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Serotonin 5-HT_{1A} Receptors Regulate AMPA Receptor Channels through Inhibiting Ca^{2+}/Calmodulin-dependent Kinase II in Prefrontal Cortical Pyramidal Neurons*

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We have studied the regulation of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor channels by serotonin signaling in pyramidal neurons of prefrontal cortex (PFC). Application of serotonin reduced the amplitude of AMPA-evoked currents, an effect mimicked by 5-HT_{1A} receptor agonists and blocked by 5-HT_{1A} antagonists, indicating the mediation by 5-HT_{1A} receptors. The serotonergic modulation of AMPA receptor currents was blocked by protein kinase A (PKA) activators and occluded by PKA inhibitors. Inhibiting the catalytic activity of protein phosphatase 1 (PP1) also eliminated the effect of serotonin on AMPA currents. Furthermore, the serotonergic modulation of AMPA currents was occluded by application of the Ca^{2+}/calmodulin-dependent kinase II (CaMKII) inhibitors and blocked by intracellular injection of calmodulin or recombinant CaMKII. Application of serotonin or 5-HT_{1A} agonists to PFC slices reduced CaMKII activity and the phosphorylation of AMPA receptor subunit GluR1 at the CaMKII site in a PP1-dependent manner. We concluded that serotonin, by activating 5-HT_{1A} receptors, suppress glutamatergic signaling through the inhibition of CaMKII, which is achieved by the inhibition of PKA and ensuing activation of PP1. This modulation demonstrates the critical role of CaMKII in serotonergic regulation of PFC neuronal activity, which may explain the neuropsychiatric behavioral phenotypes seen in CaMKII knockout mice.

The serotonergic system in prefrontal cortex (PFC)[1] is being realized as a major player in controlling emotion and cognition under normal and pathological conditions (1, 2). Changes in serotonin receptors, the serotonin transporter, and serotonin release have been found in PFC from subjects with schizophrenia and depression (3–5). Disturbed serotonergic neurotransmission as well as altered activity of PFC are considered as characteristic features of neuropsychiatric disorders (6–8). Many effective drugs that constitute a major advance in the treatment of mental disorders primarily target the serotonin system (9–11). In addition to serotonin, it has been found that glutamate receptors are altered in selective brain regions of schizophrenia patients (12, 13), and chronic administration of antipsychotic drugs causes the change of glutamate receptors (14), implying a role for cortical glutamatergic dysfunction in mental diseases (15). These studies suggest that one important target of serotonin could be the postsynaptic glutamate receptors and dysregulation of glutamatergic transmission by altered serotonin system may be responsible for the pathophysiology of neuropsychiatric disorders.

The pleiotropic functions of serotonin are afforded by the concerted actions of multiple serotonin receptor subtypes. The 5-HT receptors are composed of several families that can be grouped on the basis of conserved structures and signaling mechanisms (16). Multiple G-protein-coupled 5-HT receptors have been identified in glutamatergic PFC pyramidal neurons (17, 18). Although previous studies have shown that serotonin can play both inhibitory and excitatory roles in neuronal networks through the coupling of different 5-HT receptors to distinct ion channels (19), it remains unclear how these receptors may regulate postsynaptic glutamatergic signaling in PFC.

The fast excitatory synaptic transmission is mediated primarily by AMPA-type glutamate receptors in PFC. The AMPA receptor is an oligomeric complex composed of four subunits GluR1–4 (20). Changes in postsynaptic AMPA receptors have been implicated in synaptic plasticity (21), and one important mechanism is the alteration of the phosphorylation state of AMPA receptors (22). Multiple protein kinases and phosphatases have been shown to modulate AMPA receptor functions (23–27). Here we report that serotonin, by activating 5-HT_{1A} receptors, produced a reduction of AMPA-evoked currents in PFC pyramidal neurons. This regulation of postsynaptic AMPA receptors is through a CaMKII-mediated mechanism, which is operated by activated PP1. Given the critical role of glutamatergic transmission in controlling synaptic plasticity and neuronal activity, our results may provide a molecular and cellular mechanism for 5-HT_{1A}/CaMKII regulation of PFC functions.

EXPERIMENTAL PROCEDURES

Acute Dissociation Procedure—PFC neurons from young adult (3–5 weeks postnatal) rats were acutely dissociated using procedures similar to those described previously (28). All experiments were carried out with the approval of the State University of New York at Buffalo Animal Care Committee. In brief, rats were anesthetized by inhaling 2-bromo-2-chloro-1,1,1-trifluoroethane (1 ml/100 g, Sigma) and decapi-

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[1] The abbreviations used are: PFC, prefrontal cortex; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CaMKII, Ca^{2+}/calmodulin-dependent kinase II; 5-CT, 5-carboxamidotryptamine; EPSC, excitatory synaptic current; sEPSC, spontaneous EPSC; mEPSC, miniature EPSC; OA, okadaic acid; OAE, okadaic acid methyl ester; ANOVA, analysis of variance; K-S, Kolmogorov-Smirnov; NMDA, *N*-methyl-D-aspartic acid; PP1/2A/2B, protein phosphatases 1, 2A, and 2B; PKA, protein kinase A; PKC, protein kinase C; PSD, postsynaptic density; TTX, tetrodotoxin; 8-OH-DPAT, 8-hydroxy-DPAT; NAN-190, 1-(2-methoxyphenyl)-4-(4-[2-phthalimido]butyl)-piperazine; Sp-cAMPS, Sp-adenosine 3′,5′-cyclic monophosphothioate; Rp-cAMPS, adenosine 3′,5′-cyclic monophosphothioate; Rp-isomer; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DOI, 2,5-dimethoxy-4-iodoamphetamine; GABA, γ-amino-n-butyric acid.

tated; brains were quickly removed, iced, and then blocked for slicing. The blocked tissue was cut in 400-μm slices with a Vibrotome while bathed in a low Ca^{2+} (100 μM), HEPES-buffered salt solution (in mM: 140 isethionic acid sodium salt, 2 KCl, 4 $MgCl_2$, 0.1 $CaCl_2$, 23 glucose, 15 HEPES, 1 kynurenic acid, pH 7.4, 300–305 mosM). Slices were then incubated for 1–6 h at room temperature (20–22 °C) in a $NaHCO_3$-buffered saline bubbled with 95% O_2, 5% CO_2 (in mM: 126 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 10 glucose, 1 pyruvic acid, 0.05 glutathione, 0.1 N^G-nitro-L-arginine, 1 kynurenic acid, pH = 7.4, 300–305 mosM). All reagents were obtained from Sigma.

Slices were then removed into the low Ca^{2+} buffer, and regions of the PFC were dissected and placed in an oxygenated Cell-Stir chamber (Wheaton, Inc., Millville, NJ) containing protease (1.2–1.4 mg/ml, Sigma) in HEPES-buffered Hanks' balanced salt solution (Sigma) at 35 °C. After 30 min of enzyme digestion, tissue was rinsed three times in the low Ca^{2+}, HEPES-buffered saline and mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was then plated into a 35-mm Lux Petri dish, which was then placed on the stage of a Nikon inverted microscope.

Immunocytochemistry—Freshly dissociated neurons were precipitated on poly-L-lysine-coated coverslips. After 10 min, they were fixed in 4% paraformaldehyde in phosphate-buffered saline for 20 min and were permeabilized with 0.3% Triton X-100 for 5 min. Following 1-h incubation with 10% bovine serum albumin to block nonspecific staining, the cells were incubated with the polyclonal GluR1 antibody (Upstate Biotechnology Inc., 1:1000) and monoclonal MAP2 antibody (Upstate Biotechnology Inc., 1:1000) at 4 °C overnight. After washing off the primary antibodies, the cells were incubated with a fluorescein-conjugated and a rhodamine-conjugated secondary antibody (Sigma, 1:200) for 50 min at room temperature. For the staining of F-actin, the cells were incubated with rhodamine-conjugated phalloidin (Molecular Probes, 1:5000) for 20 min at room temperature. After washing in phosphate-buffered saline for three times, the coverslips were mounted on slides with VECTASHIELD mounting media (Vector Laboratories, Inc., Burlingame, CA). Fluorescent images were obtained using a Bio-Rad confocal microscope with a 100× oil lens.

