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PRESYNAPTIC 5-HT$_{1B}$ RECEPTOR-MEDIATED SEROTONERGIC INHIBITION OF GLUTAMATE TRANSMISSION IN THE BED NUCLEUS OF THE STRIA TERMINALIS

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Abstract—Activation of neurons in the bed nucleus of the stria terminalis (BNST) plays a critical role in stress and anxiety-related behaviors. Previously, we have shown that serotonin (5-HT) can directly modulate BNST neuronal excitability by an action at postsynaptic receptors. In this study we built upon that work to examine the effects of 5-HT on excitatory neurotransmission in an *in vitro* rat BNST slice preparation. Bath application of 5-HT reversibly reduced the amplitude of evoked excitatory postsynaptic currents (eEPSCs). These effects were mimicked by the 5-HT$_{1B/D}$ receptor agonist, sumatriptan, and by the 5-HT$_{1B}$ receptor selective agonist, CP93129. Conversely, the effects of 5-HT and sumatriptan could be blocked by the 5-HT$_{1B}$ receptor-selective antagonist, GR55562. In contrast, the 5-HT$_{1A}$ receptor agonist 8-OH DPAT or antagonist WAY 100635 could not mimic or block the effect of 5-HT on eEPSCs. Together, these data suggest that the 5-HT-induced attenuation of eEPSCs was mediated by 5-HT$_{1B}$ receptor activation. Moreover, sumatriptan had no effect on the amplitude of the postsynaptic current elicited by pressure applied AMPA, suggesting a possible presynaptic locus for the 5-HT$_{1B}$ receptor. Furthermore, 5-HT, sumatriptan and CP93129 all increased the paired pulse ratio of eEPSCs while they concomitantly decreased the amplitude of eEPSCs, suggesting that these agonists act to reduce glutamate release probability at presynaptic locus. Consistent with this observation, sumatriptan decreased the frequency of miniature EPSCs, but had no effect on their amplitude. Taken together, these results suggest that 5-HT suppresses glutamatergic neurotransmission in the BNST by activating presynaptic 5-HT$_{1B}$ receptors to decrease glutamate release from presynaptic terminals. This study illustrates a new pathway by which the activity of BNST neurons can be indirectly modulated by 5-HT, and suggests a potential new target for the development of novel treatments for depression and anxiety disorders.

Key words: serotonin, excitatory postsynaptic currents, presynaptic receptor, patch clamp recording, anxiety disorders.

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Abbreviations: ACSF, artificial cerebrospinal fluid; BLA, basolateral amygdala; BNST, the bed nucleus of the stria terminalis; BNST$_{AL}$, anterolateral BNST; CRF, corticotrophin-releasing factor; 5-CT, 5-Carboxamidotryptamine; DRN, dorsal Raphé nucleus; eEPSCs, evoked excitatory postsynaptic currents; EPSCs, excitatory postsynaptic currents; ILCx, infralimbic cortex; IPSCs, inhibitory postsynaptic currents; mEPSCs, miniature excitatory postsynaptic currents; NMDA, *N*-methyl-D-aspartic acid; PbN, parabrachial nuclei; PFC, prefrontal cortex; PPR, paired pulse ratio; PVN, paraventricular nucleus of the hypothalamus; SSRIs, selective serotonin reuptake inhibitors; ST, stria terminalis; TTX, tetrodotoxin; VTA, ventral tagmental area.

Serotonin (5-HT) neurotransmission is critically involved in stress and anxiety-like behaviors (Ressler and Nemeroff, 2000; Lowry et al., 2005), and selective serotonin reuptake inhibitors (SSRIs) are the most widely used medications for the treatment of anxiety disorders. However, the mechanisms of SSRIs are not well understood and sometimes controversial. This is partially due to the diversity of 5-HT receptors expressed in the CNS: there being seven 5-HT receptor families and 14 subtypes (Hoyer et al., 2002). Moreover, the 5-HT effects in the CNS are further complicated because 5-HT acts not only as a neurotransmitter but also a neuromodulator (see; Fink and Gothert, 2007 for a review). Hence, 5-HT not only affects neuronal excitability through activating postsynaptic receptors, including 5-HT$_{1A,2A,2C,3,4,7}$ (Rainnie, 1999a,b; Craven et al., 2001; Chapin et al., 2002; Levita et al., 2004; Xiang et al., 2005; Mlinar et al., 2006; Hashimoto and Kita, 2008; Guo et al., 2009; Hammack et al., 2009), but growing evidence indicates that 5-HT can also affect presynaptic excitatory or inhibitory neurotransmission in the CNS (Koyama et al., 2002; Bouryi and Lewis, 2003; Hashimoto and Kita, 2008).

