Serotonin Increases the Incidence of Primary Afferent-Evoked Long-Term Depression in Rat Deep Dorsal Horn Neurons

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Garraway, Sandra M. and Shawn Hochman. Serotonin increases the incidence of primary afferent-evoked long-term depression in rat deep dorsal horn neurons. J Neurophysiol 85: 1864–1872, 2001. 5-hydroxytryptamine (5-HT) is released in spinal cord by descending systems that modulate somatosensory transmission and can potentially depress primary afferent-evoked synaptic responses in dorsal horn neurons. Since primary afferent activity-induced long-term potentiation (LTP) may contribute to central sensitization of nociception, we studied the effects of 5-HT on the expression of sensory-evoked LTP and long-term depression (LTD) in deep dorsal horn (DDH) neurons. Whole cell, predominantly current clamp, recordings were obtained from DDH neurons in transverse slices of neonatal rat lumbar spinal cord. The effect of 5-HT on dorsal-root stimulation-evoked synaptic responses was tested before, during, or after high-frequency conditioning stimulation (CS). In most cells (80%), 5-HT caused a depression of the naïve synaptic response. Even though 5-HT depressed evoked responses, CS in the presence of 5-HT was not only still capable of inducing LTD but also increased its incidence from 54% in controls to 88% (P < 0.001). Activation of ligands selective for 5-HT1A/1B and 5-HT1D, but not 5-HT2A/2C or 5-HT3 receptors, best reproduced these actions. 5-HT also potently depressed postconditioning synaptic responses regardless of whether the induced plasticity was LTP or LTD. Our results demonstrate that in addition to depressing the amplitude of evoked sensory input, 5-HT can also control the direction of its long-term modifiability, favoring the expression of LTD. These findings demonstrate cellular mechanisms that may contribute to the descending serotonergic control of nociception.

INTRODUCTION

The spinal cord dorsal horn represents a nodal point for the integration of sensory information. Many studies have investigated the multi-sensory convergent properties of dorsal horn neurons of various species, particularly in relation to nociceptive input. High-intensity electrical stimulation of primary afferents recruits nociceptors (Aδ and C) and synthetically activates neurons in the dorsal horn (Jeflinia and Urban 1994; Miller and Woolf 1996). Repetitive activation of these afferents can induce alterations in spinal integrative properties that encode persistent changes in nociception. One such change is expressed as increases [long-term potentiation (LTP)] or decreases [long-term depression (LTD)] in synaptic strength. LTP and LTD have been observed in the dorsal horn (Garraway et al. 1997; Liu and Sandkühler 1995; Liu et al. 1998; Pockett 1995; Randic et al. 1993; Sandkühler and Liu 1998; Svendsen et al. 1997). These synaptic modifications probably occur at glutamatergic synapses since N-methyl-D-aspartate (NMDA) receptor activation is required for the induction of LTP (Liu and Sandkühler 1995; Randic et al. 1993; Svendsen et al. 1998).

Sandkühler and Liu (1998) demonstrated that natural activation of nociceptors in skin induced LTD of C-fiber-evoked field potentials in dorsal horn but only following spinalization, suggesting a potent inhibitory control from descending systems. Additionally, Liu et al. (1998) demonstrated that Aδ-fiber-mediated LTD of C-fiber-evoked field potentials could be switched to LTP following spinalization. Thus descending systems appear to be able to control both the induction and direction of the evoked synaptic plasticity. An identification of the mechanisms controlling synaptic plasticity is of considerable interest. For instance, the Aδ-fiber-induced LTD of nociceptor afferents in spinal cord is blocked with μ-opioid receptor antagonists (Zhong and Randic 1996), potentially linking LTD to opioid-induced analgesia. It is thus reasonable to hypothesize that synaptic plasticity participates in the physiological encoding of altered nociceptive states (e.g., hyperalgesia and allodynia).

Numerous alterations in spinal synaptic/cellular properties are observed following application of 5-hydroxytryptamine (5-HT). In dorsal horn neurons, 5-HT generally depresses primary afferent-evoked synaptic responses (Headley et al. 1978; Jordan et al. 1979; Khasabov et al. 1999; Lopez-Garcia 1998; Lopez-Garcia and King 1996; Randic and Yu 1976) although facilitation has also been observed (Jordan et al. 1979; Lopez-Garcia and King 1996), including long-lasting facilitatory responses (Hori et al. 1996; Li and Zuo 1998). Although many studies have demonstrated that 5-HT exerts antinociceptive actions in the spinal cord (for reviews, see Eide and Hole 1993; Hammond 1986; Millan 1995), details of its mechanism of action and receptor pharmacology remain incomplete.

