Dopaminergic Modulation of Spinal Neurons and Synaptic Potentials in the Lamprey Spinal Cord

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Kemnitz, Christopher P. Dopaminergic modulation of spinal neurons and synaptic potentials in the lamprey spinal cord. J. Neurophysiol. 77: 289–298, 1997. It has been shown previously that dopamine-immunoreactive cells and processes are present in the lamprey spinal cord and that dopamine modulates the cycle period of fictive swimming. The present study was undertaken to further characterize the effects of dopamine on the cellular properties of lamprey spinal neurons and on inhibitory and excitatory postsynaptic potentials to determine how dopaminergic modulation may affect the central pattern generator for locomotion. Dopamine reduced the late afterhyperpolarization (late AHP) following the action potential of motoneurons, and in three types of sensory neurons: dorsal cells, edge cells, and giant interneurons. The late AHP was not reduced in lateral interneurons or CC interneurons, both of which are part of the central motor pattern generating neural network. The reduction of the late AHP in motoneurons, edge cells, and giant interneurons resulted in an increase in firing frequency in response to depolarizing current injection. In the six cell classes examined, no changes were observed in the resting membrane potential, input resistance, rheobase, spike amplitude, or spike duration after application of dopamine. The durations of action potentials broadened by application of tetraethylammonium, atropine, and serotonin during D-glutamate-induced fictive swimming led to an initial increase in spontaneous activity and a decreased cycle period, but with time, the motor pattern became completely disrupted (Harris-Warrick and Cohen 1985). More recently, it was reported that dopamine could alter the cycle period of fictive swimming in a bimodal manner: bath application of 5–500 μM dopamine during D-glutamate-induced fictive swimming led to an initial increase in spontaneous activity and a decreased cycle period, while concentrations of dopamine > 10 μM caused a dose-dependent increase in cycle period of 5–10 times the control values (McPherson and Kemnitz 1994). In addition to its effects on fictive swimming, dopamine reduced the late afterhyperpolarization (late AHP) after the action potential in lamprey spinal motoneurons, and dopamine-immunoreactive cells and processes were localized within the lamprey spinal cord (McPherson and Kemnitz 1994). These initial reports were confirmed subsequently (Schofield et al. 1995). As part of an ongoing study of dopaminergic modulation in the lamprey spinal cord, the effects of dopamine on the cellular and synaptic properties of lamprey spinal neurons were investigated to identify possible mechanisms by which dopamine may affect network output.

It has been reported that dopamine reduces calcium currents, decreases the late AHP, and reduces monosynaptic postsynaptic potentials in lamprey spinal neurons (Wikström et al. 1995). However, it is not known which, if any, of these mechanisms are involved in altering motor output of the lamprey CPG. Serotonin (which is stored and coreleased with dopamine) has been suggested to alter motor output by reducing the late AHP of the locomotor network neurons, lateral interneurons and CC interneurons, and the selective reduction of IPSPs from CC interneurons suggest that synaptic modulation may play an important role in dopaminergic modulation of cycle period during fictive swimming in the lamprey.

INTRODUCTION

There is currently a great deal of evidence supporting a role for dopamine in the modulation of neuronal networks in the spinal cord. Dopamine and dopamine-immunoreactive cells and processes have been shown to be present in the spinal cords of several types of vertebrates including mammals, reptiles, and fish (Bennis et al. 1990; Commissiong and Neff 1979; Commissiong and Sedgwick 1974, 1975; McGeer and McGeer 1962; Roberts et al. 1989). The spinal dopaminergic system has been implicated in the modulation of spinal reflexes, nociception, and locomotion (Barasi and Roberts 1977; Barbeau and Rossignol 1991; Maitra et al. 1992). In the lamprey spinal cord, dopamine and serotonin have been shown to be colocalized and coreleased from a ventromedial plexus and the actions of these two neurotransmitters are thought to be complementary (Schofield et al. 1995; Wikström et al. 1995).

In the isolated lamprey spinal cord, the neuronal correlate of swimming can be induced by superfusion with an excitatory amino acid such as glutamate or N-methyl-D-aspartate (NMDA), and this activity is referred to as fictive swimming (Cohen and Wallén 1980). Fictive swimming in lamprey can be altered by the presence of neuromodulators such as serotonin and dopamine. Bath application of 5–500 μM dopamine during D-glutamate-induced fictive swimming led to an initial increase in spontaneous activity and a decreased cycle period, but with time, the motor pattern became completely disrupted (Harris-Warrick and Cohen 1985). More recently, it was reported that dopamine could alter the cycle period of fictive swimming in a bimodal manner: bath application of 0.1–1 μM dopamine during D-glutamate-induced fictive swimming produced a 25% decrease in the cycle period, while concentrations of dopamine > 10 μM caused a dose-dependent increase in cycle period of 5–10 times the control values (McPherson and Kemnitz 1994). In addition to its effects on fictive swimming, dopamine reduced the late afterhyperpolarization (late AHP) after the action potential in lamprey spinal motoneurons, and dopamine-immunoreactive cells and processes were localized within the lamprey spinal cord (McPherson and Kemnitz 1994). These initial reports were confirmed subsequently (Schofield et al. 1995). As part of an ongoing study of dopaminergic modulation in the lamprey spinal cord, the effects of dopamine on the cellular and synaptic properties of lamprey spinal neurons were investigated to identify possible mechanisms by which dopamine may affect network output.