Whole-cell Recordings—Whole-cell recordings of currents employed standard voltage clamp techniques (29, 30). Electrodes were pulled from Corning 7052 glass and fire-polished prior to use. The internal solution consisted of (in mM): 180 *N*-methyl-D-glucamine, 40 HEPES, 4 $MgCl_2$, 0.5 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), 12 phosphocreatine, 2 Na_2ATP, 0.2 Na_3GTP, 0.1 leupeptin, pH 7.2–3, 265–270 mosM. The external solution consisted of (in mM): 135 NaCl, 20 CsCl, 1 $MgCl_2$, 10 HEPES, 0.001 TTX, 5 $BaCl_2$, 10 glucose, pH 7.3, 300–305 mosM.

Recordings were obtained with an Axon Instruments 200B patch clamp amplifier that was controlled and monitored with a IBM PC running pCLAMP (version 8) with a DigiData 1320 series interface (Axon instruments, Union City, CA). Electrode resistances were typically 2–4 MΩ in the bath. After seal rupture, series resistance (4–10 MΩ) was compensated (70–90%) and periodically monitored. Care was exercised to monitor the constancy of the series resistance, and recordings were terminated whenever a significant increase (>20%) occurred. The cell membrane potential was held at −70 mV. The application of AMPA (100 μM) or glutamate (1 mM) evoked a fast desensitizing inward current. Peak and steady-state values were measured for generating the plot as a function of time and drug application. AMPA or glutamate was applied for 1 or 2 s every 30 s to minimize desensitization-induced decrease of current amplitude. Drugs were applied with a gravity-fed "sewer pipe" system. The array of application capillaries (approximately 150-μm inner diameter) was positioned a few hundred microns from the cell under study. Solution changes were effected by the SF-77B fast-step solution stimulus delivery device (Warner Instrument Co., Hamden, CT).

Serotonin receptor ligands serotonin, R(+)-8-OH-DPAT, NAN-190, 5-carboxamidotryptamine (5-CT), WAY-100635, 2,5-dimethoxy-4-iodoamphetamine (DOI), 5-methoxytryptamine, ketanserin, and SDZ205557 (Sigma/RBI, St. Louis, MO), as well as second messenger reagents Sp-cAMPS, Rp-cAMPS, PKI-(5–24), okadaic acid (OA), okadaic acid methyl ester (OAE), KN-93, KN-92, KN-62, calmodulin, recombinant CaMKII α subunit, and calcineurin autoinhibitory peptide (Calbiochem, San Diego, CA) were made up as concentrated stocks and stored at −20 °C. Stocks were thawed and diluted immediately prior to use. Serotonin was prepared freshly for every experiment. The amino acid sequence for the phosphorylated I-1 peptide p^{Thr35}I-1-(7–39) was: PRKIQFTVPLLEPHLDPEAAEQIRRRRP(pT)PATL.

Data analyses were performed with AxoGraph (Axon Instruments Inc.), Kaleidagraph (Albeck Software, Reading, PA), and Statview (Abacus Concepts, Inc., Berkeley, CA). Box plots were used for graphic presentation of the data because of the small sample sizes (31). The box plot represents the distribution as a box with the median as a central line and the hinges as the edges of the box (the hinges divide the upper and lower distributions in half). The inner fences run to the limits of the distribution. For analysis of statistical significance, Mann-Whitney *U* tests were performed to compare the current amplitudes in the presence or absence of various agonists. ANOVA tests were performed to compare the differential degrees of current modulation between groups subjected to different treatment.

Synaptic Current Recording in Slices and Cultures—The whole-cell voltage-clamp technique was used to evaluate the regulation of spontaneous and miniature excitatory synaptic currents by 5-HT_{1A} receptors in PFC slices. Patch electrodes (5–9 megohms) were filled with the following internal solution (in mM): 130 cesium methanesulfonate, 10 CsCl, 4 NaCl, 10 HEPES, 1 $MgCl_2$, 5 EGTA, 12 phosphocreatine, 5 MgATP, 0.2 Na_3GTP, 0.1 leupeptin, pH 7.2–7.3, 265–270 mosM. The slice (300 μm) was placed in a perfusion chamber attached to the fixed-stage of an upright microscope (Olympus) and submerged in continuously flowing oxygenated artificial cerebrospinal fluid. It takes about 1 min to change solutions completely in the perfusion chamber. Cells were visualized with a 40X water-immersion lens and illuminated with near infrared (IR) light and the image was detected with an IR-sensitive charge-coupled device camera. Multiclamp 700A amplifier was used for these recordings. Tight seals (2–10 gigohms) from visualized pyramidal neurons were obtained by applying negative pressure. The membrane was disrupted with additional suction, and the whole cell configuration was obtained. The access resistances ranged from 13 to 18 megohms. Cells were held at −70 mV for the continuous recording of EPSCs.

To evaluate the regulation of miniature excitatory synaptic currents by 5-HT_{1A} receptors in PFC cultures, the whole-cell voltage-clamp technique was used. PFC primary cultures were prepared as previously described (32). In brief, frontal cortical neurons from E18 rats were dissociated and plated onto culture dishes. After 6–8 days *in vivo*, cells were used for recording. The same internal and external solutions as those for dissociated cell recordings were used. The membrane potential was held at −70 mV.

Synaptic activities were analyzed with Mini Analysis Program (Synaptosoft, Leonia, NJ). All quantitative measurements (1 min of events) were taken 3–4 min (slice) or 1–2 min (culture) after drug application. Statistical comparisons of the amplitude of synaptic currents (mean ± S.E.) were made using the Kolmogorov-Smirnov (K-S) test.

Western Blot Analysis—After incubation, slices were transferred to boiling 1% SDS and homogenized immediately. Insoluble material was removed by centrifugation (13,000 × *g* for 10 min), and the protein concentration for each sample was measured. Equal amounts of protein from slice homogenates were separated on 7.5% acrylamide gels and transferred to nitrocellulose membranes. The blots were blocked with 5% nonfat dry milk for 1 h at room temperature. Then the blots were incubated with primary antibodies for 1 h at room temperature. Antibodies used include: anti-Thr^{286}-phosphorylated CaMKII (Promega, 1:2000), anti-CaMKII (Upstate Biotechnology Inc., 1:2000), anti-Ser^{831}-phosphorylated GluR1 (Upstate Biotechnology Inc., 1:500), and anti-GluR1 (Upstate Biotechnology Inc., 1:2000). After being rinsed, the blots were incubated with horseradish peroxidase-conjugated anti-rabbit antibodies (Amersham Biosciences, 1:2000) for 1 h at room temperature. Following three washes, the blots were exposed to the enhanced chemiluminescence substrate. Quantitation was obtained from densitometric measurements of immunoreactive bands on films. Data correspond to the mean ± S.E. values and were analyzed by ANOVA tests for statistical significance.

