Synergistic Enhancement of Glutamate-Mediated Responses by Serotonin and Forskolin in Adult Mouse Spinal Dorsal Horn Neurons

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Wang, Guo-Du and Min Zhuo. Synergistic enhancement of glutamate-mediated responses by serotonin and forskolin in adult mouse spinal dorsal horn neurons. J Neurophysiol 87: 732–739, 2002; 10.1152/jn.00423.2001. Glutamate is the major excitatory amino acid neurotransmitter in the CNS, including the neocortex, hippocampus, and spinal cord. Normal synaptic transmission is mainly mediated by glutamate AMPA and/or kainate receptors. Glutamate N-methyl-d-aspartate (NMDA) receptors are normally inactive and only activated when a sufficient postsynaptic depolarization is induced by the activity. Here we show that in sensory synapses of adult mouse, some synaptic responses (26.3% of a total of 38 experiments) between primary afferent fibers and dorsal horn neurons are almost completely mediated by NMDA receptors. Dorsal root stimulation did not elicit any detectable AMPA/kainate receptor-mediated responses in these synapses. Unlike young spinal cord, serotonin alone did not produce any long-lasting synaptic enhancement in adult spinal dorsal horn neurons. However, co-application of the adenylyl cyclase activator forskolin and serotonin (5-HT) produced long-lasting enhancement, including the recruitment of functional AMPA receptor-mediated responses. Calcium-sensitive, calmodulin-regulated adenylyl cyclases (AC1, AC8) are required for the enhancement. Furthermore the thresholds for generating action potential responses were decreased, and, in many cases, co-application of forskolin and 5-HT led to the generation of action potentials by previously subthreshold stimulation of primary afferent fibers in the presence of the NMDA receptor blocker 2-amino-5-phosphonovaleric acid. Our results suggest that pure NMDA synapses exist on sensory neurons in adult spinal cord and that they may contribute to functional sensory transmission. The synergistic recruitment of functional AMPA responses by 5-HT and forskolin provides a new cellular mechanism for glutamatergic synapses in mammalian spinal cord.

INTRODUCTION

Glutamate is the major excitatory amino acid transmitter in the CNS (Bliss and Collingridge 1993; Hollmann and Heinemann 1994; Seeberg 1993). Glutamate mediates synaptic transmission by binding to postsynaptic α-amino-3-hydroxy-5-methyl-isoxozole propionic acid (AMPA), N-methyl-d-aspartate (NMDA), and kainate receptors. In most cases, synaptic responses are primarily mediated through postsynaptic AMPA and kainate receptors as NMDA receptors are blocked by magnesium at resting membrane potential. However, there are reports that NMDA receptors contribute to synaptic transmission and modulation in brain slice preparations including the cortex, hippocampal, olfactory bulb, and spinal cord (Bardoni et al. 2000; Gil and Amitai 1996; Sah et al. 1989; Schiller et al. 2000; Schoppa et al. 1998; Yoshimura and Jessell 1990). For example, in spinal slices of young rats, it has been recently shown that high-intensity root stimulation evoked NMDA-sensitive slow synaptic activity in lamina II neurons that drove action potential firing (Bardoni et al. 2000). In thalamocortical slices, both NMDA and non-NMDA receptors contribute to excitatory synaptic transmission (Gil and Amitai 1996).

Recent studies indicate that glutamatergic synapses are not functionally homogenous; at some synapses, only functional NMDA receptors are available to respond to glutamate released from presynaptic terminals during a synaptic event (Baba et al. 2000; Bardoni et al. 1998; Durand et al. 1996; Gomperts et al. 1998; Isaac et al. 1995, 1997; Li and Zhuo 1998; Liao et al. 1995; Rumpel et al. 1998; Wu et al. 1996; see Malenka and Nicoll 1997; Malinow et al. 2000 for reviews). Assuming that dendritic resting membrane potentials are in the same range as those that can typically be observed at the soma, one would predict that in the absence of any prior postsynaptic depolarization, pure NMDA synapses would not respond to glutamate release, and thus appear functionally “silent.” What makes pure NMDA synapses important is their involvement in central synaptic plasticity. These synapses can be converted into AMPA and NMDA mixed synapses, at least functionally (Durand et al. 1996; Hayashi et al. 2000; Isaac et al. 1995; Li and Zhuo 1998; Liao et al. 1995, 1999). In the hippocampus and somatosensory cortex, long-term potentiation may involve the unsilencing of synapses (Durand et al. 1996; Issac et al. 1997; Liao et al. 1995).

