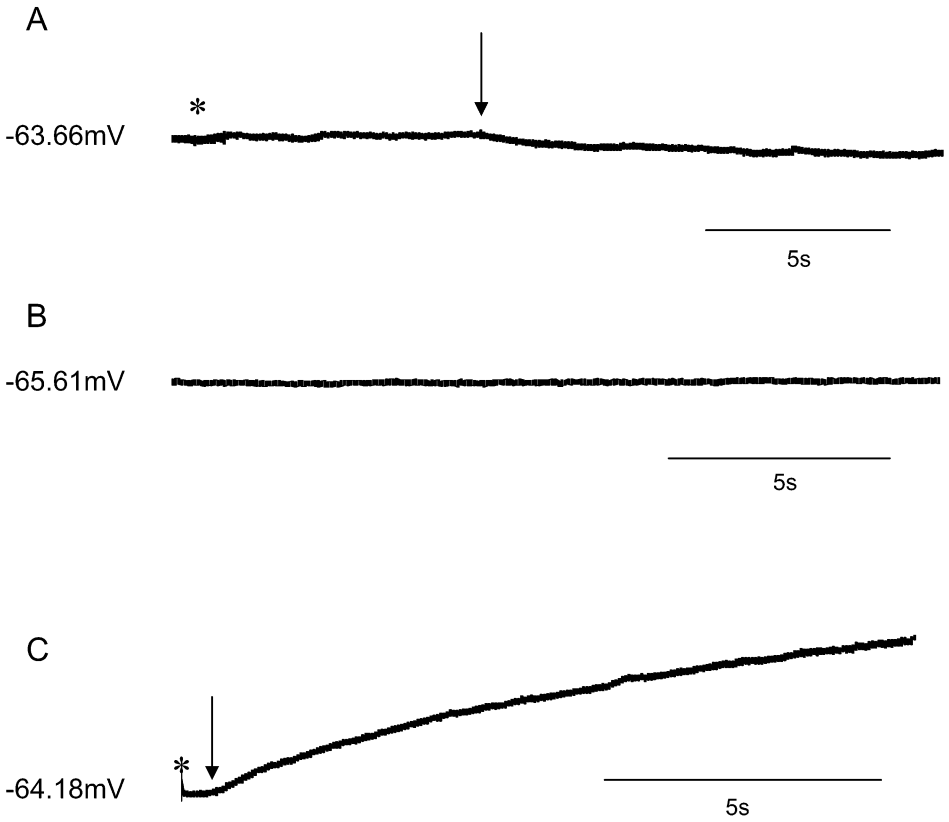

FIG. 2. The effect of the I_h antagonist ZD7288 on baseline depolarization. **A.** Initially, the preparation was bathed in a 1:1 mixture of natural seawater and isotonic (0.333 *M*) $MgCl_2$ to suppress action potential generation and synaptic input. Next, the I_h antagonist ZD7288 (200 μM) was applied (*) and the effects of this drug were observed to hyperpolarize the membrane potential (arrow) by a few millivolts. **B.** Hyperpolarization persisted for several minutes after adding ZD7288. **C.** Serotonin (1 μM) was subsequently added to the preparation (*) in the presence of ZD7288. ZD7288 did not prevent the serotonin-induced baseline depolarization (arrow).

isotonic (0.333M) $MgCl_2$ and seawater were initially applied to decrease excitability in the swim interneurons. Following addition of $MgCl_2$/seatwater, the I_h antagonist ZD7288 (200 μM) hyperpolarized the membrane potential by 1.95 mV (Fig. 2A, B). Application of serotonin (1 μM) induced a large depolarization in membrane potential in the presence of ZD7288 suggesting that I_h does not appreciably contribute to membrane depolarization.

The role of I_A in mediating baseline depolarization was tested in a similar manner using 4-Aminopyridine (4-AP) to block I_A. Activation of I_A is known to hyperpolarize the membrane potential and increase the time between action potentials of neurons in other preparations (Hille, 1992). To test the possibility that I_A contributes to baseline depolarization, 4-AP (4 mM) was applied following addition of 1:1 $MgCl_2$ as previously described. Blocking I_A initially depolarized the swim interneuron and resulted in spike activity in this cell (Fig. 3), however, 4-AP application did not cause the sustained depolarization as observed in the presence of serotonin. Therefore, it is unlikely that either I_h or I_A, acting alone, produce the baseline depolarization.

