5-HT Prolongs Ventral Root Bursting Via Presynaptic Inhibition of Synaptic Activity During Fictive Locomotion in Lamprey

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5-HT prolongs ventral root bursting via presynaptic inhibition of synaptic activity during fictive locomotion in lamprey. J Neurophysiol 93: 980–988, 2005. First published September 29, 2004; doi:10.1152/jn.00669.2004. Locomotor pattern generation is maintained by integration of the intrinsic properties of spinal central pattern generator (CPG) neurons in conjunction with synaptic activity of the neural network. In the lamprey, the spinal locomotor CPG is modulated by 5-HT. On a cellular level, 5-HT presynaptically inhibits synaptic transmission and postsynaptically inhibits a Ca\(^{2+}\)-activated K\(^+\) current responsible for the slow afterhyperpolarization (sAHP) that follows action potentials in ventral horn neurons. To understand the contribution of these cellular mechanisms to the modulation of the spinal CPG, we have tested the effect of selective 5-HT analogues against fictive locomotion initiated by bath application of spinal CPG, we have tested the effect of selective 5-HT analogues.

INTRODUCTION
Central pattern generators (CPGs) drive rhythmic motor activities such as locomotion (Grillner 2003; Grillner and Wallen 2002), respiration (Del Negro et al. 2002), and feeding (Marder 1994). The neuronal correlates to these behaviors are produced by integrating the intrinsic properties of the CPG neurons in conjunction with synaptic activity of the neural network (Alford et al. 2003; Marder and Thirumalai 2002). Several endogenous neurotransmitters have been shown to alter the output of locomotor CPGs and to modulate cellular and synaptic properties of CPG neurons (Alford et al. 2003; Barbeau and Rossignol 1991; Grillner and Wallen 2002; McLean et al. 2000; Parker 2000; Perrier et al. 2003; Schotland et al. 1996; Svensson et al. 2003). However, less is known about the mechanisms by which these cellular and synaptic changes modulate the output of the CPG. We have investigated the contribution of 5-HT–mediated presynaptic inhibition of synaptic transmission to modulating the lamprey locomotor network.

The neural network of the lamprey locomotor CPG has been well characterized. It is maintained by ipsilateral glutamatergic excitation in conjunction with contralateral inhibition (Alford and Williams 1989; Brodin et al. 1985; Buchanan 1982; Buchanan and Grillner 1991; Grillner and Wallen 1980; Hellgren et al. 1992) and comprises ventral root bursting that alternates across the spinal cord (Grillner et al. 1995). Lamprey spinal CPGs are activated by glutamate release from brain stem reticulospinal (RS) neurons (Buchanan and Cohen 1982; Buchanan et al. 1987; Ohta and Grillner 1989). The intensity of input from reticulospinal axons regulates the frequency of these bursts of activity and therefore the speed of locomotion (Brocard and Dubuc 2003; Di Prisco et al. 2000), which may range from 0.1 to 10 Hz. Experimentally, locomotor CPG activity in the spinal cord may also be activated by electrical stimulation of the lamprey brain stem in semi-intact preparations (McClellan and Grillner 1984; Sirota et al. 2000) or by application of glutamate receptor agonists in isolated spinal cords (Cohen and Wallen 1980; Grillner et al. 1981). The alternating pattern of ventral root bursting recorded under these experimental conditions is referred to as fictive locomotion (Grillner 2003) and is thought to drive the coordinated contraction of muscles necessary for lamprey swimming.

The frequency of fictive locomotion is modulated by endogenous release of neurotransmitters within the spinal cord (Christenson et al. 1989; Harris-Warrick and Cohen 1985; Parker 2000; Parker and Grillner 1998, 1999; Schotland et al. 1996; Svensson et al. 2003). Of these modulatory neurotransmitters, 5-HT reduces the frequency of ventral root bursting during fictive locomotion (Harris-Warrick and Cohen 1985). This is believed to due, in part, to 5-HT–mediated inhibition of a postsynaptic Ca\(^{2+}\)-dependant K\(^+\) current (I\(_{K(Ca)}\)) that underlies the slow afterhyperpolarization (sAHP) of synaptic transmission to modulating the lamprey locomotor network. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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finding that apamin significantly prolongs ventral root bursting during fictive locomotion (Hill et al. 1992) has been directly contradicted (Meer and Buchanan 1992). These differing results were clarified to some degree when it was suggested that the effect of apamin on fictive locomotion was dependent on the frequency of ventral root bursting. At lower burst frequencies, apamin significantly prolongs ventral root bursting, whereas at higher bursting frequencies, the effect of apamin is not significant (Buchanan 2001; Grillner et al. 2001).

