Dopamine Induces Rhythmic Activity and Enhances Postinhibitory Rebound in a Leech Motor Neuron Involved in Swimming and Crawling Behaviors

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Amine neurotransmitters play an important role in controlling motor behavior in many animals, including the medicinal leech (Hirudo medicinalis). Previous studies have established serotonin as an important modulator of swimming behavior. Serotonin levels are elevated in the blood of frequently swimming leeches and bath application of serotonin to isolated nerve cord preparations evokes fictive swimming. Serotonin alters the intrinsic electrical properties of interneurons and motor neurons involved in generating swimming behavior. In particular, serotonin increases the amplitude, but shortens the duration, of postinhibitory rebound (PIR) responses in cell DE-3, a motor neuron that innervates the dorsal longitudinal muscle cells of the body wall. More recent studies have implicated dopamine in the suppression of swimming behavior and the initiation of crawling. Here we show that bath application of dopamine to isolated leech ganglia induces rhythmic oscillatory activity in cell DE-3. The long cycle period of these oscillations is consistent with crawling, but not swimming behavior. Dopamine increases the amplitude of PIR responses in cell DE-3, but unlike serotonin does not decrease its duration. These effects provide further support for the hypothesis that dopamine promotes crawling behavior in the leech.

Key Words: Biogenic Amines; Central Pattern Generator; DE-3; Dopamine; Locomotion; Medicinal Leech; Neuromodulation; Serotonin

Introduction

The rhythmic motor behaviors of animals are strongly influenced by neuromodulatory transmitters (Harris-Warrick and Marder, 1991; Marder and Calabrese, 1996). A neuromodulator may act locally as a neurotransmitter, globally as a neurohormone, or both (Harris-Warrick and Marder, 1991). In some preparations, bath application of a transmitter induces rhythmic activity. For example, fictive swimming is evoked by glutamate in the lamprey spinal cord (Grillner et al., 1991) and rhythmic bursting in the isolated crab stomatogastric ganglion (STG) is activated by proctolin (Hooper and Marder, 1984). In Aplysia, bath application of serotonin or dopamine induces feeding movements (Kabotyanski et al., 2000). Furthermore, serotonin evokes swallowing behavior, whereas dopamine induces movements associated with biting. Thus, different transmitters may target the same motor circuitry, but configure that circuit to produce distinct motor patterns (Harris-Warrick and Marder, 1991).

Modulators may also affect ongoing rhythms. Dopamine slows the pyloric rhythm of the lobster STG by increasing firing rates in some neurons, but suppressing firing in others (Harris-Warrick et al., 1998). In the pteropod mollusk Clione limacina, bath application of serotonin produces a switch from slow to fast swimming (Satterlie et al., 2000).

Neuromodulators work by altering the intrinsic electrical properties or synaptic interactions of neurons comprising pattern-generating circuits (Harris-Warrick and Marder, 1991). In some instances, expression of intrinsic
properties important to the generation of rhythmic activity depends on the presence of a particular modulator. A prominent example is cat spinal motor neurons, which exhibit plateau potentials only after exposure to serotonin or certain other transmitters (Kiehn, 1991). Plateau potentials, postinhibitory rebound (PIR), and other intrinsic properties are altered as a result of changes in the amplitude or kinetics of voltage-gated conductances (Kiehn et al., 1997). In the pyloric constrictor neurons of the lobster STG, for example, dopamine increases the rate of PIR depolarization by suppressing an A-type K⁺ conductance (Harris-Warrick et al., 1995).