The bed nucleus of the stria terminalis (BNST) is an important forebrain region involved in the modulation of anxiety-like behaviors (Lee and Davis, 1997; Treit et al., 1998; Sullivan et al., 2004b; Davis, 2006; Meloni et al., 2006) and the mediation of the stress response (Casada and Dafny, 1991; Hammack et al., 2004, 2009). It is a pivotal relay of cortical information to the paraventricular nucleus of hypothalamus (PVN) (Herman et al., 1994; Choi et al., 2007), and lesions of the BNST decreased the stress response of rats (Fendt et al., 2003; Hammack et al., 2004). Conversely, stimulation of the BNST mimics the cardiovascular responses to stress (Dunn and Williams, 1995), and stress is a major precipitating factor in the development of anxiety-like behavior (Shekhar et al., 2005). The BNST receives substantial serotonergic innervation from the dorsal raphé nucleus (DRN), and expresses multiple 5-HT receptors (Vertes, 1991; Halberstadt and Balaban, 2008). In addition, 5-HT fibers target multiple BNST neurons including those neurons expressing the stress hormone, corticotrophin releasing factor (CRF) (Phelix et al., 1992a,b). Hence, the BNST might be a critical region for 5-HT modulation of stress-induced anxiety-like behaviors.

Previously we have shown that 5-HT has a direct postsynaptic effect on BNST neurons (Rainnie, 1999a; Levita et al., 2004; Guo et al., 2009), and can either hyperpolarize or depolarize BNST neurons, depending on the 5-HT receptor subtype(s) activated. However, the ma-

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jority of BNST neurons are inhibited by activation of postsynaptic 5-HT_{1A} receptors (Levita et al., 2004). Significantly, we have shown that local infusion of a 5-HT_{1A} agonist, 5-carboxamidotryptamine (5-CT), into the BNST attenuates the acoustic startle response in rats, suggesting that 5-HT_{1A} receptor-mediated inhibition of BNST neurons has an anxiolytic action.

In addition to a direct modulation through activation of postsynaptic 5-HT receptors, the excitability of BNST neurons could also be modulated indirectly by affecting synaptic transmission in this nucleus. The BNST receives substantial glutamatergic input from the prefrontal cortex (PFC), basolateral amygdala (BLA), and hippocampus (Weller and Smith, 1982; Dong et al., 2001a; Walker et al., 2003; Massi et al., 2008), and recent studies have shown that this input can be modulated by several neurotransmitters and neuromodulators including glutamate itself, norepinephrine, dopamine, CRF, and cannabinoids (Egli et al., 2005; Grueter and Winder, 2005; Muly et al., 2007; Kash et al., 2008; Massi et al., 2008; McElligott and Winder, 2008, 2009). However, no study to date has examined the effects of 5-HT on synaptic transmission in the BNST. In the present study, we directly addressed this issue by using patch clamp recordings from an *in vitro* BNST slice preparation to examine the effect of 5-HT on glutamatergic neurotransmission and identify the underlying 5-HT receptor subtype(s) mediating the effect.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (5–7 weeks old, Charles River, Raleigh, NC, USA) were used in this experiment. Animals were housed 4–5 per cage and had access to food and water *ad libitum*. All experimental protocols strictly conform to National Institutes of Health guidelines for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Emory University. Cares were taken to minimize the stress and suffering of rats before sacrificing.

Slice preparation

Slices containing the anterolateral BNST ($BNST_{AL}$) were obtained as previously described (Rainnie, 1999a; Muly et al., 2007). Briefly, under isoflurane anesthesia (Fisher Scientific, Hanoverpark, IL, USA), animals were decapitated and the brains rapidly removed and immersed in a cold (4 °C) 95–5% oxygen/carbon dioxide oxygenated "cutting solution" with the following composition (in mM): NaCl (130), $NaHCO_3$ (30), KCl (3.50), KH_2PO4 (1.10), $MgCl_2$ (6.0), $CaCl_2$ (1.0), glucose (10), supplemented with kynurenic acid (2.0). Slices containing the BNST were cut at a thickness of 350 μM using a Leica VTS-1000 vibratome (Leica Microsystems Inc., Bannockburn, IL, USA). Slices were kept in oxygenated "cutting solution" at room temperature for 1 h before transferring to regular artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl (130), $NaHCO_3$ (30), KCl (3.50), KH_2PO_4 (1.10), $MgCl_2$ (1.30), $CaCl_2$ (2.50), and glucose (10). Slices were kept in the regular ACSF for at least 30 min before recording.