RESULTS

Activation of 5-HT$_{1A}$ Receptors Reduces AMPA-evoked Currents in PFC Pyramidal Neurons—To test the potential impact of serotonin on postsynaptic AMPA receptors in PFC, we first examined the effect of serotonin on AMPA receptor-mediated currents in dissociated pyramidal neurons located in the intermediate and deep layers (III–VI) of the rat PFC. Acutely isolated PFC pyramidal neurons were readily distinguished from GABAergic interneurons by their distinct morphological features: a pyramidal-shaped soma and a prominent apical dendrite (18). The confocal image of double-immunocytochemical labeling with GluR1 (an AMPA receptor subunit) and MAP2 (a dendritic marker) in a representative dissociated PFC pyram-

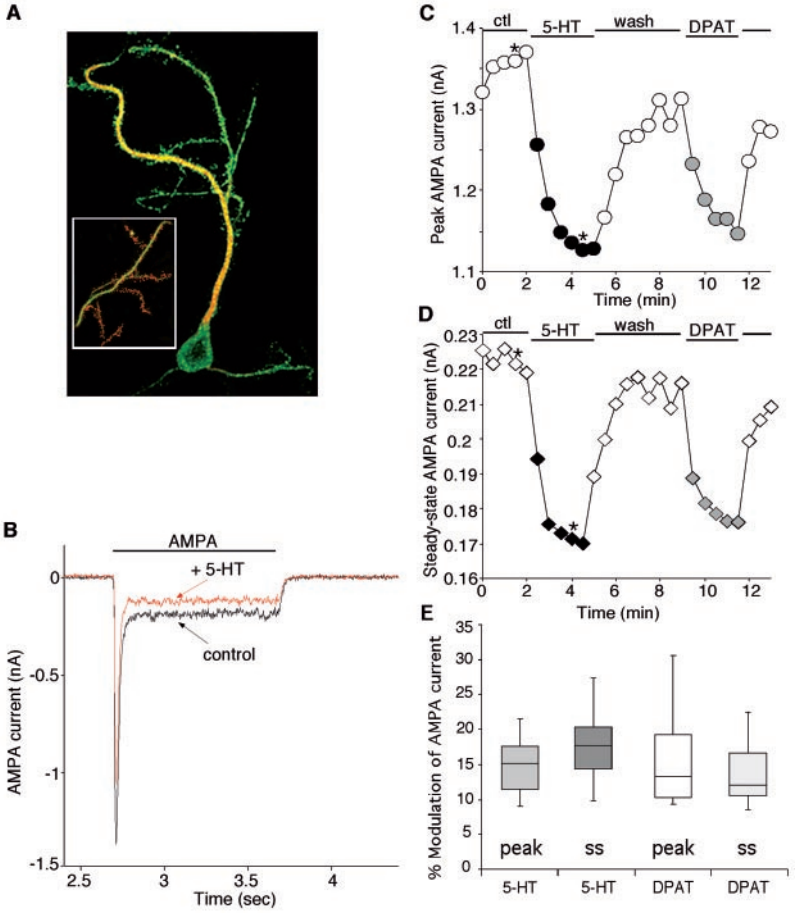

FIG. 1. **Application of 5-HT or 5-HT$_{1A}$ agonist 8-OH-DPAT caused a reduction of AMPA receptor currents in PFC pyramidal neurons.** *A*, confocal image of an acutely dissociated PFC pyramidal neuron co-immunostained with antibodies against GluR1 (*green*) and MAP2 (*red*). *Inset*, confocal image of the dendritic processes of an acutely dissociated PFC pyramidal neuron double stained with anti-MAP2 (*green*) and phalloidin (*red*). *B*, *traces* showing AMPA (100 μM)-evoked currents in the absence (control) and presence of 5-HT (20 μM) in a dissociated PFC pyramidal neuron. *C* and *D*, plot of peak (*C*) or steady-state (*D*) AMPA currents as a function of time and agonist application. *Traces* in *B* were taken from the records used to constructs *C* and *D* (at time points denoted by *asterisks*). Both 5-HT (20 μM) and 8-OH-DPAT (20 μM) reversibly reduced the amplitude of AMPA-evoked currents. *E*, box plot summary of the percent modulation of the peak and steady-state AMPA currents by 5-HT (n = 90) or 8-OH-DPAT (n = 34).

idal neuron is shown in Fig. 1*A*. It is evident that the dissociated neuron has not only elaborate dendritic processes but also densely located spines, where the majority of glutamatergic inputs is received. GluR1 is highly enriched in dendritic spines and shafts in the dissociated PFC pyramidal neuron, confirming that the subcellular localization of AMPA receptors is well preserved. Given the enrichment of F-actin in dendritic spines, we also co-stained the dissociated PFC pyramidal neurons with F-actin and MAP2, to confirm that the small protrusions along dendritic arbors were indeed spines (Fig. 1*A*, *inset*). Double staining of GluR1 and F-actin indicated that they were highly co-localized (data not shown). Application of AMPA (100 μM) elicited an inward current that consisted of two major components: a fast-desensitizing peak and a non-desensitizing sustained part (Fig. 1*B*). The AMPA-evoked current was completely blocked by the non-NMDA antagonist CNQX (10 μM, n = 3), indicating that it is mediated primarily by AMPA receptors. Serotonin decreased both the transient peak and the steady-state AMPA current (Fig. 1, *B–D*). The modulation was reversible and took 1–3 min to stabilize. In 87.5% of PFC pyramidal neurons we tested (n = 104), bath application of 5-HT (20 μM) caused a significant reduction in the amplitude of AMPA-evoked currents (peak, 15.1 ± 0.9%; steady-state, 17.9 ± 0.9%; mean ± S.E., n = 90, $p < 0.01$, Mann-Whitney U test). The desensitization kinetics of AMPA currents was not significantly altered by 5-HT. Serotonin also reduced the amplitude of glutamate (1 mM)-evoked currents that are mainly mediated by AMPA receptor activation (peak, 14.0 ± 1.5%; steady-state, 14.3 ± 0.5%; n = 22, $p < 0.01$, Mann-Whitney U test).

Since multiple 5-HT receptors are simultaneously expressed in individual PFC pyramidal neurons (18), we next used subtype-specific agonists and antagonists to examine which 5-HT receptor was involved in the modulation of AMPA currents. 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_4$ are among the most prominent serotonin receptor subtypes expressed in PFC pyramidal neurons, so we first examined the potential role of these receptors in the modulation of AMPA currents. In a sample of PFC pyramidal neurons we tested, AMPA currents were not significantly affected by the 5-HT$_2$ receptor agonist DOI (20 μM, 1.0 ± 0.6%, mean ± S.E., n = 5, $p > 0.05$, Mann-Whitney U test) or the 5-HT$_4$ receptor agonist 5-methoxytryptamine (20 μM, 2.7 ± 1.9%, n = 8, $p > 0.05$, Mann-Whitney U test), suggesting that the serotonergic effect on AMPA receptors was not mediated by 5-HT$_2$ or 5-HT$_4$ receptors. On the other hand, application of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (20 μM) significantly reduced the amplitude of AMPA currents (peak, 15.7 ± 1.8%; steady-state, 13.8 ± 1.0%; mean ± S.E., n = 34, $p < 0.01$, Mann-Whitney U test) in ~90% of the PFC pyramidal neurons tested (an example is shown in Fig. 1, *B–D*), mimicking the inhibitory effect of 5-HT. As summarized in Fig. 1*E*, 5-HT and 8-OH-DPAT decreased the amplitude of both peak and steady-state AMPA currents to similar extents.