Serotonin (5-hydroxytryptamine) is an important modulator of swimming behavior in the medicinal leech. Leeches injected with serotonin are more likely to exhibit swimming behavior (Willard, 1981). In addition, bath application of micromolar serotonin to isolated nerve cords induces spontaneous swimming episodes (Willard, 1981). Several types of serotonergic neurons have been identified in the leech central nervous system (CNS), which consists of a head brain, a chain of 21 midbody ganglia, and a tail brain (Kristan et al., 2005). Among these are the large paired Retzius (Rz) cells found in each ganglion. Rz cells can release serotonin as a neurotransmitter from axon terminals, but serotonin may also be secreted in paracrine fashion from the cell body (De-Miguel and Trueta, 2005). Intracellular stimulation of Rz cells increases the concentration of serotonin in the blood. Because the entire nerve cord is encased in a ventral blood sinus, serotonin has the opportunity to target neurons throughout the CNS.

Serotonin appears to promote swimming behavior, at least in part, by modulating ionic conductances in cell 204, a swim-gating interneuron (Angstadt and Friesen, 1993a; Angstadt and Friesen, 1993b), and in swim motor neurons (Mangan et al., 1994a; Mangan et al., 1994b). In both cell 204 and excitatory motor neurons to dorsal (cell DE-3) and ventral (cell VE-4) longitudinal muscle, 50 µM serotonin increases the peak amplitude of PIR responses (Mangan et al., 1994a). In addition, serotonin decreases the duration of the normally prolonged PIR responses of cell DE-3 (Angstadt et al., 2005).

Two additional amine modulators of leech motor behavior are octopamine and dopamine. Like serotonin, bath application of octopamine to isolated nerve cords induces fictive swimming (Hashemzadeh-Gargari and Friesen, 1989). Octopamine-synthesizing neurons are located in the head and tail brains as well as several midbody ganglia (Gilchrist et al., 1995; Crisp et al., 2002). Some of the octopamine-positive processes in the supraesophageal region of the head brain appear to release transmitter into the blood (Crisp et al., 2002). Multiple bilateral pairs of dopaminergic neuronal somata are found in the head and tail brains, but not midbody ganglia (Crisp et al., 2002). Like octopamine, some processes in the head brain appear to release dopamine into the blood (Crisp et al., 2002). An additional type of dopaminergic neuron is located in the peripheral nervous system. Anterior and posterior nerve roots, which branch into a network of peripheral nerves, extend from the left and right sides of each midbody ganglion. A small peripheral ganglion inside the anterior nerve root contains a dopaminergic anterior root ganglion (ARG) cell, whose axon enters the adjacent ganglion (Crisp et al., 2002). Thus, every neuron in a leech midbody ganglion is a potential target for dopaminergic modulation. Unlike serotonin and octopamine, dopamine appears to bias the leech toward crawling behavior. Vermiform crawling consists of alternating contraction and elongation phases coupled with the coordinated attachment and release of head and tail suckers (Eisenhart et al., 2000).

The effects of these amine transmitters on motor behavior are complex, and depend on the preparation used (e.g., nerve cord with or without brains attached) and where the transmitter is applied. Serotonin, for example, activates swimming in isolated nerve cords (brains removed), but strongly suppresses swimming when applied focally to the head brain in intact CNS preparations (Crisp and Mesce, 2004). Application of a mixture of serotonin and octopamine to intact CNS preparations suppresses swimming. However, persistent spontaneous swimming activity is evoked upon amine washout (Mesce et al., 2001). Such spontaneous swimming is rapidly and reliably suppressed by bath application of 50
µM dopamine, probably due to affects on neurons in the head brain (Crisp and Mesce, 2004). In a separate set of experiments using nerve cords with the head brain removed, spontaneous bouts of fictive crawling were observed in a majority of preparations bathed in 50 µM dopamine, but also in a majority of control preparations with no applied dopamine (Crisp and Mesce, 2004).

In this study, we sought to determine if dopamine modulates the intrinsic electrical properties of swim motor neurons, and specifically cell DE-3. Given that dopamine appears to promote crawling and suppress swimming behavior, we hypothesized that dopamine (unlike serotonin) would fail to enhance, and perhaps even suppress, PIR responses in cell DE-3 in isolated midbody ganglia.