We hypothesize that one function of descending serotonergic systems is to control the expression of activity-dependent synaptic plasticity within the spinal cord. Thus we sought to determine the effects of 5-HT and its receptor selective ligands.
on the induction and maintenance of evoked synaptic plasticity in deep dorsal horn (DDH) neurons (laminae III–VI). This was undertaken in a spinal cord slice preparation capable of evoking primary afferent-induced LTP and LTD (Garraway et al. 1997). We demonstrate that 5-HT receptor activation, in particular the 5-HT₁₆ and 5-HT₁₇ receptors, promote the induction of LTD in DDH neurons. Preliminary data were presented previously (Garraway and Hochman 1997, 1998).

METHODS

All experimental procedures complied with the Canadian Council of Animal Care guidelines. Neonatal rats (Sprague-Dawley postnatal days 3–6) were decapitated and spinal segments L₂–S₁ were removed. The isolated spinal cord was embedded in Agar, 2.5% wt/vol (Type E, Sigma), and sliced on a vibrating blade microtome in 500- to 600-μm transverse sections (Leica VT1000S or Pelco 101) in cooled (4°C) oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 25 glucose, 1.25 NaH₂PO₄, and 26 NaHCO₃ at a pH of 7.4. Bipolar tungsten electrodes were inserted into short dorsal rootlets, typically ~1 mm from the dorsal root entry zone, to allow for constant current electrical stimulation delivered from an electrically-isolated stimulator (Eide 1972). Dorsal rootlets range in length from ~1–5 mm.

Slices were then incubated at 32°C for ≥1 h in ACSF. For experimentation, spinal cord slices were affixed to a recording chamber using platinum U frames with a parallel array of nylon fibers glued across (Edwards et al. 1989). Patch electrodes were prepared from 1.5 mm OD capillary tubes (Precision Instruments or Warner) pulled in a two-stage process (Narishige PP83) producing resistance values ranging from 4 to 7 MΩ with recording electrodes containing (in mM) 140 K-glucurate, 0.2 EGTA, 10 HEPES, 4 Mg-ATP, and 1 GTP, pH 7.3. In most experiments, 2 mM N-2,6-dimethylphenylcarbamoylmethyltrithreonylgluconoammonium bromide (QX-314, RBI) was added to the recording solution to block voltage-dependent Na⁺ channels. The recording chamber was continuously superfused with oxygenated ACSF at a rate of ~2 ml/min.

The whole cell “blind” patch-clamp recording technique (Blanton et al. 1989) was undertaken at room temperature (~20°C) using the Axopatch 1D amplifier (Axon Instruments) filtered at 5 kHz (4-pole low-pass Bessel). Voltage- and current-clamp data were acquired on computer with the pCLAMP acquisition software (v 6.0; Axon Instruments). Immediately following rupture of the cell membrane, the current-clamp recording configuration was used to determine resting membrane potential. Series resistance was subtracted in current-clamp mode (bridge balance), and junction potentials were measured and accounted for. To ensure reliable recording from healthy neurons, for the duration of the experiment, leak conductance and bridge balance accounted for. To ensure reliable recording from healthy neurons, for the duration of the experiment, leak conductance and bridge balance were carefully monitored; if their values were largely unaltered, the experiments were continued. Mean electrode series resistance was 45.3 ± 13 (SD) MΩ. Additionally, a minority of experiments was undertaken in voltage-clamp mode (26/109). In these experiments, series resistance remained uncompensated.