To verify that 5-HT$_{1A}$ receptors were mediating the modulation seen with 8-OH-DPAT or 5-HT, the ability of 5-HT$_{1A}$ antagonist NAN-190 to prevent the action of 8-OH-DPAT or 5-HT was examined. As shown in Fig. 2, NAN-190 (20 μM) almost completely eliminated the effects of 5-HT (Fig. 2*A*) or 8-OH-DPAT (Fig. 2*B*). Removing the antagonist restored the ability of 5-HT or 8-OH-DPAT to modulate AMPA currents. Fig. 2*C* summarized the effects of 5-HT$_{1A}$ agonists on AMPA currents in the absence or presence of various antagonists. The median reduction of peak AMPA currents by 8-OH-DPAT was 17.1% (n = 10), similar to the 5-HT effect (median reduction: 16.4%, n = 9). In the presence of NAN-190, the inhibition of AMPA currents by 5-HT (n = 9) or 8-OH-DPAT (n = 10) was both significantly blocked (median reduction: 1.3% and 1.9%,

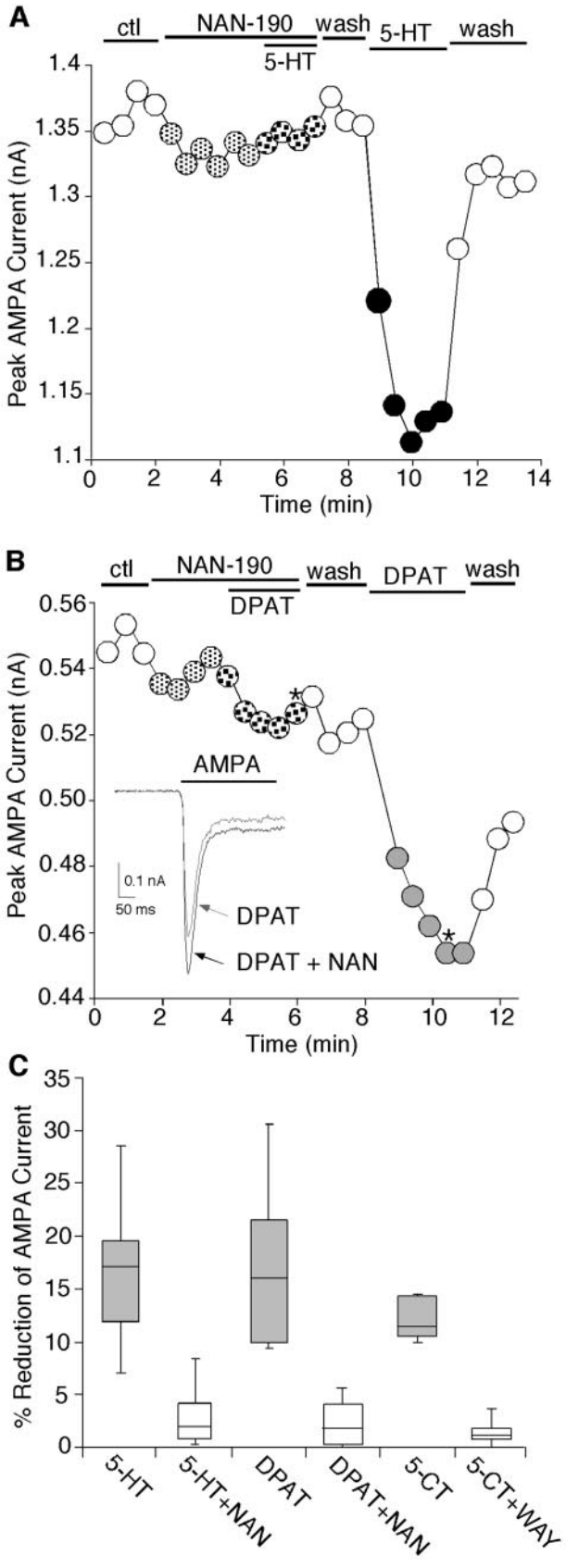

FIG. 2. **Selective 5-HT$_{1A}$ receptor antagonists blocked the effects of 5-HT or 5-HT$_{1A}$ agonists on AMPA currents.** *A* and *B*, plot of peak AMPA currents as a function of time and ligand application. In the presence of the 5-HT$_{1A}$ antagonist NAN-190 (20 μM), 5-HT (20 μM, *A*), or 8-OH-DPAT (20 μM, *B*) failed to reduce AMPA currents, and washing off the antagonist led to recovery of the effect of 5-HT (*A*) or 8-OH-DPAT (*B*). *Inset* of *B*: representative current traces taken from the records used to construct B (at time points denoted by asterisks). *C*, box plots showing the percent modulation of peak AMPA currents by the 5-HT$_{1A}$ agonist (5-HT, 8-OH-DPAT, or 5-CT) in the absence or presence of the 5-HT$_{1A}$ antagonist (NAN-190 or WAY-100635). Note that NAN-190 significantly blocked the reduction of AMPA currents by 5-HT or 8-OH-DPAT, whereas WAY-100635 (20 μM) significantly blocked 5-CT (20 μM)-induced reduction of AMPA currents.

respectively, $p < 0.01$, ANOVA). However, in the presence of the 5-HT$_{2A}$ antagonist ketanserin (20 μM, $n = 5$) or the 5-HT$_4$ antagonist SDZ205557 (20 μM, $n = 7$), 5-HT reduction of AMPA currents was not altered (median reduction of peak currents: 16.5% and 13.2%, respectively, data not shown). Another 5-HT$_{1A}$ receptor agonist 5-CT (20 μM) also decreased the amplitude of AMPA currents (median reduction of peak currents: 11.4%, $n = 7$), and this effect was significantly attenuated in the presence of another 5-HT$_{1A}$ antagonist WAY-100635 (20 μM, median reduction of peak currents: 1.0%, $n = 5$, $p < 0.01$, ANOVA). These results suggest that the serotonergic effect on AMPA receptor channels is mediated by 5-HT$_{1A}$ receptors, consistent with the abundant expression of 5-HT$_{1A}$ receptors in almost all PFC pyramidal neurons detected with the single cell mRNA profiling method (18).

Activation of 5-HT$_{1A}$ Receptors Decreases AMPA Receptor-mediated Synaptic Transmission in PFC Pyramidal Neurons—To understand the impact of 5-HT$_{1A}$ receptors on glutamatergic synaptic transmission, we examined the effect of 8-OH-DPAT on AMPA receptor-mediated excitatory synaptic currents (EPSCs). Spontaneous EPSCs (sEPSCs) and miniature EPSCs (mEPSCs) were recorded in PFC pyramidal neurons in acute slices and primary cultures. Application of CNQX (10 μM) blocked both sEPSCs and mEPSCs ($n = 5$), indicating that these synaptic currents are mainly mediated by AMPA receptors. As shown in Fig. 3 (*A* and *B*), bath application of 8-OH-DPAT to the PFC slice reversibly reduced the sEPSC amplitude by 20.1% ($p < 0.01$, K-S test). In nine PFC pyramidal neurons we examined, 8-OH-DPAT decreased the mean amplitude of sEPSCs by 19.5 ± 1.8% (mean ± S.E., $n = 7$, $p < 0.01$, K-S test). The frequency of sEPSCs was also reduced by 8-OH-DPAT (57.1 ± 4.1%, $n = 7$, $p < 0.001$, K-S test), suggesting the existence of a presynaptic mechanism as well. To better isolate the postsynaptic effect of 5-HT$_{1A}$ receptors, we exposed PFC slices to TTX (0.5 μM), and mEPSCs were measured. Bath application of 8-OH-DPAT to the PFC slice reversibly reduced the mEPSC amplitude by 18.6% (Fig. 3, *C* and *D*, $p < 0.01$, K-S test). In eight PFC pyramidal neurons we examined, 8-OH-DPAT decreased the mean amplitude of mEPSCs by 15.9 ± 2.7% (mean ± S.E., $n = 6$, $p < 0.01$, K-S test).