Materials and Methods

*Hirudo medicinalis* (Leeches, Westbury, NY) were maintained in artificial pond water at 11 °C. Our experiments were restricted to midbody ganglia G7-G14. Following anesthetization in ice-cold leech saline, individual ganglia were removed by dissection. Each ganglion was pinned in a 60 mm petri dish containing clear Sylgard resin (Dow Corning, Midland, MI). Immediately prior to experimentation, the thin connective tissue sheath overlying the neuronal cell bodies was removed using fine scissors. The ganglion was superfused with saline solution at a rate of ~2.5 mL/min using a gravity-feed system. Total bath volume surrounding the ganglion was approximately 2 ml. All experiments were conducted at room temperature (~22°C).

Normal leech saline (NS) contains (in mM): 115 NaCl, 4 KCl, 1.8 CaCl₂, 10 D-Glucose, and 10 HEPES. The pH was set to 7.4 with NaOH. A 100 mM stock solution of dopamine hydrochloride in NS was prepared each day immediately prior to experimentation. Aliquots of this stock were added to saline solutions to yield a final dopamine concentration of 50 or 100 µM. In most experiments, 2 mM Mg²⁺ was added to NS to suppress rhythmic electrical activity induced by dopamine. Leech saline containing Mg²⁺ is hereafter referred to as control saline solution. All chemicals described above were obtained from Sigma-Aldrich.

Microelectrodes were made from thin-wall (0.1 mm o.d. and 0.75 mm i.d.) borosilicate glass (A-M Systems, Carlsberg, WA) using a Flaming/Brown P-97 puller (Sutter Instruments, Novato, CA). Electrodes were filled with 3 M KCl, and then dipped in dimethylpolysiloxane to reduce capacitance. Electrode resistances ranged from 16 to 35 MΩ. Intracellular recordings were made in discontinuous current clamp (DCC) mode with an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA). A separate oscilloscope was used to monitor the electrode voltage during current injection cycles (sample rate approximately 3 kHz) to ensure complete recovery between pulses. Data were digitized and current pulses controlled using a DigiData 1200 and Clampex software (Axon Instruments, Foster City, CA). In some experiments rhythmic electrical activity induced by dopamine was recorded with a PowerLab 4SP and Chart software (AD Instruments, Colorado Springs, CO).

The resting membrane potential (RMP) of leech motor neurons is approximately -40 mV. Following impalement, the cell was injected with a steady current of -0.1 to -0.2 nA for several minutes. Current injection was continued as required to "hold" the membrane potential (Vₘ) near -40 mV during DCC experiments. Cells requiring more than -0.25 nA to hold Vₘ at -40 mV were considered damaged and excluded from the study. In a typical experiment, the neuron was injected with a series of 1 sec current pulses ranging from -2.0 to +2.0 nA in 0.5 nA increments. In some experiments, data were collected from the same neuron both before and after exposure to DA. In the remaining experiments, data were collected from separate groups of neurons bathed in control solution or solution containing dopamine or serotonin.
After data collection, the electrode was removed from the neuron and the bath voltage recorded. Only when this final bath voltage was within ±5 mV of 0 mV were the data accepted.

Each leech ganglion contains about 400 neurons, most occurring as bilateral pairs (Muller et al., 1981). Experiments were conducted on cell DE-3, an identified motor neuron that excites dorsal muscle. A bilateral pair of DE-3 cell bodies is located at the medial edge of each posterior cell packet on the ganglion’s dorsal surface (Ort et al., 1974). Data were obtained from only one of the two DE-3 motor neurons in each isolated ganglion. However, in most experiments multiple ganglia from the same leech were used. Thus, n values reported in this study are equivalent to the number of ganglia tested, but not the number of leeches.

In NS, DE-3 and other swim motor neurons exhibit spontaneous attenuated action potentials. Cell DE-3 also generates a characteristic PIR response that persists for several seconds (Mangan et al., 1994a). PIR is defined as excitation evoked at the offset of a strong hyperpolarizing stimulus (Harris-Warrick and Marder, 1991).

