Activation of Serotonin Receptors Modulates Synaptic Transmission in Rat Cerebral Cortex

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Zhou, Fu-Ming and John J. Hablitz. Activation of serotonin receptors modulates synaptic transmission in rat cerebral cortex. J. Neurophysiol. 82: 2989–2999, 1999. The cerebral cortex receives an extensive serotonergic (5-hydroxytryptamine, 5-HT) input. Immunohistochemical studies suggest that inhibitory neurons are the main target of 5-HT innervation. In vivo extracellular recordings have shown that 5-HT generally inhibited cortical pyramidal neurons, whereas in vitro studies have shown an excitatory action. To determine the cellular mechanisms underlying the diverse actions of 5-HT in the cortex, we examined its effects on cortical inhibitory interneurons and pyramidal neurons. We found that 5-HT, through activation of 5-HT_3_ receptors, induced a massive enhancement of spontaneous inhibitory postsynaptic currents (sIPSCs) in pyramidal neurons, lasting for ~6 min. In interneurons, this 5-HT-induced enhancement of sIPSCs was much weaker. Activation of 5-HT_2A_ receptors also increased spontaneous excitatory postsynaptic currents (sEPSCs) in pyramidal neurons. This response desensitized less and at a slower rate. In contrast, 5-HT slightly decreased evoked IPSCs (eIPSCs) and eEPSCs. In addition, 5-HT via 5-HT_3_ receptors evoked a large and rapidly desensitizing inward current in a subset of interneurons and induced a transient enhancement of sIPSCs. Our results suggest that 5-HT has widespread effects on both interneurons and pyramidal neurons and that a short pulse of 5-HT is likely to induce inhibition whereas the prolonged presence of 5-HT may result in excitation.

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is an important endogenous neuromodulator in the CNS (Baumgarten and Grozdanovic 1997; Jacobs and Azmitia 1992). Since the discovery of brain 5-HT pathways in the 1960s (Dahlstrom and Fuxe 1964), 5-HT has been implicated in drug-induced psychoses and a number of psychiatric disorders, major depression, and schizophrenia in particular (Abi-Dargham et al. 1997; Blier and De Montigny 1997). Histochemical studies have shown that the mammalian cerebral cortex receives an extensive 5-HT input originating from midbrain raphe 5-HT neurons (Jacobs and Azmitia 1992; Tork 1990). GABAergic inhibitory neurons appear to be the principal cortical target of 5-HT fibers (DeFelipe et al. 1991; Hornung and Celio 1992; Smiley and Goldman-Rakic 1996), suggesting a potential role of GABAergic neurons in functioning of the 5-HT system.

5-HT receptors comprise a complex family. On the basis of their pharmacology, signal transduction mechanisms and molecular structure, more than a dozen types of 5-HT receptors have been identified (Hoyer et al. 1994). Most of these receptors are coupled to various G proteins with the exception of the 5-HT_3_ receptor, which is a ligand gated cation channel (Derakch et al. 1989; Jackson and Yakel 1995; Maricq et al. 1991). Multiple 5-HT receptor subtypes are expressed in the cerebral cortex (Mengod et al. 1997). In cerebral cortex, 5-HT_3_ receptors are only expressed in inhibitory neurons (Morales and Bloom 1997) whereas 5-HT_2A_ receptors are heavily expressed in pyramidal cells and to a lesser extent in inhibitory neurons (Hamada et al. 1998; Jakab and Goldman-Rakic 1998; Willins et al. 1997).

Since the 1960s, many experiments using in vivo microiontophoretic methods have characterized how 5-HT affects neuronal behavior. The predominant effect of 5-HT on cerebral cortical pyramidal neurons is an inhibition of spontaneous spiking, but the underlying mechanism is not clear (see Jacobs and Azmitia 1992; Phillis 1984; Reader and Jasper 1984 for review). However, intracellular studies in rat cortical slices suggested that 5-HT induces depolarization and action potential firing in pyramidal cells (Araneda and Andrade 1991; Davies et al. 1987; Tanaka and North 1993). Furthermore, Aghajanian and Marek (1997) reported that 5-HT enhances spontaneous excitatory postsynaptic currents (sEPSCs) without significantly changing spontaneous inhibitory postsynaptic currents (sIPSCs) in frontal pyramidal neurons. These in vitro results suggest that 5-HT is mainly excitatory in cortical neuronal circuitry.

The present study directly compares the effects of 5-HT on EPSCs and IPSCs in cortical inhibitory neurons and pyramidal cells. We paid particular attention to layer I neurons because these neurons are mostly GABAergic and layer I receives a dense 5-HT innervation (DeFelipe et al. 1991; Gabbott and Somogyi 1986; Hornung and Celio 1992; Hornung et al. 1990; Lidov et al. 1980; Mulligan and Tork 1988; Smiley and Goldman-Rakic 1996). We found that in pyramidal neurons 5-HT induces a robust and desensitizing enhancement of sIPSCs and a weaker and longer-lasting enhancement of sEPSCs through 5-HT_2A_ receptor activation. 5-HT also induced a rapidly desensitizing direct inward current through activation of 5-HT_3_ receptors in a small subset of cortical interneurons. Our results suggest that a short pulse of 5-HT released under physiological conditions is likely to have an inhibitory effect on cortical neuronal circuitry.

