

Serotonergic Modulation of GABAergic and Glutamatergic Synaptic Transmission in Mechanically Isolated Rat Medial Preoptic Area Neurons

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The medial preoptic area (MPOA) of the hypothalamus is critically involved in the regulation of male sexual behavior and has been implicated in several homeostatic processes. Serotonin (5-hydroxytryptamine, 5-HT) inhibits sexual behavior via effects in the MPOA, where there are high densities of 5-HT_{1A} and 5-HT_{1B} receptor subtypes. We used whole-cell recordings under voltage-clamp conditions to investigate the serotonergic modulation of γ -aminobutyric acid (GABA)ergic and glutamatergic synaptic transmission in mechanically dissociated rat MPOA neurons with native presynaptic nerve endings. Spontaneous GABAergic miniature inhibitory postsynaptic currents (mIPSCs) in the MPOA were completely blocked by bicuculline. Serotonin reversibly reduced the GABAergic mIPSC frequency without affecting the mean current amplitude. Serotonergic inhibition of mIPSC frequency was mimicked by (\pm)-8-hydroxy-2-dipropylaminotetralin hydrobromide, a specific 5-HT_{1A} receptor agonist, and blocked by 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)-butyl] piperazine hydrobromide, a specific 5-HT_{1A} receptor antagonist. 6-Cyano-7-nitroquinoxaline-2,3-dione completely blocked spontaneous glutamatergic miniature excitatory postsynaptic currents (mEPSCs) in the MPOA. Serotonin reversibly decreased the glutamatergic mEPSC frequency without affecting the mean current amplitude. Serotonergic inhibition of mEPSC frequency was mimicked by CGS 12066B, a specific 5-HT_{1B} receptor agonist, and blocked by SB 216641, a specific 5-HT_{1B} receptor antagonist. Stimulation of adenylyl cyclase with forskolin increased the frequencies of GABAergic mIPSCs and glutamatergic mEPSCs, and blocked the inhibitory effects of 5-HT. H-89, a selective protein kinase A (PKA) inhibitor, decreased the frequencies of GABAergic mIPSCs and glutamatergic mEPSCs, and blocked their reduction by 5-HT. These findings suggest that 5-HT reduces the frequency of GABAergic mIPSCs and glutamatergic mEPSCs through 5-HT_{1A} and 5-HT_{1B} receptor-mediated inhibition, respectively, of the PKA-dependent pathway in the presynaptic nerve terminals of MPOA neurons.

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INTRODUCTION

The medial preoptic area (MPOA) of the hypothalamus is critically involved in the regulation of male sexual behavior (Meisel and Sachs, 1994) and has been implicated in several homeostatic processes (Swanson and Mogenson, 1981; van Gemert *et al*, 1975). Damage to the MPOA impairs sexual behavior (Klaric and Hendricks, 1986; Paredes *et al*, 1998), whereas stimulation of the MPOA facilitates sexual behavior (Malsbury, 1971; Rodriguez-Manzo *et al*, 2000). The MPOA exerts its various functions via widespread neuronal

connections in the central nervous system (CNS) (Simerly and Swanson, 1986, 1988). Neuroanatomical investigation of the MPOA demonstrated projections to this region from neurons releasing many putative neurotransmitters and neuromodulators (Simerly *et al*, 1986), and electrophysiological studies showed that MPOA neurons can generate currents mediated by α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl-D-aspartate, γ -aminobutyric acid (GABA)_A, and glycine receptors (Karlsson *et al*, 1997a,b).

Serotonin (5-hydroxytryptamine, 5-HT), a neurotransmitter and neuromodulator, is widely distributed in the mammalian CNS, where it plays a role in sleep, cognition, sensory perception, motor activity, temperature regulation, appetite, hormone secretion, nociception, and sexual behavior (Jacobs and Azmitia, 1992). The cellular actions of 5-HT are mediated by up to 14 distinct receptor subtypes

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located in the cell membrane (Hoyer and Martin, 1997). Regulation of male sexual behavior by 5-HT has been studied in humans, primates, and rodents. In most cases, pharmacological manipulation of 5-HT has shown an inhibitory effect on sexual motivation and performance (Gorzalka *et al.*, 1990; Hull *et al.*, 2004; Zajecka *et al.*, 1991). Microinjection of 5-HT into the MPOA of male rats has been shown to impair sexual activity (Fernandez-Guasti *et al.*, 1992; Verma *et al.*, 1989). Conversely, decreasing serotonergic activity, by either lesioning the brain or inhibiting 5-HT synthesis, has been shown to facilitate sexual behavior (Hull *et al.*, 2004; Gorzalka *et al.*, 1990).

The brain localization of 5-HT₁ receptor subtypes is well described. A high density of 5-HT_{1A} receptors is found in the hippocampus, whereas the highest concentrations of 5-HT_{1B} receptors are found in the substantia nigra, globus pallidus, and dorsal subiculum (Pazos and Palacios, 1985). Both receptor subtypes are present at high densities in the MPOA (Pazos and Palacios, 1985), but it is not clear which one mediates the inhibitory effects of serotonin. In addition, electrophysiological studies have revealed that 5-HT suppressed the transmitter release probability in GABA-containing as well as in glutamate-containing presynaptic nerve terminals on MPOA neurons (Malinina *et al.*, 2005). However, the presynaptic mechanisms of serotonergic inhibition of the GABAergic and glutamatergic synaptic transmission in the MPOA neurons have not yet been elucidated. Thus, the present study investigated the electrophysiological and pharmacological mechanisms for serotonergic inhibition of sexual function by performing conventional whole-cell patch-clamp recordings in mechanically isolated rat MPOA neurons under voltage-clamp conditions.

METHODS

Preparation

All experiments conformed to the guiding principles for the care and use of animals approved by the Council of the Korean Physiological Society and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals as well as suffering.

Single MPOA neurons with presynaptic boutons were isolated using techniques described previously (Akaike and Moorhouse, 2003; Hahn *et al.*, 2004). In brief, 10- to 15-day-old Sprague-Dawley rats of both sexes were decapitated under Zoletil 50[®] anesthesia (50 mg kg⁻¹, i.p.). Brains were quickly removed and transversely sliced at a thickness of 400 μ m using a vibratome (Series 1500, Vibratome, St Louis, MO). Before mechanical dissection, slices were preincubated for at least 1 h at room temperature (22–25°C) in solution well saturated with 95% O₂ and 5% CO₂. For dissociation, slices were transferred to a 35-mm plastic culture dish (Falcon Primaria 3801, Becton Dickinson, Rutherford, NJ), and the MPOA was identified under a binocular microscope (SZ-ST, Olympus, Tokyo, Japan). Mechanical dissociation was accomplished using a custom-built vibration device and a fire-polished glass pipette oscillating at approximately 50–60 Hz. The tip of the pipette was lightly placed on the surface of the MPOA and vibrated

horizontally (0.1–0.2 mm displacement) for approximately 2 min. Slices were removed, and the mechanically dissociated neurons were allowed to settle and adhere to the bottom of the dish for at least 15 min before commencement of recording. The neurons undergoing dissociation retained short portions of their proximal dendrites.

Electrophysiological Recordings

Electrophysiological recordings were performed in the conventional whole-cell patch-clamp recording mode (Hammill *et al.*, 1981) under voltage-clamp conditions. Patch pipettes were prepared from glass capillaries (1B150F-4, World Precision Instruments Inc., Sarasota, FL) on a vertical pipette puller (PP-830, Narishige, Tokyo, Japan) in two stages. The patch pipette was positioned on the neuron using a water-driven micromanipulator (WR-60, Narishige, Tokyo, Japan). The resistance between the recording electrode filled with the internal pipette solution and the reference electrode was 5–7 M Ω . The neurons were visualized with phase-contrast equipment on an inverted microscope (IX-70, Olympus, Tokyo, Japan). Electrical stimulation, current recordings, and current filtration (at 1 kHz) were obtained with an EPC-10 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany) linked to an IBM-compatible PC controlled by HEKA software. Data were digitized with a LIH 1600 board (HEKA Elektronik, Lambrecht, Germany) and stored on a hard disk. The currents were monitored on a thermal linear corder (WR3320-3CL, Graphtec Co., Yokohama, Japan). All experiments were performed at room temperature (22–25°C).