Sensory transmission in the spinal cord dorsal horn receives biphasic descending modulation from supraspinal structures including the rostroventral medulla (RVM) (Fields et al. 1991; Zhuo and Gebhart 1992, 1997). Serotonin-containing neurons in the RVM send descending projecting terminals to the spinal cord, and serotonin released from descending projecting fibers regulates spinal sensory transmission in a dose-related, biphasic manner (Li and Zhuo 1998). In the young rat spinal cord, serotonin at a low dose or a selective 5-HT2 receptor agonist induced long-lasting synaptic enhancement, and activation of silent synapses at least in part contributes to the enhancement (Li and Zhuo 1998; Li et al. 1999). No similar study has been carried out in adult spinal slices.
Several studies clearly indicate that pure NMDA synapses may be involved in development-related synaptic plasticity (Isaac et al. 1997; Liao et al. 1999; Petralia et al. 1999). What about the role of pure NMDA synapses in adult animal physiology? Less information is available related to this question. We wanted to determine if pure NMDA synapses contribute to sensory plasticity in adult neurons. Considering potential advantages of genetically manipulated mice, we selected adult mice (greater than 8 wk) in this study. We demonstrate here that there are pure NMDA receptor-mediated responses in these sensory synapses and reveal a signaling synergistic pathway for recruiting functional AMPA receptor-mediated responses.

METHODS

Slice preparation

Adult male 8–14 wk-old mice (C57/Bl; Jackson Laboratory) were used. AC1, AC8 and AC1, and AC8 double knockout and littermate wild-type mice were generously provided by Dr. Louis Muglia. Mice were anesthetized with urethan. Transverse lumbar spinal cord slices, 450–500 μm with 7–12 mm of attached dorsal root, were obtained from male adult mice. Slices were kept in a plastic chamber containing oxygenated saline (which was composed of (in mM) 124 NaCl, 4 KCl, 26 NaHCO3, 2 NaH2PO3, 2 CaCl2, 1 MgSO4, and 10 d-glucose) at 32°C for at least 2–3 h. Experimental protocols were approved by the Animal Studies Committee at Washington University.

Intracellular recordings

Intracellular recordings of synaptic responses were performed from neurons located in the dorsal horn Lamina I and II with a 3 M potassium chloride-filled glass microelectrode (DC impedance, 75–200 MΩ). The synaptic responses were activated by electrical stimulation of dorsal roots with a bipolar electrode or a suction electrode. We did not find any obvious difference in responses to stimulation using a bipolar electrode or a suction electrode. Postsynaptic excitatory postsynaptic potentials (EPSPs) were evoked at 0.01–0.02 Hz. Monosynaptic EPSPs were identified using two criteria: the response latency did not change with increasing intensities of electrical stimulation and repetitive stimulation did not change response latency. EPSPs of the neurons were evoked with electrical stimulation at different intensities (0.2 ms, 1–30 V) and recorded through a high-input impedance bridge circuit of amplifier (Intracellular Electrometer IE-210, Warner Instrument, or Axonclump 2B, Axon Instruments) and stored in PClamp (Axon Instruments) files. The perfusion medium contained (in mM) 124 NaCl, 4 KCl, 26 NaHCO3, 1 NaH2PO4, 1 MgSO4, 2 CaCl2, and 10 glucose was oxygenated with 95% O2–5% CO2. The temperature and perfusion rate of recording were kept at 34°C and 2–5 ml/min, respectively. All drugs were purchased from RBI-Sigma. In all experiments, bicuculline methiodide (10 μM) and strychnine hydrochloride (1 μM) were added to the perfusion solution to block inhibitory transmission.

Data and statistical analysis

Data are presented as means ± SE. Statistical comparisons were made with the use of one-way ANOVAs (Dunnett test for post hoc comparison) or Student’s t-test. P < 0.05 was considered significant.