METHODS

Slice preparation

Brain slices were prepared according to methods described previously (Zhou and Hablitz 1996a). Briefly, 9– to 28-day-old Sprague-Dawley rats were decapitated, and the brains were dissected out in <1 min. The isolated brain was immersed immediately in ice-cold saline. Brain slices

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data were pooled. Because no difference was observed among cells from the two areas, were in the anterior cingulate cortex (Paxinos and Watson 1986). in the shoulder region or Fr2 region of the frontal cortex and the rest expressed as mean according to R(chamber at room temperature (22–24°C). During recordings individual were perfused at a rate of ~3 ml/min. The normal bathing solution contained (in mM) 125 NaCl, 3.5 KCl, 2.5 CaCl2, 1.3 MgCl2, 26 NaHCO3, and 10 d-glucose. The bathing solution was continuously bubbled with 95% O2-5% CO2 to maintain pH around 7.4.

Whole cell recording

Individual neurons were visualized using an Olympus BX50WI upright microscope equipped with Nomarski optics, a ×40 water immersion lens and a Hamamatsu Newvicon video camera. Layer I neurons were identified reliably during recording by their location below the pial surface (Zhou and Hablitz 1996a). Fast-spiking interneurons in layers II/III were identified by their nonpyramidal appearance and fast-spiking characteristics. Pyramidal neurons (layers II–VI) were identified by their pyramidal shape, prominent apical dendrites, and regular spiking properties. All recordings were made at room temperature. Patch electrodes were prepared from Garner KG-33 glass tubing using a Narishige PP-83 puller. Electrodes were coated with silicone elastomer (Sylgard). Series resistance (R) was estimated according to R = 10 mV/ where I was the current (filtered ≥10 kHz) evoked by a 10-mV pulse when the pipette capacitance was fully compensated. During recordings, R was 4–15 MΩ among different cells and was not compensated. Care was exercised to monitor the constancy of the series resistance, and recordings were terminated whenever R was >15 MΩ or a significant increase (>20%) occurred. The intracellular solutions contained (in mM) 135 CsCl or KCl, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, and 0.5 EGTA. pH and osmolarity were adjusted to 7.3 and 280 mOsm, respectively. About 50% of voltage-clamp experiments were performed using the CsCl-based intracellular solution, and the rest were conducted with the KCl-based intracellular solution. No difference was observed in 5-HT effects with the use of these two intracellular solutions. Current-clamp experiments used only the KCl-based intracellular solution. Tight seals (~5 GΩ before breaking into whole cell mode) were achieved without cleaning the cell. Before forming tight seals, all os wells were cutoff using the offset feature of the patch amplifier. To examine evoked synaptic responses in layers IV and V pyramidal neurons, a stainless steel bipolar electrode was placed ~200 μm lateral to the recorded cells. Electrical stimuli were controlled by a Grass stimulator-isolator system.

Electrical signals were recorded using an Axopatch-200A amplifier (Axon Instruments), stored on videotape, and analyzed off-line. Digitization and analysis of the records were achieved using SCAN software (courtesy of J. Dempster, University of Strathclyde, Glasgow, UK). Spontaneous and miniature synaptic events were defined as those recorded in the absence and presence of 0.3 μM tetrodotoxin (TTX), respectively. Frequencies of synaptic events were calculated as the reciprocals of interevent intervals. The decay of synaptic currents was fitted to double exponential functions. Statistical comparisons of the frequency and amplitude of synaptic currents before, during, and after 5-HT were made using the Kolmogorov-Smirnov (K-S) test. P < 0.01 was considered significant. Numerical values are expressed as mean ± SD. About 20% of the cells were recorded in the shoulder region or Fr2 region of the frontal cortex and the rest were in the anterior cingulate cortex (Paxinos and Watson 1986). Because no difference was observed among cells from the two areas, data were pooled.

Bicuculline methiodide (Bic) (10 μM) was used to block GABAa receptor-mediated synaptic events whereas ionotropic glutamate receptors were blocked by 6-cyano-7-nitroquininoxaline-2,3-dione (CNQX) (10 μM) and d(−)-2-amino-5-phosphonovaleric acid (d-APV) (20 μM). 5-HT, α-methyl-5-HT, 1-(m-chlorophenyl)biguanide (mCPBG), and risperidone were obtained from Research Biochemical International (Natick, MA).