Data Analysis

Spontaneous miniature inhibitory and excitatory postsynaptic currents (mIPSCs and mEPSCs) were counted and analyzed using the MiniAnalysis program (Synaptosoft Inc., Leonia, NJ). For curve fitting, Kaleida Graph software (Synergy Software, Reading, PA) was used. To set the adequate threshold for mIPSCs and mEPSCs, we analyzed mIPSCs and mEPSCs at the threshold of 1 pA, and then manually accepted or rejected each event according to the characteristics of rise time and decay time kinetics. Then, we plotted the histogram of amplitude distribution of mIPSCs and mEPSCs. Almost all events of mIPSCs and mEPSCs were larger than 10–20 and 5–10 pA, respectively (Figures 1c and 5c). Thus, we set the threshold for event detection at 10–20 pA for mIPSC and 5–10 pA for mEPSC. Spontaneous events were initially detected automatically using an amplitude threshold of 10–20 pA for the recording of mIPSCs and 5–10 pA for mEPSCs at the holding voltage (V_H) of –60 mV. After automatic detection, all events were manually accepted or rejected on the basis of the rise and decay times. Events with brief rise times (0.5–1.5 ms) that were well fitted by a single-exponential function were selected for analysis. The amplitudes and inter-event intervals of large numbers of mIPSCs and mEPSCs obtained from a single neuron were examined by constructing all-point cumulative probability distributions and compared using the Kolmogorov–Smirnov test with Stat View software (SAS Institute Inc., Cary, NC). Mean amplitudes and

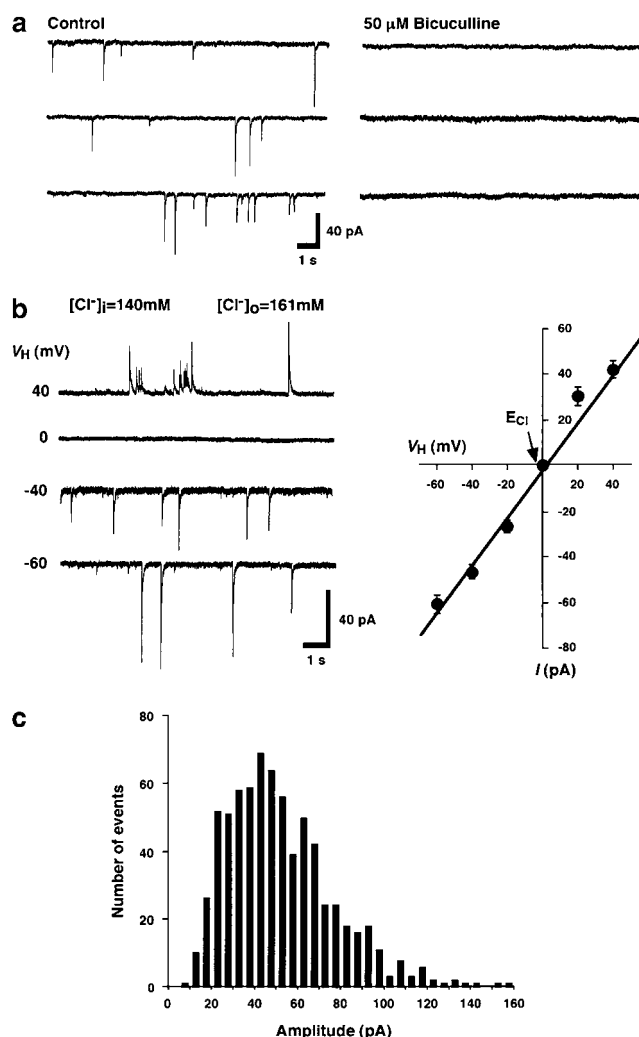


Figure 1 GABAergic mIPSCs recorded from mechanically dissociated MPOA neurons. (a) In the presence of 300 nM TTX, 3 μ M CNQX, and 10 μ M AP5, superfusion of 50 μ M bicuculline completely and reversibly blocked mIPSCs in isolated MPOA neurons ($V_H = -60$ mV). (b) Traces of mIPSCs recorded at V_H of -60 , -40 , 0 , and $+40$ mV. Intracellular and extracellular Cl^- concentrations were 140 and 161 mM, respectively. I-V curve for the mean amplitude of mIPSCs recorded at various V_H s. Each point is the mean of four neurons. (c) A histogram showing typical amplitude distribution of mIPSCs. The amplitudes of 717 events detected at the threshold of 1 pA were plotted. Amplitudes of almost all events were larger than 10 pA. The bin size was 5. $V_H = -60$ mV.

frequencies of mIPSCs and mEPSCs were normalized to the control conditions and are reported as means \pm SEM. Differences in mean amplitude and frequency of mIPSCs and mEPSCs were tested with Student's paired two-tailed *t*-test using absolute rather than normalized values. Values of $P < 0.05$ were considered significant.

Solutions

The ionic composition of the incubation solution was (in mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, glucose 10, and $NaHCO_3$ 24. The pH was adjusted to 7.4 by continuous bubbling with 95% O_2 and 5% CO_2 . The

standard external solution was (in mM): NaCl 150, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) 10. The pH was adjusted to 7.4 with tris-hydroxymethyl aminomethane (Tris-base). The composition of the internal pipette solution for mIPSC recording was (in mM): CsCl 110, TEA-Cl 30, ethylene glycol-bis (β -aminoethyl ether)-*N,N,N'*-tetraacetic acid (EGTA) 5, adenosine 5'-triphosphate magnesium salt (Mg-ATP) 5, guanosine 5'-triphosphate trisodium salt (Na_3 -GTP) 0.4, and HEPES 10. The pH was adjusted to 7.2 with Tris-base. For mEPSC recording, external solutions contained 300 nM TTX, 3 μ M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and 10 μ M DL-2-amino-5-phosphonovaleic acid (AP5) to block voltage-dependent Na^+ channels and glutamatergic excitatory synaptic currents. The composition of the internal pipette solution for mEPSC recording was (in mM): cesium methanesulfonate 120, TEA-Cl 10, EGTA 5, NaCl 10, Mg-ATP 5, Na-GTP 0.4, and HEPES 10. The pH was adjusted to 7.2 with Tris-base. For mEPSC recording, external solutions contained 300 nM TTX and 10 μ M (-)-bicuculline methochloride (bicuculline) to block voltage-dependent Na^+ channels and GABAergic inhibitory synaptic currents.

Drugs and Chemicals

Zoletil 50[®] (tiletamine HCl 125 mg/5 ml + zolazepam HCl 125 mg/5 ml) was purchased from Virbac laboratory (06516 Carros, France). Tetrodotoxin, (-)-bicuculline methochloride, CNQX, AP5, EGTA, Mg-ATP, Na_3 GTP, methanesulfonic acid, HEPES, dimethyl sulfoxide, *N*-methyl-D-glucamine, $BaCl_2$, CsCl, $CdCl_2$, cesium methanesulfonate, 4-aminopyridine (4-AP), forskolin, *N*-(2-[p-bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide hydrochloride (H-89), and 5-HT HCl were purchased from Sigma (St Louis, MO). (\pm)-8-Hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT), α -methyl-5-hydroxytryptamine maleate (α -methyl-5-HT), 7-trifluoromethyl-4-(4-methyl-1-piperazinyl)pyrrolo[1,2-a]-quinoxaline dimaleate (CGS 12066B), NAN-190 hydrobromide, ketanserin tartrate, and *N*-4-methoxyphenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-4-carboxamide hydrochloride (SB 216641) were purchased from Tocris Cookson (Bristol, UK). CNQX, bicuculline, and forskolin were dissolved in dimethyl sulfoxide at 10 mM as a stock solution. Drugs were added to external solutions at the final concentrations shown in the text and the vehicle concentrations never exceeded 0.01%. Drugs were applied using a rapid application system termed the 'Y-tube method,' described elsewhere (Akaike and Harata, 1994).

RESULTS

GABAergic mIPSCs in Isolated MPOA Neurons

Mechanically isolated MPOA neurons were bipolar or pyramidal in shape and retained short portions of their proximal dendrites. When the neurons were voltage-clamped at $V_H -60$ mV in the presence of 300 nM TTX, 3 μ M CNQX, and 10 μ M AP5, spontaneous mIPSCs were observed in dissociated MPOA neurons. After rupture of the patch membrane, it took 10–20 min for synaptic currents to stabilize. Upon stabilization, recording of

mIPSCs commenced. In most neurons, mIPSCs were stable for approximately 60 min. These results indicate that presynaptic nerve terminals attached to the dissociated neurons were functional and their spontaneous activity stable within 60 min. Superfusion of 50 μ M bicuculline completely and reversibly blocked spontaneous mIPSCs (Figure 1a). Figure 1b shows typical spontaneous GABAergic mIPSCs at various V_H values. The reversal potential (-5 mV) of these mIPSCs, estimated from the current-voltage ($I-V$) relationship, was very similar to the theoretical Cl^- Nernst equilibrium potential (E_{Cl}) of -4 mV calculated using the extracellular and intracellular Cl^- concentrations of 161 and 140 mM, respectively. These results indicate that the spontaneous mIPSCs were mediated by GABA_A receptors. Figure 1c shows that the typical amplitude distribution of mIPSCs was roughly unimodal and somewhat skewed. The amplitudes of almost all mIPSC events in the present study were larger than 10–20 pA.

5-HT_{1A} Receptors Mediate Serotonergic Presynaptic Inhibition of GABAergic mIPSCs

Superfusion of 5-HT (100 nM) decreased the frequency of GABAergic synaptic events in almost MPOA neurons. The effect of 5-HT was reversible (Figure 2b). Figure 2b shows cumulative probability plots for inter-event intervals and

current amplitudes of mIPSCs. Serotonin shifted the distribution curve of mIPSC frequency to the right, indicating a reduction of mIPSC frequency. The amplitude distribution was not affected. Serotonin decreased the mean mIPSC frequency to $62.4 \pm 3.2\%$ of the mean control frequency ($P < 0.01$, $n = 23$; Control, 2.20 ± 0.57 Hz; 5-HT, 1.58 ± 0.47 Hz), but the mean amplitude was not affected ($98.5 \pm 4.4\%$ of the control amplitude, $P = 0.739$, $n = 23$; Control, 61.66 ± 3.74 pA; 5-HT, 60.38 ± 4.42 pA; Figure 2c). These results indicate that serotonin acts presynaptically to inhibit the release probability of GABA at the synapse.