RESULTS

Pure NMDA receptor-mediated sensory responses

To examine if pure NMDA receptor synapses may exist in adult sensory synapses between primary afferent fibers and dorsal horn neurons, we recorded EPSPs from neurons in the superficial dorsal horn of the spinal cord, which receive afferent inputs from peripheral sensory neurons. EPSPs were induced by electrical stimulation delivered to the dorsal root, and the selective NMDA receptor antagonist inhibitor 2-amino-5-phosphonovaleric acid (AP5, 50 or 100 μM) was used to identify the NMDA receptor-mediated component of an EPSP. In a total of 38 dorsal horn neurons tested, 10 of them were completely blocked by bath application of AP5 (26.3% of the total population; Fig. 1A). The blockade was reversible, as the EPSP recovered after washout of AP5. The resting membrane potentials were not significantly affected throughout the experiments. The mean stimulation intensity (0.2-ms duration) was 19.0 ± 2.1 V (n = 10, ranged from 10.0 to 30.0 V). In the other 28 neurons, AP5 only partially blocked the synaptic responses, and subsequent co-application of CNQX and AP5 completely blocked the response (Fig. 1, B and D). Dorsal horn neurons containing only NMDA receptor-mediated EPSPs had the similar resting membrane potential (mean –74.7 ± 2.1 mV, n = 10) as neurons exhibiting mixed AMPA/NMDA responses (–71.0 ± 2.0 mV, n = 28), indicating that pure NMDA receptor-mediated EPSPs did not require depolarization at the soma.

We also tested if pure NMDA receptor-mediated EPSPs may be affected by changing extracellular Mg2+ concentrations. After obtaining pure NMDA EPSPs in normal Mg2+ (1 mM), we then perfused the slices with the solution with less Mg2+ (0.2 mM). In a total of eight neurons tested, no significant

![FIG. 1. Pure N-methyl-D-aspartate (NMDA) receptor and mixed NMDA/AMPA receptor-mediated sensory synaptic transmission in adult neurons. A: examples of pure NMDA receptor-mediated excitatory postsynaptic potentials (EPSPs) showing synaptic responses before, during, and after treatment with 50 μM 2-amino-5-phosphonovaleric acid (AP5). EPSPs recovered during the washout of AP5. B: examples of mixed AMPA/NMDA receptor-mediated EPSPs showing synaptic responses before and during perfusion of 50 μM AP5 or 50 μM AP5 plus 20 μM 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX). Data shown in A and B were collected from 2 different neurons. C: the effect of AP5 on the EPSP slopes in the experiment shown in A. D: the effects of AP5 and CNQX on the EPSP slopes in the experiment shown in B.](image-url)
difference in the slopes of NMDA receptor-mediated EPSPs were found (the ratio of EPSP slopes at 1 vs. 0.2 mM Mg$^{2+}$: 0.9 ± 0.1, n = 8), indicating that these NMDA receptors are less sensitive to the Mg$^{2+}$ blockade than the normal NMDA receptors.

Pure NMDA receptor-triggered action potentials

Can pure NMDA receptor-mediated EPSPs generate action potentials in adult dorsal horn neurons in response to stimulation of the dorsal root? Bardoni et al. (2000) reported that in young rat dorsal horn neurons, repetitive stimulation could generate action potentials in neurons with pure NMDA synapses. We thus wanted to see if action potentials can be induced at pure NMDA synapses in adult dorsal horn neurons. As shown in Fig. 2, adult dorsal horn neurons containing pure NMDA receptor-mediated synapses generated spike responses in response to stimulation of the dorsal root. Bath application of the NMDA receptor antagonist AP5 completely blocked the EPSP and action potentials. These results indicate that at adult sensory synapses (between primary afferent fibers and dorsal horn neurons), pure NMDA receptor-mediated EPSPs can mediate sensory transmission. The results also provide direct evidence that pure NMDA receptor synapses can drive action potentials in adult dorsal horn neurons.

Serotonin predominantly inhibited sensory synaptic responses in adult spinal cord slices

5-HT is a key transmitter of descending pain modulatory systems (Fields et al. 1991; Willis 1982). 5-HT biphasically regulates spinal sensory synaptic transmission in young spinal cord slices, causing facilitation of responses at lower doses and inhibition at higher doses by acting on different 5-HT receptor subtypes (Hori et al. 1996; Li and Zhuo 1998; Li et al. 1999). To explore if similar modulatory effects can be observed in adult spinal cord slices, we applied 5-HT through the bath solution during recordings of EPSPs in which AMPA and NMDA components were not distinguished. In contrast to spinal slices from young animals, all doses of 5-HT used (5, 10, and 100 μM; n = 5–8) produced predominantly inhibitory effects on EPSPs (Fig. 3) in 18 of 20 experiments. In the other two experiments, 5-HT produced slight increases in the EPSPs (5 μM 5-HT, 114% of control; 10 μM 5-HT, 136% of control), and the EPSPs returned to the baseline after the washout of 5-HT. Resting membrane potentials were not significantly affected in these neurons by 5-HT application.