To compare the effects of 5-HT (or receptor-selective ligands) and conditioning stimulation (CS) on evoked synaptic responses, postnatal day 3–6 neonates were used since both LTD and LTP are evocable in DDH neurons in transverse slices at this age, whereas LTD dominates in transverse slices obtained from postnatal day 10–14 neonates (Garraway et al. 1997). One problem with these early neonates, however, is that myelination of many afferent fibers is incomplete (Friede and Samorajski 1968; see also Fitzgerald 1985). Hence, we observed that only 13% of cells received synaptic responses at primary afferent stimulation intensities <500 μA, 100 μs. Sixty-nine percent of neurons were observed to have synaptic events recruited at intensities ranging from 500 μA, 100 μs to 500 μA, 500 μs, while the remaining 18% of neurons had their first synaptic events recruited at intensities >500 μA, 500 μs. Thus we used high stimulation intensities to recruit the highest threshold unmyelinated afferents, and hence, the majority of afferent fiber types, irrespective of age (typically ≥500 μA, 500 μs) (see Thompson et al. 1990).

Generally, the evoked synaptic responses were first characterized as predominantly excitatory by determining their reversal potential prior to collection of baseline events. This was accomplished by recording primary afferent evoked synaptic responses at holding potentials ranging from ~90 to +30 mV (Fig. 2D). Neurons having obvious inhibitory synaptic responses were not included in this study. To further characterize the excitatory synaptic responses, in some cells, the ionotropic glutamate receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10–20 μM) and (±)-2-amino-5-phosphono-pentanoic acid (o, l-APV, 50 μM) were added to determine whether the evoked synaptic responses were mediated by (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate and N-methyl-d-aspartate (NMDA) receptors, respectively.

To assess the effects of conditioning stimulation on synaptic plasticity, the following protocol was used. Baseline synaptic responses were recorded for 10–25 min by stimulating dorsal rootlets at low frequency (generally every minute) at a holding potential of ~94 mV. This was followed by a high-frequency CS (5 100-Hz tetani of 1-s duration at 5-s intervals), often at a higher intensity stimulation, at a holding potential of ~54 mV, approximately at a cell’s resting membrane potential. Following CS, the synaptic response was then recorded at the preconditioning baseline intensity, frequency, and holding potential (~94 mV). Synaptic plasticity, expressed as LTD or LTP, was defined as a ≥20% change in amplitude maintained for ≥20 min post CS and always for the duration of the recording. Unlike the hippocampus, the DDH is heterogeneous in nature, thereby making it more difficult for a given cell to be activated by individual stimuli. To reliably elicit LTD or LTP, however, high-frequency electrical stimulation of the dorsal roots (Randic et al. 1993) or the dorsomedial white matter (Pockett 1995) has been previously demonstrated to induce both LTD and LTP in the spinal cord.

5-HT was applied at 10 μM (in 100 μM ascorbic acid, an antioxidant). The following 5-HT receptor ligands were used: 5-carboxamidotryptamine (5-CT) in the presence of the 5-HT₃ receptor antagonist, clozapine, for selective activation of 5HT₁₆ receptors; 7-trifluoromethyl-4-(4-methyl-1-piperazinyl)-pyrrolo[1,2-a]quinoxaline maleate (CGS) to activate 5-HT₁₃ receptors; 1-(2,5-dimethoxy-4-iodo-phenyl)-2-amino-propane (DOI) to activate 5-HT₁₂ receptors; and 1-(m-chlorophenyl)-biguanide (CPBG) for activation of 5-HT₄ receptors. Ligands were applied at 1 μM. All drugs were purchased from RBI (Natick, MA).

The relationship between primary afferent-evoked responses and 5-HT application was studied using the three paradigms outlined in Fig. 1. These three experimental procedures were used to evaluate the effects of 5-HT on the “naïve” synaptic response, the post-conditioning response, and the induction of synaptic plasticity respectively. To determine the effects of specific activation of 5-HT receptor subtypes on the induction of synaptic plasticity, all experiments involving specific 5-HT receptor ligands were conducted as outlined in Fig. 1C. Recordings were analyzed using pCLAMP (v 6.0, Axon Instruments). The maximum amplitude of the synaptic response of individual traces was measured. Figures were constructed using Sigma Plot (SPSS) and/or CorelDRAW (Corel). Values are reported as means ± SE in Figs. 3–5 and means ± SD elsewhere.