5-HT alters the output of CPGs in several species (Schmidt and Jordan 2000). In adult chronic spinal (t13) cats, 5-HT modulates treadmill-induced locomotor patterns (Barbeau and Rossignol 1990; Edgerton et al. 1997) and depresses the sAHP in motoneurons (White and Fung 1989). Application of 5-HT also modulates fictive locomotion in turtles, presumably by activation of plateau potentials by 5-HT1 receptors (Alaburda et al. 2002) and inhibition of K+ conductance by 5-HT1A receptors (Perrier et al. 2003).

In addition to activating a postsynaptic 1A, 5-HT presynaptically inhibits synaptic transmission in the lamprey spinal cord (Blackmer et al. 2001; Buchanan and Grillner 1991; El Manira et al. 1994; Shupliakov et al. 1995; Takahashi et al. 2001). As in lamprey, inhibition of synaptic transmission by 5-HT has been observed in several vertebrate CPGs. 5-HT presynaptically inhibits midcycle glycineergic inputs and prolongs ventral root bursting during Xenopus larval swimming (Sillar et al. 1998). In neonatal rat, activation of 5-HT receptors presynaptically decreases inspiratory modulated synaptic currents (Di Pasquale et al. 1997; Hilaire et al. 1997; Lindsay and Feldman 1993) and suppresses descending glutamatergic responses (Skagerberg and Bjorklund 1985). Furthermore, both glutamatergic and glycineergic synaptic transmission to rat hypoglossal motoneurons is inhibited by activation of 5-HT1B receptors (Singer et al. 1996; Umemiya and Berger 1995). Despite this evidence, less is known about the contribution of presynaptic inhibition of synaptic transmission to the modulation of the CPG output. We now show in the lamprey that the 5-HT1D agonist, L694-247, acts presynaptically to inhibit synaptic transmission without activating the known postsynaptic locus. Using this selective 5-HT1D agonist, we show that presynaptic inhibition of synaptic transmission is sufficient to slow the rhythm of fictive locomotion. Furthermore, our results reveal that 5-HT modulates locomotor activity during fictive locomotion by presynaptically decreasing glutamatergic synaptic drive within the CPG.

METHODS

Lamprey preparation

Experiments were performed on isolated spinal cords of both adult and larval lampreys (Petromyzon marinus and Ichthyomyzon unicuspis). The animals were anesthetized with tricaine methanesulfonate (MS222), decapitated in accordance with institutional guidelines, and dissected in a cold saline solution (Ringer) of the following composition (mM): 100 NaCl, 2.1 KCl, 2.6 CaCl2, 1.8 MgCl2, 4 glucose, and 26 NaHCO3, bubbled with 95% O2–5% CO2 to a pH of 7.60 (modified from Wickelgren 1977). The spinal cord (12–20 segments) was isolated and removed from the protective meninx primitiva and placed in a cooled small-volume Sylgard-lined chamber. The recording chamber was continually superfused with cold oxygenated Ringer (8–10°C) or solutions of pharmacological agents bath-applied at a perfusion rate of ~1 ml/min. In experiments involving whole cell patch recording, a 10- to 20-μm slice of tissue was removed from the surface of the spinal cord superior to the ventral horn using a vibrotome tissue slicer. Patch pipettes were readily introduced to the cut ventral surface. Fictive locomotion was induced by application of 100–150 μM N-methyl-D-aspartate (NMDA).