RESULTS

5-HT induces an enhancement of sIPSCs in neocortical pyramidal cells

Bath application of 40 μM 5-HT induced an enhancement of sIPSCs recorded in pyramidal cells. Specimen records under control conditions and during application of 5-HT are shown in Fig. 1, A and B, respectively. The effects of 5-HT lasted for ~6 min (range 3–10 min), as shown in Fig. 1C. During the 2-min period of peak 5-HT enhancement, sIPSC frequency and amplitude were increased by 810 ± 260% and 110 ± 45%, respectively (n = 38). The kinetics of sIPSCs were not altered by 5-HT. Depending on recording conditions, the 10–90% rise time was 0.5–1 ms, and the double exponential decay had a fast time constant of 2–4 ms and a slower time constant of 10–20 ms, similar to those described previously (Zhou and Hablitz 1997b). In the presence of 5-HT, there were more large-amplitude events although small-amplitude events also were increased. The effect of 5-HT declined in the continued presence of the agonist. Dose-response relations were not studied due to the long time needed for a full recovery. However, 10 μM 5-HT was not able to reliably induce a large sIPSC enhancement, whereas the responses induced by 40 and 100 μM 5-HT were indistinguishable, indicating that 40 μM was a nearly saturation concentration. Events recorded under our recording conditions (20 μM d-APV, 10 μM CNQX, up to 100 μM 5-HT, symmetric Cl− and a holding potential of −70 mV) were sIPSCs because all events were blocked by 10 μM Bic in three cells tested (see also Zhou and Hablitz 1997b).

5-HT2A receptors enhance sIPSCs in pyramidal cells

5-HT2A receptors are expressed abundantly in the cortex (Wright et al. 1995). To examine if activation of these receptors could increase sIPSC frequency in pyramidal cells, the 5-HT2-specific agonist α-methyl-5-HT (Baxter et al. 1995; Sheldon and Aghajanian 1990) was bath applied. In all pyramidal neurons tested (n = 25), 20 μM α-methyl-5-HT produced an enhancement of sIPSCs similar to that induced by 5-HT (Fig. 2). The mean frequency and amplitude of sIPSCs were increased by 820 ± 180% and 115 ± 42% over control levels, respectively. The kinetics of sIPSCs were not altered by α-methyl-5-HT. The enhancing effect of α-methyl-5-HT also declined over time, lasting from 3 to 10 min among different cells. We also tested the selective 5-HT2A antagonist, risperidone (Baxter et al. 1995). Because of the desensitization, experiments attempting to apply risperidone (10 μM) after an α-methyl-5-HT-induced enhancement of sIPSCs were inconclusive. Therefore we preincubated brain slices with 5 or 10 μM risperidone. With this treatment, α-methyl-5-HT failed to induce any significant change of sIPSCs in six pyramidal neurons from six slices. In two additional pyramidal neurons, pretreatment with risperidone also rendered 40 μM 5-HT ineffective in enhancing sIPSCs. These results indicate that 5-HT en-
hancement of sIPSCs in pyramidal cells was mediated by activation of 5-HT$_{2A}$ receptors on GABAergic interneurons.

We sought to determine the mechanism underlying the 5-HT$_{2A}$ receptor enhancement of sIPSCs. Under the somatic recording conditions employed, $\alpha$-methyl-5-HT induced no detectable direct inward current in layer I neurons ($n = 20$) or layer II/III fast-spiking interneurons ($n = 3$). In current-clamp recordings, $\alpha$-methyl-5-HT failed to induce a significant depolarization or increase in spontaneous firing in seven layer I neurons tested. $\alpha$-methyl-5-HT also failed to alter input resistance under either voltage- or current-clamp conditions. No hyperpolarization or outward current was induced by 5-HT or $\alpha$-methyl-5-HT in any of the cells recorded in this study. Furthermore despite the enhancement of sIPSCs, bath application of 5-HT ($n = 4$) or $\alpha$-methyl-5-HT ($n = 2$) did not induce any detectable change in the frequency or amplitude of miniature IPSCs (mIPSCs) recorded in the presence of 0.3 $\mu$M TTX (Fig. 3).

**Activation of 5-HT$_{2A}$ receptors enhances sIPSCs in interneurons**

Layer I neurons receive GABAergic inputs (Zhou and Hablitz 1997b). Our results presented in the preceding section suggest that serotonergic activation may enhance this GABAergic input to layer I neurons. To test this idea, we studied the effects of 5-HT on sIPSCs recorded in layer I neurons. 5-HT (40–100 $\mu$M) produced an enhancement of sIPSCs in 37/52 layer I neurons. As shown in Fig. 4, $\alpha$-methyl-5-HT (20 $\mu$M) also increased sIPSCs in layer I neurons (15/20). The sIPSC enhancement in layer I neurons was also desensitizing. However, the percentage increases in frequency (90 $\pm$ 48%) and amplitude (46 $\pm$ 37%) in layer I neurons were lower than those in pyramidal neurons. Similarly, $\alpha$-methyl-5-HT did not induce any significant enhancement of sIPSCs in four layer I neurons from four slices preincubated with 10 $\mu$M risperidone, indicating an involvement of 5-HT$_{2A}$ receptors.