RESULTS

A total of 109 DDH (laminae III–VI) neurons were recorded having a mean resting membrane potential of ~58 ± 10 mV and input resistance of 635 ± 358 MΩ. The location of a subpopulation of neurons where the topographic location was mapped is presented in Fig. 2A.
Characterization of primary afferent-evoked synaptic responses in the DDH

We used an invariable synaptic delay as an indicator of a monosynaptic linkage (Fitzgerald and Wall 1980). Variability in excitatory postsynaptic potential (EPSP) latency in a subpopulation of neurons is presented (Fig. 2B) with representative individual values also provided (Fig. 2B, inset). Generally, synaptic events whose onset occurred before 6 ms following the stimulus artifact had relatively constant latencies. At room temperature, synaptic delay in spinal cord slices from rats in the present age range is 3 ms (Jonas et al. 1998; Takahashi 1992), suggesting a minimal value of 6 ms for disynaptic actions. Hence, it is probable that synaptic responses with latencies $\leq 6$ ms were evoked monosynaptically, originating directly from primary afferents. These were the majority of responses.

Figure 2C depicts representative synaptic responses recorded from DDH neurons. Evoked EPSPs were observed to have three general appearances; single peak with slow decay (Ci), an early and late peak (Cii), and EPSPs with fast rate of rise and decay (Ciii). The longer-latency synaptic responses are APV sensitive (Ci) and thus due to activation of NMDA receptors, while the early response is CNQX-sensitive due to AMPA/kainate receptor activation (C, ii and iii). Application of CNQX and APV largely blocked evoked responses in eight of nine cells tested. Thus primary afferent-evoked responses in the neonatal DDH are predominantly glutamatergic (cp. Gerber et al. 1991; Randic et al. 1993; Sandkühler et al. 1997; Yoshimura and Jessell 1990).
5-HT INDUCED INCREASE IN SPINAL LTD

TABLE 1. Effect of 5-HT and specific 5-HT receptor ligands on evoked naïve synaptic responses

<table>
<thead>
<tr>
<th>5-HT</th>
<th>Decrease ↓</th>
<th>Increase ↑</th>
<th>No Change</th>
<th>Total</th>
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<tr>
<td>5-HT</td>
<td>55 ± 21 (37)*</td>
<td>28 ± 10 (3)</td>
<td>(6)</td>
<td>46</td>
</tr>
<tr>
<td>5-HT₁A/B</td>
<td>56 ± 16 (5)</td>
<td>—</td>
<td>(2)</td>
<td>7</td>
</tr>
<tr>
<td>5-HT₁A</td>
<td>22 ± 16 (3)</td>
<td>—</td>
<td>(2)</td>
<td>5</td>
</tr>
<tr>
<td>5-HT₁D</td>
<td>22 ± 8 (4)</td>
<td>—</td>
<td>(4)</td>
<td>8</td>
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<tr>
<td>5-HT₁B</td>
<td>24 ± 16 (2)</td>
<td>20 ± 7 (7)</td>
<td>(4)</td>
<td>13</td>
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</table>

Values (means ± SD) are percentage changes in the amplitude of evoked synaptic responses in the presence of the agonists. The sample sizes are in parentheses. CGS, 7-trifluoromethyl-1-(4-methyl-1-piperazinyl)-pyrrolo[1,2-a]quinazoline maleate; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propanesulfonic acid; CPBG, 1-(m-chloro-phenyl)-bi-guanide. *5-hydroxytryptamine (5-HT) significantly depressed the evoked synaptic responses in most neurons (P < 0.01).

Effects of 5-HT on evoked synaptic responses

The action of 5-HT on membrane properties was assessed at the cell’s resting membrane potential. 5-HT did not significantly alter resting membrane potential or cell input resistance. However, 5-HT significantly (P < 0.01; Student’s t-test) decreased the peak amplitude of evoked excitatory synaptic responses in 37 of 46 cells (Table 1). This depression was largely reversible following drug washout (88 ± 28% of initial amplitude, tested in 17/37 neurons). 5-HT also increased EPSP amplitude in three neurons, while the remaining six neurons were relatively unaffected by 5-HT (Table 1). The percent change in peak amplitude corresponded well with changes in synaptic charge transfer calculated as the integral of the synaptic response (area under the curve). For the cells depressed by 5-HT, both the early (presumably AMPA/kainate) and longer latency (presumably NMDA) synaptic components were equally depressed. For example, the area under the curve of the EPSP was decreased identically for synaptic events occurring <200 ms to those ≥200 ms. Hence, hereafter only peak amplitude values were compared.