It has been reported that dopamine reduces calcium currents, decreases the late AHP, and reduces monosynaptic postsynaptic potentials in lamprey spinal neurons (Wikström et al. 1995). However, it is not known which, if any, of these mechanisms are involved in altering motor output of the lamprey CPG. Serotonin (which is stored and coreleased with dopamine) has been suggested to alter motor output by
its actions on the late AHP (Wallén et al. 1989). The data presented here suggest that dopamine alters motor output via synaptic modulation rather than through the late AHP. It appears that the actions of dopamine and serotonin are complementary and distinct.

**METHODS**

**Animals and preparation**

The experiments were performed using in vitro preparations of spinal cord from adult silver lampreys (*Ichthyomyzon unicuspis*, 20–33 cm in length) and adult sea lampreys (*Petromyzon marinus*, 35–48 cm in length). The animals were anesthetized by immersion in 100 mg/L tricaine methane sulfonate (Sigma), decapitated, and the notochord with spinal cord attached was dissected in cooled Ringer solution (Rovainen 1974a). Normal Ringer solution consisted of (in mM) 91 NaCl, 2.1 KCl, 2.6 CaCl$_2$, 1.8 MgCl$_2$, 20 NaHCO$_3$, and 4 glucose and was bubbled with 95% O$_2$/5% CO$_2$ to pH 7.4. Four- to 30-segment long sections of spinal cord were prepared for recording by splitting the notochord ventrally and then pinning the tissue dorsal side up to the bottom of a Sylgard-lined recording chamber (Sylgard 184, Dow Corning). The spinal cord was exposed dorsally by removing the overlying cartilage and meninges. The recording chamber was perfused with cooled Ringer solution (8–10°C; flow rate 2–3 ml/min; bath volume 10 ml) and illuminated from below. Individual cells and axons were viewed using a stereomicroscope.

**Intracellular and extracellular recordings**

Intracellular recordings were made with glass microelectrodes, which usually were filled with 4 M potassium acetate. Occasionally, the micropipette contained 5% Lucifer yellow CH (Sigma) in 1 M LiCl for pressure injection and subsequent identification of neurons using an epifluorescence microscope. Microelectrode resistances with 4 M potassium acetate were from 50 to 90 MΩ. Intracellular signals were amplified using an Axoclamp 2A electrometer (Axon Instruments) in either bridge mode or discontinuous current clamp (DCC) mode. Extracellular ventral root electrodes and spinal cord electrodes for stimulation and recording consisted of glass suction electrodes filled with Ringer. Extracellular recordings were amplified with AC differential amplifiers (Bak). Recordings were stored on a digital tape recorder (Biologic) and analyzed on a personal computer using a Cambridge Electronics Design 1401 data acquisition system and associated software (Spike2).

**Identification of neurons**

The effects of dopamine on six classes of lamprey spinal neurons were tested. 1) Dorsal cells are primary mechanosensory neurons that respond to touch and pressure (Christenson et al. 1988). Dorsal cells were readily identified anatomically by their large size (40–60 μm diam), circular shape, and characteristic position dorsolateral to the central canal of the spinal cord (Rovainen 1967). 2) Giant interneurons have crossed ascending axons and their large cell bodies in the caudal half of the spinal cord are easily identified visually. These cells relay signals from dorsal cells and from caudal giant interneurons to the rostral spinal cord and brain stem (Rovainen 1967). 3) Edge cells are intraspinal stretch-sensitive neurons localized along the ventrolateral edge of the spinal cord; they are easily identified by their unique shape and location (Grillner et al. 1984). 4) Lateral interneurons are inhibitory interneurons and have been proposed to be part of the locomotor central pattern generator network (Buchanan and Grillner 1987). Lateral interneurons are the largest interneurons in the rostral half of the spinal cord and were recognized by their large transversely oriented soma located at the border of the grey matter and lateral axon tract. The identity of each lateral interneuron was confirmed by recording its caudally projecting ipsilateral axon in the spinal cord while stimulating the lateral interneuron intracellularly (Rovainen 1974a). 5) Interneurons with contralaterally and caudally projecting axons (CC) also have been proposed to be involved in the locomotor network (Buchanan and Grillner 1987). These cells have medium-sized cell bodies and were identified physiologically by recording one-for-one action potentials extracellularly from the cut contralateral, caudal end of the spinal cord while stimulating each CC interneuron intracellularly (Buchanan 1982). 6) Motor-neurons were identified by one-for-one correlation of action potentials elicited via intracellular stimulation, with spikes recorded from extracellular suction electrodes placed against the ventral roots. To aid neuron identification, retrograde labeling techniques were also used in some experiments. To apply retrograde tracers, lampreys were anesthetized in 100 mg/L tricaine methanesulfonate and placed in a shallow, Ringer-filled, Sylgard-lined tray. A small region of spinal cord was exposed dorsally along the midline. For rhodamine-conjugated latex microspheres (0.02–0.2 μm diam, Lumafuor), pressure was used to inject the microspheres from a glass micropipette into the spinal cord. For fluorescein dextran amines (MW 3000; Molecular Probes), a hemisection was made in the spinal cord, and a dye-soaked piece of gelfoam was inserted into the cut. The animals were allowed to survive for 2–4 wk to allow time for retrograde transport, after which they were killed for physiological experiments as described above. Labeled interneurons were visualized during recording with a modified Nikon epifluorescent microscope using a 100-W mercury light source (McPherson and Buchanan 1995). No differences were noted in the properties of labeled neurons versus neurons in nonlabeled spinal cords.