Because mEPSCs of pyramidal neurons in PFC slices often had small sizes (mean amplitude: 14.1 pA; $n = 6$), we further examined the modulation of mEPSC by 5-HT$_{1A}$ receptors in cultured PFC pyramidal neurons. A representative example is shown in Fig. 3 (*E* and *F*). Bath application of 8-OH-DPAT caused a reversible reduction of the mEPSC amplitude by 22.0% in the cultured neuron ($p < 0.01$, K-S test). Similar to the results in PFC slices, 8-OH-DPAT decreased the mean amplitude of mEPSCs by 17.1 ± 1.9% in cultured PFC pyramidal neurons (mean ± S.E., $n = 10$, $p < 0.01$, K-S test). These results indicate that activation of 5-HT$_{1A}$ receptors could downregulate AMPA receptor function by a postsynaptic mechanism. The frequency of mEPSCs recorded from PFC pyramidal neurons in slices and cultures was also reduced by 8-OH-DPAT (slice: 23.9 ± 3.1%, $n = 6$, $p < 0.01$, K-S test; culture: 53.1 ± 6.1%, $n = 9$, $p < 0.001$, K-S test), confirming that 5-HT$_{1A}$ receptors could also regulate excitatory transmission by a presynaptic mechanism.

The 5-HT$_{1A}$ Modulation of AMPA Currents in PFC Neurons

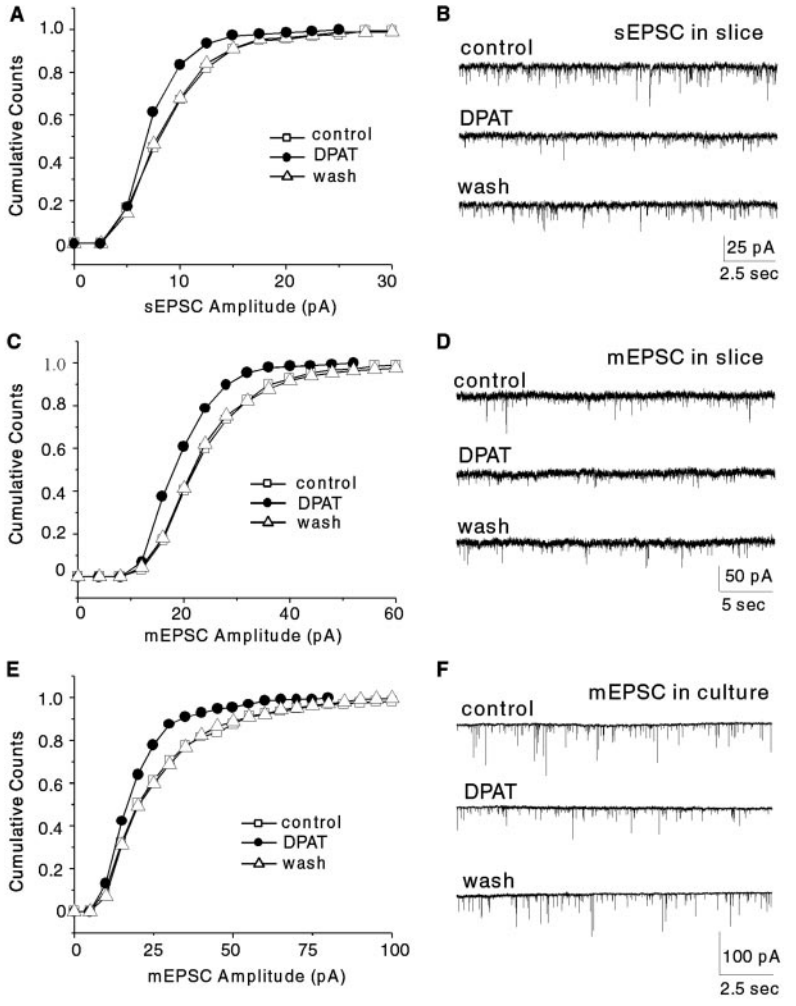

FIG. 3. **Activation of 5-HT$_{1A}$ receptors reduced the amplitude of sEPSCs and mEPSCs recorded from pyramidal neurons in PFC slices and PFC cultures.** *A* and *C*, cumulative plots of spontaneous EPSCs (*A*) or miniature EPSCs (*C*) recorded from a pyramidal neuron in the PFC slice demonstrating that the distribution of sEPSC (*A*) or mEPSC (*C*) amplitude was decreased by 8-OH-DPAT (20 μM). *B* and *D*, representative traces of sEPSCs (*B*) or mEPSCs (*D*) recorded in the slice before (control), during bath application of 8-OH-DPAT, and after washout of the agonist. The slice was incubated in artificial cerebrospinal fluid solution with TTX (0.5 μM) for the recording of mEPSCs. *E*, cumulative plots of miniature EPSCs recorded from a cultured PFC pyramidal neuron showing that the distribution of mEPSC amplitude was decreased by 8-OH-DPAT (20 μM). The culture was incubated in external recording solution with TTX (0.5 μM). *F*, representative traces of mEPSCs recorded in the culture before (control), during application of 8-OH-DPAT, and after washout of the agonist.

Is Dependent on the Inhibition of PKA—We next examined the signal transduction pathways mediating the modulation of AMPA currents by 5-HT$_{1A}$ receptors. A classic pathway for 5-HT$_{1A}$ receptors is to couple to G_i/G_o proteins to inhibit adenylate cyclase and cAMP formation (33). This led us to speculate that the 5-HT$_{1A}$ reduction of AMPA currents is through the inhibition of PKA. If that is the case, then the effect of 5-HT$_{1A}$ on AMPA receptor currents should be blocked by stimulating PKA and occluded by inhibiting PKA. To test this, we applied selective PKA activators and inhibitors.

As shown in Fig. 4*A*, application of the membrane-permeable PKA activator Sp-cAMPS (50 μM) blocked the modulatory effect of 5-HT. Removing Sp-cAMPS restored the ability of 5-HT to modulate AMPA currents. On the other hand, in the presence of the membrane-permeable PKA inhibitor Rp-cAMPS (50 μM), application of 5-HT failed to further reduce AMPA currents (data not shown). To confirm the involvement of PKA in 5-HT modulation of AMPA currents, we dialyzed neurons with the specific PKA inhibitory peptide PKI-(5–24) (34) and then examined 5-HT effects. The AMPA currents were reduced by 17.4 ± 1.2% (n = 5) during the dialysis of PKI-(5–24), which was similar to the effect of 5-HT treatment. After ~5 min of dialysis to allow PKI-(5–24) to enter the cell to inhibit PKA activity, subsequent application of 5-HT had little effect on AMPA currents, whereas a control peptide with the scrambled sequence sPKI-(5–24) did not affect 5-HT-induced reduction of AMPA currents (Fig. 4*B*). Shown in Fig. 4*C* is a summary to compare the effects of 5-HT in the absence or presence of various PKA activators and inhibitors. 5-HT caused little change in AMPA currents in the presence of Sp-cAMPS (1.8 ± 0.8%, mean ± S.E., n = 12, $p > 0.05$, Mann-Whitney U test), or Rp-cAMPS (1.4 ± 0.8%, n = 10, $p > 0.05$, Mann-Whitney U test), or PKI-(5–24) (1.3 ± 0.8%, n = 8, $p > 0.05$, Mann-Whitney U test), which was significantly different from the 5-HT effect in the absence of these agents (14.8 ± 1.8%, n = 22, $p < 0.01$, ANOVA). These results suggest that 5-HT reduction of AMPA currents depends on the inhibition of PKA.