Comparison of the actions of 5-HT to the induction of synaptic plasticity

Conditioning stimulation evoked LTP, LTD, or was without effect in the population of neurons sampled. In the absence of 5-HT, LTD was evoked in the majority of neurons (54%), while LTP was induced in 20% of neurons sampled. EPSP amplitude in the remaining neurons was unchanged (see Table 2, left column). These findings are consistent with our earlier study (Garraway et al. 1997). The ensuing results compare the actions of 5-HT before, during, and after conditioning stimulation of primary afferents.

RELATIONSHIP BETWEEN 5-HT’S EFFECT ON THE NAÏVE SYNAPTIC RESPONSE AND SYNAPTIC PLASTICITY. In 9 of 10 neurons, 5-HT depressed evoked responses, which then returned to baseline amplitude following drug withdrawal (to 101 ± 27%; also see Fig. 3). Thereafter, following CS, evoked synaptic responses could be observed to undergo LTD (n = 5; avg. of 45% ↓) or LTP (n = 3; avg. of 153% ↑), suggesting that the effect of 5-HT in a given cell is independent of the type of synaptic plasticity evoked (Fig. 3, A and B, respectively). The average magnitude of synaptic depression caused by 5-HT (55%) was very similar to the average magnitude of LTD produced following high-frequency CS (58%).

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FIG. 3. 5-HT can depress the naïve synaptic response irrespective of whether synapse will undergo CS-induced potentiation or depression. A1 and B1: 5-HT-evoked depression of synaptic responses prior to CS-induced LTD (n = 5) and CS-induced LTP (n = 3), respectively. Aii: an example of 5-HT-evoked depression of the raw synaptic responses (74% ↓). The evoked response, which returns to pre-5-HT values following washout, underwent CS induced LTD (66% ↓). Bii: following washout of the synaptic depression evoked by 5-HT (42% ↓), CS induced LTD (178% ↑) in this cell. Scale bars are 10 mV, 100 ms. In this and the following figures: the timing of 5-HT application is indicated with a horizontal bar and conditioning stimulation (CS) is indicated with a vertical bar, the circled numbers in graphs coincide with the panel of raw superimposed traces presented below them, graphs present normalized response amplitude ± SE, and cells were held at −94 mV during collection of EPSPs.
tioned response in 8 of 10 neurons by an average of 62%. This occurred regardless of the conditioning-evoked response in these neurons; three underwent LTD (Fig. 4A), two underwent LTP (Fig. 4B), and three were unaffected by CS (not illustrated). Thus 5-HT can cause depression in addition to CS-induced LTD and can also depress a potentiated synaptic response. After 5-HT washout, evoked responses returned to 76% of their pre-5-HT values (7 cells).

EFFECTS OF 5-HT ON THE INDUCTION OF SYNAPTIC PLASTICITY. In 16 cells, following a 10- to 15-min baseline of evoked responses, 5-HT was applied and its action recorded for an additional 10–15 min. These cells then underwent CS in the continued presence of 5-HT and for ≥20 min post conditioning (Fig. 5). In the presence of 5-HT, 88% of cells underwent CS-induced LTD. In 11 of the 14 cells undergoing LTD following CS, the naïve synaptic response was already depressed by preapplied 5-HT (48% ↓). These cells underwent an additional 51% depression following CS in the presence of 5-HT. Thus LTD could be induced on top of, and in addition to, the depression evoked by 5-HT. Table 3 compares the incidence of CS-induced LTP and LTD observed in the absence and presence of 5-HT. Significantly, CS of primary afferents in the presence of 5-HT caused an increased incidence of LTD compared with controls (cells conditioned in the absence of 5-HT) from 54 to 88% (χ²; P < 0.001; see Table 2). The presence of 5-HT, however, did not affect the magnitude of the LTD produced. The average CS-induced LTD was 54 ± 24% in the presence of 5-HT and 58 ± 24% in the controls.