To determine which 5-HT receptor subtypes mediated the decrease in mIPSC frequency, the effects of selective 5-HT_{1A} and 5-HT₂ receptor agonists and antagonists were examined. The selective 5-HT_{1A} receptor agonist 8-OH-DPAT (100 nM) reversibly decreased the mIPSC frequency in a manner similar to 5-HT itself. For example, 8-OH-DPAT reduced the mean mIPSC frequency to $61.9 \pm 9.0\%$ of the control frequency ($P < 0.01$, $n = 6$; Control, 0.88 ± 0.33 Hz; 8-OH-DPAT, 0.56 ± 0.23 Hz) without affecting the mean amplitude ($97.6 \pm 13.3\%$ of the control amplitude, $P = 0.769$, $n = 6$; Control, 43.16 ± 4.32 pA; 8-OH-DPAT, 43.03 ± 7.31 pA; Figure 2c). In contrast, α -methyl-5-HT (100 nM), a selective 5-HT₂ receptor agonist, did not affect mean mIPSC frequency ($97.7 \pm 13.4\%$ of the control frequency, $P = 0.836$, $n = 5$; Control, 0.44 ± 0.12 Hz;

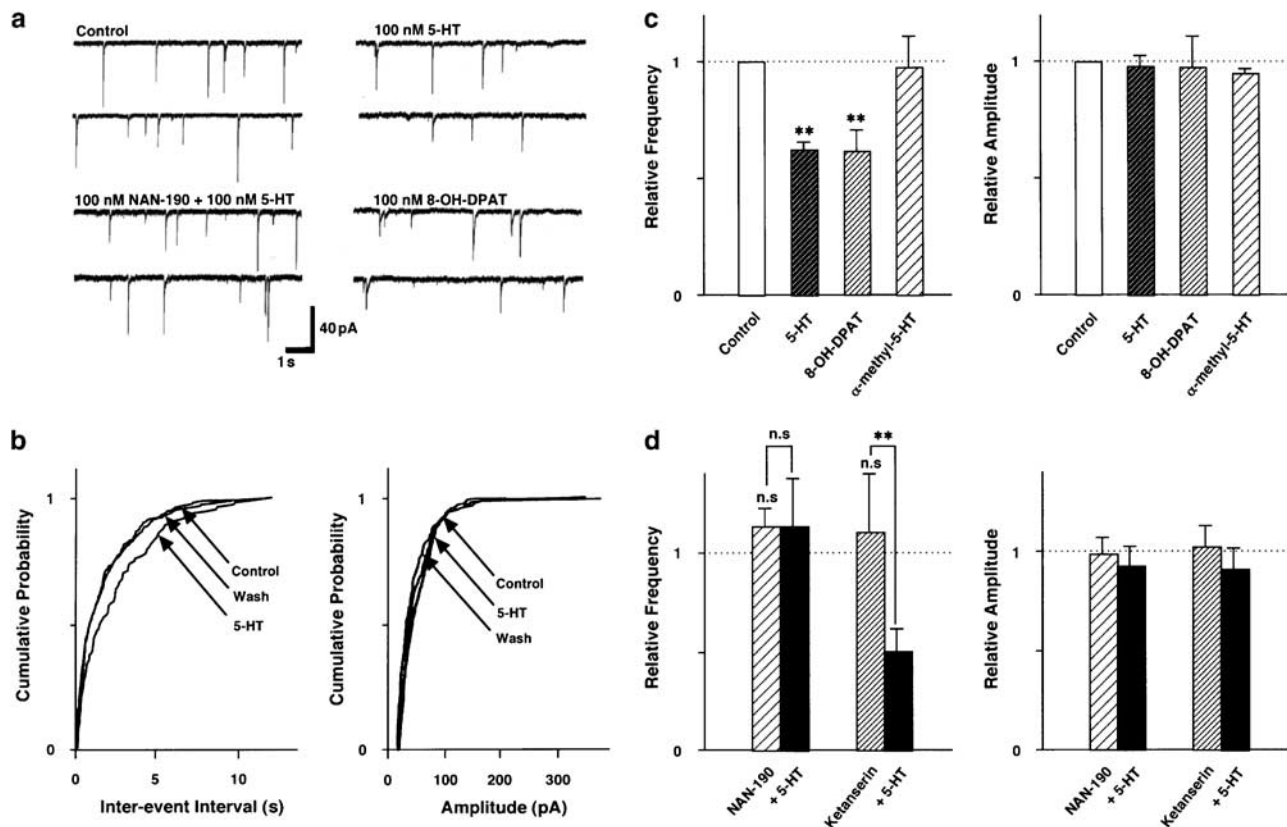


Figure 2 5-HT_{1A} receptor-mediated presynaptic inhibition of GABAergic mIPSCs. (a) Representative recording traces of mIPSCs observed before and during the application of 100 nM 5-HT in the absence or presence of 100 nM NAN-190, and during the application of 100 nM 8-OH-DPAT. (b) Cumulative distributions for inter-event intervals and amplitudes of mIPSCs recorded from the same neuron. (c) All frequencies and amplitudes are normalized to those of control mIPSCs. 5-HT ($n = 23$), 8-OH-DPAT ($n = 6$), 100 nM α -methyl-5-HT ($n = 5$). (d) All frequencies and amplitudes are normalized to those of control mIPSCs. NAN-190 + 5-HT ($n = 4$) and 100 nM ketanserin + 5-HT ($n = 4$). Dashed lines in each histogram indicate the relative control. In this and subsequent figures, asterisks represent a statistically significant difference (** $P < 0.01$, paired two-tailed t -test). n.s. indicates $P > 0.05$.

α -methyl-5-HT, 0.44 ± 0.14 Hz) or amplitude ($94.8 \pm 2.1\%$ of the control amplitude, $P = 0.524$, $n = 5$; Control, 51.35 ± 6.27 pA; α -methyl-5-HT, 48.81 ± 6.39 pA; Figure 2c).

The neurons were superfused with external solution containing each 5-HT antagonist for 5 min before simultaneous superfusion of 100 nM 5-HT with one of the antagonists. The selective 5-HT_{1A} receptor antagonist NAN-190 (100 nM) blocked the inhibitory effect of 5-HT on mIPSC frequency ($100.6 \pm 19.9\%$ of the NAN-190 condition, $P = 0.210$, $n = 4$; NAN-190, 0.41 ± 0.10 Hz; NAN-190 + 5-HT, 0.49 ± 0.12 Hz) without affecting mIPSC amplitude ($95.8 \pm 4.3\%$ of the NAN-190 condition, $P = 0.261$, $n = 4$; NAN-190, 55.39 ± 9.51 pA; NAN-190 + 5-HT, 52.38 ± 7.87 pA; Figure 2d). NAN-190 itself did not affect mIPSC frequency ($113.3 \pm 9.3\%$ of the control frequency, $P = 0.416$, $n = 4$; Control, 0.49 ± 0.15 Hz; NAN-190, 0.41 ± 0.10 Hz) or amplitude ($99.1 \pm 8.0\%$ of the control amplitude, $P = 0.651$, $n = 4$; Control, 60.38 ± 13.95 pA; NAN-190, 55.39 ± 9.51 pA; Figure 2d). Ketanserin (100 nM), a selective 5-HT₂ receptor antagonist, did not alter the inhibitory effect of 5-HT on mIPSC frequency ($39.2 \pm 8.5\%$ of the ketanserin condition, $P < 0.01$, $n = 4$; Ketanserin, 0.66 ± 0.10 Hz; Ketanserin + 5-HT, 0.26 ± 0.06 Hz) or amplitude ($94.7 \pm 2.3\%$ of the ketanserin condition, $P = 0.081$, $n = 4$; Ketanserin, 44.78 ± 4.81 pA; Ketanserin + 5-HT, 42.40 ± 4.51 pA; Figure 2d). Ketanserin itself also did not affect the frequency ($110.8 \pm 28.9\%$ of the control frequency, $P = 0.777$, $n = 4$; Control, 0.58 ± 0.15 Hz; Ketanserin, 0.66 ± 0.10 Hz) or amplitude ($102.4 \pm 10.4\%$ of the control amplitude, $P = 0.837$, $n = 4$; Control, 48.56 ± 7.63 pA; Ketanserin, 44.78 ± 4.81 pA; Figure 2d) of mIPSCs. These results suggest that serotonergic modulation of GABAergic synaptic transmission in the MPOA is mediated by presynaptic 5-HT_{1A} receptors.

Blockade of Presynaptic K⁺ Channels does not Affect Serotonergic Inhibition of GABAergic mIPSCs

It was previously suggested that 5-HT_{1A} receptor activation inhibits neuronal excitability through activation of G-protein-coupled inwardly rectifying K⁺ (GIRK) channels (Penington *et al.*, 1993; Katayama *et al.*, 1997; Jeong *et al.*, 2001). To determine whether presynaptic activation of GIRK channels is responsible for the inhibitory effect of 5-HT on GABAergic mIPSC frequency, we tested the effects of Ba²⁺, which is known to block GIRK channels. Ba²⁺ (1 mM) significantly and reversibly increased mIPSC frequency to $345.7 \pm 48.8\%$ of the control frequency ($P < 0.05$, $n = 4$; Control, 0.49 ± 0.09 Hz; Ba²⁺, 1.65 ± 0.37 Hz) without affecting the mean amplitude ($100.5 \pm 7.7\%$ of the control amplitude, $P = 0.961$, $n = 4$; Control, 70.46 ± 3.25 pA; Ba²⁺, 71.51 ± 9.14 pA; Figure 3b). This increase in mIPSC frequency is consistent with the expected depolarization of presynaptic nerve terminals, which should activate voltage-dependent Ca²⁺ channels (VDCCs). However, in the presence of Ba²⁺, 5-HT still reversibly decreased mIPSC frequency to $62.5 \pm 8.0\%$ of the frequency in the Ba²⁺ condition ($P < 0.05$, $n = 4$; Ba²⁺, 1.65 ± 0.37 Hz; Ba²⁺ + 5-HT, 1.01 ± 0.27 Hz), not affecting the mean mIPSC amplitude ($90.7 \pm 4.3\%$ of the Ba²⁺ condition, $P = 0.148$, $n = 4$; Ba²⁺, 71.51 ± 9.14 pA; Ba²⁺ + 5-HT, 64.22 ± 7.47 pA; Figure 3b).