Electrophysiology

Ventral horn neurons (motoneurons or interneurons) were whole cell clamped (Axopatch 200A amplifier, Axon Instruments) using a modified blind technique (Blanton et al. 1989; Cochilla and Alford 1997). Cell types were identified by their location in the tissue, and neurons were distinguished from the non-neuronal cells and axons by their membrane properties and their capacitative transients in response to a 10-ms, 10-mV step. Paired recordings were made between presynaptic reticulospinal axons and postsynaptic spinal neurons, and action potentials were evoked in the presynaptic axons at 5–10 Hz intervals. Pipettes had open-tip resistances of 5–10 MΩ. Series resistance was monitored continuously by giving a 10-mV voltage step before each episode, and if the change exceeded 15%, the cell was discarded. Microelectrode (sharp) recordings were made conventionally with thin-walled glass. Tip resistances of 20–50 MΩ when filled with 3 M potassium methane sulfonate allowed recording from either postsynaptic somata or presynaptic axons. Ventral root recordings were performed with glass extracellular suction electrodes and amplified with a differential AC amplifier from A-M systems.

Solutions

Patch pipette solution contained (mM): 102.5 cesium methane sulfonate, 1 NaCl, 1 MgCl2, 5 EGTA, and 5 HEPES, pH adjusted to 7.2 with CsOH. Microelectrode pipette solution was either 3 M potassium methane sulfonate or 3 M potassium acetate. External solution contained (in mM) 100 NaCl, 2.1 KCl, 2.6 CaCl2, 1.8 MgCl2, 26 NaHCO3, and 4 glucose, bubbled with 95% O2–5% CO2, NMDA and all 5-HT analogues were obtained from Tocris; all other chemicals were from Sigma unless otherwise noted. Drugs were applied to the superfuse or applied over the spinal cord by pressure ejection from a fine-tipped pipette (patch pipette) with a 200- to 800-ms pulse of pressure (100 kPa).

Data analysis

For paired recordings, the mean was taken of ±12 traces of excitatory postsynaptic currents (EPSCs) for each condition for each animal. For biphasic ESPCs, the decay of the electrical component was measured to a 10-ms, 10-mV step. Paired recordings were made between presynaptic reticulospinal axons and postsynaptic spinal neurons, and action potentials were evoked in the presynaptic axons at 5–10 Hz intervals. Pipettes had open-tip resistances of 5–10 MΩ. Series resistance was monitored continuously by giving a 10-mV voltage step before each episode, and if the change exceeded 15%, the cell was discarded. Microelectrode (sharp) recordings were made conventionally with thin-walled glass. Tip resistances of 20–50 MΩ when filled with 3 M potassium methane sulfonate allowed recording from either postsynaptic somata or presynaptic axons. Ventral root recordings were performed with glass extracellular suction electrodes and amplified with a differential AC amplifier from A-M systems.

Statistics

Data are given as means ± SE. Student paired two-tailed t-test was used to calculate the significance of the data unless otherwise noted.

RESULTS

Effect of L694-247 on fictive locomotion

Application of 5-HT to the lamprey spinal cord prolongs ventral root bursting and is known to activate at least two pharmacologically distinct receptors (El Manira et al. 1997; Wikstrom et al. 1995). One receptor, identified as 5-HT1A-like, inhibits K(Ca) (Wikstrom et al. 1995), but does not alter synaptic transmission at sensory synapses in the lamprey spinal cord (El Manira et al. 1997). A second unclassified 5-HT receptor is known to presynaptically inhibit synaptic transmission (in mM): 100 NaCl, 2.1 KCl, 2.6 CaCl2, 1.8 MgCl2, 4 glucose, and 26 NaHCO3, bubbled with 95% O2–5% CO2, NMDA and all 5-HT analogues were obtained from Tocris; all other chemicals were from Sigma unless otherwise noted. Drugs were applied to the superfuse or applied over the spinal cord by pressure ejection from a fine-tipped pipette (patch pipette) with a 200- to 800-ms pulse of pressure (100 kPa).