**Contribution of 5-HT$_{3}$ receptors**

In other cell systems, 5-HT$_{3}$ receptors are coupled to cation channels conducting inward currents under physiological conditions (Jackson and Yakel 1995; Kawa 1994; McMahon and Kauer 1997). A recent immunohistochemical study indicated that 5-HT$_{3}$ receptors are expressed only in a subset of inhibitory neurons in cerebral cortex (Morales and Bloom 1997). Therefore we rea-
soned that activation of these 5-HT<sub>3</sub> receptors might induce a transient enhancement of sIPSCs through a direct excitation of inhibitory neurons. Indeed, in 4 of 52 layer I cells, bath application of 40–100 µM 5-HT induced a burst-like enhancement of sIPSCs, lasting for 5–20 s. The rapid desensitization of this enhancement is indicative of 5-HT<sub>3</sub> receptor involvement. Therefore we tested the specific 5-HT<sub>3</sub> receptor agonist, mCPBG. In 2 of 21 layer I cells and in 2 of 19 pyramidal neurons, bath application of 40 or 100 µM mCPBG also induced a brief enhancement of sIPSCs in the absence TTX (Fig. 5). The similarity of 5-HT and mCPBG responses suggests that the transient enhancement of sIPSCs was mediated by activation of 5-HT<sub>3</sub> receptors in inhibitory neurons.

**FIG. 2.** α-methyl-5-HT enhances sIPSCs in pyramidal neurons. Recordings were made from a 17-day-old frontal pyramidal neuron at −70 mV in the presence of 20 µM D-APV and 10 µM CNQX but no TTX. A: sIPSCs recorded under control condition. Mean interevent interval and amplitude during the control period were 0.6 s (1.7 Hz) and 40 pA, respectively. B: bath application of 20 µM α-methyl-5-HT enhanced sIPSCs. Mean interval was decreased to 65 ms (15 Hz). Mean amplitude was only increased to 78 pA. C: enhancing effect of α-methyl-5-HT on sIPSCs started to decline in ~6 min; sIPSCs declined to near control level in ~10 min. D and E: scatter plots showing the effects of α-methyl-5-HT on sIPSC interevent interval and amplitude during the entire recording.

**FIG. 3.** 5-HT had no effect on miniature IPSCs in cortical neurons. Recordings were made from a 17-day-old anterior cingulate pyramidal neuron at −70 mV in the presence of 20 µM D-APV, 10 µM CNQX, and 0.3 µM TTX. A: cumulative probability plot indicating that the distribution of mIPSC interevent intervals was not altered by 100 µM 5-HT. B: cumulative probability plot indicating that the distribution of mIPSC amplitude was not altered by 100 µM 5-HT.
Activation of 5-HT$_3$ receptors induces a desensitizing inward current in interneurons

Interneurons were examined for a 5-HT$_3$-receptor-mediated inward current. Indeed, as shown in Fig. 6A, bath application of 5-HT produced an inward current in the absence or presence of 0.3 μM TTX. Similar currents were observed in response to mCPBG (Fig. 6B). In layer I neurons, 40–100 μM 5-HT (10 of 108) and 40–100 μM mCPBG (4 of 43 cells) induced a direct inward current (315 ± 319 pA, ranging from 30 to 1,000 pA) at a holding potential of −70 mV (Fig. 6, A and B). In the remainder of the layer I cells, 5-HT and mCPBG did not induce detectable direct inward currents or changes in input resistance. On application of 5-HT or mCPBG to responding cells, the inward current developed rapidly. In the continued presence of 5-HT or mCPBG, the current declined, presumably due to desensitization. The exact kinetics of both activation and desensitization were not examined because bath application of drugs was employed. In the responding cells, 5-HT induced a decrease in input resistance, ranging from 10 to 75% (Fig. 6, A and B). The decrease in input resistance was correlated with the magnitude of the inward current. The relatively large amplitude of the observed 5-HT$_3$-receptor-mediated current suggests that the low number of responsive cells was due to a paucity of 5-HT$_3$-receptor-expressing cells not a problem in current detection. Furthermore neither 5-HT nor mCPBG was able to induce any direct inward current in the pyramidal neurons tested (n = 82).

The reversal potential of the 5-HT$_3$-receptor-mediated current was examined by voltage-ramp experiments. A CsCl-based intracellular pipette solution was used to partially block voltage-dependent K$^+$ currents in these experiments. Currents were evoked by ramping cells from 35 to −65 mV in 10 ms in the absence and presence of 100 μM 5-HT. The difference current, obtained by subtracting the control current from the current evoked in the presence of 5-HT, represents the current induced by 5-HT. The current reversed polarity between −5 and −2 mV in the three layer I cells tested (Fig. 7). An inward rectification was evident in the current-voltage relation in the voltage range −65 to 35 mV. A similar inward rectification of 5-HT$_3$-receptor-medi-
ated currents has been observed in other cell types (Kawa 1994; Yang et al. 1992).