We also examined the effect of 4-AP, a K⁺ channel blocker, on 5-HT-induced inhibition of mIPSCs. Superfusion of 100 μ M 4-AP reversibly increased mIPSC frequency to $470.7 \pm 70.4\%$ of the control frequency ($P < 0.01$, $n = 5$; Control, 0.69 ± 0.14 Hz; 4-AP, 3.12 ± 0.59 Hz) without altering mIPSC amplitude ($137.7 \pm 21.7\%$ of the control amplitude, $P = 0.157$, $n = 5$; Control, 50.59 ± 3.13 pA; 4-AP, 67.23 ± 5.58 pA; Figure 3d). In the presence of 4-AP, 5-HT reversibly decreased mIPSC frequency to $70.4 \pm 8.7\%$ of the 4-AP condition ($P < 0.05$, $n = 5$; 4-AP, 3.12 ± 0.59 Hz; 4-AP + 5-HT, 2.17 ± 0.42 Hz) without affecting mIPSC amplitude ($98.0 \pm 3.6\%$ of the 4-AP condition, $P = 0.598$, $n = 5$; 4-AP, 67.23 ± 5.58 pA; 4-AP + 5-HT, 66.32 ± 7.23 pA; Figure 3d). Together, these results indicate that activation of presynaptic K⁺ channels is unlikely to contribute to 5-HT_{1A} receptor-mediated inhibition of GABAergic mIPSCs.

Presynaptic Ca²⁺ Channels do not Mediate Serotonergic Inhibition of GABAergic mIPSCs

As Ca²⁺ influx through VDCCs plays an important part in neurotransmitter release from presynaptic nerve terminals (Wu and Saggau, 1994), we tested whether the serotonergic inhibition of GABAergic mIPSCs was mediated by modulation of VDCCs, investigating the effect of Cd²⁺, a general VDCC blocker. Superfusion of 100 μ M Cd²⁺ reversibly decreased not only mIPSC frequency ($37.3 \pm 6.3\%$ of the control frequency, $P < 0.01$, $n = 6$; Control, 0.78 ± 0.19 Hz; Cd²⁺, 0.33 ± 0.14 Hz) but also mIPSC amplitude ($75.5 \pm 8.7\%$ of the control amplitude, $P < 0.05$, $n = 6$; Control, 57.24 ± 4.80 pA; Cd²⁺, 43.34 ± 6.14 pA; Figure 3f). In the presence of Cd²⁺, 5-HT reversibly decreased mIPSC frequency ($77.8 \pm 2.9\%$ of the Cd²⁺ condition, $P < 0.05$, $n = 6$; Cd²⁺, 0.33 ± 0.14 Hz; Cd²⁺ + 5-HT, 0.25 ± 0.10 Hz) but not amplitude ($83.3 \pm 10.2\%$ of the Cd²⁺ condition, $P = 0.135$, $n = 6$; Cd²⁺, 43.34 ± 6.14 pA; Cd²⁺ + 5-HT, 33.48 ± 1.56 pA; Figure 3f). These results indicate that presynaptic VDCCs do not contribute to the inhibitory effect of 5-HT on mIPSC frequency.

Serotonergic Inhibition of GABAergic mIPSCs Involves Activation of the Adenylyl Cyclase-cAMP and PKA Pathways

In some regions, 5-HT_{1A} receptor activation is negatively coupled to cAMP formation through inhibition of adenylyl cyclase (AC) (Koyama *et al.*, 1999). Thus, we tested the effect of forskolin, an AC activator, on 5-HT_{1A} receptor-mediated inhibition of GABAergic mIPSCs. Forskolin (10 μ M) significantly and reversibly increased mIPSC frequency to $150.9 \pm 18.8\%$ of the control frequency ($P < 0.05$, $n = 7$; Control, 0.46 ± 0.15 Hz; Forskolin, 0.63 ± 0.16 Hz), without affecting the mean mIPSC amplitude ($99.6 \pm 5.5\%$ of the control amplitude, $P = 0.944$, $n = 7$; Control, 66.38 ± 5.88 pA; Forskolin, 64.95 ± 4.28 pA; Figure 4c). In addition, forskolin completely and reversibly blocked 5-HT-induced inhibition of GABAergic mIPSC frequency ($128.4 \pm 23.7\%$ of the forskolin condition, $P = 0.277$, $n = 7$; Forskolin, 0.63 ± 0.16 Hz; Forskolin + 5-HT, 0.84 ± 0.30 Hz) without altering the current amplitude ($102.4 \pm 5.7\%$ of the forskolin condition, $P = 0.689$, $n = 7$; Forskolin, 64.95 ± 4.28 pA;

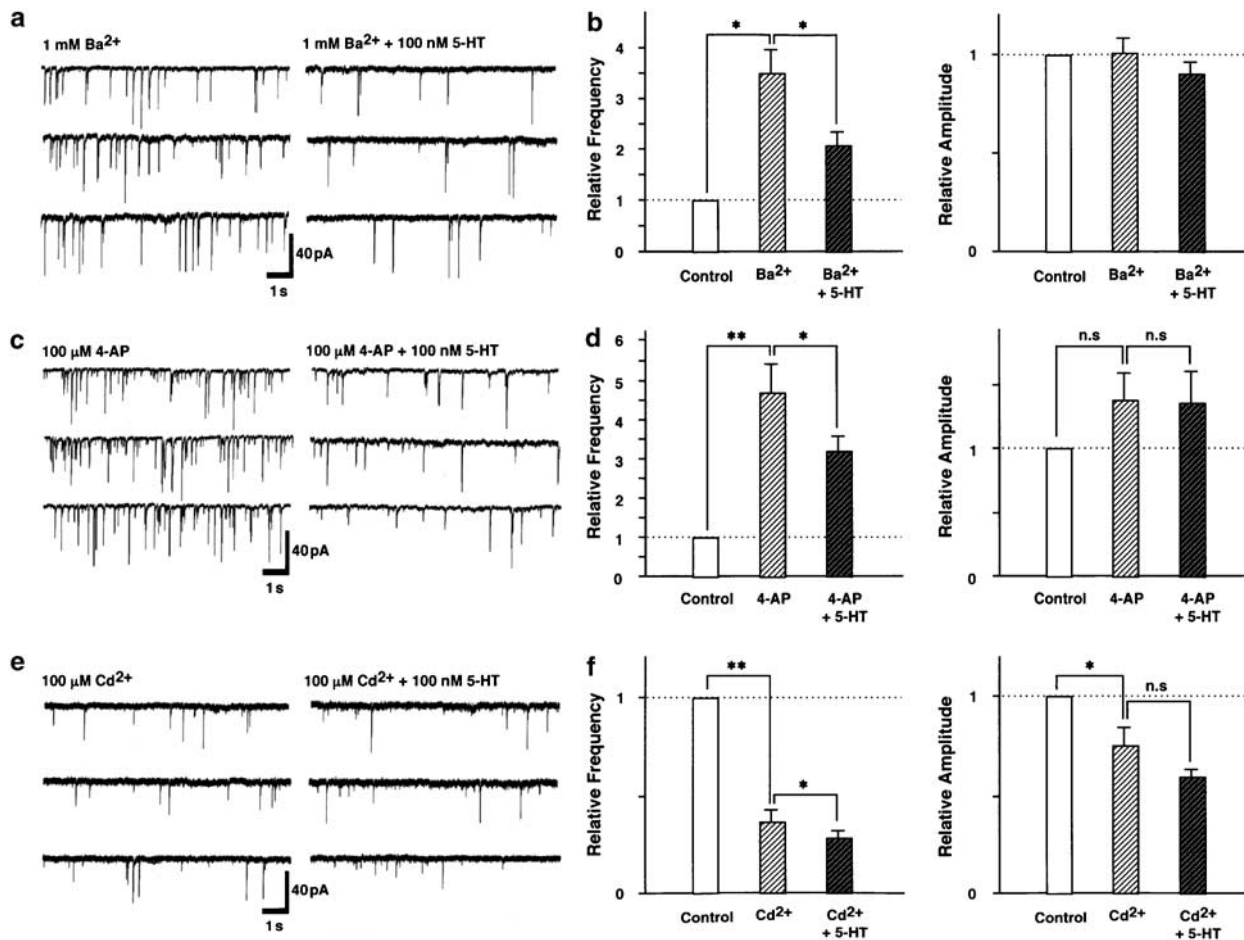


Figure 3 5-HT_{1A} receptor-mediated presynaptic inhibition of GABAergic mIPSCs is not related to the presynaptic K⁺ channels and the presynaptic VDCCs. (a) Representative recording traces of mIPSCs show the change in the 5-HT effect in the presence of 1 mM Ba²⁺. (b) All amplitudes and frequencies are normalized to the control mIPSCs. Each column is the mean of four neurons. (c) Representative recording traces of mIPSCs show the change in the 5-HT effect in the presence of 100 μ M 4-AP. (d) All amplitudes and frequencies are normalized to the control mIPSCs. Each column is the mean of five neurons. (e) Representative recording traces of mIPSCs show the change in the 5-HT effect with external solution containing 100 μ M Cd²⁺. (f) All amplitudes and frequencies are normalized to the control mIPSCs. Each column is the mean of six neurons. Dashed lines in each histogram indicate the relative control. Asterisks represent a statistically significant difference (* P < 0.05; ** P < 0.01, paired two-tailed t -test). n.s. indicates P > 0.05.