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sion at several sites in the spinal cord, including the reticulo-spinal synapse and sensory inputs (Blackmer et al. 2001; Buchanan and Grillner 1991; El Manira et al. 1994; Shupliakov et al. 1995; Takahashi et al. 2001). Thus to study the effect of 5-HT–mediated presynaptic inhibition of synaptic transmission on fictive locomotion, it is necessary to identify a specific agonist that selectively inhibits synaptic transmission without activating postsynaptic 5-HT receptors that inhibit I\(_{\text{K(Ca)}}\).

Previous studies have shown that the action of 5-HT to inhibit postsynaptic I\(_{\text{K(Ca)}}\) prolongs ventral root bursting during fictive locomotion (El Manira et al. 1994; Grillner et al. 2001; Wallen et al. 1989). Should presynaptic inhibition of synaptic transmission also contribute to modulation of fictive locomotion, we would expect that selectively activating presynaptic receptors that inhibit synaptic transmission would also prolong ventral root bursting. To investigate this hypothesis, we assayed the effects of various 5-HT receptor agonists and antagonists versus fictive locomotion. Simultaneous recordings of contralateral ventral roots were made to monitor fictive locomotion induced by 100–150 μM NMDA (Fig. 1, Ai and Bi). Of all the 5-HT analogues tested, only the 5HT\(_{1D}\) agonist, L694-247, significantly prolonged fictive locomotion ventral root bursting (Table 1). In 11 of 17 preparations, application of the 5-HT\(_{1D}\) agonist, L694-247 (100 nM), slowed the frequency of fictive locomotion and prolonged ventral root bursting (Aii). In 3 of 17 animals, L694-247 caused the ventral root bursting pattern to become disorganized (Bii).

![Fig. 1. Activation of 5-HT\(_{1D}\)-like receptors slows the frequency of N-methyl-D-aspartate (NMDA)-induced fictive locomotion. Application of NMDA (100–150 μM) induced fictive locomotion (Ai and Bi). Fictive locomotion ventral root bursting was monitored by performing extracellular ventral root recordings on opposite sides of the spinal cord (vrl and vr2). In 11 of 17 animals, application of the 5-HT\(_{1D}\) agonist, L694-247 (100 nM), slowed the frequency of fictive locomotion and prolonged ventral root bursting (Aii). In 3 of 17 animals, L694-247 caused the ventral root bursting pattern to become disorganized (Bii).](http://jn.physiology.org/)

<table>
<thead>
<tr>
<th>Receptor Name of Drug</th>
<th>Concentration</th>
<th>n</th>
<th>Effect</th>
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<tbody>
<tr>
<td>5-HT(_{1D}) agonist L694-247</td>
<td>100 nM</td>
<td>17</td>
<td>Prolonged bursting*</td>
</tr>
<tr>
<td>5-HT(_{1D}) antagonist BRL 15572</td>
<td>100 nM</td>
<td>7</td>
<td>No effect on locomotion</td>
</tr>
<tr>
<td>5-HT(_{2}) antagonist Cyproheptadine</td>
<td>100 μM</td>
<td>6</td>
<td>No effect on locomotion</td>
</tr>
<tr>
<td>5-HT(_{3}) antagonist SB216641</td>
<td>100 nM</td>
<td>6</td>
<td>No effect on locomotion</td>
</tr>
<tr>
<td>5-HT(_{1A}) antagonist Alpha-methyl-5-hydroxytryptamine maleate</td>
<td>1 μM</td>
<td>4</td>
<td>No effect on locomotion</td>
</tr>
<tr>
<td>5-HT(_{1B}) antagonist SB224289</td>
<td>100 nM</td>
<td>2</td>
<td>No effect on syn. trans.†</td>
</tr>
<tr>
<td>5-HT(_{1D}) antagonist GR 55562</td>
<td>500 nM</td>
<td>3</td>
<td>No effect on syn. trans.†</td>
</tr>
</tbody>
</table>