We also tested both mCPBG and α-methyl-5-HT in 10 pyramidal neurons. In each of these cells, bath application of 40 or 80 μM mCPBG for ≤5 min failed to induce any visible change in sIPSCs, whereas subsequent application of 20 μM α-methyl-5-HT was able to induce a strong enhancement of sIPSCs (Fig. 8). These results suggest that there are more interneurons expressing 5-HT₂A than 5-HT₃ receptor in cerebral cortex, as indicated by recent anatomic studies (Jakab and Goldman-Rakic 1998; Morales and Bloom 1997; Willins et al. 1998).

5-HT enhances sEPSCs but not mEPSCs in pyramidal cells

It has been reported that 5-HT can increase pyramidal cell excitability (Araneda and Andrade 1991; Sheldon and Aghajanian 1991). Accordingly, we reasoned that 5-HT might induce an increase in sEPSCs. To test this idea, we recorded sEPSCs from pyramidal neurons after blockade of GABAergic inhibition with 10 μM Bic. Bath application of 5-HT (50–100 μM, n = 7) or α-methyl-5-HT (20 μM, n = 8) enhanced sEPSCs in each of these cells (Fig. 9). The mean frequency of sEPSCs was increased to 880 ± 230% of control levels. Most of the increase in frequency was due to an augmentation in the number of small- and medium-amplitude events. The mean amplitude of sEPSCs was not or only minimally enhanced (±20%). The 5-HT- and α-methyl-5-HT-induced enhancement of sEPSC frequency also displayed desensitization. However, this process was slower than that observed with sIPSCs: the frequency started to decline ≥10 min after applying 5-HT or α-methyl-5-HT. Further, this desensitization was not complete. The frequency of sEPSCs was still higher in the prolonged presence of 5-HT or α-methyl-5-HT than that under control conditions and was sustained as long as the recording condition was stable. Preincubation of brain slices with 10 μM risperidone prevented α-methyl-5-HT from having any effect on sEPSCs in two pyramidal neurons from two slices, suggesting an involvement of 5-HT₂A receptors.

In the presence of 0.3 μM TTX, neither 5-HT nor α-methyl-5-HT induced any change in mEPSCs (n = 6). These results suggested that 5-HT and α-methyl-5-HT might induce action potential firing in pyramidal neurons. However, bath application of 5-HT or α-methyl-5-HT did not induce any detectable direct inward current in the absence (n = 53) or presence (n = 6) of TTX or depolarization (n = 4) in pyramidal neurons. Input resistance of these pyramidal neurons was also not changed by 5-HT or α-methyl-5-HT.

5-HT did not enhance sEPSCs in layer I neurons

To further characterize the effects of 5-HT on cortical neuronal circuits, we studied the modulation of excitatory synaptic inputs to layer I neurons, a type of cortical interneuron. In contrast to pyramidal neurons, 5-HT (50 or 100 μM, n = 6) and α-methyl-5-HT (20 μM, n = 5) were unable to induce any detectable change in the frequency or amplitude of sEPSCs (Fig. 10). Kinetics of these sEPSCs were also not altered by 5-HT or α-methyl-5-HT and were similar to those reported previously (Zhou and Hablitz 1997a).

FIG. 6. 5-HT and 1-(m-chlorophenyl)-biguanide (mCPBG) induced direct transient inward currents in a minority of layer I neurons. Recordings were made from a 17-day-old frontal layer I neuron at ~70 mV in the presence of 20 μM d-APV and 10 μM CNQX but no TTX. A: bath application of 50 μM 5-HT induced a large direct inward current of ~550 pA at peak. Current declined in the continued presence of 5-HT. Note that there was an increase in sIPSCs on the declining phase of the direct inward current, indicating that there was a slower 5-HT receptor mediating the delayed enhancement of sIPSCs. B: after 30 min of washing out 5-HT, bath application of 50 μM mCPBG induced a similar direct inward current of ~450 pA at peak in the same layer I neuron. The smaller amplitude was likely a result of less optimal recording conditions. Current also showed a desensitization in the continued presence of mCPBG. Note that there was no increase in sIPSCs.