Forskolin + 5-HT, 66.29 ± 5.15 pA; Figure 4c). These results indicate that reduction of cAMP formation by 5-HT_{1A} receptor activation is closely related to serotonergic inhibition of GABAergic mIPSCs.

It is well known that in the CNS a change in intracellular cAMP concentration affects a variety of cellular signaling via cAMP-dependent protein kinase A (PKA) (Bouron, 2001). To address the possible involvement of PKA in the serotonergic modulation of GABA release, we examined the effects of H-89, a selective PKA inhibitor, on 5-HT_{1A} receptor-mediated inhibition of GABAergic mIPSCs. H-89 (100 nM) significantly and reversibly decreased basal GABAergic mIPSC frequency to $61.7 \pm 5.3\%$ of the control frequency (P < 0.01, n = 10; Control, 0.45 ± 0.11 Hz; H-89, 0.29 ± 0.09 Hz) without affecting the mean mIPSC amplitude ($123.9 \pm 13.4\%$ of the control amplitude, P = 0.108, n = 10; Control, 53.99 ± 3.88 pA; H-89, 64.52 ± 6.60 pA; Figure 4f). However, H-89 completely blocked 5-HT-induced inhibition of GABA release; in the presence of both 5-HT and H-89 the mean mIPSC frequency was $95.4 \pm 8.8\%$ of the H-89 control frequency (P = 0.612, n = 10; H-89, 0.29 ± 0.09 Hz;

H-89 + 5-HT, 0.28 ± 0.09 Hz; Figure 4f). This finding indicates that 5-HT_{1A} receptor-mediated inhibition of GABAergic mIPSCs is likely to be dependent on a decrease in PKA activity.

Glutamatergic mEPSCs in Isolated MPOA Neurons

When dissociated MPOA neurons were voltage-clamped at V_H -60 mV in the presence of 300 nM TTX and 10 μ M bicuculline, spontaneous mEPSCs were observed. These spontaneous mEPSCs were completely and reversibly blocked by the superfusion of 10 μ M CNQX, an antagonist of the AMPA-type glutamate receptor (Figure 5a). In the I-V relationship, the reversal potential of the spontaneous mEPSCs was close to 0 mV (Figure 5b). These results indicate that the spontaneous mEPSCs were generated by currents passing through AMPA receptor-activated cation channels. Figure 5c shows that the mEPSC amplitude distribution was deviated from a Gaussian and skewed to the right. The amplitudes of almost all mEPSC events in the present study were larger than 5–10 pA.

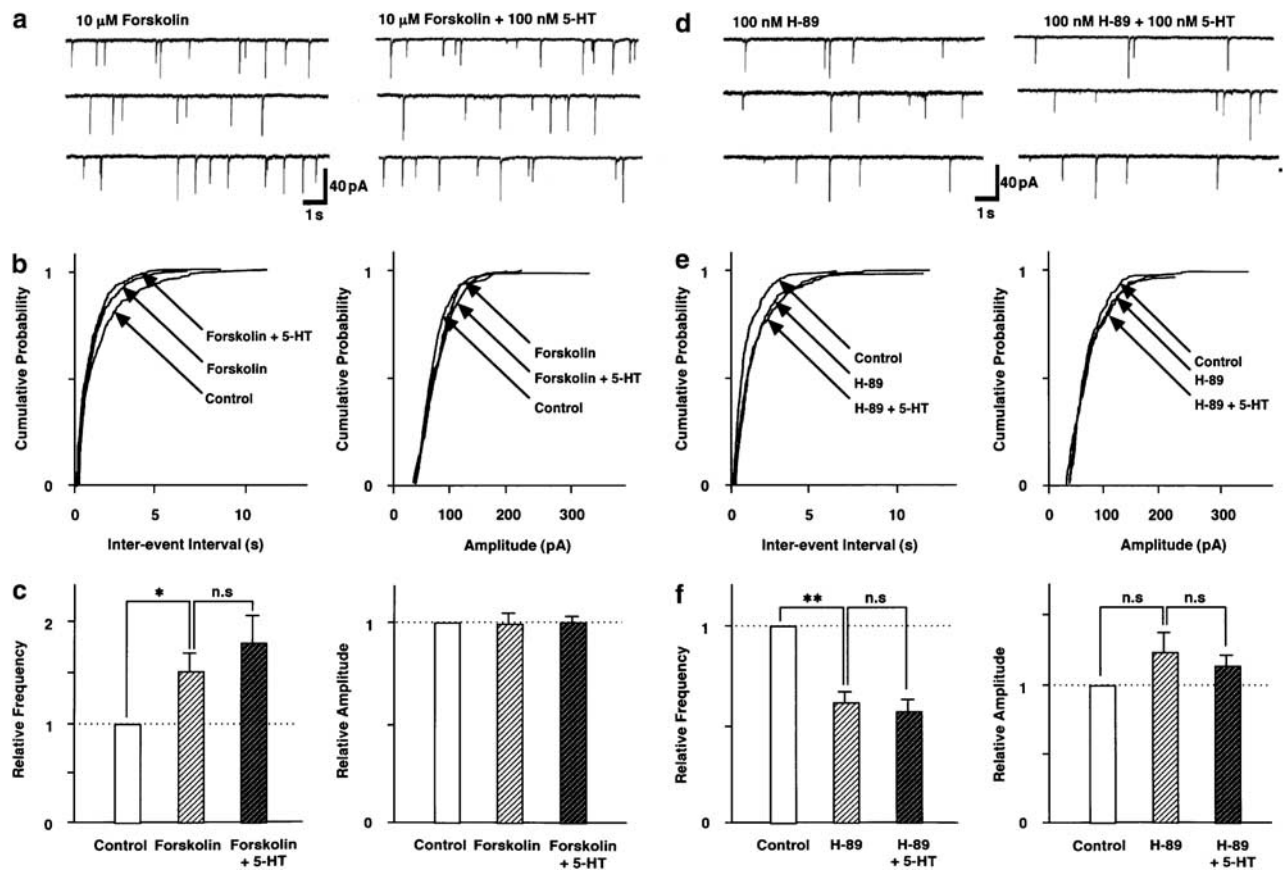


Figure 4 5-HT_{1A} receptor-mediated presynaptic inhibition of GABAergic mIPSCs is coupled to the AC-cAMP signal-transduction pathway. (a) Representative recording traces of mIPSCs show the change in the 5-HT effect in the presence of 10 μ M forskolin. (b) Cumulative distributions for mIPSC inter-event intervals and amplitudes recorded from the same neuron. (c) Each column is the mean of seven neurons. All frequencies and amplitudes are normalized to those of control mIPSCs. (d) Representative recording traces of mIPSCs show the change in the 5-HT effect in the presence of 100 nM H-89. (e) Cumulative distributions for mIPSC inter-event intervals and amplitudes recorded from the same neuron. (f) Each column is the mean of 10 neurons. All frequencies and amplitudes are normalized to those of control mIPSCs. Dashed lines in each histogram indicate the relative control. Asterisks represent a statistically significant difference (* $P < 0.05$; ** $P < 0.01$, paired two-tailed t -test). n.s. indicates $P > 0.05$.

5-HT_{1B} Receptors Mediate Serotonergic Presynaptic Inhibition of Glutamatergic mEPSCs

Superfusion of 5-HT (100 nM) reversibly decreased the frequency of glutamatergic synaptic events in MPOA neurons (Figure 6b). Figure 6b shows cumulative probability plots for mEPSC inter-event intervals and current amplitudes. Serotonin shifted the distribution curve of mEPSC frequency to the right, indicating a reduction in frequency. The amplitude distribution was not affected. Serotonin reversibly decreased the mean mEPSC frequency to $64.4 \pm 8.4\%$ of the control frequency ($P < 0.01$, $n = 11$; Control, 0.18 ± 0.06 Hz; 5-HT, 0.12 ± 0.03 Hz) but did not affect the mean amplitude ($97.1 \pm 5.2\%$ of the control, $P = 0.595$, $n = 11$; Control, 21.29 ± 1.93 pA; 5-HT, 20.33 ± 1.61 pA; Figure 6c). These results indicate that serotonin acts presynaptically to inhibit the release probability of glutamate at the synapse.