The 5-HT$_{1A}$ Modulation of AMPA Currents in PFC Neurons Requires the Activation of PP1—The 5-HT$_{1A}$-induced inhibition of PKA could directly reduce AMPA currents through decreased phosphorylation of GluR1 subunit on the PKA site (35). Alternatively, the inhibition of PKA could cause the disinhibition of protein phosphatase 1 (PP1) via decreased phosphorylation of the inhibitory protein I-1 (36), leading to the down-regulation of AMPA currents. To test which is the potential signaling mechanism, we examined the serotonin effect on AMPA currents in the presence of phosphatase inhibitors. As shown in Fig. 5*A*, bath application of the PP1/2A inhibitor okadaic acid (OA, 1 μM) eliminated the ability of 5-HT to inhibit AMPA currents. After washing off OA, the 5-HT modulation emerged. In cells dialyzed with OA, application of 5-HT failed to reduce AMPA currents (Fig. 5*B*). On the contrary, injecting with OA methyl ester (OAE), a compound with a similar structure as OA but lacking the ability to inhibit PP1/2A, did not affect the 5-HT inhibition of AMPA currents (Fig. 5*B*). To test the potential involvement of PP2B (calcineurin), we also examined the effect of 5-HT on AMPA currents when the activity of this phosphatase was inhibited. In a sample of neurons dia-

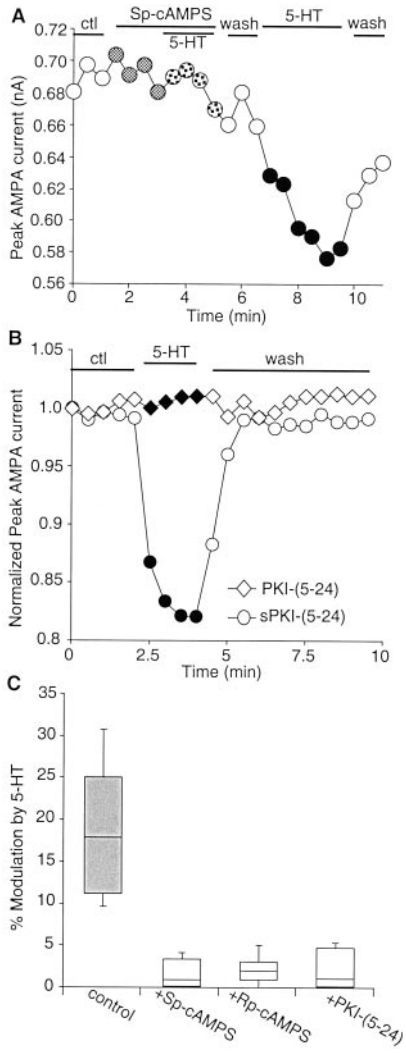

FIG. 4. **The effects of 5-HT on AMPA currents were blocked by PKA activators and occluded by PKA inhibition.** *A*, plot of peak AMPA currents as a function of time and drug application. In the presence of the membrane-permeable PKA activator Sp-cAMPS (50 μM), 5-HT failed to reduce AMPA currents. Following washing off the Sp-cAMPS, the 5-HT effect emerged. *B*, plot of peak AMPA currents as a function of time and drug application in neurons dialyzed with PKI-(5–24) or sPKI-(5–24). The specific PKA inhibitory peptide PKI-(5–24) (20 μM), but not the scrambled control peptide sPKI-(5–24) (20 μM), eliminated 5-HT-induced reduction of AMPA currents. *C*, box plot summary of the percent modulation of AMPA currents by 5-HT in the absence (control, $n = 22$) or presence of Sp-cAMPS ($n = 12$), Rp-cAMPS (50 μM, $n = 10$), or PKI-(5–24) ($n = 8$).

lyzed with the calcineurin autoinhibitory peptide (20 μM), the 5-HT-induced reduction of AMPA currents was still intact (15.4 ± 1.7%, $n = 9$, $p < 0.01$, Mann-Whitney *U* test). These results suggest that activation of PP1 or PP2A, but not PP2B, is required for serotonergic regulation of AMPA receptors.

We then tried to determine the identity of the phosphatase involved in 5-HT$_{1A}$ regulation of AMPA currents. I-1, once it is phosphorylated by PKA at Thr^{35}, acts as a specific inhibitor of PP1 (36). To test the role of PP1 in serotonergic modulation of AMPA currents, we dialyzed PFC pyramidal neurons with the phosphorylated I-1 peptide p^{Thr35}I-1-(7–39), derived from the PP1 interaction region. Biochemical analysis demonstrated that the phospho-I-1 peptide p^{Thr35}I-1-(7–39) potently inhibited PP1 catalytic activity with an IC$_{50}$ at the nanomolar range, whereas the dephospho-I-1 peptide I-1-(7–39), was much less effective (37, 38). As shown in Fig. 5*C*, dialysis with the active p^{Thr35}I-1-(7–39)-peptide (40 μM), but not the inactive peptide I-1-(7–39) (40 μM), abolished the ability of 5-HT to modulate AMPA currents. The effects of 5-HT on AMPA currents in the presence of PP1 inhibitors or their inactive analogs are summarized in Fig. 5*D*. 5-HT had little effect on AMPA currents in the presence of OA (3.6 ± 1.4%, mean ± S.E., $n = 16$, $p > 0.05$, Mann-Whitney *U* test), which was significantly different from the 5-HT effect in the presence of OAE (13.6 ± 1.4%, $n = 13$; $p < 0.01$, ANOVA). Moreover, in cells dialyzed with the p^{Thr35}I-1-(7–39)-peptide, 5-HT caused little change in AMPA currents (3.3 ± 1.6%, mean ± S.E., $n = 17$, $p > 0.05$, Mann-Whitney *U* test), which was significantly different from the 5-HT effect in cells dialyzed with the I-1-(7–39)-peptide (14.3 ± 0.2%, $n = 5$; $p < 0.01$, ANOVA). These results suggest that 5-HT reduction of AMPA currents requires the activation of PP1.

The 5-HT$_{1A}$ Modulation of AMPA Currents in PFC Neurons Is Mediated by the Inhibition of CaMKII—The 5-HT$_{1A}$-induced activation of PP1 could lead to the down-regulation of AMPA currents by two potential mechanisms. One is through the increased dephosphorylation of AMPA receptors directly (26). The other is through the decreased phosphorylation of GluR1 subunit on the CaMKII site (24, 39), because the activated PP1 could reduce CaMKII Thr^{286} autophosphorylation and its consequent Ca^{2+}-independent activity (40, 41). To test which is the potential signaling mechanism, we examined the serotonin effect on AMPA currents of PFC pyramidal neurons in which CaMKII is inhibited or activated.

Bath application of the selective CaMKII inhibitor KN-93 (10 μM) produced a similar effect as 5-HT; *i.e.* decreasing the AMPA current amplitude (14.6 ± 3.3%, $n = 13$). In the presence of KN-93, 5-HT failed to further reduce currents (Fig. 6, *A* and *B*), suggesting that KN-93-induced inhibition of CaMKII occluded the ability of 5-HT to modulate AMPA receptors (occlusion by KN-93: 83.7 ± 5.3%, $n = 21$; Fig. 6*C*). In contrast, the inactive analog KN-92 had little effect on 5-HT modulation (occlusion by KN-92: 9.6 ± 4.1%, $n = 10$; Fig. 6*C*; $p < 0.01$, ANOVA, KN-93 *versus* KN-92). Another structurally different CaMKII inhibitor KN-62 (5 μM) gave results resembling KN-93: occluded most of the 5-HT effect on AMPA currents (occlusion by KN-62: 92.3 ± 5.1%, $n = 8$; Fig. 6*C*).