To confirm cell identity, each neuron was injected with fluorescent dye after the collection of physiological data. This was accomplished by repenetrating the cell with an electrode containing the fluorescent dye Alexa 568 (Invitrogen) at a concentration of 10 mM in 200 mM KCl. Dye was injected iontophoretically using a continuous –5 nA of current passed for 4-5 min. After dye injection, the ganglion was mounted on a glass microscope slide, and a cover slip was applied. Cell DE-3 was identified based on the following criteria: 1) a single axon projecting to the contralateral posterior nerve root, and 2) a unique pattern of ipsilateral neurites consisting of several thin neurites oriented along the anterior-posterior axis adjacent to a tuft of neurites ending abruptly at the ganglion mid-line (Figure 1). Stained cells were examined and photographed as described previously (Angstadt et al., 2005).
**Data Analysis**

Figure 2 illustrates the methods used to quantify the raw electrophysiological data. The change in $V_m$ from -40 mV was measured as shown and graphed versus current pulse amplitude to produce current–voltage (I/V) plots. The peak amplitude of PIR responses evoked at the offset of negative current pulses was measured and plotted similarly. Rebound responses (usually hyperpolarizing) sometimes occurred at the offset of depolarizing current pulses. Mangan et al. (1994a) referred to these as afterhyperpolarizing potentials, or AHPs. Because AHP responses were often quite small, we found it difficult to make accurate measurements of their peak amplitude. Similarly, it was difficult to measure the duration of both AHP and PIR responses. In some instances (e.g., Figure 2), the PIR response persisted beyond the 5 sec data collection window. An alternate and completely objective means of quantification is to determine the area under the rebound response (Angstadt et al., 2005). In this study, we determined rebound response areas for the first 1 sec after current pulse termination. During this time, the difference between PIR responses in control and serotonin solutions is greatest (see Figure 7). Also, the rebound responses (especially the small AHPs) are less likely to be contaminated by random $V_m$ fluctuations during this time.

All analysis of data collected using Clampex was performed with associated Clampfit software (Axon Instruments). In those experiments where rhythmic electrical activity induced by dopamine was recorded with PowerLab, the cycle period and amplitude were quantified using Chart software. Selected means were compared with t-tests. Paired t-tests were used for experiments in which the same cell was exposed to both control solution and dopamine. All other comparisons utilized unpaired t-tests. Differences were considered significant when P values were less than 0.05. Means are expressed as ± standard error (S.E.M.).

**Results**

In normal saline (NS), cell DE-3 has a RMP of approximately -40 mV, and spontaneously fires action potentials (Figure 3A). However, several minutes after switching to solution containing 100 μM dopamine, a majority of cells (10/12) exhibited rhythmic oscillatory activity (Figure 3B,C). Cycle periods, which were measured between 5 and 16 min after switching to dopamine, ranged from 4.5 to 15.1 sec, with a mean of 9.6 ± 1.1 sec. A scatter plot (not shown) revealed no correlation between cycle period and time of exposure to dopamine ($R^2 = 0.14$). The dopamine-evoked oscillations were quite robust, usually persisting for the duration of the experiment (over 20 min in several instances). However, the voltage range of dopamine-evoked oscillations varied from cell to cell. Oscillations peaked at voltages ranging from -35 mV to -43 mV.
Figure 3. Dopamine induces rhythmic activity in cell DE-3. (A) Spontaneous attenuated impulses recorded in a cell DE-3 bathed in normal leech saline. (B) Oscillatory activity in the same cell approximately 10 min after switching to solution containing 100 μM dopamine (DA). (C) Spontaneous rhythmic activity from the same neuron shown at a slower sweep speed. Upper scale bars are for panels (A) and (B). Lower scale bars are for panel (C).