**Cellular studies**

After impalement and identification of a neuron, the effect of dopamine was tested on action potentials, input resistance, rheobase, and the spike frequency versus input current (F-I) relationship. For action potentials, short (2–3 ms) intracellular current pulses were used to elicit action potentials in bridge mode. The resting membrane potential, the potential of the peak of the spike, the spike duration at one-half peak amplitude, and the amplitude of the late AHP with respect to resting membrane potential were measured. Input resistance was determined in discontinuous current clamp mode by intracellular injection of small hyperpolarizing currents, 300 ms in duration. The voltage responses for several current levels were recorded, and the steady-state voltage responses were plotted against the injected current to yield input resistance (slope). Spike frequency versus input current was measured in discontinuous current-clamp mode using pulses of 400 ms duration given at 2-s intervals starting at threshold, and incremented until the action potentials attenuated to 67% of the initial action potential’s amplitude. The instantaneous spike frequency (1/interval) was measured for the first spike intervals and plotted versus the injected current (normalized to rheobase).

The effect of dopamine on calcium action potentials was tested in dorsal cells and giant interneurons. After stable impalement of a neuron in normal Ringer, the perfusion medium was switched to modified high-calcium Ringer containing (in mM) 107.5 NaCl, 2 KCl, 10 CaCl$_2$, 2 N-2-hydroxyethylpipperazine-N’-2-ethanesulfonic acid, 4 glucose, 15 tetraethylammonium-Cl (TEA), and 5 4-amino-pyridine (4-AP) and 1 μM tetrodotoxin (TTX) (Leonard and Wickelgren 1985). Calcium action potentials were elicited at a rate of 0.1–0.5 spikes/s depending on the cell and the stability of the calcium action potential. Once stable calcium action potentials were obtained, dopamine was tested. To test the effect of dopamine
on calcium entry into motoneurons, action potentials broadened by TEA (5 mM) were used. The broadening of the action potential in TEA has been shown to be due to increased calcium influx (Wallén et al. 1989). In motoneurons, calcium action potentials obtained as described for dorsal cells and giant interneurons were too unstable for testing the effects of dopamine. Addition of 500 µM CdCl₂ eliminated calcium action potentials and TEA broadened action potentials.

The effect of dopamine on TTX-resistant, NMDA-induced membrane potential oscillations was tested by impaling a motoneuron, and then perfusing with 0.2 mM N-methyl-D,L-aspartate (NMA) to induce oscillatory membrane potential activity. After adding 1 µM TTX to the perfusion fluid to block action potentials, membrane potential oscillations were observed, although in some instances, it was necessary to inject a small hyperpolarizing current (−0.5 to −3 nA) to stabilize the oscillatory activity.

Dopamine application

Dopamine was applied either by bath perfusion or by local pressure ejection. All dopamine solutions contained 0.1% sodium metabisulfite (Sigma) as an antioxidant, and solutions for pressure ejection also contained Fast Green dye to visualize application following pressure ejection. For bath perfusion, 1–100 µM dopamine was used; for local pressure ejection, 10 µM dopamine was used. For pressure ejection, the tip of the micropipette was lowered to within 100 µm of the spinal cord surface, slightly upstream from the impaled neuron while continuously perfusing the experimental chamber.

Synaptic potentials

Polysynaptic excitatory postsynaptic potentials (EPSPs) were elicited in motoneurons by electrically stimulating the contralateral half of the spinal cord in the presence of 5 µM strychnine (stimulation rate 0.2 pulses/s). It was the intent of this experiment to activate crossed excitatory synaptic input, rather than descending reticulospinal input to these spinal neurons. For this, the spinal cord was split along the midline for 2–3 segments at one end and drawn into a glass electrode using suction. Excitatory interneurons with crossing axons were thus activated to produce polysynaptic EPSPs in the contralateral motoneuron, while blocking any glycinergic inhibited crossed input. Polysynaptic inhibitory postsynaptic potentials (IPSPs) were elicited in motoneurons via similar electrical stimulation of the contralateral spinal cord in the absence of strychnine. In this case, both inhibitory and excitatory interneurons with crossing axons were activated, but the IPSPs dominated the responses.

Paired intracellular recordings between Müller axons and motoneurons were made to assess the effects of dopamine on the combined electrotonic-chemical monosynaptic EPSP produced by Müller axons (Rovainen 1974b). Müller axons were identified by their ventromedial position in the spinal cord and their fast conduction velocities (3.2–4.1 m/s). Once a paired recording was established, the EPSPs were elicited by stimulating the presynaptic axon at 1 pulse/s.