To further test the role of CaMKII in serotonergic regulation of AMPA currents, we dialyzed PFC neurons with a recombinant constitutively active form of CaMKII α subunit. The basal AMPA currents were not significantly affected by the injected active CaMKII ($n = 5$, data not shown), suggesting that endogenous active CaMKII has kept AMPA receptors at a highly phosphorylated state in PFC pyramidal neurons. However, the ability of 5-HT to reduce AMPA currents was abolished in CaMKII-loaded cells (Fig. 6*D*), but was not affected in cells loaded with the heat-inactivated enzyme (control, Fig. 6*D*). Moreover, dialysis with purified calmodulin also eliminated the ability of 5-HT to decrease AMPA currents (Fig. 6*E*). The effects of 5-HT on AMPA currents in cells loaded with or without CaMKII activators are summarized in Fig. 6*F*. 5-HT had little effect on AMPA currents in the presence of active CaMKII (3.2 ± 0.9%, mean ± S.E., $n = 10$, $p > 0.05$, Mann-Whitney *U* test) or calmodulin (1.8 ± 0.5%, $n = 9$, $p > 0.05$, Mann-Whitney *U* test), which was significantly different from the 5-HT effect under control conditions (16.3 ± 2.1%, $n = 7$; $p < 0.01$, ANOVA). These results suggest that 5-HT reduction of AMPA currents is mediated by the inhibition of CaMKII.

The 5-HT$_{1A}$ Receptors Decrease Autophosphorylated CaMKII and CaMKII Phosphorylation of GluR1 Subunit in a PP1-dependent Manner—To provide further evidence showing that the serotonergic regulation of AMPA currents is mediated by CaMKII phosphorylation of AMPA receptors, we performed biochemical experiments to examine whether 5-HT$_{1A}$ receptor

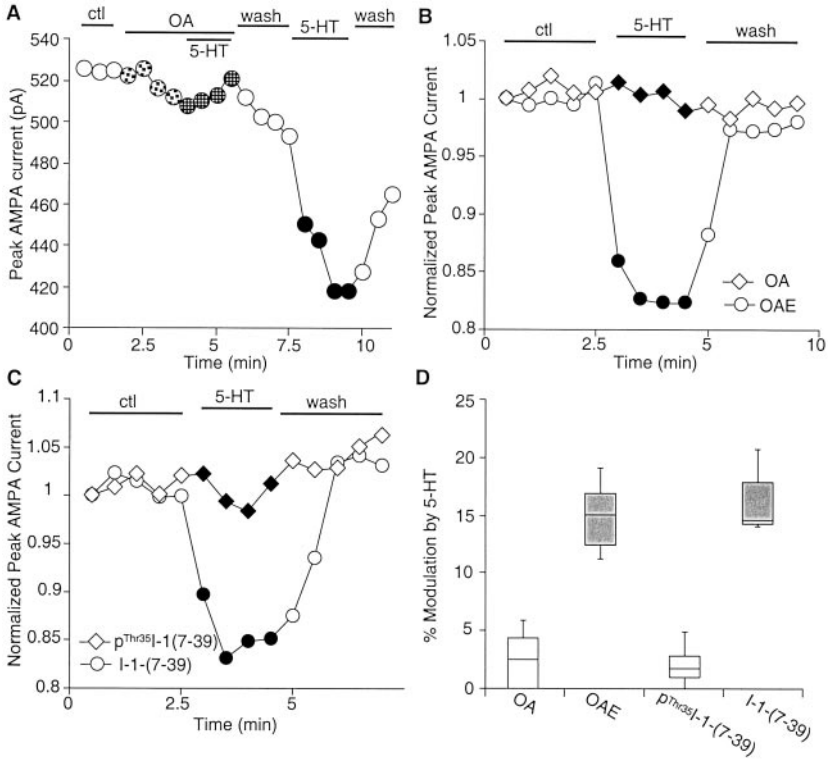

FIG. 5. **The effects of 5-HT on AMPA currents were blocked by PP1 inhibition.** *A*, plot of peak AMPA currents as a function of time and drug application. In the presence of the membrane-permeable PP1 inhibitor okadaic acid (OA), 5-HT failed to reduce AMPA currents. Washing off OA led to recovery of the 5-HT effect. *B*, plot of peak AMPA currents as a function of time and drug application in neurons dialyzed with OA or OA methyl ester (OAE). OA (1 μM), but not the inactive analog OAE (1 μM), eliminated 5-HT-induced reduction of AMPA currents. *C*, plot of peak AMPA currents as a function of time and drug application in neurons dialyzed with the phosphorylated I-1 peptide p^{Thr35}I-1-(7–39) or the dephosphorylated I-1-(7–39)-peptide. The constitutively active p^{Thr35}I-1-(7–39)-peptide (40 μM), but not the inactive I-1-(7–39)-peptide (40 μM), blocked 5-HT modulation of AMPA currents. *D*, box plot summary of the percent modulation of AMPA currents by 5-HT in the presence of OA (n = 16), OAE (n = 13), p^{Thr35}I-1-(7–39) (n = 17), or I-1-(7–39) (n = 5).

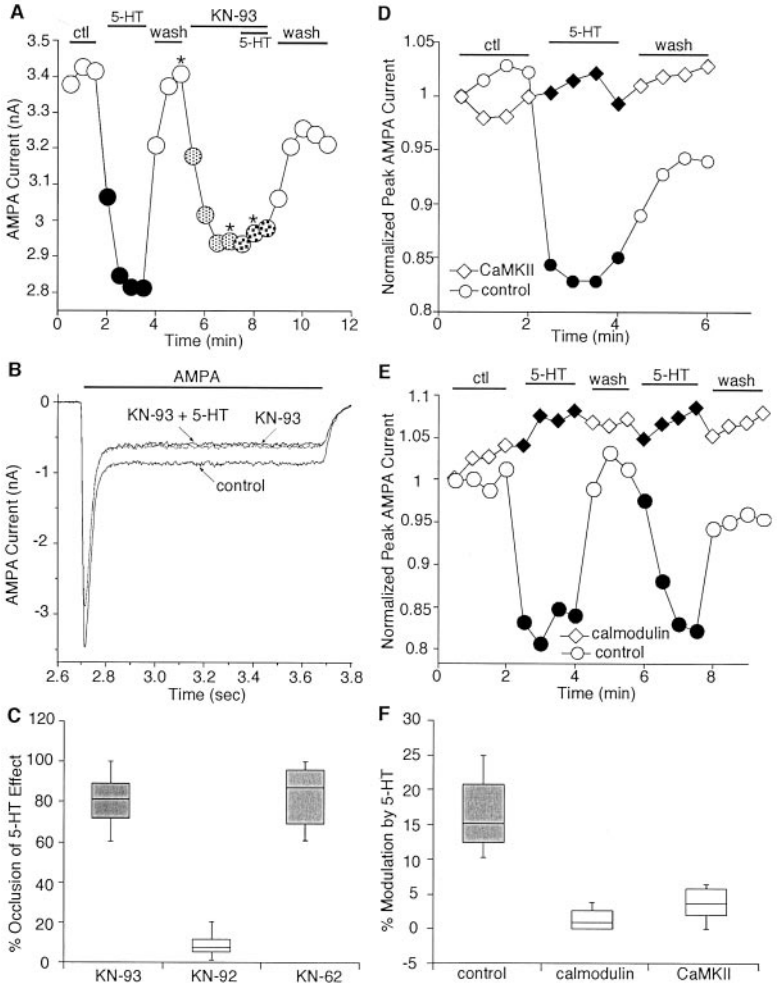

FIG. 6. **The effects of 5-HT on AMPA currents were occluded by CaMKII inhibitors and blocked by CaMKII activation.** *A*, plot of peak AMPA currents as a function of time and drug application. Similar to 5-HT (20 μM), application of the membrane-permeable CaMKII inhibitor KN-93 (10 μM) reduced AMPA currents. In the presence of KN-93, 5-HT had no further effect (occluded). *B*, representative current traces taken from the records used to construct A (at time points denoted by asterisks). *C*, box plot summary of the percent occlusion of 5-HT effects by KN-93 (10 μM, n = 21) or its inactive analog KN-92 (10 μM, n = 10), or another CaMKII inhibitor KN-62 (5 μM, n = 8). *D*, plot of peak AMPA currents as a function of time and drug application in neurons dialyzed with the recombinant active CaMKII α subunit (2 units/μl) or the heat-inactivated enzyme (control, 2 units/μl). The active CaMKII blocked the ability of 5-HT to reduce AMPA currents. *E*, plot of peak AMPA currents as a function of time and drug application in neurons dialyzed with the purified calmodulin (1 μM). Calmodulin eliminated the 5-HT effect on AMPA currents. *F*, box plot summary of the percent modulation of AMPA currents by 5-HT in the absence (control, n = 7) or presence of calmodulin (n = 9) or CaMKII (n = 10).