Synergistic effects between 5-HT and forskolin

Adult in vivo electrophysiological, pharmacological, and behavioral studies have consistently demonstrated that the spinal serotonergic system mediates descending facilitation as well as inhibition from the raphe nuclei and adjacent nuclei (Zhuo and Gebhart 1992, 1997). Intrathecal administration of 5-HT or 5-HT receptor subtype-selective agonists facilitates spinal nociceptive reflexes in adult awake animals (see Millan 1999 for a review). What could be the cellular mechanism explaining the difference between in vivo physiological findings and our in vitro spinal cord slice electrophysiology? We hypothesized that the facilitatory effect of 5-HT in adult animals may depend on the cAMP signal pathway. Evidence from several different studies suggests cAMP may serve as a bifunctional signal transduction molecule in central glutamatergic synapses and contribute to synaptic potentiation and depression.
(Blitzer et al. 1995; Brandon et al. 1995; Lee et al. 2000; Lisman 1989; Qi et al. 1996). In the spinal dorsal horn, sensory transmitters such as glutamate and neuropeptides can increase postsynaptic calcium and may raise the cAMP level through calcium-sensitive adenylyl cyclases (Gu et al. 1996; Parsons and Seybold 1997).

To test this hypothesis, we examined the effect of an activator of adenylyl cyclase, forskolin (10 μM), on sensory synaptic transmission. Unlike at hippocampal synapses (Chavez-Noriega and Stevens 1992; Weisskorf et al. 1994), 10 μM forskolin did not significantly affect synaptic responses (n = 6, 96.2 ± 6.5% of control; Fig. 4B). Co-application of 5 μM 5-HT with 10 μM forskolin produced significant enhancement of EPSPs (mean 263.8 ± 40.1% of control; P < 0.05, n = 6; Fig. 4A). The duration of EPSPs were also increased after the application. This enhancement sustained after washout. Resting membrane potentials were not significantly affected by 5 μM 5-HT and forskolin co-application in the same neurons (−74.5 ± 1.8 mV before and −75.8 ± 2.2 mV after). The synergistic effect of 5-HT and forskolin depended on the dose of 5-HT. Co-application of 10 μM forskolin with 100 μM 5-HT produced significant inhibition of synaptic responses (25.5 ± 9.5% of control; n = 4) and synaptic responses recovered during washout.

Two major forms of calcium-calmodulin-sensitive adenylyl cyclases type 1 and 8 (AC1 and AC8) are found in the CNS (Xia and Storm 1997). To examine if the effects of forskolin depends on activation of AC8 or AC1, we performed experiments in wild-type mice and mutant mice lacking AC1, AC8, and AC1 and AC8. Consistently, co-application of 5-HT (5 μM) and forskolin (10 μM) produced significant enhancement of synaptic responses in wild-type mice (n = 6, 221.1 ± 59.9% of control, P < 0.05). However, the enhancement of 5-HT and forskolin was absent in mice lacking AC1 (n = 9, 92.5 ± 7.7% of control) and AC8 (n = 7; 88.2 ± 8.0% of control) or mice lacking both AC1 and AC8 (n = 2, 100.2 and 95.4% of control, respectively; Fig. 5). These findings indicate that forskolin acts through AC1 and AC8.

Due to enhanced EPSP amplitudes after the application of 5-HT and forskolin, dorsal horn neurons showed enhanced spike responses to the dorsal root stimulation. In three of six experiments, following treatment with 5-HT plus forskolin,
dorsal horn neurons demonstrated spike responses to stimulation at an intensity that did not induce any spike response before drug application (Fig. 4A). Spike responses to stimulation of primary afferent fibers had a late onset and often appeared during washout. In the other three cells, synaptic responses were enhanced without any spike response.