Figure 4. Voltage responses of a representative DE-3 motor neuron before and after exposure to 100 μM DA. The cell was injected with a series of current pulses ranging from -2.0 nA to +2.0 nA. All solutions in this and subsequent figures contained 2 mM Mg²⁺ to suppress rhythmic activity. (A) Voltage responses in control solution (no DA). (B) Same cell 14 min after switching to 100 μM DA.
Rhythmic activity in dopamine solutions prevented accurate measurements of voltage responses to injected current pulses. We found that dopamine-induced oscillations were prevented by adding 2 mM Mg$^{2+}$ to normal leech saline. With Mg$^{2+}$ present (control solution), cell DE-3 was injected with a series of current pulses before, and again 14-17 min after bath application of dopamine. Figure 4A shows the voltage responses of a representative cell DE-3 in control solution. Depolarization increased the firing rate, with a gradual depolarizing trajectory observed in response to the larger current pulses. Such trajectories are consistent with those reported previously for cell DE-3 in control solution (Mangan et al., 1994a). In this cell, the membrane potential returned immediately to −40 mV following current pulse termination. Hyperpolarizing pulses suppressed spontaneous firing, and responses PIR were evoked at current pulse offset. Figure 4B shows the same neuron 14 minutes after switching to control solution containing 100 µM dopamine. The trajectory and amplitude of voltage responses during current injection was little changed. The most obvious effect of dopamine was on rebound responses. In this cell, small after-hyperpolarizing responses (AHPs) occurred following termination of depolarizing current pulses, and the peak amplitude of PIR increased approximately two-fold. It should be noted that in the two preparations where dopamine (in the absence of Mg$^{2+}$) failed to evoke rhythmic activity, similar changes in rebound responses were observed (data not shown). This indicates that Mg$^{2+}$ does not alter, at least qualitatively, the modulatory effects of dopamine.

We quantified the effects of dopamine by measuring the amplitude of voltage responses to injected current pulses, the peak amplitude of PIR responses, and rebound response areas (see Materials and Methods). Dopamine had relatively minor effects on cell DE-3 current-voltage (I/V) plots (Figure 5A). Responses to depolarizing pulses appeared to be slightly larger in dopamine, but this increase was statistically significant only for the +0.5 nA pulses (P<0.05). In contrast, voltage responses to hyperpolarizing pulses tended to be smaller in dopamine, but only one of these differences (at -1.0 nA) was significant.

**Figure 5.** Effect of DA on the electrical properties of cell DE-3. (A) Current voltage (I/V) plots before and after 100 µM DA (n = 7 cells). (B) Peak amplitude of PIR responses evoked at the offset of hyperpolarizing current pulses. (C) Rebound response areas (RRAs) for the same set of cells. The left side shows PIR area and the right AHP area (see Materials and Methods). Error bars in this and subsequent figures represent standard error (S.E.M.). * P<0.05, significant difference between means.
The peak amplitude of PIR was increased by dopamine in six of the seven cells tested. A plot of mean peak PIR revealed statistically significant increases following -1.0 and -1.5 nA current pulses (Figure 5B). The failure to obtain a significant difference for -2 nA pulses (P = 0.07) was likely due to the large variability of peak PIR amplitudes in dopamine, which ranged from 6.5 to 24 mV in the seven cells examined. We also quantified rebound responses by measuring their areas (see Materials and Methods). Unlike its effect on PIR amplitude, dopamine failed to alter PIR area in cell DE-3 (Figure 5C, left). Finally, five of seven cells treated with dopamine, including the one illustrated in Figure 2, showed an increase in AHP responses following +2.0 nA pulses. However, statistical analysis revealed that none of the apparent increases in mean AHP area (Figure 5C, right) was significant. Again, this was likely due to the substantial variability of AHP responses in dopamine solution, as reflected by the large error bars.