Effects of 5-HT receptor agonists on naïve synaptic responses

Serotonin mediates its effect through various classes of receptors, many of which are located in the spinal cord. To determine some of the 5-HT receptors involved in 5-HT-induced alterations of evoked synaptic responses in DDH neurons, we compared the effects of ligands specific to 5-HT₁A/₁B, 5-HT₁B, 5-HT₂A/₂C, and 5-HT₃ receptors on the naïve synaptic responses. The effects of these ligands on neuronal passive membrane properties were first assessed at the cell’s resting membrane potential. Like 5-HT, none of the ligands had any significant effect on resting membrane potential or measured input resistance. Table 1 summarizes the effects of these agonists on EPSPs. Briefly, while selective activation of the 5-HT₁A/₁B receptor agonist 5-CT produced depressant actions similar to those observed for 5-HT, modulatory actions at 5-HT₁B, 5-HT₂, and 5-HT₃ receptors were modest.

Effects of 5-HT receptor agonists on the incidence of synaptic plasticity

The effects of selective 5-HT receptor ligands on the induction of synaptic plasticity was conducted using the experimental conditions described above.

<table>
<thead>
<tr>
<th>Table 3. Effect of 5-HT and specific receptor ligands on CS-induced synaptic plasticity</th>
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<tr>
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<tr>
<td>5-HT</td>
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<tr>
<td>5-HT₁A/₁B (5-CT/Claz)</td>
</tr>
<tr>
<td>5-HT₁B (CGS)</td>
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<tr>
<td>5-HT₂ (DOI)</td>
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<td>5-HT₃ (CPBG)</td>
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Values (means ± SD) are percentage changes in excitatory postsynaptic potential amplitude following conditioning stimulation (CS), in the presence of drug. The sample sizes, representing the relative incidence of LTP, LTD, or no change, are presented in parentheses.
The effects of these ligands on the induction and incidence of plasticity are summarized in Table 3. CS of primary afferents during selective 5-HT₁A/₁B receptor activation (with 5-CT/ cloz) induced only LTD (Fig. 6A). Similarly, CS induced LTD in all three neurons tested during 5-HT₁B receptor activation (with CGS; Fig. 6B). In comparison, CS of primary afferents in the presence of agonists at 5-HT₂ or 5-HT₃ receptors (DOI and CPBG, respectively) produced both LTP and LTD (not illustrated).

DISCUSSION

The ability of descending serotonergic systems to depress synaptic transmission in the dorsal horn provides for control of sensory transmission at the first CNS site of synaptic integration. Hence, it is important to elucidate the manner in which sensory synaptic transmission is regulated in the DDH particularly since this spinal cord region has the greatest longitudinal spread of nociceptor-induced activity (Coghill et al. 1991; Mao et al. 1993; Porro et al. 1991). Our experiments have compared the effects of 5-HT and specific 5-HT receptor ligands on CS-induced synaptic plasticity. Consistent with previous studies, 5-HT generally depressed primary afferent-evoked naïve synaptic response within neurons of the spinal dorsal horn (Jordan et al. 1979; Khasabov et al. 1999; Lopez-Garcia 1998; Lopez-Garcia and King 1996; Randic and Yu 1976). Following washout of 5-HT, CS of primary afferents could induce LTP or LTD, indicating that there was no association between the effect of 5-HT in a given neuron and the direction of induced plasticity. 5-HT also depressed synaptic responses following CS-induced LTP or LTD. Thus 5-HT can further depress synaptic responses that have already undergone LTD and can potently depress potentiated synapses. Of particular significance, CS of primary afferents in the presence of 5-HT significantly increased the incidence of LTD, indicating that 5-HT can alter the direction of plasticity, strongly favoring LTD. Given the correspondence of nociceptor activity to the induction of LTP (Liu et al. 1998; Sandkühler and Liu 1998), these results suggest that 5-HT may prevent the induction of nociceptor-induced LTP as well as depress existing “sensitized” (potentiated) synapses. In an intact system, descending serotonergic systems may use both methods to attenuate somatosensory input.

Possible mechanism for 5-HT-induced increases in LTD

A critical trigger underlying virtually all forms of synaptic plasticity is related to changes occurring in the concentration of postsynaptic calcium (Ca²⁺) (Artola and Singer 1993; Lisman 1989). The “calcium hypothesis” proposes that a large postsynaptic Ca²⁺ influx favors LTP, whereas moderate increases in postsynaptic Ca²⁺ favor LTD. For example, in the hippocampus, Cummings et al. (1996) showed that brief tetanic stimulation (which normally produced LTP) is able to elicit LTD if NMDA channels were partially blocked by moderate concentrations of d-APV or cells were voltage clamped at hyperpolarized potentials, thereby limiting postsynaptic Ca²⁺ influx. The induction of LTP and LTD in the spinal cord may also depend on the magnitude of evoked increases in Ca²⁺ and hence would tend to induce LTD rather than LTP. There are numerous examples of an inhibition of Ca²⁺ channels by 5-HT₁-like receptors (e.g., Scroggs and Anderson 1990; for review see Anwyl 1990).