Monosynaptic and polysynaptic EPSPs and IPSPs were evaluated using paired intracellular recordings made between presynaptic axons or presynaptic interneurons and postsynaptic motoneurons. Synapses were considered to be monosynaptic if they had a short, constant synaptic delay (<3 ms) and had the ability to follow stimulation of the presynaptic cell at 10 pulses/s. Polysynaptic responses had synaptic delays >3 ms and generally did not follow presynaptic stimulation with a constant latency at 10 Hz stimulation. Synaptic delay was estimated by subtracting the conduction time of the action potential from the presynaptic cell to the postsynaptic cell based on measurement of the conduction velocity and the distance between the two cells.

Statistics

Data were analyzed using a two-tailed Student’s paired t-test to test for differences between means before and after experimental manipulation. Values are reported as means ± SE. Percent changes are given to indicate the difference between the values being compared such that a reduction of 30% indicates the mean control values were normalized to 100% and the mean experimental value was decreased by 30–70% of control. The level of significance chosen was P < 0.05.

RESULTS

Effect of dopamine on the cellular properties of lamprey spinal neurons

For the six classes of neurons examined, application of dopamine produced no significant changes in the mean values for resting membrane potential, spike amplitude, spike duration, rheobase, or input resistance (Fig. 1, A, C, and D; n = 75 neurons). The late AHP, however, was reduced significantly in motoneurons by 37 ± 10%, giant interneurons by 32 ± 11%, in edge cells by 30 ± 8%, and dorsal cells by 39 ± 12% (Figs. 1B and 2). The late AHPs of lateral interneurons and CC interneurons were not significantly altered by dopamine.

Spike frequency versus input current relationship

The late AHP is important in regulating the firing frequency of neurons (Gustaffson 1974). To determine whether the reductions of the late AHP observed here altered the spike frequency versus input current relationship, dopamine was applied to neurons while injecting long (400 ms) supra-threshold current pulses. Instantaneous spike frequency (1/first interspike interval) was plotted versus input current, normalized to rheobase (F-I plot). Dopamine produced an upward shift of the F-I plots of neurons that showed a reduction of the late AHP; motoneurons, giant interneurons, and edge cells (Fig. 3, A–C). Thus for the same input current, an affected cell exhibited a higher firing rate after application of dopamine. The F-I plots for 4/5 motoneurons, 4/6 giant interneurons, and 7/8 edge cells were shifted upward after application of dopamine. No shifts in the F-I plots were observed after dopamine application to CC interneurons (Fig. 3D; n = 4) or to lateral interneurons (Fig 3E; n = 5), consistent with the lack of an effect of dopamine on the late AHP in these cell types. The F-I relationships for dorsal cells were not tested with dopamine.

Calcium influx

The late AHP in lamprey spinal neurons is produced by a Ca⁺⁺-dependent K⁺ current (Hill et al. 1985). To determine whether the reduction of the late AHP was due to a reduction of calcium currents, the effect of dopamine on calcium entry was assessed. The action potentials of motoneurons were broadened by adding 5 mM TEA to the perfusion Ringer to block voltage-gated potassium currents. This broadening has been shown to be dependent upon calcium influx (Hill et al. 1985; Wallén et al. 1989). The mean duration of TEA-broadened action potentials was 21 ± 8 ms (n = 5) measured at one-half maximal spike amplitude, ~10 times the normal duration (Fig. 1C). Bath
FIG. 1. Summary of cellular effects of dopamine on lamprey spinal neurons. Values shown represent means ± SE for all neurons tested before (●) and after (○) dopamine application. Dopamine was applied via bath perfusion or pressure ejection for motoneurons. In all other cases, dopamine was applied by pressure ejection. A: resting potential (RP) and peak of action potential (AP). B: amplitude of late after-spike hyperpolarization (lAHP) as measured from resting potential to peak of lAHP. C: spike duration as measured at one-half peak amplitude. D, top: rheobase is depolarizing current required to reach spike threshold when injecting long (400 ms) pulses. Ordinate is expressed logarithmically. D, bottom: input resistance of neuron as measured using small hyperpolarizing current pulses. MN, motoneurons (n = 23); LIN, lateral interneurons (n = 10); CC, contralaterally and caudally projecting neurons (n = 6); EC, edge cells (n = 13); GI, giant interneurons (n = 13); DC, dorsal cells (n = 13). *P < 0.05 level, Student’s t-test.

application of 10 μM dopamine reversibly reduced the duration of TEA-broadened action potentials in motoneurons by an average of 23 ± 4% (n = 5; Fig. 4A). These results are consistent with earlier reports showing that dopamine reduces calcium influx into lamprey spinal neurons (Kemnitz et al. 1994; Schotland et al. 1995). It has been suggested that decreased calcium influx may be involved in reduction of the late AHP in motoneurons by reducing the calcium available for activation of Ca²⁺-dependent K⁺ currents (Kemnitz et al. 1994; Schotland et al. 1995).