Role of I_h and I_A in changing the trajectory of PIR

To test the hypothesis that a serotonin-induced decrease in I_A of *Clione* swim interneurons contributes to enhanced locomotory speed we applied 4-AP to reduced preparations. The serotonin antagonist, mianserin (10 μM) was added to the preparation to preclude any serotonin effects. Administration of 4-AP (4 mM) in the presence of minaserin significantly increased the swim cycle frequency (39%) from $1.41 \pm 0.194\ s^{-1}$ to $1.960 \pm 0.205\ s^{-1}$ ($n = 50$, average of ten consecutive cycles from five preparations, paired sample *t*-test, $P < 0.05$, Fig. 4).

To examine PIR of *Clione* swim interneurons it was necessary to synaptically isolate the swim interneurons by applying high-magnesium-high-calcium seawater (HIDI), by applying tetrodotoxin (TTX, 10 μM), or by applying a combination of TTX, the cholinergic antagonist, atropine (1 mM), and the glutaminergic antagonist, CNQX (10 μM). By suppressing the synaptic activity in the preparation it was possible to clearly examine the effects of I_A and I_h channel antagonists on PIR. The effect of 4-AP (4 mM) on the timing of PIR is shown in Figure 5A–C. 4-AP increases the duration range of hyperpolarizing current pulses that produce

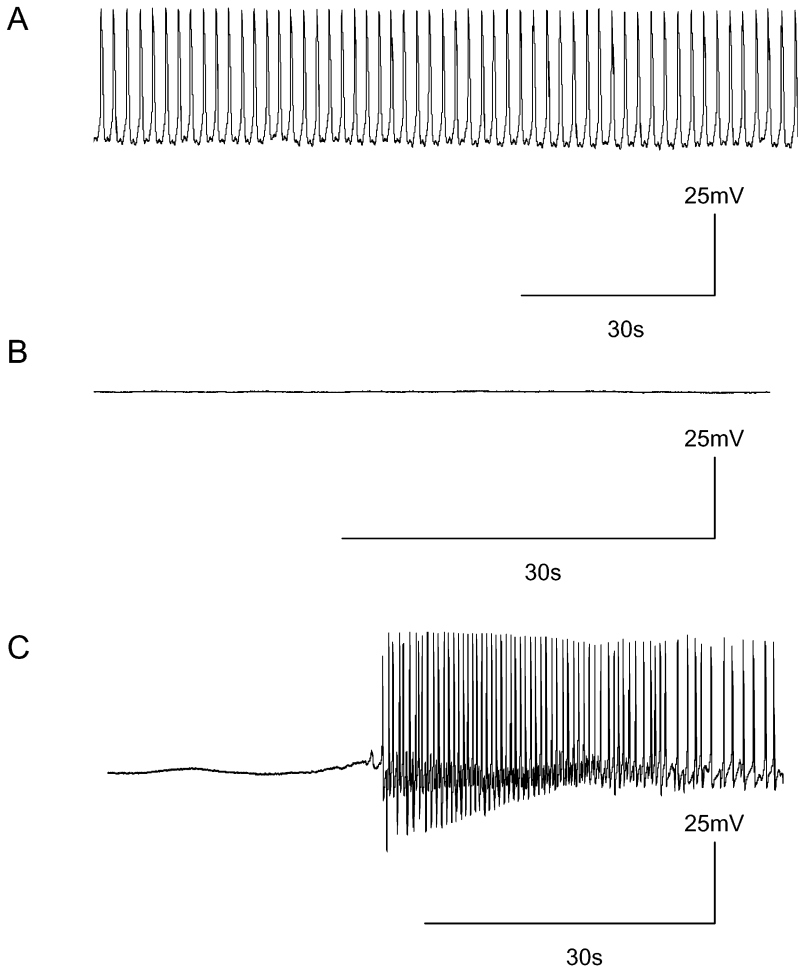

FIG. 3. The effect of the I_A antagonist 4-aminopyridine (4 mM) on baseline depolarization. **A.** Intracellular record of swim interneuron activity in normal seawater. **B.** Addition of a 1:1 mixture of natural seawater and isotonic (0.333 *M*) $MgCl_2$ is applied to suppress action potential generation and synaptic input. **C.** Addition of 4-aminopyridine (4 mM) depolarized the cell and initiated action potentials. However, 4-aminopyridine did not result in the sustained depolarization observed in the presence of serotonin.