5-HT receptor subtypes and synaptic plasticity

Pharmacological experiments demonstrated that activation of the 5-HT₁A and 5-HT₁B, but not the 5-HT₂A/₂C or 5-HT₃ receptors best compared with the effects of 5-HT in supporting LTD. LTP was never produced following CS in the presence of these 5-HT₁ receptor ligands. It is not surprising that agonists of both 5-HT₁A and 5-HT₁B receptors depress naïve synaptic responses and produce CS-induced LTD. Both subtypes are negatively coupled to adenylyl cyclase and have been previously associated with synaptic depression throughout the CNS (see Anwyl 1990). 5-HT₁A and 5-HT₁B receptors account for 27 and 18% of high-affinity 5-HT binding sites in the spinal cord, respectively (Huang and Peroutka 1987), and are present on both primary afferent terminals and postsynaptic dorsal horn neurons (see Daval et al. 1987), suggesting that pre- and/or postsynaptic mechanisms contribute to synaptic depression. Since we failed to observe any significant changes in intrinsic properties of the postsynaptic cell in the presence of 5-HT and receptor selective ligands, depressant actions probably occur at the glutamatergic synapse (see Lopez-Garcia 1998). Like 5-HT₁A and 5-HT₁B receptors, the 5-HT₁D-₁F receptors also negatively couple to adenylyl cyclase and hence, may also mediate synaptic depression. However, details of these receptor subtypes are not well known (Barnes and Sharp 1999).

In contrast to the 5-HT₁ receptors, activation of the 5-HT₂A/₂C receptors with DOI did not appear to favor the expression of LTD. While relatively few 5-HT₂A/₂C receptors are found in the dorsal horn (Corneà-Hébert et al. 1999; Mae-
shima et al. 1998; Pompeiano et al. 1994), activation of the 5-HT\textsubscript{2A/2C} receptors in this region can facilitate glutamatergic responses in some neurons (Hori et al. 1996), and may be involved in pronociceptive processes (e.g., Eide and Hole 1991).

The 5-HT\textsubscript{3} ionotropic receptor agonist CPBG evoked only a modest facilitation of naïve synaptic responses in 7 of 13 cells tested (at 1 \(\mu\)M), and there was no clear shift toward LTD following CS. The observed EPSP facilitation is consistent with an increase in number of evoked spikes in dorsal horn neurons (Ali et al. 1996) but opposes the attenuation of afferent-evoked neurotransmission observed by Khasabov et al. (1999). Khasabov et al. (1999) observed that higher concentrations of CPBG (10–50 \(\mu\)M) favor synaptic depression. 5-HT\textsubscript{3} receptors are present on primary afferent terminals (Hamon et al. 1989; Kidd et al. 1993) where they can mediate primary afferent depolarization (Khasabov et al. 1999), an indicator of presynaptic inhibition. 5-HT\textsubscript{3} receptors are also found on dorsal horn neurons (see Hamon et al. 1989) where they can cause direct excitation of GABAergic (Morales et al. 1998) and enkephalinergic interneurons (Tsuchiya et al. 1999). Both pronociceptive (Ali et al. 1996; Oyama et al. 1996) and antinociceptive effects (Alhaider et al. 1991; Bardin et al. 1997; Giordano 1997) have been reported following activation of 5-HT\textsubscript{3} receptors.

Importance of the DDH and synaptic connectivity

Neurons in the DDH represent a functionally heterogeneous population. Most receive convergent input from both low- and high-threshold afferent fibers and hence are classified as wide dynamic range (WDR) neurons, many of which are ascending tract cells conveying nociceptive information to the brain (Chung et al. 1979; Herrero and Headley 1995; Lopez-Garcia and King 1994; Willis and Coggeshall 1991).