*P < 0.05; no effect, P > 0.05. †Drugs did not to reverse inhibition of synaptic transmission by 1 μM 5-HT.
The 5-HT$_{1D}$ agonist L694-247, which is thought to be mediated by 5-HT$_{1D}$ receptors in rabbit, inhibits synaptic transmission at the reticulospinal (RS) synapse in a manner similar to the known presynaptic effect of 5-HT. This effect is consistent with a current carried through gap junctions (this electrical component was observed in most, but not all recordings), while the slower variable phase is mediated through a chemical synaptic component (Fig. 2). The initial fast invariant phase of the EPSC (Fig. 2A) resulted in a 16% reduction in burst frequency, similar to the effect of 5-HT (Harris-Warrick and Cohen 1985) and of the 5-HT$_{1A}$ agonist 8-OH-DPAT ([±]-8-hydroxy-dipropylaminotetralin hydrobromide) (Wikstrom et al. 1995).

To probe the site of action of this 5-HT$_{1D}$ agonist, we used the RS synapse to assay the effects of L694-247 on synaptic transmission. The lamprey spinal preparation is uniquely advantageous for studying synaptic transmission electrophysiologically since descending RS axons are unmyelinated and form en passant synapses. The lack of myelin allows for microelectrode penetration of presynaptic axons. Moreover, recording from anywhere along the length of an en passant axon is electrophysiologically equivalent to recording directly from the terminal. Thus the lamprey RS synapse is one of the few known synapses that allow electrophysiological access to both the pre- and postsynaptic neurons.

To assay the effect of L694-247 on synaptic transmission, paired recordings were made between presynaptic RS axons and postsynaptic spinal neurons (Fig. 2A). Action potentials evoked in the presynaptic RS axon (Fig. 2B) resulted in biphasic EPSCs, containing both electrical and chemical components (Fig. 2C). The initial fast invariant phase of the EPSC is consistent with a current carried through gap junctions (this electrical component was observed in most, but not all recordings), while the slower variable phase is mediated through synaptic release of glutamate and activation of AMPA and NMDA receptors (Fig. 2C) (Buchanan et al. 1987). We found that the 5-HT$_{1D}$ agonist, L694-247 (100 nM), inhibited the chemical component of evoked EPSCs to $37 \pm 16\%$ of control (Fig. 2C; $P < 0.05, n = 3$). L694-247 did not alter either the impedance of the cell or the electrical component of the EPSCs, consistent with a presynaptic mechanism of inhibition (Fig. 2C). This result is in accord with the conclusions of previous studies that 5-HT presynaptically inhibits synaptic transmission (Blackmer et al. 2001; Buchanan and Grillner 1991; El Manira et al. 1994; Shupliakov et al. 1995; Takahashi et al. 2001). Thus L694-247, a specific 5-HT$_{1D}$ agonist, inhibits chemical synaptic transmission at this synapse in a manner similar to the known presynaptic effect of 5-HT.

Since we were unable to antagonize the effect on L694-247 on locomotion, we probed for a specific antagonist to 5-HT receptor–mediated inhibition of synaptic transmission. Nevertheless, neither SB224289 (5-HT$_{1B}$ antagonist) nor GR 55562 (5-HT$_{1D}$ antagonist) reversed inhibition of synaptic transmission mediated by 1 $\mu$M 5-HT (Table 1).