PIR and also significantly reduces the latency to PIR in *Clione* swim interneurons (from 202.3 ± 23.7 ms to 89.2 ± 15.5 ms). In these experiments, we normalized the latency data by dividing the latency in the presence of 4-AP (the experimental value) by the corresponding latency in normal saline (the control value). This was done to eliminate variability between preparations; a paired sample *t*-test was used to determine significance (n = 5, $P < 0.05$).

The I_h antagonist, CsCl, has the opposite effect on the latency to PIR (Fig. 6). Application of CsCl (5 mM) significantly increases the latency to PIR (from 54.5 ± 8.80 ms to 105.4 ± 20.6 ms, n = 6, paired sample *t*-test, $P < 0.05$). In current clamp recordings, I_h activity is represented by a sag potential, a steady depolarization of the membrane potential that lasts the duration of hyperpolarizating current injection. The sag of *Clione* swim interneurons is blocked by CsCl, a blocker of I_h. A Cs-sensitive depolarizing sag that occurs during hyperpolarizing input is characteristic of I_h other preparations (Pape, 1996; Robinson, 2003). The I_h antagonist, ZD7288 (200 mM) had similar effects on the latency and sag potential (data not shown).

Role of $I_{K(Ca)}$ in mediating spike narrowing

Spike narrowing is involved in serotonin-induced swim acceleration in *Clione*, but only as a permissive change (Satterlie *et al.*, 2000). Serotonergic-induced spike narrowing is sensitive to TEA suggesting that activation or modulation of a potassium current mediates this response. Spike narrowing is affected by apamin, an antagonist of the small conductance calcium-activated potassium current ($I_{K(Ca)}$), and this blocker significantly affects swim speed. Figure 7 illustrates the effects of serotonin and apamin on swim frequency and spike duration. Initially, addition of serotonin causes a significant increase in swim frequency accompanied by a decrease in spike duration. Serotonin significantly increased the mean cycle from 3.07 ± 0.103 s^{-1} to 4.65 ± 0.332 s^{-1} (51%, n = 30, ten consecutive cycles from three preparations, one-way ANOVA with post hoc multiple comparison test, F =