DDH neurons project dendrites into superficial laminae and receive direct monosynaptic connections presumably from nociceptive primary afferents in laminae II (Naim et al. 1998; Todd 1989; Willis and Coggeshall 1991). On the other hand, low-threshold A fibers project monosynaptically onto DDH neurons via collaterals located in laminae III–V (Fitzgerald et al. 1994; Willis and Coggeshall 1991; see also Miller and Woolf 1996). Since our study was undertaken in neonates at a period when myelination is incomplete (Friede and Samorajski 1968), we did not determine the relative contribution of low- and high-threshold afferents to our evoked synaptic responses. However, the observed effects of 5-HT must be partly produced in WDR neurons since even at postnatal days 3–6, neuronal firing in response to depolarizing current injection is functionally differentiated (Hochman et al. 1997) and corresponds predominantly to WDR neurons that tend to fire repetitively in response to current injection (Lopez-Garcia and King 1994).

Descending monoaminergic transmission, antinociception, and development

Descending serotonergic systems exert a critical inhibitory control on spinal cord nociceptive transmission (for reviews, see Basbaum and Fields 1984; Fields and Basbaum 1978; Fitzgerald 1986; Hammond 1986; Millan 1995). For example, serotonergic fibers originating from brain stem raphe nuclei (Dahlström and Fuxe 1965) innervate the dorsal horn and comprise the best-described descending anti-nociceptive pathway. The inhibition of dorsal horn neurons caused by stimulation of brain stem regions is antagonized by the administration of 5-HT receptor antagonists, implicating 5-HT in mediating these antinociceptive effects (e.g., Chitour et al. 1982; Yaksh and Wilson 1978). Thus the prevention of LTP in spinal sensory systems with 5-HT is consistent with antinoceptive actions of some serotonergic descending systems.

Although bulbo spinal serotonergic axon terminals are abundant in the rat spinal cord at birth (Steinbusch 1981), modifications in the pattern and density occur postnatally (Bregman 1987). In relation to functional synaptic connections, Fitzgerald and Koltzenburg (1986) reported that despite the early anatomical presence of serotonergic fibers descending in the dorsolateral funiculus, there is no functional descending inhibition until P10–12. However, several other studies provide evidence for the existence of descending inhibition much earlier than P10. For instance, Miyata et al. (1987), Wallis and Wu (1993), and Wallis et al. (1993a) demonstrated that stimulation of the lateral or latero-ventral thoracic cord resulted in strong inhibition of the segmental monosynaptic reflex (MSR) in neonatal rats (P1–9), an effect mediated by serotonin (Wallis et al. 1993a). Similarly, Brocard et al. (1999) demonstrated that in the newborn rat, motoneurons are excited and/or inhibited by stimulating the ventral funiculus, while Magnuson et al. (1995) and Magnuson and Trinder (1997) showed that ventral root reflexes are evoked following stimulation of the ventrolateral funiculus in the neonatal rat (P1–8). Although none of these studies directly investigated the function of the dorsolateral funiculus, clearly, bulbospinal systems including the serotonergic innervations of the spinal cord are present and functional at birth, though presumed to be immature. In addition, many 5-HT receptor subtypes are clearly present and functional in the spinal cord of embryonic and newborn rats (e.g., Hentall and Fields 1983; Hochman and Garraway 1998; Wallis et al. 1993b; Ziskind-Conhaim et al. 1993) and there is evidence of endogenous release of serotonin at this stage (Wallis and Wu 1993). Therefore the use of 5-HT receptor agonists, even in neonates, may be effective in mediating anti-nociception.

In conclusion, we demonstrate that 5-HT acting at least partly via the 5-HT\textsubscript{1A} and \textsubscript{1B} receptors can influence the induction of afferent-evoked synaptic plasticity in spinal cord, favoring depression. Currently little is known of the modulatory properties of descending monoamine transmitters on the control of the spinal sensory integrative apparatus (see Jankowska et al. 1997). However, the emergence of syndromes following spinal cord injury that involves abnormally high-gain sensory processing (spasticity and chronic pain) attest to the importance of descending inhibitory control on spinal cord function (Ashby and McCrea 1987; Schouenbourg et al. 1992). Clearly a better understanding of the serotonergic modulation on spinal activity is required, including an identification of the actions of specific receptor subtypes on sensorimotor integration.

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