Recruitment of AMPA/kainate receptor-mediated responses at pure NMDA synapses

We tested if the synergistic interaction between 5-HT and forskolin recruits AMPA receptors to pure NMDA synapses. We picked recordings where 100 μM AP5 blocked EPSPs completely (Fig. 6A). We then perfused both 5-HT (5 μM) and forskolin (10 μM) in the continuous presence of AP5. Interestingly, in all three experiments, co-application of 5-HT and forskolin caused AMPA/kainate receptor-mediated EPSPs to appear (Fig. 6A). The effect was long-lasting and persisted during the washout (in the continuous presence of AP5). Spike responses were also observed after the treatment. Both newly recruited EPSPs and spike responses were completely blocked by bath application of 20 μM CNQX in the continuous presence of 100 μM AP5 (Fig. 6). These results indicate that recruited AMPA receptor-mediated EPSPs were large enough to excite the neurons and generate spike responses to primary afferent fiber stimulation at intensities that were previously unable to activate action potentials. Our results are in agreement with previous studies in young animals that the recruitment of AMPA responses in dorsal horn neurons are NMDA receptor-independent (Li and Zhuo 1998; Li et al. 1999), a different form of plasticity from that reported in the hippocampus (Durand et al. 1996; Isaac et al. 1995; Liao et al. 1995).

**DISCUSSION**

We provide strong evidence that pure NMDA receptor-mediated synapses exist on adult dorsal horn sensory neurons. More importantly, we show here that these synapses can indeed mediate somatosensory synaptic transmission between primary afferent fibers and dorsal horn neurons. Because dorsal horn neurons containing pure NMDA synapses have normal resting membrane potentials, we believe that pure NMDA receptors contribute to normal sensory synaptic transmission in adult mammalian spinal cord. Due to the fact that the sensitivity of intracellular recording is lower than that of patch clamping, it is quite possible that there may be some AMPA receptor-mediated responses below the detectable level in these synapses. Baba et al. (2000) reported that in adult rat dorsal horn there are a few pure NMDA synapses by using the whole cell patch-clamp recording technique. One obvious difference is that adult mice were used in the present study. We think that our studies will allow us to investigate the contribution of different subtypes of NMDA receptors to sensory synaptic EPSPs in the spinal cord using genetically manipulated mice in future. Our current results do not exclude the possible presynaptic effects of serotonin and forskolin and future studies are needed to determine the pre- versus postsynaptic contribution to the enhancement. It is also important to determine what serotonin receptor subtypes are involved in the present experiments by using both pharmacological and genetic approaches.

**Pure NMDA receptor-mediated synaptic responses**

The existence of pure NMDA synapses has been reported in many different regions of the CNS, including the neocortex, hippocampus, and spinal cord (see **INTRODUCTION**). Studies from brain slices from young rats as well as neuronal cultures indicate that the recruitment of AMPA receptors into pure NMDA synapses occurs during development and may contribute to development-related synaptic plasticity. In some areas of the brain, pure NMDA synapses disappear in late developmental stages with the loss of ability to undergo synaptic potentiation (or long-term potentiation). However, in other regions of the brain, long-term potentiation or long-lasting synaptic enhancement of synaptic transmission happen in adult animals and are thought to contribute to different functions of the brain, such as learning, memory in physiological conditions and persistent pain after tissue injury (see Bliss and Collingridge 1993; Malenka and Nicoll 2000; Sandkühler 2000). One critical question is whether pure NMDA synapses are still present in adult neurons and, if so, whether the functional recruitment

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**FIG. 6.** Synergistic recruitment of AMPA/kainate receptor-mediated responses at pure NMDA synapses. A: examples of EPSPs showing synaptic responses before, during, and after co-application of 5 μM 5-HT and 10 μM forskolin in the presence of 100 μM AP5. B: the effect of 5-HT and forskolin on the EPSP slopes in the experiment shown in A. Spike responses to stimulation of the dorsal root nerves at the subthreshold intensity were observed during the washout (indicated by arrows). C: in a separate experiment, 20 μM CNQX completely blocked both EPSPs and action potentials induced by 5 μM 5-HT and 10 μM forskolin in the presence of AP5. D: summary data of 5 μM 5-HT and 10 μM forskolin. Data contain 2 sets of experiments: pure NMDA synapses (n = 3) and mixed AMPA/kainate and NMDA synapses (n = 6). In both cases, significant enhancement was observed, and data were pooled together.
of AMPA receptors into these synapses also occurs under certain circumstances. Indeed, early studies by Bardoni et al. (1998) and Li and Zhuo (1998) in the spinal cord were able to detect pure NMDA synapses in slices from postnatal 21-day-old rats; this is different from what is observed in the neocortex. Our present studies provide strong evidence that indeed pure NMDA synapses exist in adult spinal cord dorsal horn neurons. About 26% of synaptic responses between primary afferent fibers and dorsal horn neurons are mediated purely by NMDA receptors. The stimulation paradigm used in the current study is sufficient to activate $A_\beta$ and even C fibers in some cases (see Yoshimura and Jessell 1989). This is in consistent with the fact that neurons in superficial dorsal horn of adult animals primarily receive nociceptive $A_\beta$ and C fiber inputs (Light et al. 1979). Our results suggest that some of pure NMDA synapses exist between nociceptive primary afferent fibers and dorsal horn neurons.