It has been shown previously that sensory neurons within the lamprey spinal cord are capable of producing calcium action potentials (Leonard and Wickelgren 1985). Calcium action potentials were elicited in the present study in both sensory dorsal cells and in giant interneurons using a high-calcium Ringer containing TTX to block voltage-gated sodium currents, and TEA and 4-AP to block potassium currents. After switching the perfusion solution to the high-calcium Ringer, the cells typically hyperpolarized 5–10 mV during the first few minutes and then stabilized. The membrane potentials of the neurons recorded from ranged from 0.59 to 0.68 mV in dorsal cells and 0.72 to 0.78 mV in giant interneurons. Calcium action potentials were elicited by short, depolarizing current pulses. The amplitudes of the calcium action potentials were similar in dorsal cells and giant interneurons, ranging from 60 to 80 mV. The durations of the calcium action potentials, however, were quite variable from cell to cell, ranging from 70 to 400 ms, but stable in a given cell. Bath application of dopamine reduced the duration of the calcium action potentials in dorsal cells by 32 ± 7% (n = 7; Fig. 4B) and in giant interneurons by 26 ± 9% (n = 6; Fig. 4C). The effects of dopamine on the calcium action potentials were partially reversible in most cases (Fig. 4, A–C). Thus as for motoneurons, decreased calcium influx may be responsible for reduction of the late AHP in these cells.

TTX-RESISTANT NMDA OSCILLATIONS. Activation of NMDA receptors induces fictive swimming in the lamprey spinal cord and also produces TTX-resistant membrane potential oscillations in lamprey spinal neurons. The repolarization phase of these oscillations may depend in part on the activation of Ca²⁺-dependent K⁺ channels (Wallén and Grillner 1987). These membrane potential oscillations are believed to be important in helping to shape neuronal output during fictive swimming (Wallén and Grillner 1987). Because dopamine was found to reduce calcium influx and the late AHP in several classes of spinal neurons, the possibility of dopaminergic modulation of TTX-resistant, NMDA-induced membrane potential oscillations was explored.
DOPAMINERGIC MODULATION IN THE LAMPREY SPINAL CORD

POLYSYNAPTIC EPSPS AND IPSPS. Compound polysynaptic EPSPs were elicited in motoneurons by electrically stimulating the contralateral spinal cord in the presence of 5 μM strychnine. These EPSPs thus relied on excitatory commissural interneurons. Bath application of 10 μM dopamine caused little or no change in the amplitude of the polysynaptic EPSPs (Fig. 6A; n = 10). Compound polysynaptic IPSPs were elicited in motoneurons by stimulating the contralateral spinal cord in the absence of strychnine. These PSPs are mixed EPSPs and IPSPs, but the IPSPs dominate the records. The IPSPs probably are due mainly to inhibitory commissural interneurons. The mean amplitude of polysynaptic IPSPs was reduced by 33 ± 6% after bath application of 10 μM dopamine (Fig. 6B; n = 11). The effects of dopamine on polysynaptic IPSPs suggest that dopaminergic modulation of synaptic interactions may be occurring in the lamprey spinal cord, possibly at the output synapses of inhibitory CC interneurons.

Dorsal cells produce monosynaptic and polysynaptic EPSPs and polysynaptic IPSPs in the second-order sensory giant interneurons (Rovainen 1974b). Paired recordings between dorsal cells and giant interneurons evoked either polysynaptic EPSPs or IPSPs in the giant interneurons, as indicated by the long synaptic latencies and the inability to follow high stimulation rates. Therefore low rates of stimulation (0.2 Hz) were used. The dorsal cell to giant interneuron polysynaptic IPSPs were reduced by 39 ± 14% (n = 2) after bath application of 10 μM dopamine (Fig. 7A). The dorsal cell to giant interneuron polysynaptic EPSPs were not affected by 10 μM bath-applied dopamine (n = 3; Fig. 7B).

ELECTROTONIC-CHEMICAL EPSPS. Müller axons of the descending reticulospinal system activate many types of spinal neurons via a monosynaptic combined electrotonic-chemical EPSP (Rovainen 1974b). To test whether dopamine was modulating descending EPSPs in motoneurons, paired recordings were made between Müller axons and motoneurons. No changes in the amplitude or time course of either the early or late component of the EPSPs were observed after bath application of 10 μM dopamine (n = 5) or pressure ejection of 10 mM dopamine (n = 6; Table 1). Dopamine, therefore, does not appear to modulate the Müller axon to motoneuron EPSP in the lamprey spinal cord.

Giant interneurons synapse directly upon more rostral giant interneurons on either side of the spinal cord via combined electrotonic-chemical synapses (Rovainen 1974a). Paired recordings between giant interneurons were made to further explore possible effects of dopamine on the combined electrotonic-chemical excitatory synapse. In the eight pairs of giant interneuron EPSPs tested, no change in the amplitude or time course of the EPSPs were observed after application of dopamine (Table 1).