FIG. 7. Current-voltage relation for the 5-HT-induced current in a layer I neuron. Control bathing solution contained 0.3 μM TTX, 20 μM d-APV, 10 μM CNQX, and 10 μM bicculline methiodide (Bic). Intracellular pipette solution contained 135 mM CsCl. A. top: command voltage protocol used. Cell was held at ~65 mV, stepped to 35 mV for 1 s to inactivate voltage-dependent currents, and then ramped back to ~65 mV. Middle and bottom: currents evoked by ramping the cell from 35 to ~65 mV in the absence and presence of 100 μM 5-HT. B: difference current, obtained by subtracting the control current from the current evoked in the presence of 5-HT, represents the 5-HT-induced current. This current, when plotted against voltage change, reverses its polarity near 0 mV and shows a modest inward rectification.
5-HT decreased eIPSCs and eEPSCs in pyramidal neurons

In the nucleus accumbens, Nicola and Malenka (1997) reported that dopamine inhibited evoked IPSCs by depressing Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels. Therefore we reasoned that the enhancement of sIPSCs and sEPSCs induced by 5-HT\(_{2A}\) receptor activation might be mediated by increased Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels. To test this idea, we examined the effects of 5-HT on evoked IPSCs (eIPSCs) and eEPSCs in pyramidal neurons. As shown in Fig. 11, monosynaptic eIPSCs were reliably evoked in the presence of 10 μM CNQX and 40 μM N-APV. A modest paired-pulse synaptic depression was observed at intervals of 100–200 ms. Bath application of 40 μM 5-HT induced a robust increase in sIPSCs each of the 5 pyramidal neurons tested (Fig. 11). However, 5-HT simultaneously decreased the amplitude of eIPSCs to ~80% of control level in these cells. Paired-pulse depression was not significantly changed. Similarly, eEPSCs in pyramidal neurons were reduced, whereas sEPSCs were enhanced in frequency (n = 4 cells).

The strong enhancement of sIPSCs and sEPSCs induced by 5-HT\(_{2A}\) receptor activation does not appear to be mediated by increased Ca\(^{2+}\) influx. Instead, a decrease in eEPSCs and eIPSCs suggests that 5-HT might modulate the availability of transmitter vesicles (Wang and Zucker 1998). Similarly, Kondo and Marty (1998) reported that norepinephrine increased sIPSCs but decreased eIPSCs in cerebellar stellate neurons.

DISCUSSION

The main findings of this study are that 5-HT induces a large, desensitizing enhancement of sIPSCs and a weaker longer-lasting enhancement of sEPSCs through 5-HT\(_{2A}\) receptors and that 5-HT\(_{3}\) receptors appear to be present exclusively on interneurons. These results may provide a cellular explanation to the predominantly inhibitory 5-HT effect in cerebral cortex.

Activation of 5-HT\(_{2A}\) receptors induces a desensitizing enhancement of sIPSCs in pyramidal neurons

In the present study, carried out in a neocortical in vitro slice preparation, the most prominent effect of 5-HT was a strong...
enhancement of sIPSCs in pyramidal neurons. This effect appeared to be mediated predominantly by 5-HT$_{2A}$ receptors because it was mimicked by the 5-HT$_{2}$-agonist a-methyl-5-HT and blocked by the specific 5-HT$_{2A}$-antagonist risperidone. This is consistent with the fact that 5-HT$_{2A}$ receptors are the most abundant 5-HT receptors in the cerebral cortex (Burnet et al. 1995; Jakab and Goldman-Rakic 1998; Mengod et al. 1997; Willins et al. 1997; Wright et al. 1995). A similar action of 5-HT$_{2A}$ receptors has been reported in the hippocampus (Piguet and Galvan 1994; Shen and Andrade 1998). This 5-HT enhancement of sIPSCs fits well with the generally inhibitory effects of 5-HT repeatedly observed in cerebral cortex in vivo (Reader and Jasper 1984). However, Aghajanian and Marek (1997) recently reported that, in rat neocortical pyramidal neurons in vitro, 5-HT$_{2A}$ receptor activation induced only a minimal enhancement of sIPSCs with a strong enhancement of sEPSCs, indicating an excitatory 5-HT effect on cerebral cortex. The reason(s) for this discrepancy is unknown.

How 5-HT$_{2A}$ receptor activation leads to an enhancement of sIPSCs is not clear. The increase in both frequency and amplitude of sIPSCs and its TTX sensitivity suggested an enhanced excitability of inhibitory neurons. However, our somatic recordings failed to reveal a 5-HT$_{2A}$-receptor-mediated direct inward current or depolarization. This is similar to the situation with 5-HT-induced enhancement of sEPSCs (Aghajanian and Marek 1997; present study). However, we cannot rule out the possibility that a subset of GABAergic neurons were depolarized by 5-HT but might have evaded our detection, especially most of our interneurons were from layer I and laminar differences may exist. Cox et al. (1998) recently reported that glutamate locally activates dendritic terminals and induces dendritic transmitter release, which is not detected by somatic recording in thalamic interneurons. 5-HT may act on axon and/or dendritic terminals in a similar fashion. Furthermore, because we used relatively large recording pipettes, intracellular factor(s) might be dialyzed such that certain 5-HT responses including the 5-HT-induced depolarization (Araneda and Andrade 1991) were washed out in the recorded cells (Yakel et al. 1988). On the other hand, we found that 5-HT decreased the amplitude of eIPSCs, indicating that 5-HT did not increase action potential-evoked Ca$^{2+}$ influx. Therefore it seems that 5-HT$_{2A}$ receptor activation may instead affect transmitter vesicles through other signaling pathways. This is consistent with studies showing that 5-HT$_{2A}$ receptor activation