Pure NMDA synapses in adult neurons are functional. At normal soma resting membrane potentials, we detected pure NMDA receptor-mediated EPSPs. Our results are thus unlikely due to tonic soma depolarization, which removes the magnesium blockade of the NMDA receptor channel. One likely possibility is that the magnesium blockade is reduced at NMDA receptors at distal synapses sites. There are at least two mechanisms for reduced magnesium blockade: first, pure NMDA synapses may be primarily situated in distal dendrites and local postsynaptic potentials may be depolarized. Therefore the magnesium blockade of NMDA receptors at these sites may be reduced. Second, they may represent a subpopulation of NMDA subtype receptors such as NMDA2C and NMDA2D that are less sensitive to the magnesium blockade (Burnashev et al. 1992; Momiyama 2000; Momiyama et al. 1996; Seeburg 1993). Our results using a low concentration of magnesium support the second possibility. Consistent with this finding, in young spinal dorsal horn neurons, Bardoni et al. (2000) observed a slow, NMDA receptor-mediated excitatory postsynaptic current (EPSC) at $-70$ mV evoked by stimulation, implying that not all current through NMDA receptors is blocked by magnesium at negative membrane potentials. Furthermore, these NMDA receptor-mediated EPSCs can generate action potentials during repetitive stimulation in young animals (Bardoni et al. 2000) and after chemical treatments in adult dorsal horn neurons in the present study. This suggests that pure NMDA synapses can indeed contribute to sensory transmission in the spinal cord. It is worthy to mention that indeed NMDA receptor-mediated spikes has been reported in cortical pyramidal neuronal dendrites (Schiller et al. 2000). In their experiments using fluorescence confocal microscopy and mimicking EPSPs by ultraviolet laser glutamate uncaging, Schiller et al. (2000) showed that NMDA receptors on fine basal dendrites can be activated by glutamate and contribute to initiating local dendritic spikes.

**Effects of serotonin on sensory synaptic transmission and nociception**

Pharmacological and electrophysiological studies have provided evidence for spinal 5-HT system exerts biphasic effects on spinal nociceptive transmission. 5-HT or 5-HT receptor agonists produced both inhibitory and excitatory effects on spinal dorsal horn neurons including ascending spinothalamic tract cells (see Millan 1999 for a review). In behavioral tests, biphasic modulatory effects of 5-HT are also reported, and different subtypes of 5-HT receptors are thought to contribute to biphasic modulation (Ali et al. 1994, 1996; Solomon and Gebhart 1988; Zelman et al. 1983). One major source of spinal 5-HT is from descending projection fibers from the raphe nucleus and adjacent nuclei in the brain stem (Bowker and Abbott 1990; Fields et al. 1991), and stimulation at high intensities in these areas or indirectly in the periaqueductal gray released 5-HT in the lumbar spinal cord (Cui et al. 1999; Sorkins et al. 1993). Indeed, electrical or chemical stimulation in brain stem areas produces biphasic modulation in spinal nociceptive transmission and the tail-flick reflex (Haber et al. 1980; Light et al. 1986; McCreery et al. 1979; Zhuo and Gebhart 1990–1992, 1997). Descending facilitation and descending inhibition are mediated by different spinal 5-HT subtype receptors (Zhuo and Gebhart 1991).