Patch clamp recording

Individual slices were transferred to a recording chamber mounted on the fixed stage of a Leica DMLFS microscope (Leica Microsystems Inc., Bannockburn, IL, USA), where they were maintained fully submerged and continuously perfused with oxygenated 32 °C ACSF at a flow rate of 1–2 ml/min. Individual BNST neurons were identified by using differential interference contrast (DIC) optics and infrared (IR) illumination with an IR sensitive CCD camera (Orca ER, Hamamatsu, Tokyo Japan). All cells recorded were confined to the $BNST_{AL}$ as previously reported (Levita et al., 2004; Hammack et al., 2007). Patch pipettes were pulled from borosilicate glass and had a resistance of 4–6 MΩ. The patch recording solution had the following composition (in mM): 130 K-gluconate, 2 KCl, 10 HEPES, 3 $MgCl_2$, 2 K-ATP, 0.2 NaGTP, and 5 phosphocreatine, titred to pH 7.3 with KOH, and 290 mOsm. Data acquisition and analysis were performed using a MultiClamp 700B amplifier in conjunction with pClamp 10.0 software and a DigiData 1320A AD/DA interface (Molecular Devices, Burlingame, CA, USA). Whole cell patch clamp recordings were obtained and whole cell currents were filtered at 2 kHz and digitized at 10–20 kHz. The membrane potential was held at −60 mV for all neurons if not specified. Only those BNST neurons which had a stable membrane potential more negative than −55 mV and an action potential that overshot by >10 mV were used. Access resistance was monitored throughout the experiments and neurons showing more than a 15% change of access resistance were discarded.

Recording of evoked EPSCs

Excitatory postsynaptic currents (EPSCs) onto BNST neurons were evoked as previously described (Muly et al., 2007). In brief, a concentric bipolar stimulation electrode (FHC, Bowdoinham, ME, USA) was placed on the afferent fibers of the stria terminalis (ST). Evoked EPSCs (eEPSCs) were recorded in the presence of the $GABA_A$ receptor antagonist SR 95531 (5 μM) to block $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs). One train of five single square wave pulses (150 μs, 0.2 Hz) was delivered every 2 min throughout the experiment to induce EPSCs. For analysis, the peak eEPSC amplitude was calculated as the mean response to each series of five stimulations. The mean of three stable eEPSCs obtained immediately before drug treatment was considered as baseline eEPSCs. All eEPSCs values were normalized to the baseline amplitude and expressed as the percentage of baseline.

Paired pulse paradigm

To examine the potential involvement of presynaptic 5-HT receptors, a paired pulse paradigm was employed in which two stimuli were delivered with an inter-stimulus-interval (ISI) of 50 ms. Five pairs of stimuli were delivered with an interval of 5 s between each pair and were averaged to measure the peak amplitude of both eEPSCs. The paired pulse ratio (PPR) was then calculated as the peak amplitude of the second EPSC (P2) divided by the first, EPSC1 (P1). Alterations in the PPR are thought to represent changes in release probability in the presynaptic terminal (Hess et al., 1987; Manabe et al., 1993).

Miniature EPSCs recording

Miniature EPSCs (mEPSCs) were examined in the presence of tetrodotoxin (TTX) (1 μM) and SR 95531 (5 μM). Two sessions of 1 min recording captured before 5-HT application were used as baseline. Another two sessions of 1 min recordings were captured during 5-HT application. All mEPSCs were detected offline using the MiniAnalysis program 6.0 (Synaptosoft Inc., Decatur, GA, USA). The mEPSCs frequency and amplitude were represented as the mean values of two sessions.

Drug application

The following drugs were obtained from (1) Sigma-Aldrich (St. Louis, MO, USA): serotonin, TTX, DNQX (6,7-dinitroquinoxaline-2,3-dione),