**FIG. 9.** a-Methyl-5-HT enhances sEPSCs in pyramidal neurons. Recordings were made from an 18-day-old frontal pyramidal neuron at −70 mV in the presence of 10 μM Bic but no TTX. A: sEPSCs recorded under control condition. Mean interevent interval and amplitude during the control period were 2.1 s and 24 pA, respectively. B: bath application of 20 μM a-methyl-5-HT decreased the mean interevent interval of sEPSCs to 80 ms. Mean amplitude was only increased to 29.2 pA. Enhancing effect of a-methyl-5-HT on sEPSCs showed little decline. C: addition of 20 μM D-APV and 10 μM CNQX blocked all synaptic events. D and E: scatter plots of sEPSC interevent interval and amplitude during the entire recording.
Recordings were made from an 18-day-old frontal layer I neuron at −70 mV in the presence of 10 μM Bic but no TTX. A: sEPSCs recorded under control condition. B: bath application of 20 μM α-methyl-5-HT induced no visible change in sEPSCs. C and D: cumulative plots showing that the amplitude (P = 0.99) and interevent interval (P = 0.2) distributions of sEPSCs are not different under control and α-methyl-5-HT.

Increases intracellular Ca²⁺ concentration by releasing Ca²⁺ from intracellular stores in nonneuronal cell types (Hagberg et al. 1998; Ozaki et al. 1997; Saucier and Albert 1997) and that 5-HT might increase the number of transmitter vesicles available for release (Wang and Zucker 1998).

The mechanism(s) underlying the desensitization of 5-HT₂ₐ receptors-mediated enhancement of sIPSCs was not examined in this paper. However, studies in nonneuronal cell systems have indicated that agonist-induced desensitization was associated with decreased numbers of 5-HT₂ₐ receptors (Berry et al. 1996; Roth et al. 1995; Saucier and Albert 1997). Because our observed desensitization of 5-HT₂ₐ-receptor-mediated enhancement of sIPSCs showed a time course comparable with the agonist-induced internalization of 5-HT₂ₐ receptors in a cell line (Berry et al. 1996), a similar mechanism might be operational in cortical interneurons. A tonic desensitization may exist in cerebral cortex due to the physiological release of 5-HT, which may underlie 5-HT-sensitivity of cortical pyramidal neurons after 5-HT denervation (Ferron et al. 1982).

We also found that 5-HT enhancement of sIPSCs in layer I neurons was much weaker than in pyramidal neurons, indicating that GABAergic neurons innervating interneurons versus pyramidal neurons are differentially modulated by 5-HT. Therefore our results that 5-HT can greatly increase GABAergic inhibitory inputs to pyramidal neurons are consistent with the large body of in vivo studies showing that 5-HT inhibits pyramidal neurons.

Activation of 5-HT₂ₐ receptors enhances sEPSC frequency in pyramidal neurons

5-HT and α-methyl-5-HT greatly increased the frequency but not the amplitude of sEPSCs in every pyramidal neuron tested, as reported by Aghajanian and Marek (1997). This is consistent with Jakab and Goldman-Rakic (1998) that 5-HT₂ₐ receptors are expressed uniformly in all cortical pyramidal neurons. The enhancing effect was TTX sensitive. In a large sample of pyramidal neurons, no detectable direct inward current or input conductance change was produced by 5-HT or α-methyl-5-HT. 5-HT also decreased the amplitude of eEPSCs. This situation is quite similar to that of 5-HT-induced enhancement of sIPSCs. Therefore the possibilities envisioned for sIPSC enhancement are also applicable to sEPSCs.

Recent data have shown that 5-HT₂ₐ receptors are highly expressed in cortical pyramidal neurons, the proximal apical dendrites in particular (Hamada et al. 1998; Jakab and Goldman-Rakic 1998; Roth et al. 1997). This suggests that dendrites, the major postsynaptic membrane, are the major site of 5-HT action. However, the strong enhancement of sEPSC frequency suggests that 5-HT was acting presynaptically. This apparent contradiction may be reconciled by the following possibilities. First, dendritic 5-HT₂ₐ receptors might induce a local depolarization (Cox et al. 1998). Such local effects can cause either dendritic transmitter release or a release of retrograde messengers with subsequent effects on presynaptic axon terminals (Maletic-Savatic and Malinow 1998; Morishita et al. 1998). Second, 5-HT₂ₐ receptors have been detected in a minority of axon terminals (Jakab and Goldman-Rakic 1998) and activation of these terminal 5-HT receptors may be responsible for the observed sEPSC enhancement.