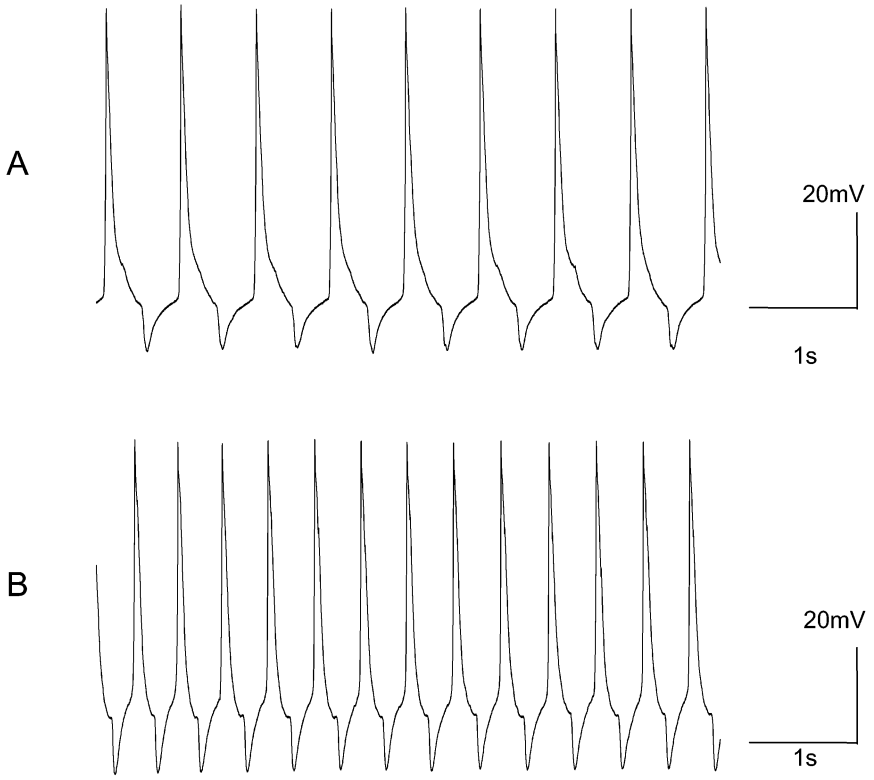

FIG. 4. Application of 4-aminopyridine (4 mM) in the presence of mianserin (10 μM) increased swim speed. **A.** Swim frequency in normal saline (seawater) **B.** Increase in swim frequency occurred with the addition of 4 mM 4-aminopyridine.

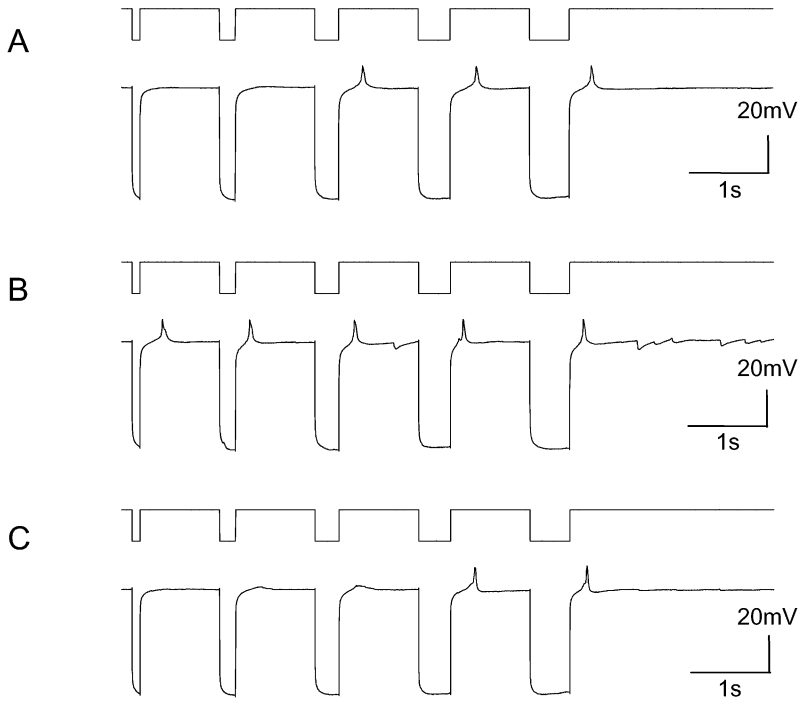

FIG. 5. Application of the I_A antagonist 4-aminopyridine affected the timing of PIR. **A.** PIR elicited by −2.5 nA hyperpolarizing current injection of different durations (in HI-DI seawater). PIR first appeared with 300 ms duration hyperpolarizing current injection. **B.** Administration of 4-aminopyridine (4 mM) increased the probability of PIR at lower duration pulses and decreased the latency to peak PIR response relative to control. **C.** Wash in normal saline (HI-DI seawater).