To identify the subtypes of 5-HT receptors mediating the decrease in mEPSC frequency, we examined the effects of selective 5-HT_{1A}, 5-HT_{1B}, and 5-HT₂ receptor agonists and antagonists. The selective 5-HT_{1B} receptor agonist CGS 12066B (100 nM) reversibly decreased the mEPSC frequency in a manner similar to 5-HT itself. CGS 12066B reduced the

mean EPSC frequency to $75.3 \pm 9.1\%$ of the control frequency ($P < 0.05$, $n = 8$; Control, 0.38 ± 0.08 Hz; CGS 12066B, 0.28 ± 0.07 Hz) without affecting the mean amplitude ($107.3 \pm 4.8\%$ of the control amplitude, $P = 0.169$, $n = 8$; Control, 25.16 ± 2.22 pA; CGS 12066B, 26.66 ± 2.39 pA; Figure 6c). In contrast, 8-OH-DPAT (100 nM), a selective 5-HT_{1A} receptor agonist, and α -methyl-5-HT (100 nM), a selective 5-HT₂ receptor agonist, did not affect mEPSC frequency (8-OH-DPAT: $82.7 \pm 9.6\%$ of the control frequency, $P = 0.104$, $n = 11$; Control, 0.23 ± 0.07 Hz; 8-OH-DPAT, 0.20 ± 0.07 Hz; α -methyl-5-HT: $95.9 \pm 7.4\%$ of the control frequency, $P = 0.602$, $n = 7$; Control, 0.25 ± 0.08 Hz; α -Methyl-5-HT, 0.23 ± 0.07 Hz) or amplitude (8-OH-DPAT: $97.2 \pm 2.5\%$ of the control amplitude, $P = 0.285$, $n = 11$; Control, 27.12 ± 3.42 pA; 8-OH-DPAT, 26.42 ± 3.37 pA; α -methyl-5-HT: $98.5 \pm 3.4\%$ of the control amplitude, $P = 0.683$, $n = 7$; Control, 22.24 ± 3.74 pA; α -methyl-5-HT, 21.85 ± 3.71 pA; Figure 6c).

The neurons were superfused with external solution containing each 5-HT antagonist for 5 min before simultaneous superfusion of 100 nM 5-HT with one of the antagonists. The selective 5-HT_{1B} receptor antagonist SB 216641 (100 nM) blocked the action of 5-HT on mEPSC frequency ($110.8 \pm 12.9\%$ of the SB 216641 condition,

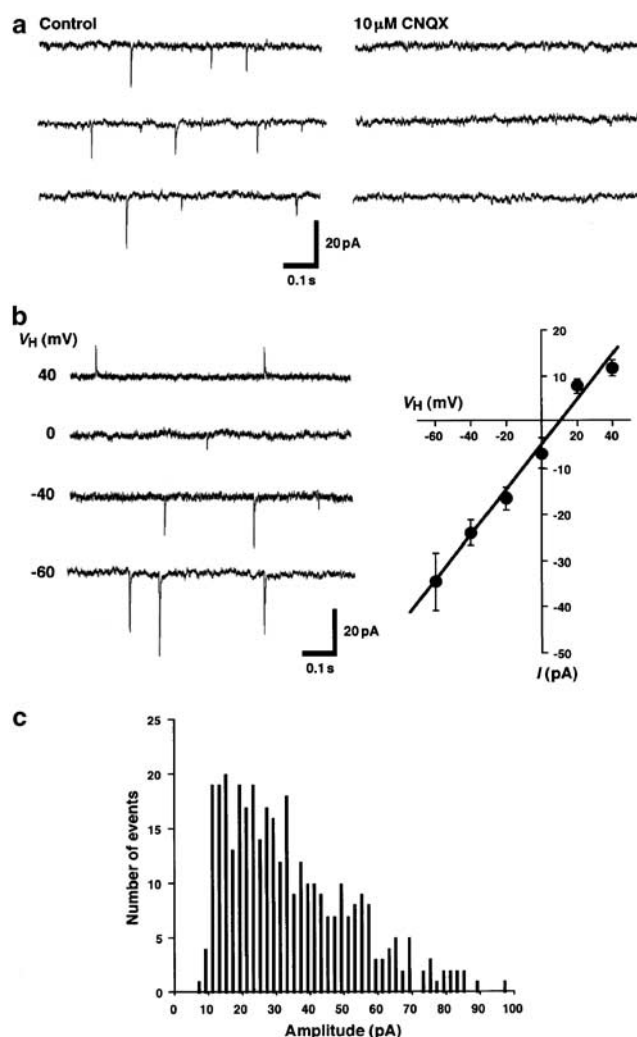


Figure 5 Glutamatergic mEPSCs in mechanically isolated MPOA neurons. (a) In the presence of 300 nM TTX and 10 μ M bicuculline, superfusion of 10 μ M CNQX completely and reversibly blocked mEPSCs in isolated MPOA neurons ($V_H = -60$ mV). (b) Traces of mEPSCs recorded at V_H of -60 , -40 , 0 , and $+40$ mV. I-V curve for the mean amplitude of mEPSCs recorded at various V_H s. Each point represents the mean of four neurons. (c) A histogram showing typical amplitude distribution of mEPSCs. The amplitudes of 353 events detected at the threshold of 1 pA were plotted. Amplitudes of almost all events were larger than 5 pA. The bin size was 2. $V_H = -60$ mV.

$P = 0.449$, $n = 5$; SB 216641, 0.20 ± 0.07 Hz; SB 216641 + 5-HT, 0.22 ± 0.07 Hz) without affecting mEPSC amplitude ($95.0 \pm 7.6\%$ of the SB 216641 condition, $P = 0.545$, $n = 5$; SB 216641, 26.85 ± 4.07 pA; SB 216641 + 5-HT, 24.50 ± 2.65 pA; Figure 6d). SB 216641 itself did not affect mEPSC frequency ($102.9 \pm 7.8\%$ of the control frequency, $P = 0.730$, $n = 5$; Control, 0.19 ± 0.05 Hz; SB 216641, 0.20 ± 0.07 Hz) or amplitude ($109.4 \pm 4.7\%$ of the control amplitude, $P = 0.115$, $n = 5$; Control, 24.34 ± 3.13 pA; SB 216641, 26.85 ± 4.07 pA; Figure 6d). In contrast, NAN-190 (100 nM), a selective 5-HT_{1A} receptor antagonist, did not alter the inhibitory effect of 5-HT on mEPSC frequency ($58.5 \pm 7.4\%$ of the NAN-190 condition, $P < 0.01$, $n = 7$; NAN-190, 0.38 ± 0.22 Hz; NAN-190 + 5-HT, 0.20 ± 0.09 Hz) or affect mEPSC amplitude ($95.7 \pm 6.9\%$ of the NAN-190

condition, $P = 0.559$, $n = 7$; NAN-190, 27.46 ± 2.15 pA; NAN-190 + 5-HT, 25.92 ± 2.13 pA; Figure 6d). NAN-190 itself did not affect mEPSC frequency ($82.0 \pm 6.7\%$ of the control frequency, $P = 0.198$, $n = 5$; Control, 0.43 ± 0.21 Hz; NAN-190, 0.38 ± 0.22 Hz) or amplitude ($84.0 \pm 4.5\%$ of the control amplitude, $P = 0.159$, $n = 5$; Control, 33.52 ± 3.47 pA; NAN-190, 27.46 ± 2.15 pA; Figure 6d). These findings indicate that serotonergic modulation of glutamatergic synaptic transmission might be mediated by presynaptic 5-HT_{1B} receptors.

PKA Mediates the Serotonergic Inhibition of Glutamatergic mEPSCs

As the 5-HT_{1B} receptor is a G-protein-coupled receptor, and its activation decreases AC activity (Maroteaux *et al.*, 1992; Ng *et al.*, 1993), we tested the effect of forskolin, an AC activator, on 5-HT_{1B} receptor-mediated inhibition of glutamatergic mEPSCs. Forskolin (10 μ M) significantly and reversibly increased mEPSC frequency to $162.2 \pm 25.5\%$ of the control frequency ($P < 0.05$, $n = 9$; Control, 0.11 ± 0.02 Hz; Forskolin, 0.17 ± 0.04 Hz) without affecting the mean mEPSC amplitude ($101.3 \pm 6.7\%$ of the control amplitude, $P = 0.848$, $n = 9$; Control, 30.04 ± 3.50 pA; Forskolin, 31.78 ± 4.04 pA; Figure 7c). In addition, forskolin completely blocked 5-HT-induced reduction of glutamatergic mEPSC frequency ($112.6 \pm 19.1\%$ of the forskolin condition, $P = 0.530$, $n = 9$; Forskolin, 0.17 ± 0.04 Hz; Forskolin + 5-HT, 0.18 ± 0.04 Hz) without altering the current amplitude ($99.9 \pm 3.7\%$ of the forskolin condition, $P = 0.979$, $n = 9$; Forskolin, 31.78 ± 4.04 pA; Forskolin + 5-HT, 32.53 ± 4.18 pA; Figure 7c). These results suggest that reduction of cAMP formation by 5-HT_{1B} receptor activation is closely related to serotonergic inhibition of glutamatergic mEPSCs.

To investigate the possibility that PKA is involved in the serotonergic modulation of glutamate release, we tested the effects of H-89, a selective PKA inhibitor, on 5-HT_{1B} receptor-mediated inhibition of glutamatergic mEPSCs. H-89 (100 nM) significantly and reversibly decreased basal glutamatergic mEPSC frequency to $64.4 \pm 8.6\%$ of the control frequency ($P < 0.01$, $n = 7$; Control, 0.16 ± 0.03 Hz; H-89, 0.11 ± 0.03 Hz) without affecting the mean mEPSC amplitude ($97.4 \pm 9.2\%$ of the control amplitude, $P = 0.787$, $n = 7$; Control, 24.32 ± 3.74 pA; H-89, 22.41 ± 2.49 pA; Figure 7f). However, H-89 completely blocked serotonergic inhibition of glutamate release; in the presence of 5-HT and H-89 the mean mEPSC frequency was $111.1 \pm 13.1\%$ of the H-89 control value ($P = 0.432$, $n = 7$; H-89, 0.11 ± 0.03 Hz; H-89 + 5-HT, 0.11 ± 0.03 Hz; Figure 7f). These results suggest that 5-HT_{1B} receptor-mediated inhibition of glutamatergic mEPSCs is dependent on a decrease in PKA activity.