5-HT does not affect sEPSCs in layer I neurons

5-HT and α-methyl-5-HT had no effect on sEPSCs in layer I neurons. Even though sampling bias might have contributed to this observation, the fact that activation of 5-HT₂ₐ receptors induced robust enhancement of sIPSCs in all pyramidal neurons tested suggests that this differential modulation of sIPSCs in the two cell types was real. 5-HT₂ₐ receptor expression is high in pyramidal neuron proximal apical dendrites and low in distal parts (Jakab and Goldman-Rakic 1998; Willins et al. 1997). It is possible that activation of dendritic 5-HT₂ₐ receptors may induce dendritic transmitter release and/or release of retrograde messenger(s). Therefore a lack of proximity of layer I neurons (Zhou and Hablitz 1996b) to pyramidal proximal apical dendrites may lead to a lack of 5-HT effect on sEPSCs in layer I neurons.

FIG. 10. α-Methyl-5-HT had no effect on sEPSCs in layer I neurons. Recordings were made from an 18-day-old frontal layer I neuron at −70 mV in the presence of 10 μM Bic but no TTX. A: sEPSCs recorded under control condition. B: bath application of 20 μM α-methyl-5-HT induced no visible change in sEPSCs. C and D: cumulative plots showing that the amplitude (P = 0.99) and interevent interval (P = 0.2) distributions of sEPSCs are not different under control and α-methyl-5-HT.

5-HT decreased evoked IPSCs (eIPSCs) in pyramidal neurons. Recordings were made from a 16-day-old frontal pyramidal neuron at −70 mV in the presence of 10 μM CNQX and 40 μM D-APV. Left: monosynaptic eIPSCs recorded under control condition. Paired stimuli (5 V × 0.1 ms) were delivered at 100-ms interval. eIPSCs were small and relatively infrequent. Right: bath application of 40 μM 5-HT induced a large increase in sIPSCs but a small decrease (by −20% in amplitude) in eIPSCs. Paired-pulse depression was not significantly changed.
5-HT$_3$ receptors were observed only in interneurons

Our present results show that 5-HT and mCPBG induced a large desensitizing inward current in fast spiking interneurons. This current had a reversal potential near 0 mV and showed a modest inward rectification. The induction of this current was accompanied by a large increase in input conductance, indicating that 5-HT was opening ion channels. These biophysical and pharmacological properties suggest that this inward current was mediated by 5-HT$_3$ receptors (Derkach et al. 1989; Jackson and Yakel 1995; Kawa 1994; McMahon and Kauer 1997; Yang et al. 1992). Consistent with a recent immunohistochemical study (Morales and Bloom 1997), this 5-HT$_3$-receptor-mediated current was recorded exclusively in a small number of cortical interneurons. However, it is possible that our relatively slow drug application system might have caused a certain amount of underestimation of 5-HT$_3$ receptor expression in cortical neurons.

The functional significance of such a highly selective expression of 5-HT$_3$ receptors remains to be established. However, it appears certain that a short pulse of 5-HT released from 5-HT nerve terminals can result in a transient excitation of fast-spiking interneurons and a subsequent transient inhibition of targets innervated by these interneurons. It will be important to determine if GABAergic neurons receiving 5-HT baskets (Hornug and Celio 1992) express 5-HT$_3$ receptors. If that is the case, then 5-HT baskets are suited ideally to rapidly deliver large amounts of 5-HT to 5-HT$_3$ receptors on these neurons.

**Functional considerations**

Our data clearly show that 5-HT$_{2A}$-receptor-mediated effects are widespread in cerebral cortex. Because of the low density of 5-HT terminals and synapses, our results also suggest that nerve terminal-released 5-HT may spread and act on nonsynaptic 5-HT receptors (Audet et al. 1989; Descarries and Umbracio 1995; Seguela et al. 1989). Further, our data indicate that in cerebral cortex, the predominantly inhibitory effect of 5-HT might be affected by multiple factors; i.e., precise spatial and temporal location and quantity of 5-HT released, the rate of 5-HT clearance, and the state of the 5-HT receptors. As shown by Blier and De Montigny (1997), most antidepressants increase brain 5-HT levels, whereas our results suggest that such treatments may alter the sensitivity of 5-HT receptors on GABAergic neurons and thereby the excitation-inhibition balance in cortical neuronal circuitry. Therefore 5-HT modulation of GABAergic neurons may play an important role in the pathophysiology and treatment of major psychiatric disorders (Abi-Dargham et al. 1997).

In conclusion, our study demonstrated that 5-HT preferentially induced a large desensitizing enhancement of sIPSCs in cortical pyramidal neurons; 5-HT also induced a weaker but longer-lasting enhancement of sEPSCs in pyramidal neurons. Therefore short 5-HT pulses may inhibit cortical circuitry, whereas prolonged 5-HT presence may result in excitation.

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