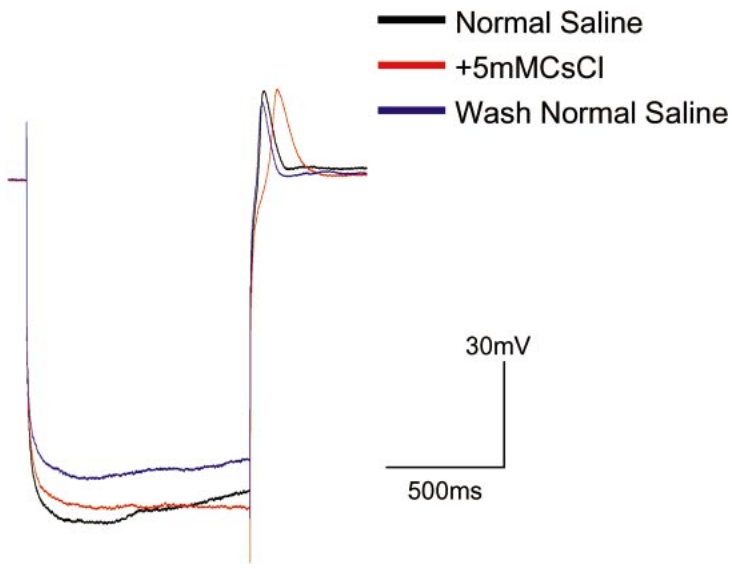

FIG. 6. Application of the I_h antagonist CsCl affects the timing of PIR. **A.** Overlay of PIR elicited by −1 nA hyperpolarizing current injection. Black: normal saline [TTX Thomas Pirtle 25 (10 μM), atropine (1 mM) and CNQX (10 μM)]; Red: +CsCl (5 mM); Blue wash in normal saline. Application of CsCl increased the latency to PIR and eliminated the sag potential.

25.25, $P < 0.05$), and the decreased spike duration from 87.7 ± 9.55 ms to 55.9 ± 12.6 ms (36%, n = 30, ten consecutive spikes from three preparations, one-way ANOVA with post hoc multiple comparison test, F = 36.177, $P < 0.05$). Addition of apamin reverses the effects of serotonin, causing a significant reduction in mean swim frequency from 4.65 ± 0.332 s^{-1} to 2.44 ± 0.179 s^{-1} (48%, n = 30, ten consecutive cycles from three preparations, one-way ANOVA with post hoc multiple comparison test, F = 25.25, $P < 0.05$, Fig. 7) and a significant increase in mean spike duration 55.9 ± 12.6 ms to 79.1 ± 20.5 ms (42%, n = 30, ten consecutive spikes from three preparations, one-way ANOVA with post hoc multiple comparison test, F = 36.177, $P < 0.05$) in comparison to values for serotonin.

DISCUSSION

Three subthreshold ionic currents are potential targets for serotonergic modulation of CPG interneurons and may contribute to altered swim speed in *Clione limacina*. I_A and I_h affect the timing of the PIR response, and $I_{K(Ca)}$ affects spike narrowing. Neither I_A nor I_h alone can account for the type of sustained depolarization observed when serotonin is applied. I_A and I_h may contribute to baseline depolarization, but only in concert with each other or other currents. This contrasts with work done in other systems. Using a model LP neuron of the stomatogastric system, Golowasch *et al.* (1992) demonstrate that a decrease in the $I_{K(Ca)}$ conductance causes the model cell to depolarize by 5 mV. Current clamp experiments with *Clione* suggest that serotonin enhances $I_{K(Ca)}$ thus ruling out the possibility that this ionic current plays a similar role in baseline depolarization. A specific neuromodulator may modulate multiple ionic currents in a single neuron. For example, SCP_b modulates multiple ion channels in identified neurons of *Hermissenda* to enhance excitability. SCP_b reduces I_A and I_K but enhances $I_{K(Ca)}$, I_{Ca}, and I_{Na} with the net effect of causing depolarization (Acosta-Urquidi, 1988). On the other hand, Kiehn *et al.* (2000) found that modulation of a single ionic current, I_h, influences the motor output in the mammalian spinal cord by tonic depolarization. Their data suggests that I_h enhancement acts as a leak conductance that depolarizes the membrane potential thereby increasing the rate of action potential production in neonatal rat spinal motoneurons.

The main property of *Clione* swim interneurons that is affected by I_h and I_A is the timing of PIR. Blocking I_A using 4-AP resulted in a shortening of the latency to PIR, while blocking I_h using CsCl resulted in a lengthening of the latency to PIR (Figs. 5–6). Serotonin had previously been shown to decrease the latency to PIR in *Clione* swim interneurons (Satterlie *et al.*, 2000). Data presented here suggests that serotonin simultaneously inhibits I_A and enhances I_h to shorten the latency to peak PIR. Reduction of I_A and enhancement of I_h have not been directly demonstrated in voltage clamp experiments. Nonetheless, the changes in latency to PIR revealed in current clamp experiments when I_A and I_h specific antagonists are applied suggest their role as modulation targets. Modulation of PIR latency brings the membrane potential of the swim interneurons to threshold more quickly, thereby speeding the onset of spike production in these cells increasing locomotor speed. Similar effects have been observed in the stomatogastric preparation of the lobster. Harris-Warrick *et al.* (1995) have shown that dopaminergic modulation reduces I_A and enhances I_h to affect timing in the stomatogastric rhythm by shortening the latency to PIR in the isolated LP neuron. Furthermore, Klop-