MONOSYNAPTIC IPSPS. The reduction of polysynaptic IPSPs in motoneurons by dopamine suggested that inhibitory synaptic transmission may be a site for dopaminergic modulation. To further investigate the effects of dopamine on IPSPs, paired recordings were made between inhibitory presynaptic...
FIG. 4. Bath application of dopamine (10 μM) reduced duration of tetraethylammonium (TEA)-broadened action potential in motoneurons (A) and reduced duration of calcium action potentials in dorsal cells (B) and giant interneurons (C). TEA-broadened action potentials were elicited by bath perfusion with 5 mM TEA. Calcium action potentials were elicited by bath perfusion with a high-calcium Ringer containing tetrodotoxin (TTX), TEA, and 4-aminopyridine (4-AP).

Axons and postsynaptic motoneurons and between presynaptic inhibitory CC interneurons and postsynaptic motoneurons. After bath application of 10 μM dopamine, the amplitudes of the IPSPs from unidentified presynaptic axons to motoneurons were reduced by an average of 41 ± 20% (n = 3; Table 1). The IPSPs from CC interneurons to motoneurons also were tested. After bath application of 10 μM dopamine, the CC interneuron to motoneuron IPSPs were reduced by 48 ± 15% (n = 6; Table 1; Fig. 8A). The decreases in the CC interneuron to motoneuron IPSP were reversible in three cases; the recordings were lost during wash-out of dopamine in the remaining pairs. Inhibitory CC interneurons synapse upon contralateral interneurons and contralateral motoneurons and form a reciprocal inhibitory network that has been proposed to be the core of the lamprey locomotor network (Buchanan 1986; Buchanan and Grillner 1987). The reduction of monosynaptic IPSPs and in particular the CC interneuron to motoneuron IPSPs suggests that dopamine may be acting at inhibitory synapses within the lamprey locomotor network to modulate network activity.

Another important inhibitory synapse in the lamprey locomotor network is the IPSP produced in CC interneurons by presynaptic inhibitory lateral interneurons. Lateral interneurons have been proposed to be important for burst termination during fictive swimming by inhibiting ipsilateral CC interneurons (Buchanan 1986; Buchanan and Grillner 1987). Three lateral interneuron to CC interneuron pairs were tested with bath-applied 10 μM dopamine. Dopamine had no effect on these IPSPs (Table 1; Fig. 8B). The lack of modulation by dopamine at the lateral interneuron to CC interneuron synapse suggests that dopamine acts preferentially at crossed inhibitory synapses. However, more lateral interneuron to CC interneuron pairs will be needed to confirm this possibility.

MONOSYNAPTIC EPSPS. Three excitatory CC interneurons that produced monosynaptic EPSPs in unidentified contralaterally and caudally located neurons were tested with bath-applied 10 μM dopamine, with no effect (Table 1). One excitatory neuron with a contralateral ascending axon was impaled that produced a monosynaptic EPSP in an unidentified rostrally located neuron. After bath perfusion of 10 μM dopamine, the EPSP was reduced by 19%, and this reduction was reversed following washout of dopamine with normal Ringer (Fig. 8D; Table 1).

FIG. 5. Dopamine increases duration of TTX-resistant, N-methyl-d-aspartate (NMDA)-induced membrane potential oscillations in motoneurons. Each trace in this figure represents average of 30 oscillations for time periods before (A), during 10 μM dopamine perfusion (B), and after wash-out of dopamine (C). TEA was not present in bath, therefore oscillations do not show a distinct depolarized plateau phase.
DISCUSSION

It has been shown previously that dopamine alters the cycle period of fictive swimming in the isolated lamprey spinal cord (McPherson and Kemnitz 1994; Schotland et al. 1995). The present study was undertaken to further explore possible cellular and synaptic mechanisms of dopaminergic neuromodulation of the locomotor network. Dopamine also has been shown to modulate sensory neurotransmission in the spinal cord of several vertebrate species (Barbeau and Rossignol 1991; Maitra et al. 1992). Therefore the effects of dopamine on the cellular and synaptic properties of sensory-related spinal neurons also were assessed in the present study.

Cellular properties

The late AHP after the action potential plays an important role in regulating neuronal firing frequency (Gustaffson 1974). In the lamprey, the late AHP has been shown to be the site of modulation by \( \gamma \)-aminobutyric acid (GABA) and serotonin (Matsushima et al. 1993; Van Dongen et al. 1986; Wallén et al. 1989). In the present study, it was found that the late AHP was reduced by dopamine in motoneurons, giant interneurons, edge cells, and dorsal cells (Figs. 1 and 2). However, the late AHP was not reduced by dopamine in either CC interneurons or lateral interneurons (Fig. 1), suggesting a selective action of dopamine on different cell classes. Reduction of the late AHP in lamprey spinal neurons has been proposed to be a mechanism for increasing the cycle period of fictive swimming by delaying burst termination in CC interneurons (Grillner et al. 1988; Wallén et al. 1989). It has been suggested that dopamine may reduce the cycle frequency of fictive swimming via reduction of the late AHP (Grillner et al. 1995; Schotland et al. 1995). If so, it may be acting on CPG interneurons not tested in this study.

Lamprey motoneurons are proposed to act only as output elements for the locomotor network and appear not to participate in rhythmogenesis (Wallén and Lansner 1984). Reduction of the late AHP in motoneurons therefore would not be expected to affect the cycle period of fictive swimming but would increase the number of action potentials fired during the excitatory phase of each locomotor cycle (Fig. 3). Consistent with this prediction, apomorphine, a dopamine receptor agonist, has been shown to increase ventral root burst intensity during fictive swimming (Kemnitz et al. 1994).