Synaptic mechanisms for 5-HT-produced inhibition and facilitation have been studied in spinal cord slices from young animals (Hori et al. 1996; Khasabov et al. 1999; Li and Zhuo 1998; Lopes-Garzia and King 1996). 5-HT at high doses produces inhibition of synaptic EPSCs or EPSPs by acting through postsynaptic and/or presynaptic 5-HT receptors (see Grudt et al. 1996; Hori et al. 1996; Khasabov et al. 1999; Li and Zhuo 1998, 2001; Lopes-Garzia and King 1996). For 5-HT-induced excitatory or facilitatory effects, the recruitment of postsynaptic silent synapses is critical for the facilitation (Li and Zhuo 1998; Li et al. 1999). Furthermore, the interaction between AMPA receptor and PDZ-domain-containing proteins are important for the effects of 5-HT (Li et al. 1999).

Unlike slices from young animals, a few reports were performed in slices of adult animals. Both postsynaptic excitatory and inhibitory effects on dorsal horn neurons have been reported in adult frog spinal cord, although synaptic responses to stimulation ofafferent fibers had not been examined (Tan and Miletic 1990). In adult rats spinal cord, 5-HT produced inhibition or no effects on primary afferent-evoked synaptic responses (Ito et al. 2000). Consistent with this report in adult rats, we showed here that 5-HT produced predominant inhibition of synaptic responses in dorsal horn neurons of adult mice. Furthermore, we provided a new synergistic mechanism between 5-HT and cAMP for explaining the facilitatory effect of spinal 5-HT system.

**Synergistic effect between cAMP and serotonin**

cAMP signal pathways haven been implicated in the function of spinal dorsal horn neurons. Activation of several receptors for sensory transmitters such as glutamate and CGRP has been reported to raise cAMP levels. In slices or isolated cells from young animals, cAMP analogue enhanced glutamate receptor-mediated synaptic responses (Cerne et al. 1992, 1993) or no effect on AMPA/kainate receptor-mediated synaptic responses (Hori et al. 1996). In the present studies, application of forskolin did not significantly affect synaptic responses induced by dorsal root stimulation in slices of adult mice. However, co-application of 5-HT and forskolin produced long-lasting facilitation of synaptic responses. Possible contributors to the increases in the cAMP levels are the calcium-sensitive adenylyl cyclases. We found that the facilitatory effect induced by 5-HT and forskolin was completely blocked in mice lacking...
AC1 or AC8, indicating that calcium-sensitive adenyl cyclases are important. Our results demonstrate that in adult sensory synapses, cAMP-signaling pathways determine whether activation of 5-HT receptors causes facilitatory or inhibitory effects on synaptic responses. Unlike synapses from young animals, 5-HT alone did not induce reliable and long-lasting facilitation of synaptic responses (see Li and Zhuo 1998). Instead, 5-HT at the same low dose induced no effects, short-lasting increases or inhibition (see RESULTS) in adult neurons. However, co-application of the same dose of 5-HT with forskolin produced significant long-lasting enhancement of synaptic responses. This finding provides a possible scenario for regulation of two different signaling pathways under physiological or pathological conditions. Postsynaptic increases in cAMP levels by sensory transmitters may favor 5-HT-induced facilitation. The interaction between cAMP and 5-HT may provide an associative heterosynaptic form of central plasticity in the spinal dorsal horn to allow sensory inputs from the periphery to act synergistically with central descending modulatory influences. We think that it is unlikely that cAMP acts additionally to 5-HT signal pathways. First, 5-HT at a higher dose produce opposite effects, that is inhibition of synaptic responses. Forskolin alone did not produce any facilitation. Second, in young neurons, it has been reported that PKC is required for the effects of 5-HT (Li et al. 1999).

Physiological implications

Our results may provide a synaptic mechanism for the recruitment of ineffective synapses in adult animals after tissue or nerve injury (Wall 1977, 1988; Zhuo 2000). Recruitment of functional AMPA responses may allow subthreshold stimulation of primary afferent fibers, which alone were not sufficiently strong to raise the cells above their firing threshold, to fire action potentials. A recent study revealed that AMPA receptor-PDZ domain interactions are important for the 5-HT induced facilitation in young sensory neurons (Li et al. 1999). Considering rapid developments in research on glutamate receptors and advanced mouse genetics (Garner et al. 2000; Hollmann and Heinemann 1994; Seeburg 1993), we believe that this mouse study will facilitate future dissection of molecular mechanisms for plasticity of sensory glutamatergic synapses in the spinal cord.

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