5-HT$_{1D}$ agonist, L694-247, does not inhibit the postsynaptic sAHP

In addition to presynaptically inhibiting synaptic transmission, 5-HT also inhibits a postsynaptic apamin-sensitive Ca$^{2+}$-dependent K$^+$ current ($I_{\text{K(Ca)}}$) (El Manira et al. 1994; Wallen et al. 1989; Wikstrom et al. 1995). To test whether this 5-HT$_{1D}$ agonist (L694-247) inhibits postsynaptic $I_{\text{K(Ca)}}$, current–microelectrode recordings were made from spinal neurons. We rapidly applied L694-247 by pressure ejection since subtle changes in the membrane potential that occur during prolonged bath application of drugs are capable of altering the sAHP. Two pressure ejection pipettes, filled with either 5-HT (300 $\mu$M) or L694-247 (1 $\mu$M), were positioned within 1 mm of the recording pipette. To compensate for dilution of L694-247 during pressure ejection, we used a concentration 100-fold higher than the $K_d$ (Glennon et al. 1996) (10-fold higher than a dose that significantly inhibits synaptic transmission, see Fig. 2). To
visualize ejection of the drugs, the dye Fast Green (Fisher) was included in the pressure ejection pipettes, and the spread of dye from the pipette was observed through a stereo-microscope. Fifteen action potentials were evoked at 0.33 Hz by brief current injection. Following the fourth action potential, either 5-HT or L694-247 was alternately pressure ejected (200–800 ms, 100 kPa). Comparison of the sAHP in control versus in L694-247 showed that L694-247 did not alter the sAHP (Fig. 3, Bi and Bii; 103 ± 0.5% of control, P > 0.05, n = 3). This result is consistent with the findings of other studies, which showed that the 5-HT₁₄ agonist, sumatriptan, also did not alter the sAHP (Wikstrom et al. 1995). As previously shown, 5-HT inhibited the sAHP (Fig. 3, Ai and Aii; 34 ± 12% of control, P < 0.05, n = 3) (Wallen et al. 1989). Thus the 5-HT₁₄ agonist L694-247 acts at the presynaptic locus of 5-HT without affecting the known postsynaptic locus.

5-HT inhibits synaptic transmission in a dose-dependent manner

To understand the activity of endogenous 5-HT on fictive locomotion, we assayed the apparent affinity of 5-HT at the presynaptic locus by making paired recordings at the RS synapse in the presence of varying doses of 5-HT. Action potentials evoked in the presynaptic RS axon resulted in EPSCs recorded in motor neurons (Fig. 4A). We found that 5-HT inhibited synaptic transmission in a dose-dependent manner (Fig. 4B, 600 nM = 48.65 ± 8.05%, n = 9, 100 nM = 63.11 ± 17.46% of control, n = 3, P < 0.05). Furthermore, saturating doses of 5-HT inhibited synaptic transmission to ~20% of control (Fig. 4B, 1 μM = 16.9 ± 6.6%, n = 5, 30 μM = 19.8 ± 7.5% of control, n = 4, P < 0.05). By generating a dose-response curve and fitting the data with a Hill plot, we found that 5-HT inhibits synaptic transmission with an apparent affinity of 143.5 ± 75 nM (Fig. 4B). This is a significantly higher apparent affinity compared with previous reports, which found that 5-HT presynaptically inhibits synaptic transmission with an apparent Kᵦ of 2.3 μM and a maximal inhibition of 49% of control (30 μM 5-HT) (Takahashi et al. 2001). The dichotomy is likely due to previous studies using extracellular stimulation to evoke action potentials in a mixed population of both excitatory and inhibitory axons in the spinal cord. Additionally, the different location of axons within the spinal cord may affect the concentration of 5-HT that reaches the synapse. For example, RS synapses are very close to the spinal cord surface, whereas axons embedded deeper in the tissue are likely to be protected by endogenous 5-HT uptake. Our findings show a similar apparent affinity of 5-HT for the presynaptic locus as for modulation of fictive locomotion (~100 nM) (Harris-Warrick and Cohen 1985).
How might inhibition of neurotransmitter release alter fictive locomotion?

5-HT inhibits synaptic transmission at the lamprey RS synapse. Specific activation of presynaptic 5-HT$_{1D}$–like receptors inhibits synaptic transmission and leads to a prolongation of burst duration during fictive locomotion. This result is consistent with the effects of 5-HT itself on fictive locomotion. However, probing the effects of L694-247 on synaptic transmission at the RS synapse has indirect implications on the effect of 5-HT during locomotion since this synapse is quiescent during fictive locomotion stimulated by bath-applied NMDA. It is therefore important to study the effect of 5-HT (1 μM) on monosynaptic projections from intraspinal excitatory interneurons (EINs) to other neurons of the central pattern generator. Unfortunately, stable recordings of EINs are hard to maintain, and positive identification of these cells is difficult.

Alternatively, it is possible to identify and directly evaluate excitatory synaptic drive from EINs by voltage clamping spinal ventral horn neurons during fictive locomotion.