Previous studies showed that PIR area in cell DE-3 increases over time during prolonged intracellular recordings (Angstadt et al., 2005). We therefore performed a second set of experiments using separate groups of cells, one from ganglia bathed in control solution (n=13) and the other from ganglia incubated in solution containing 100 µM dopamine (n=12). Dopamine solution was added prior to removing the overlying connective tissue sheath. Thus, incubation time varied (from 11 to 26 min) depending on how quickly the DE-3 was located and successfully penetrated in each ganglion. Each DE-3 motor neuron was injected with a series of current pulses identical to that described above. Dopamine again had little effect on the amplitude of voltage responses to depolarizing current pulses (Figure 6A). As in the previous experiment, voltage responses to hyperpolarizing current pulses tended to be smaller in the presence of dopamine, and this reduction was statistically significant at all current pulses levels except -0.5 nA.

Figure 6. Comparison of electrical properties from separate groups of cells, one incubated in control solution (n=13) and the other in DA (n=12) prior to penetration with the intracellular microelectrode. (A) Current-voltage (I/V) plots. (B) Peak PIR amplitude. (C) Rebound response areas.
Peak PIR amplitude was larger in cells treated with dopamine (Figure 6B). Following the -2.0 nA current pulses, for example, peak PIR increased from approximately 6 mV to 10 mV in dopamine-treated cells. Despite the increase in peak amplitude, dopamine again had no effect on the area of PIR responses (Figure 6C, left). However, AHPs were enhanced in dopamine-treated cells as indicated by a significant increase in AHP area following all depolarizing current pulses (Figure 6C, right).

Angstadt et al. (2005) showed that 50 µM serotonin increases peak PIR amplitude and decreases PIR area. We sought to compare the effects of serotonin with those of dopamine using equivalent concentrations. Thus, separate sets of neurons were incubated in control solution or solution containing 100 µM serotonin. Neurons were exposed to serotonin for at least 15 min prior to data collection. Representative examples of raw data are shown in Figure 7. In control solution (Figure 7A), PIR responses of long duration were evoked. In contrast, PIR responses of cells incubated in serotonin were truncated, ending within about 500 msec (Figure 7B). In addition, the serotonin-treated cells exhibited a characteristic change in the voltage trajectory during injection of depolarizing current. This “relaxation” response has been reported in previous studies using 50 µM serotonin (Mangan et al., 1994a).

I/V plots revealed significantly smaller voltage changes at all current pulse levels in serotonin-treated cells (Figure 8A). In contrast to the effect of 100 µM dopamine, as well as previous studies with 50 µM serotonin (Mangan et al., 1994a), peak PIR amplitudes were unchanged in the presence of 100 µM serotonin (Figure 8B). PIR area, however, was significantly reduced at all current pulse levels (Figure 8C, left), consistent with the truncated appearance of PIR in the raw data. Finally, 100 µM serotonin had no significant effect on the area of AHP responses occurring at the offset of depolarizing current pulses (Figure 8C, right).

In a final set of experiments, we compared cells incubated in control solution (n = 7) with a separate set of cells (n = 7) exposed

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**Figure 7.** Effect of serotonin (5-HT) on the voltage responses of cell DE-3. (A) Voltage responses of a cell in control solution. (B) Voltage responses of a different cell incubated for 15 min in 100 µM 5-HT. Note the decreased duration of PIR responses.
to 50 µM dopamine for 17-28 min (data not shown). At this lower concentration, dopamine had no effect on I/V plots at any current pulse amplitude. Mean peak PIR amplitude tended to be larger in cells exposed to 50 µM dopamine. At the offset of -2 nA current pulses, for example, mean peak PIR was 7.1 mV for control cells and 9.7 mV for cells treated with 50 µM dopamine. The increase in peak PIR was statistically significant following the -1.5 and -2.0 nA pulses. There was no significant effect of 50 µM dopamine on the area of PIR or AHP responses.