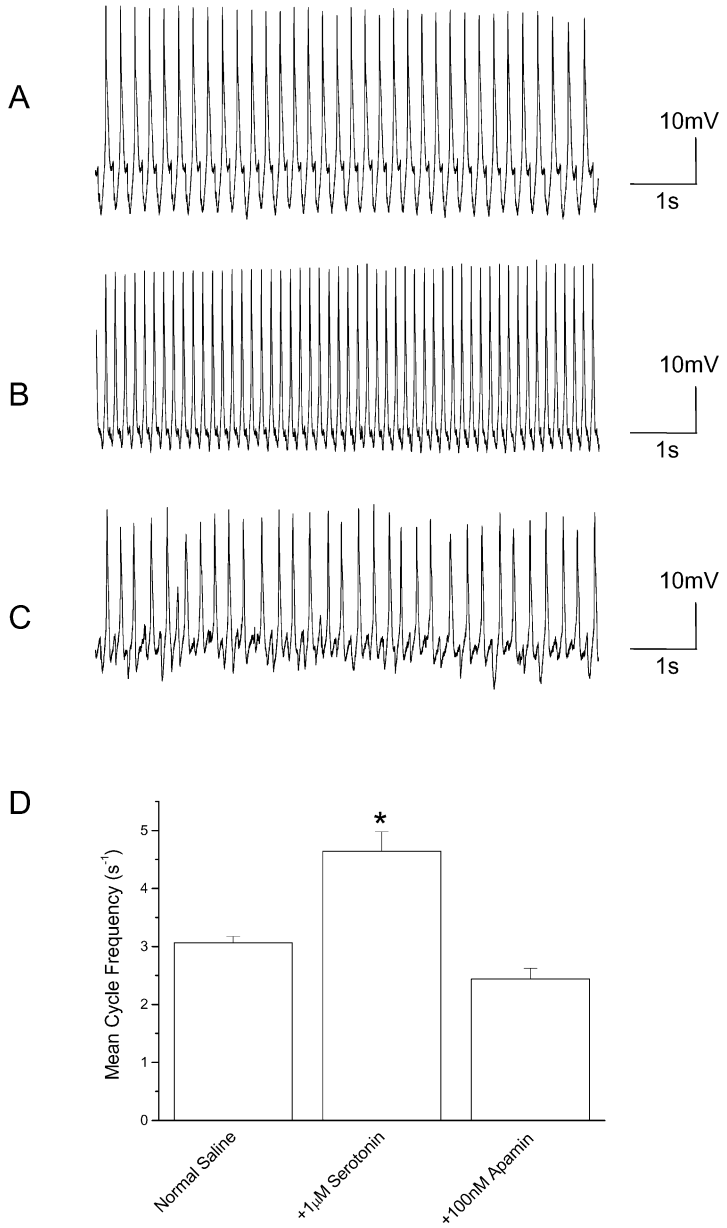

FIG. 7. Effects of serotonin (1 μM) and apamin (100 nM) on swim interneuron cycle frequency and spike duration. **A.** Intracellular record of swim interneuron activity in normal seawater. **B.** Addition of serotonin increased swim frequency. **C.** Addition of apamin reduced swim frequency and caused spike broadening. **D.** Mean cycle frequency (mean of ten consecutive cycles from three different preparations) in normal seawater, serotonin, and apamin.

penburg *et al*. (1999) have shown that the opposite occurs in isolated PD neurons of the stomatogastric system. Here, enhancement of I_A in isolated PD neurons leads to an increase in the latency to PIR in these cells. Thus, it appears that dopamine differentially modulates I_A in different cells of the stomatogastric system to affect the timing of PIR in opposite ways.