During fictive locomotion driven by bath application of NMDA, membrane potential oscillations of any recorded neuron are driven both by synaptic drive from excitatory and inhibitory interneurons and from activation of NMDA receptors directly from the bath-applied NMDA. We hypothesize that 5-HT significantly inhibits excitatory drive during fictive locomotion but that it will not alter NMDA receptor–mediated membrane potential oscillations, which will continue to be activated by the bath-applied NMDA. Indeed, in cells recorded under current-clamp conditions, the addition of 5-HT to the superfusate significantly reduced the frequency of fictive locomotion but did not alter the amplitude of membrane potential oscillations (Fig. 5, A and Aii; n = 10). To characterize the currents that underlie these slower membrane potential oscillations, we voltage clamped the ipsilateral neurons at −65 mV and recorded current oscillations. Voltage clamping the cell inhibits NMDA receptor–mediated current oscillations, as membrane potential excursions needed for Mg$^{2+}$ unblock of the receptor channel are no longer possible. Furthermore, we do not expect Cl$^{-}$ currents to contribute to current oscillations since −65 mV is near the Cl$^{-}$ reversal potential (Grillner and Wallen 1980). Thus any remaining depolarizing current oscillations observed under voltage clamp would be predominantly mediated by AMPA receptors. Under voltage-clamp conditions, current oscillations observed during fictive locomotion were abolished by addition of 1 μM 5-HT (n = 3), indicating that 5-HT inhibits AMPA-mediated synaptic currents during fictive locomotion (Fig. 5, Bi and Bii). Furthermore, the slower membrane potential oscillations that persist under current clamp in 5-HT are likely driven by NMDA receptor currents activated by bath-applied NMDA.

DISCUSSION

Locomotor CPGs integrate the intrinsic oscillatory properties of spinal neurons with the synaptic activity of the network to produce coordinated neuromuscular excitation and movement. The frequency of fictive locomotion is slowed by application of 5-HT in the lamprey (Harris-Warrick and Cohen 1985). On a cellular level, 5-HT is known to activate two distinct receptors. Postsynaptically, activation of 5-HT$_{1A}$ receptors causes inhibition of an apamin sensitive $I_{K(Ca)}$. Inhibiting this current is thought to prolong ventral root bursting during fictive locomotion by blocking the sAHP of spinal neuron action potentials (El Manira et al. 1994; Wallen et al. 1989; Wikstrom et al. 1995). Additionally, activation of presynaptic 5-HT receptors inhibits synaptic transmission, but the contribution of this 5-HT–mediated inhibition on prolonging ventral root bursting during locomotion has not previously been investigated.

While it is known that activation of 5-HT receptors markedly slows fictive locomotion, the site of action of 5-HT may be postsynaptic, presynaptic, or both. We now show that a selective 5-HT$_{1D}$ receptor agonist (L694-247) slows fictive locomotion, qualitatively mimicking the effects of 5-HT and of a selective 5-HT$_{1A}$ agonist known to inhibit postsynaptic $I_{K(Ca)}$.  

FIG. 4. Characterization of 5-HT–mediated inhibition of synaptic transmission at the RS synapse. A: presynaptic action potential was evoked in the RS axon by current injection (2 ms) through the microelectrode. This evoked EPSCs that were recorded through the patch-clamp electrode. 5-HT (1 μM) reduces the chemical component of the EPSC (gray). B: dose-response curve of synaptic transmission vs. 30 μM, 1 μM, 600 nM, and 100 nM 5-HT. Fitting the data with a Hill plot showed 5-HT inhibits synaptic transmission with an apparent $K_{d}$ of 143 ± 75 nM, with saturating doses causing a maximal inhibition to ~20% of control.
At the same dose (100 nM) that profoundly reduced the frequency of fictive locomotion, L694-247 markedly inhibits synaptic transmission, but leaves the sAHP of postsynaptic action potentials unaffected. There is extensive evidence that 5-HT acts presynaptically to inhibit synaptic transmission in the lamprey spinal cord. 5-HT–mediated inhibition of synaptic responses is prevented by selectively blocking G protein signaling in presynaptic terminals (Blackmer et al. 2001). Furthermore, it is unlikely that 5-HT acts postsynaptically to alter synaptic currents since postsynaptic responses to pressure ejection of glutamate are not affected by doses of 5-HT that inhibit synaptic transmission (Buchanan and Grillner 1991). Additionally, both the impedance of postsynaptic neurons and the electrical component of EPSCs are left unaffected by 5-HT (El Manira et al. 1997; Takahashi et al. 2001). Since L694-247 is a 5-HT receptor agonist and does not alter either the electrical component of the EPSC or the input impedance of the postsynaptic cell, it follows that L694-247 inhibits synaptic transmission by activating the previously identified presynaptic 5-HT receptor.