**Discussion**

We have shown that 100 µM dopamine evokes rhythmic electrical activity in DE-3 motor neurons in isolated ganglia. In a majority of cells, these membrane potential oscillations persisted for many minutes and exhibited cycle periods ranging from 4 to 17 sec. This is much longer than the cycle period of fictive swimming in isolated nerve cords (0.5 to 1 sec), but is consistent with the mean cycle period (~14 sec) reported for fictive crawling (Eisenhart et al., 2000; Kristan et al., 2005). Bouts of fictive swimming have been reported in isolated ganglia exposed to 50 µM serotonin (Hashemzadeh-Gargari and Friesen, 1989), but to our knowledge this is the first demonstration that dopamine may activate components of the crawling circuit in an isolated ganglion. Preliminary experiments in our lab indicate that dopamine induces similar rhythmic activity in CV cells, excitatory motor neurons to ventrolateral circular muscles. Importantly, this rhythmic CV activity was antiphasic to concomitant bursting in DE-3 motor neurons (recorded extracellularly). Such a phase relationship is consistent with that reported for these cell types during crawling (Eisenhart et al., 2000).

The addition of 2 mM Mg$^{2+}$ to normal leech saline was sufficient to suppress dopamine-induced rhythmic activity in most ganglia. This suppression is likely due to the nonspecific effect of divalent ions on the local electric field, which causes ion channels to sense...
a more hyperpolarized membrane voltage (Hille, 2001). In fact, several laboratories routinely add millimolar concentrations of Mg$^{2+}$ to normal saline in order to dampen spontaneous contraction of muscle fibers embedded in connective nerves (Weeks, 1981). In previous studies of motor neuron PIR and its modulation by serotonin (Mangan et al., 1994a), control solutions contained 1.8 mM Mg$^{2+}$, a concentration similar to that used in our experiments. The stabilizing effect of Mg$^{2+}$ allowed us to establish a stable holding potential of -40 mV from which to assess the effect of dopamine on voltage responses to injected current pulses.

Dopamine produced only minor shifts in cell DE-3 I/V plots. We found no consistent effect on the amplitude of voltage responses to depolarizing current pulses. Responses to hyperpolarizing pulses tended to be smaller in the presence of dopamine. While in some instances the differences were statistically significant, the absolute magnitude of dopamine’s effect was relatively small (on the order of 3-4 mV). Qualitatively, the effects of 100 µM dopamine were similar to those reported previously for 50 µM serotonin, which produced no significant change in the depolarizing quadrant, but a significant decrease in voltage responses to hyperpolarizing current pulses (Angstadt et al., 2005). Our experiments with 100 µM serotonin revealed a significant decrease in response amplitudes in both quadrants at all current pulse levels. Thus, a higher concentration of serotonin appears to strengthen the trend toward a decreased input resistance. Dopamine also tended to decrease cell DE-3 input resistance, at least for hyperpolarizing pulses. However, this change is unlikely to be explained by an effect on leak currents. The mean bias current needed to hold $V_m$ at -40 mV (about -0.1 nA) was not affected by dopamine. This suggests that dopamine is instead altering voltage-dependent currents activated in response to the injected current pulses.

Contrary to our initial hypothesis, the effect of dopamine (50 or 100 µM) was to increase the peak amplitude of PIR responses in cell DE-3. This is qualitatively similar to the effect of 50 µM serotonin (Mangan et al., 1994a), suggesting that both dopamine and serotonin may target the same voltage-dependent Ca$^{2+}$ and Na$^+$ conductances that underlie PIR responses (Angstadt et al., 2005). To our surprise, 100 µM serotonin failed to increase the peak amplitude of PIR. One possible explanation is that the overall decrease in cell input resistance associated with serotonin short-circuited the ability of the PIR current to depolarize $V_m$. However, this seems unlikely to be the principal explanation given the minimal nature of the resistance decrease. An alternate explanation is related to the decreased duration of PIR in the presence of serotonin (see below).