Increases in swim speed are accompanied by spike narrowing in *Clione* swim interneurons. This was previously attributed to an enhancement of an unidentified potassium conductance (Satterlie *et al*., 2000) since application of the potassium channel blocker, tetraethylammonium (TEA, 10 mM), blocks serotonin-induced spike narrowing. Apamin, a blocker of the small conductance calcium activated potassium current ($I_{K(Ca)}$), reverses serotonin-induced spike narrowing and causes a decrease in swim speed. Thus, serotonergic enhancement of an $I_{K(Ca)}$ contributes to both spike narrowing and increased swim speed in *Clione*. There could be other potassium currents that contribute to spike narrowing since TEA is a relatively nonspecific blocker of outward potassium currents in other molluscan preparations. However, the concentration range for TEA to block an appreciable amount of $I_{K(Ca)}$ in other mollusks can be considerably high—100 mM to block 20% of $I_{K(Ca)}$ in *Tritonia* (Thompson, 1982). While it

is likely that the 10 mM concentration used in experiments by Satterlie *et al.* (2000) had only a slight effect on $I_{K(Ca)}$, we cannot rule out the possibility that TEA has a larger affect on $I_{K(Ca)}$. $I_{K(Ca)}$ of different neurons within a given species respond differently to TEA administration. For example, in some cells of *Helix* high concentrations of TEA (greater than about 100 mM) are necessary to block $I_{K(Ca)}$ while in other *Helix* neurons lower concentrations of TEA (less than about 1 mM) block $I_{K(Ca)}$ (Hermann and Hartung, 1983). Because apamin is a highly specific blocker of $I_{K(Ca)}$, it appears that serotonergic enhancement of this current plays a role in mediating swim speed changes.

$I_{K(Ca)}$ activates within a large range of both positive and negative membrane potentials, and thus participates in many neurophysiological processes. $I_{K(Ca)}$ may contribute to the resting membrane potential, to spike frequency adaptation, and to pacemaking (Hermann and Hartung, 1983). Hyperpolarization caused by IPSPs or activity of outward currents including $I_{K(Ca)}$ may result in activation of I_h (Lüthi and McCormick, 1998). Modulation of this type of interaction between I_h and $I_{K(Ca)}$ affects the oscillatory frequency of vertebrate inferior olive neurons (Bal and McCormick, 1997). Modulation of $I_{K(Ca)}$ also affects the output of neurons in motor systems. In the lamprey, application of N-methyl-d-aspartic acid (NMDA) increases the frequency of plateau potential oscillations characteristic of motoneurons and interneurons. This increase in frequency is inhibited by subsequent application of apamin (El Manira *et al.*, 1994; Tegner *et al.*, 1998). Addition of apamin also increases the duration of the plateau potential and lengthens the duration of hyperpolarization between plateau potentials. This suggests a role for $I_{K(Ca)}$ in mediating output frequency, at least in the low frequency range. $I_{K(Ca)}$ shortens the duration of the plateau potential, and by doing so increases the frequency of plateau potential oscillation (El Manira *et al.*, 1994). Similar mechanisms may be involved in mediating speed changes in *Clione*. However, we do not know if serotonin directly affects $I_{K(Ca)}$. Serotonin could enhance calcium entry into the cell and this could have an indirect modulatory effect on $I_{K(Ca)}$.

Differential modulation of outward currents in *Clione* swim interneurons contribute to increased locomotor speed—inhibition of I_A and enhancement of $I_{K(Ca)}$ and possibly I_K. Furthermore, serotonin differentially modulates two currents, I_A and I_h, to affect the latency to PIR. Finally, serotonin may directly or indirectly modulate $I_{K(Ca)}$ to enhance swim speed by contributing to spike narrowing, and enhancement of $I_{K(Ca)}$ may lead to an enhancement of I_h to circuitously contribute to increases in swim speed. It is obvious that serotonin targets multiple ionic conductances to trigger an increase in firing frequency in these cells. Most important, individual variables at the current clamp level—baseline depolarization, decreased PIR latency, and spike narrowing—are each altered through a collaborative modulation of more than a single ionic conductance.

ACKNOWLEDGMENTS

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