In addition to its action on motoneurons, dopamine also reduced the late AHP in sensory-related neurons in lamprey. This is in contrast to the neuromodulator serotonin, which reduces the late AHP of motoneurons but not of edge cells or dorsal cells, suggesting that serotonin and dopamine have different sites of action (Wallén et al. 1989). Dopamine modulates sensory and reflex behaviors in the spinal cord of rats, mice, and cats (Fleetwood-Walker et al. 1988; Hasegawa et al. 1990; Liu et al. 1992; Maitra et al. 1993). Thus as in higher vertebrates, dopamine may be involved in modulating the sensitivity of sensory-related neurons in the lamprey.

The late AHP in lamprey spinal neurons is due to a \( \text{Ca}^{2+} \)-dependent \( K^+ \) current that is sensitive to apamin, a selective SK \( \text{Ca}^{2+} \)-dependent \( K^+ \) channel blocker (Hill et al. 1985; Meer and Buchanan 1992). Dopamine could act on the late...
### Table 1. Summary of the effects of dopamine on monosynaptic postsynaptic potentials

<table>
<thead>
<tr>
<th>Presynaptic Cell</th>
<th>CV, m/s</th>
<th>Postsynaptic Cell</th>
<th>CV, m/s</th>
<th>Synaptic Control</th>
<th>Postsynaptic Cell</th>
<th>Control Delay, ms</th>
<th>PSP Type</th>
<th>Amplitude, mV</th>
<th>Post-Dopamine Amplitude, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidentified axon</td>
<td>1.80 ± 0.30</td>
<td>Motoneuron</td>
<td>+</td>
<td>2.10 ± 0.20</td>
<td>IPSP (3)</td>
<td>1.30 ± 0.50</td>
<td>0.70 ± 0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC interneuron</td>
<td>1.47 ± 0.50</td>
<td>Motoneuron</td>
<td>+</td>
<td>2.99 ± 0.37</td>
<td>IPSP (6)</td>
<td>0.80 ± 0.70</td>
<td>0.40 ± 0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC interneuron</td>
<td>1.33 ± 0.49</td>
<td>Unidentified neuron</td>
<td>+</td>
<td>3.00 ± 0.57</td>
<td>EPSP (3)</td>
<td>0.87 ± 0.39</td>
<td>0.88 ± 0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral interneuron</td>
<td>2.10 ± 0.20</td>
<td>CC interneuron</td>
<td>1.45 ± 0.21</td>
<td>3.42 ± 0.35</td>
<td>IPSP (3)</td>
<td>1.13 ± 0.31</td>
<td>1.13 ± 0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified axon</td>
<td>1.3</td>
<td>Lateral interneuron</td>
<td>1.9</td>
<td>4.10</td>
<td>IPSP (1) †</td>
<td>1.07</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuron with crossed ascending axon</td>
<td>2.96</td>
<td>Unidentified neuron</td>
<td>+</td>
<td>3.71</td>
<td>EPSP (1) ‡</td>
<td>1.73</td>
<td>1.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giant interneuron</td>
<td>3.81 ± 0.30</td>
<td>Giant interneuron</td>
<td>+</td>
<td>0.71 ± 0.53 ‡</td>
<td>EPSP</td>
<td>3.12 ± 1.89 ‡</td>
<td>3.08 ± 1.79 ‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muller Axon</td>
<td>2.80 ± 0.73</td>
<td>Motoneuron</td>
<td>+</td>
<td>1.12 ± 0.41 ‡</td>
<td>EPSP</td>
<td>2.59 ± 1.72 ‡</td>
<td>2.58 ± 1.88 ‡</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE except for two cases (†) where only one pair was tested; number of pairs tested is in parentheses. Conduction velocities (CV) are reported in m/s. Postsynaptic potential (PSP) amplitude is reported in millivolts. † Parameter not measured. ‡ Electrical component of combined electrotonic-chemical PSP. § Chemical component of combined electronic-chemical PSP.

AHP by either reducing the influx of calcium or by directly reducing the potassium current. The results here confirm earlier reports that dopamine may reduce the late AHP by reducing calcium influx (Kemnitz et al. 1994; Schotland et al. 1995). It was found that dopamine reduced the duration of the TEA-broadened action potential in motoneurons (Fig. 4). Because the broadening of the action potential in TEA has been shown to be dependent on calcium (Wallén et al. 1989), it is likely that dopamine decreased the influx of calcium during the action potential. This could decrease activation of Ca$_{2+}$-dependent K$^+$ channels underlying the late AHP. Serotonin also reduces the late AHP in lamprey motoneurons, but does not reduce the duration of the TEA-broadened action potential. Thus serotonin may act directly on the potassium current or on calcium handling in the cell. It would seem, then, that dopamine and serotonin modulate the late AHP through different mechanisms (Schotland et al. 1995). Dopamine also reduced calcium action potentials in both dorsal cells and giant interneurons (Fig. 4), again suggesting that decreased calcium influx during the action potential decreases the activation of the Ca$_{2+}$-dependent K$^+$ channels. However, the possibility that dopamine has direct effects upon Ca$_{2+}$-dependent K$^+$ channels cannot be ruled out by the present experiments. Modulation of calcium currents by dopamine has been shown in a variety of cells including retinal cells, chromaffin cells, and molluscan neurons (Artajelo et al. 1990; Gershenfeld et al. 1986; Marchetti et al. 1986; Pfeiffer-Linn and Lasater 1993).