PIR responses in control cells persist for several seconds. Dopamine and serotonin had different effects on PIR duration, which we assessed indirectly by determining the rebound response area. Dopamine (50 or 100 µM) had no statistically significant effect on PIR area. This result is consistent with observations of the raw data, where PIR responses in both control and dopamine-treated cells persisted for at least 1-2 seconds. In contrast, PIR responses in serotonin-treated cells terminated within about 500 msec. This was reflected in the smaller PIR areas obtained for cells exposed to 100 µM serotonin, which were less than 40% of those obtained in control cells. This suppression of PIR area by 100 µM serotonin was similar to that reported previously for 50 µM serotonin (Angstadt et al., 2005). It may be that the mechanism responsible for terminating PIR in the presence of serotonin also serves to limit its amplitude when the concentration is increased to 100 µM. The precise mechanism by which serotonin truncates PIR response has not been determined, but could involve a more effective, and thus more rapid, inactivation of the underlying PIR conductances (see Angstadt et al., 2005).

In addition to increasing PIR amplitude, dopamine also tended to evoke AHP responses in cell DE-3. Because the peak amplitude of AHP responses is less than 3 mV in NS (Mangan et al., 1994a), we chose to quantify AHPs by measuring their area. At 50 µM, dopamine had no effect on AHP area, but significant increases were detected using 100
µM. Mangan et al. (1994a) reported that AHP responses were increased by 50 µM serotonin. In a subsequent study, however, no significant difference was found between AHP areas from control cells and those treated with 50 µM serotonin (Angstadt et al., 2005). In this study, 100 µM serotonin also had no effect on AHP area. In summary, when a significant effect of serotonin or dopamine on AHPs was detected, it was to increase this rebound response. This change could be due to enhancement of depolarization-activated K⁺ currents, but more detailed studies will be required to determine the precise mechanisms.

Overall, we have shown that the modulatory effects of serotonin and dopamine on DE-3 motor neurons in isolated ganglia are similar, but not identical. Our working hypothesis is that these amines work through a common signaling pathway that increases the amplitude of PIR responses, and perhaps also AHP responses. However, our data suggest that serotonin activates an additional pathway that results in a decreased duration of PIR responses. There are numerous descriptions of the complex and varied actions of neuromodulators on rhythmic motor circuits (Marder and Calabrese, 1996). One interesting example relevant to the present study is amine modulation of the AB neuron, a conditional oscillator in the pyloric circuit of the lobster STG. Bath application of dopamine, serotonin or octopamine induces rhythmic bursting in the isolated AB cell. However, each transmitter produces activity with a characteristic amplitude and frequency (Harris-Warrick and Flamm, 1987). These differences occur in part because the modulators target distinct combinations of ionic conductances in the AB cell. Our data are consistent with a similar explanation for the distinct effects of dopamine and serotonin on cell DE-3.

There is no evidence that DE-3 motor neurons are directly involved in generating the rhythmic electrical activity underlying either swimming or crawling behavior (Kristan et al., 2005). However, rhythmic activity produced by the pattern generating circuit must be transferred through motor neurons to muscle, and thus cell DE-3 exhibits Vₘ oscillations with a cycle period appropriate for each respective behavior (Kristan et al., 2005). It seems reasonable to assume that PIR and AHP rebound responses could affect this information transfer, either by reinforcing the rhythm, or interfering with it. In fact, Mangan et al. (1994a) concluded that serotonergic modulation of PIR and other intrinsic properties of excitatory swim motor neurons contributed to the effective implementation of swimming behavior. The shorter duration of PIR associated with serotonin is well-suited for the relatively rapid frequency of swim oscillations. Conversely, PIR responses of long duration (several seconds), which occur in NS and persist in the presence of dopamine, seem inappropriate for swimming. However, prolonged PIR responses are consistent with, and may even help promote, the slow rhythmic activity associated with crawling. In summary, by augmenting PIR amplitude and promoting AHPs, each modulator appears to prime cell DE-3 for rhythmic activity. However, by also shortening PIR duration, serotonin prepares the cell for effective transfer of swim oscillations occurring in the 1 Hz range. Dopamine need not induce this change in PIR duration because prolonged PIR responses are appropriate for the rhythmic activity underlying crawling behavior.

References


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