Interestingly, L694-247 did not exhibit the same effect on fictive locomotion in all experiments. Application of L694-247 led to a disorganized pattern of ventral root bursting in three of the preparations. If selective activation of presynaptic 5-HT receptors inhibits glutamate release, we may consider a number of alternative possible outcomes to its effect on fictive locomotion. First, we must consider the method of activation used to maintain fictive locomotion. In the isolated lamprey spinal cord, this has generally been achieved by bath application of a glutamate receptor agonist, typically either NMDA or δ-glutamate. Either of these methods will effectively activate postsynaptic NMDA receptors. NMDA stimulates fictive locomotion by a direct action as an agonist, whereas δ-glutamate acts by inhibiting glutamate uptake and by raising intraspinal extracellular glutamate concentrations to levels that will primarily activate NMDA receptors because of their relatively high affinity to glutamate (Dunlop 2001). In addition, δ-glutamate also acts as a weak NMDA receptor agonist (Olverman et al. 1988). Thus fictive locomotion activated in this way will be driven by a combination of NMDA receptor–mediated membrane potential oscillations that are augmented and phase-locked by excitatory and inhibitory synaptic drive (Grillner 2003). If synaptic drive were severely inhibited by 5-HT, we may hypothesize that spinal neurons, while still excited by NMDA, would no longer be phase-locked by synaptic drive, and their ventral root output would be random. We believe this was the paradigm we observed in the three preparations where L694-247 caused ventral root bursting to become disorganized. However, the mean reduction in synaptic transmission mediated by L694-247 was ~37% of control. We may hypothesize that, under these more moderate inhibitory conditions, the frequency of fictive locomotion will be coupled with the remaining synaptic drive, but will be dominated by the resonant frequency of the NMDA receptor–mediated membrane potential oscillations driven by bath application of NMDA. In fact, such an outcome has been directly predicted by computer models of fictive locomotion in the lamprey spinal cord (Hellgren et al. 1992; Lansner and Ekeberg 1994) and is supported by physiological studies of the effects of glutamatergic agonists and antagonists on fictive locomotion (Brodin and Grill-
ner 1986; Brodin et al. 1985). Indeed, we show clearly that selective activation of a presynaptic 5-HT1D receptor very significantly slows fictive locomotion. We therefore conclude that 5-HT slows fictive locomotion by reducing synaptic release of glutamate in the spinal cord.

This conclusion is predicated on a model in which 5-HT activates presynaptic receptors to inhibit transmitter release from CPG neurons during fictive locomotion. Thus 5-HT must inhibit synaptic transmission at doses of 5-HT that also prolong ventral root bursting. By generating a dose-response curve of 5-HT versus inhibition of synaptic transmission, we show that 5-HT inhibits synaptic release of glutamate with an apparent $K_d$ of 140 nM. This dose is entirely consistent with doses of 5-HT known to modulate fictive locomotion (Harris-Warrick and Cohen 1985). We have also shown that 5-HT significantly reduces excitatory synaptic drive onto neurons of the CPG during NMDA-activated fictive locomotion. Thus neurons show rhythmic membrane potential oscillations during fictive locomotion in NMDA in phase with ipsilateral ventral root bursting activity and similarly show these oscillations in the presence of 5-HT. However, when we whole cell voltage clamp the neurons to isolate AMPA-mediated rhythmic synaptic drive, addition of 5-HT blocks this synaptic drive, and we see no further rhythmic oscillations in current across the cell membrane.

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