The activation of NMDA receptors can induce fictive swimming in the isolated lamprey spinal cord and also has been shown to produce TTX-resistant membrane potential oscillations in lamprey spinal neurons (Wallén and Grillner 1987). These membrane potential oscillations are believed to be important in shaping neuronal output during fictive swimming (Wallén and Grillner 1987). The repolarization phase of these TTX-resistant, NMDA-induced oscillations...
has been proposed to be due in part to \(\text{Ca}^{2+}\)-dependent \(\text{K}^{+}\) channels activated by calcium that enters the cell during the depolarizing phase of the oscillation (Wällén and Grillner 1987). Dopamine increased the duration of the plateau of these membrane potential oscillations in motoneurons (Fig. 5), and this result is consistent with the reduction of the late AHP in motoneurons. Reduction of the \(\text{Ca}^{2+}\)-dependent \(\text{K}^{+}\) current would delay the repolarization, leading to a prolonged plateau phase (Wällén and Grillner 1987). During fictive swimming, a prolongation of the plateau phase of the oscillations may result in greater excitability and spike firing during the plateau.

**Synaptic effects of dopamine**

Synaptic sign and strength are important factors in determining network output, and modulation of synaptic transmission is a common site of neuromodulatory action (Getting 1989; Harris-Warrick and Marder 1991). Dopamine reduced polysynaptic IPSPs in lamprey motoneurons but had no effect upon polysynaptic EPSPs (Fig. 6). This finding suggests that dopamine may act selectively at inhibitory synapses. In these experiments, the polysynaptic IPSPs in motoneurons were elicited by stimulation of the contralateral half of the cord, presumably activating inhibitory CC interneurons. The CC interneuron output synapses would affect the locomotor output because CC interneurons are proposed to form a reciprocal inhibitory network that is the core of a proposed model of the locomotor pattern generator (Buchanan and Grillner 1987). A selective decrease in CC interneuron output synapses would produce a speeding of the locomotor network (McPherson et al. 1994), whereas a selective decrease in lateral interneuron outputs would slow the network. The IPSPs in motoneurons elicited by stimulation of either presynaptic axons or intrasomatic stimulation of inhibitory CC interneurons were reduced by application of dopamine (Fig. 8). However, the IPSPs produced by lateral interneurons in CC interneurons were not affected by dopamine (Fig. 8). It therefore appears that dopamine may exert its actions selectively at specific types of inhibitory synapses. The IPSPs produced by lateral interneurons and CC interneurons have been shown to be glycineric (Buchanan 1982). It therefore seems unlikely that dopamine is directly altering the responsiveness of these postsynaptic receptors. Selective dopaminergic modulation of inhibitory synaptic transmission would provide a mechanism for changing the output of the locomotor network via alteration of synaptic strength. However, the mechanism of this modulation in lamprey is unknown. In other preparations, presynaptic D2 dopamine receptors have been shown to reduce the release of acetylcholine from nerve terminals in the nucleus accumbens and at motoneuronal axon collateral terminals in the spinal cord of rat (Maitra et al. 1993; Wedzony et al. 1988). It has been recently suggested that presynaptic D2 receptors may be involved in modulating synaptic transmission in the lamprey spinal cord (Wikström et al. 1995).

Unfortunately, the findings reported here do not explain the previously reported decrease in the rate of fictive locomotor cycling following application of higher concentrations of dopamine. Several possibilities exist that may explain this discrepancy: 1) it may be possible that the higher doses of dopamine used were not in a physiologically relevant range. 2) Higher doses of dopamine may have led to nonspecific cross-reactivity, activation of multiple dopamine receptor subtypes, or, cause dopamine-induced release from dopamine/serotonin containing neurons in the ventromedial plexus. Release of endogenous serotonin then would cause reduction of the late AHP in network interneurons. 3) Dopamine may have been acting on the late AHP of CPG interneurons not tested here. 4) Reduction of calcium influx may lead to long-term changes in calcium-mediated second messenger systems.

In summary, in an effort to further characterize dopaminergic modulation in the lamprey spinal cord, the effects of dopamine on the cellular and synaptic properties of known classes of neurons in the lamprey spinal cord were assessed. This study demonstrated that dopamine reduced the late AHP of sensory-related neurons but not CC interneurons and lateral interneurons, suggesting a role for dopamine in modulating sensory function. In addition, dopamine reduced the late AHP of motoneurons and prolonged their TTX-resistant, NMDA-induced membrane potential oscillations; this may increase motoneuron firing rates during fictive swimming. Finally, the output synapses of inhibitory CC interneurons were selectively reduced by dopamine. The reduction of CC interneuron output synapses could account for the speeding of fictive swimming observed with low concentrations of dopamine.

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