DISCUSSION

The present study was designed to investigate the mechanisms underlying the inhibitory effect of 5-HT on pre-synaptic GABA and glutamate release in MPOA neurons. Our principal finding is that 5-HT reversibly suppressed both inhibitory and excitatory transmission in MPOA

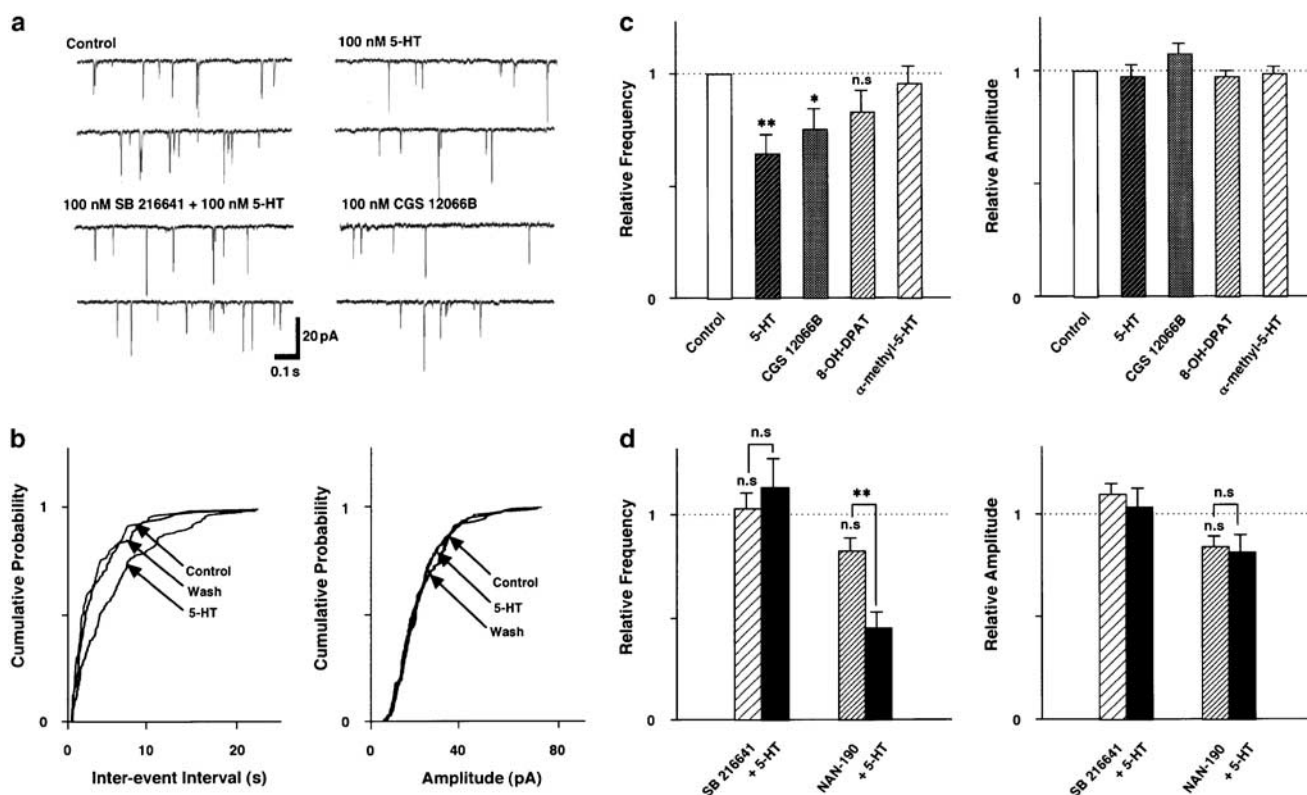


Figure 6 5-HT_{1B} receptor-mediated presynaptic inhibition of glutamatergic mEPSCs. (a) Representative recording traces of mEPSCs observed before and during the application of 100 nM 5-HT in the absence or presence of 100 nM SB 216641, and during the application of 100 nM CGS 12066B. (b) Cumulative distributions for mEPSC inter-event intervals and amplitudes recorded from the same neuron. (c) All frequencies and amplitudes are normalized to those of control mEPSCs. 5-HT ($n = 11$), CGS 12066B ($n = 8$), 100 nM 8-OH-DPAT ($n = 11$), 100 nM α -methyl-5-HT ($n = 7$). (d) All frequencies and amplitudes are normalized to those of control mEPSCs. SB 216641 + 5-HT ($n = 5$) and 100 nM NAN-190 + 5-HT ($n = 7$). Dashed lines in each histogram indicate the relative control. Asterisks represent a statistically significant difference (* $P < 0.05$; ** $P < 0.01$, paired two-tailed t -test). n.s. indicates $P > 0.05$.

neurons. The inhibitory response of 5-HT was not different between male and female rats. It appears that serotonin inhibits GABAergic mIPSCs by activation of 5-HT_{1A} receptors and inhibition of PKA-dependent signaling, whereas it inhibits glutamatergic mEPSCs by activation of 5-HT_{1B} receptors in the presynaptic nerve terminals of MPOA neurons and inhibition of PKA-dependent signaling.

5-HT_{1A} Receptor-Mediated Inhibition of Spontaneous GABA Release

It has been suggested that activation of presynaptic 5-HT_{1A} receptors mediates a decrease in GABA release in the rat locus coeruleus, rat hippocampal CA1, and guinea-pig enteric neurons (Bobker and Williams, 1989; Pan and Galligan, 1994; Schmitz *et al.*, 1995). In the present study, 5-HT reversibly decreased GABAergic mIPSC frequency without affecting the amplitude distribution (Figure 2), indicating that 5-HT acts presynaptically to inhibit spontaneous GABA release from the presynaptic nerve terminals of MPOA neurons. This effect was completely blocked by NAN-190, a selective 5-HT_{1A} receptor antagonist, and mimicked by 8-OH-DPAT, a 5-HT_{1A} receptor agonist (Figure 2). Thus, the effect of 5-HT on GABAergic presynaptic nerve terminals in the MPOA seems to be mediated primarily by 5-HT_{1A} receptors.

Intracellular Signal-Transduction Pathway of 5-HT_{1A} Receptor-Mediated Inhibition of GABAergic Transmission

Serotonin_{1A} receptors commonly couple to G-proteins, and activation of these receptors leads to inhibition of AC. At least three possible mechanisms can be proposed for the G-protein-coupled presynaptic inhibition of GABA release by 5-HT. First, activation of K⁺ channels could hyperpolarize the presynaptic membrane, resulting in decreased GABA release (Umehiya and Berger, 1995; Bijak and Misgeld, 1997). Second, inhibition of Ca²⁺ influx into the nerve terminal via the closing of VDCCs could decrease exocytosis (Umehiya and Berger, 1995). Third, inhibition of the AC-cAMP signal-transduction pathway could decrease neurotransmitter release (Dumuis *et al.*, 1988).

Serotonin is known to directly modulate K⁺ channels via G-proteins (Oh *et al.*, 1995). However, the present findings do not suggest a role of K⁺ channels in the presynaptic serotonergic modulation of GABAergic mIPSCs (Figure 3). This is consistent with previous results showing that serotonin does not affect presynaptic K⁺ channels in the hippocampus (Schmitz *et al.*, 1995). VDCCs are also directly modulated by 5-HT via G-proteins (Rhee *et al.*, 1996) and are known to trigger neurotransmitter release. But in the present study, modulation of GABAergic transmission occurred independent of Ca²⁺ (Figure 3), which is

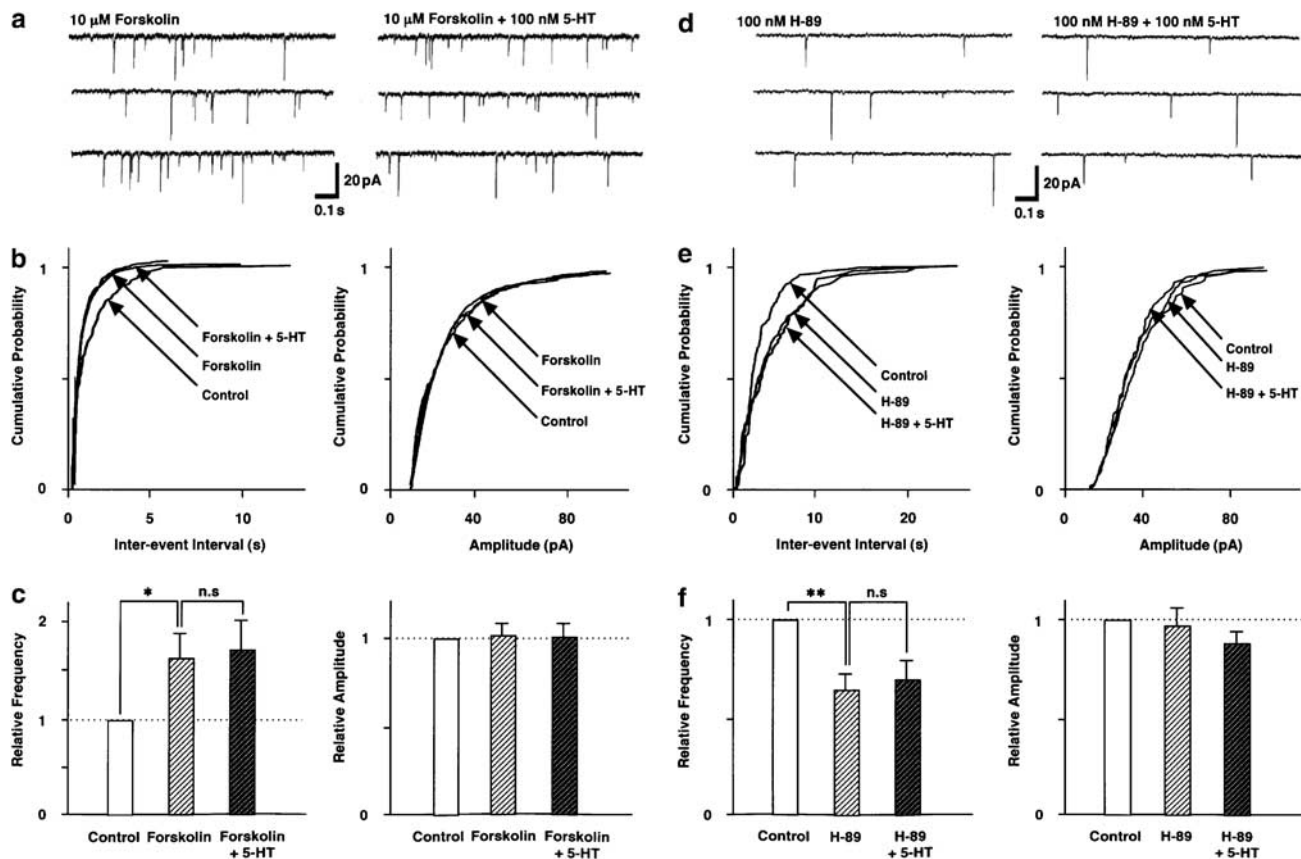


Figure 7 5-HT_{1B} receptor-mediated presynaptic inhibition of glutamatergic mEPSCs is coupled to the AC-cAMP signal-transduction pathway. (a) Representative recording traces of mEPSCs show the change in the 5-HT effect in the presence of 10 μ M forskolin. (b) Cumulative distributions for mEPSC inter-event intervals and amplitudes recorded from the same neuron. (c) Each column is the mean of nine neurons. All frequencies and amplitudes are normalized to those of control mEPSCs. (d) Representative recording traces of mEPSCs show the change in the 5-HT effect in the presence of 100 nM H-89. (e) Cumulative distributions for mEPSC inter-event intervals and amplitudes recorded from the same neuron. (f) Each column is the mean of seven neurons. All frequencies and amplitudes are normalized to those of control mEPSCs. Dashed lines in each histogram indicate the relative control. Asterisks represent a statistically significant difference (* $P < 0.05$; ** $P < 0.01$, paired two-tailed t -test). n.s. indicates $P > 0.05$.

consistent with studies of presynaptic inhibition in the hippocampus (Scanziani *et al.*, 1992; Thompson *et al.*, 1993).

Activation of the AC-cAMP signal-transduction pathway directly facilitates presynaptic neurotransmitter release in rat central neurons (Chavez-Noriega and Stevens, 1994; Chen and Regehr, 1997; Capogna *et al.*, 1995; Trudeau *et al.*, 1996). The proteins synapsin I and II, which are active in the vesicular release process, are phosphorylated by cAMP-dependent kinase, and synapsin phosphorylation increases the number of synaptic vesicles in the releasable pool (Greengard *et al.*, 1993). Reduction of cAMP might reduce PKA activity, which in turn would decrease the amount of phosphorylated synapsins. In light of this fact and the current results (Figure 4), we propose that inhibition of AC-cAMP signal transduction by 5-HT might lead to dephosphorylation of proteins on synaptic vesicles in presynaptic nerve terminals, causing a decrease in presynaptic GABA release.

5-HT_{1B} Receptor-Mediated Inhibition of Spontaneous Glutamate Release

Our finding of 5-HT_{1B} receptor-mediated presynaptic inhibition of glutamate release in the MPOA adds to a

growing literature indicating powerful presynaptic inhibitory effects of 5-HT mediated by 5-HT_{1B} receptors on axonal processes (Boschert *et al.*, 1994) in several regions of the CNS, including the cerebellum (Raiteri *et al.*, 1986), spinal cord (Wu *et al.*, 1991), midbrain (Johnson *et al.*, 1992), cortex (Tanaka and North, 1993), tectum (Mooney *et al.*, 1994), brainstem (Singer *et al.*, 1996), and basal forebrain (Muramatsu *et al.*, 1998). In the present study, 5-HT reversibly decreased glutamatergic mEPSC frequency without affecting the amplitude distribution (Figure 6), indicating that 5-HT acts presynaptically to inhibit spontaneous glutamate release from the presynaptic nerve terminals of MPOA neurons. Moreover, this effect was completely blocked by SB 216641, a selective 5-HT_{1B} receptor antagonist, and mimicked by CGS 12066B, a 5-HT_{1B} receptor agonist (Figure 6). Thus, 5-HT seems to act primarily on 5-HT_{1B} receptors on the glutamatergic presynaptic nerve terminals in the MPOA.

Although the majority of proximal dendrites are still attached to the neurons, the mechanical dissociation can alter the majority of distal dendrites. Although the remaining dendritic as well as somatic synapses are well elucidated to be still functioning (Akaike and Moorhouse, 2003), it can not be ruled out that the dendritic synapses

may be modulated in a different manner shown in the present study.

Intracellular Signal-Transduction Pathway of 5-HT_{1B} Receptor-Mediated Inhibition of Glutamatergic Transmission

The mechanism by which activation of 5-HT_{1B} receptors in the presynaptic terminals modulates transmitter release is unknown. 5-HT_{1B} receptors are negatively coupled to AC (Maroteaux *et al*, 1992; Ng *et al*, 1993). Thus, a decrease in the production of cAMP may be presumed to regulate the availability of free Ca²⁺ at the site of neurotransmitter release or alter the sensitivity of the Ca²⁺ release machinery by a variety of possible mechanisms. The ability of cAMP to serve as a long-range second messenger provides a mechanism for modulation of neurotransmitter release via activation of 5-HT_{1B} receptors in the presynaptic terminal (Kasai and Petersen, 1994). Together with the current results (Figure 7), this suggest that serotonergic inhibition of AC-cAMP signal transduction might lead to dephosphorylation of proteins on synaptic vesicles in the presynaptic nerve terminals, decreasing synaptic glutamate transmission. This conclusion is partly supported by previous findings showing that 5-HT_{1B} activation reduces forskolin-stimulated cAMP production in cultured cell lines (Maroteaux *et al*, 1992; Ng *et al*, 1993). However, further studies are needed to definitively identify the signal-transduction mechanism for 5-HT_{1B} receptor-mediated inhibition of glutamatergic transmission.

Physiological Implications

MPOA neurons receive a moderately dense serotonergic innervation from the dorsal raphe nucleus (Vertes, 1991). Moreover, *in vivo* studies have shown that local application of 5-HT or selective 5-HT agonists to the MPOA inhibits or facilitates male sexual behavior in rats (Fernandez-Guasti *et al*, 1992; Verma *et al*, 1989). These observations raise the possibility that 5-HT released from the dorsal raphe nuclei may differentially affect male sexual behavior by interacting with different 5-HT receptor subtypes in the MPOA. The results of the present study may provide information regarding the mechanism and 5-HT receptor subtypes underlying raphe modulation of MPOA neuron activity. For example, 5-HT may activate or inhibit synaptic influence to MPOA neurons by modulating presynaptic release of excitatory and inhibitory transmitters. A presynaptic action of 5-HT implies that the fibers containing 5-HT contribute to axo-axonic synapses on glutamatergic and GABAergic nerve terminals in the MPOA (Malinina *et al*, 2005). Our findings are consistent with earlier reports of serotonergic suppression of glutamatergic and GABAergic transmission via presynaptic 5-HT_{1A/B} receptors in brain areas such as the amygdala (Cheng *et al*, 1998; Koyama *et al*, 1999), nucleus accumbens (Muramatsu *et al*, 1998), and entorhinal cortex (Schmitz *et al*, 1998). Our results are also consistent with and can show some explanatory clues to the results of a previous *in vivo* study indicating that 5-HT can facilitate and inhibit sexual behavior according to the receptor subtypes (Fernandez-Guasti *et al*, 1992). However, some *in vivo* studies have revealed that the effect of 5-HT on the

MPOA is generally inhibitory to sexual behavior (Gorzalka *et al*, 1990; Hull *et al*, 2004; Zajecka *et al*, 1991). Thus, modulatory effects of 5-HT on synaptic transmission may be generally inhibitory to neural activity in the MPOA. In addition, we previously observed that 5-HT hyperpolarizes and inhibits MPOA neuronal excitability via activation of 5-HT_{1A} receptors (unpublished data). However, factors such as proximity between the release sites and different 5-HT receptors and the concentration of 5-HT attainable at different receptor sites may dictate whether 5-HT has a predominantly postsynaptic or presynaptic site of action.

Although the findings of the present study were mostly consistent with the previous *in vivo* studies, the animals used in the present study were 10- to 15-day old. It is possible that the young animals might be sexually immature and that the physiology of young animals might not be same to that of sexually mature animals. To rule out the possibility, the action mechanism of 5-HT on MPOA neurons with synaptic boutons of adult rat should be performed.

ACKNOWLEDGEMENTS

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