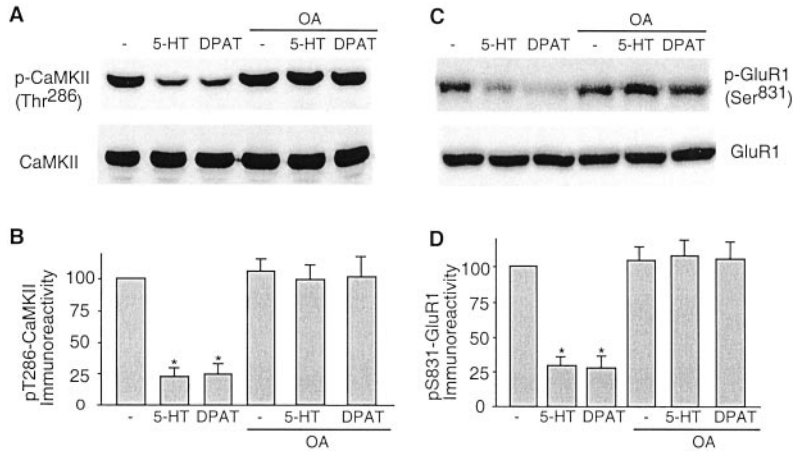

FIG. 7. **5-HT$_{1A}$ receptors decreased the autophosphorylation of CaMKII and CaMKII phosphorylation of GluR1 subunit in a PP1-dependent manner in PFC slices.** *A*, immunoblots of autophosphorylated CaMKII and total CaMKII. PFC slices were pretreated with or without OA (0.5 μM) for 15 min, followed by incubation with or without 5-HT (20 μM) or 8-OH-DPAT (20 μM) for 2 min. Half of the slice lysates was blotted with an antibody specific for Thr^{286}-phosphorylated CaMKII (*upper panel*), and the other half was blotted with an antibody recognizing total CaMKII. *B*, quantification of phosphorylation of CaMKII at Thr^{286} under three treatments (control, 5-HT, or 8-OH-DPAT) in the absence (n = 8) or presence (n = 5) of OA (*, $p < 0.01$, ANOVA). *C*, immunoblots of GluR1 phosphorylated at the CaMKII site Ser^{831} and total GluR1. PFC slices were treated in the same way. Half of the slice lysates was blotted with an antibody recognizing the GluR1 Ser^{831} phosphorylation site (*upper panel*), and the other half was blotted with an antibody against total GluR1 (*lower panel*). *D*, quantification of phosphorylation of GluR1 at Ser^{831} under three treatments (control, 5-HT, or 8-OH-DPAT) in the absence (n = 8) or presence (n = 5) of OA (*, $p < 0.01$, ANOVA).

activation can indeed change CaMKII activity and its phosphorylation of GluR1 subunit. The Thr^{286} autophosphorylation state of CaMKII determines its Ca^{2+}-independent activity (41), therefore we first compared the phosphorylation of CaMKII at Thr^{286} in PFC slices treated with or without 5-HT or 8-OH-DPAT. A representative example is shown in Fig. 7*A*. A short (2–5 min) treatment of 5-HT or 8-OH-DPAT potently reduced the Thr^{286} phosphorylation state of CaMKII, and this effect was completely abolished in the presence of OA (0.5 μM). The expression levels of total CaMKII were not changed by any of these treatments. The effects of 5-HT or 8-OH-DPAT on CaMKII Thr^{286} phosphorylation are summarized in Fig. 7*B*. Under control conditions, 5-HT decreased CaMKII Thr^{286} phosphorylation to 24 ± 6% of basal level (n = 8), and 8-OH-DPAT gave similar results: decreased CaMKII Thr^{286} phosphorylation to 25 ± 7% of basal level (n = 8). In PFC slices in which PP1 was inhibited with OA, both 5-HT and 8-OH-DPAT failed to inhibit CaMKII Thr^{286} phosphorylation (96 ± 14% and 98 ± 16% of basal level, respectively, n = 5). These results suggest that 5-HT$_{1A}$ receptors can significantly reduce the kinase activity of CaMKII in a PP1-dependent manner ($p < 0.01$, ANOVA, 5-HT or 8-OH-DPAT effects in the absence *versus* presence of OA).

We next examined the impact of 5-HT or 8-OH-DPAT on the phosphorylation of GluR1 at the CaMKII site Ser^{831} (39, 42) in PFC slices. A representative example is shown in Fig. 7*C*. A short (2–5 min) treatment of 5-HT or 8-OH-DPAT decreased the GluR1 Ser^{831} phosphorylation level, and this effect was eliminated in the presence of OA (0.5 μM). The expression levels of total GluR1 were not changed by any of these treatments. The effects of 5-HT or 8-OH-DPAT on GluR1 Ser^{831} phosphorylation are summarized in Fig. 7*D*. Under control conditions, 5-HT or 8-OH-DPAT decreased GluR1 Ser^{831} phosphorylation to 33 ± 8% or 36 ± 9% of basal level, respectively (n = 8). In PFC slices in which PP1 was inhibited with OA, both 5-HT and 8-OH-DPAT had little effect on GluR1 Ser^{831} phosphorylation (99 ± 15% and 96 ± 17% of basal level, respectively). These results suggest that 5-HT$_{1A}$ receptors can significantly reduce the CaMKII phosphorylation of GluR1 in a PP1-dependent manner ($p < 0.01$, ANOVA, 5-HT or 8-OH-DPAT effects in the absence *versus* presence of OA).

Because GluR1 can also be phosphorylated by PKA at Ser^{845} (35), we further examined the effect of 8-OH-DPAT on the phosphorylation of GluR1 at the PKA site in PFC slices. Application of 8-OH-DPAT did not significantly inhibit GluR1 Ser^{845} phosphorylation (97 ± 12% of basal level, n = 6, data not shown, $p > 0.05$, ANOVA), suggesting that 5-HT$_{1A}$-mediated PKA cannot directly regulate the GluR1 phosphorylation state in PFC pyramidal neurons.

DISCUSSION

Among the multiple G-protein-coupled serotonin receptor subtypes, 5-HT$_{1A}$ receptors are highly enriched in prefrontal cortex (43, 18). Attention is increasingly being directed toward developing pharmacological agents that target 5-HT$_{1A}$ receptors for the treatment of schizophrenia, anxiety, depression, and cognition disorders (44, 45). Increased prefrontal 5-HT$_{1A}$ receptor density has been found in schizophrenia patients (3, 4). Mice lacking 5-HT$_{1A}$ receptors show consistently elevated anxiety alongside antidepressant-like response (46, 47).

A potential mechanism underlying these actions of 5-HT$_{1A}$ receptors is the change of synaptic plasticity and neuronal activity through regulation of ion channels. Postsynaptic 5-HT$_{1A}$ receptors are found only in the dendritic compartment and associated exclusively with dendritic spines (48) where glutamate receptors are concentrated, raising the possibility that 5-HT$_{1A}$ receptors may exert some of their functions by modulating glutamatergic signaling. In this study, we revealed the 5-HT$_{1A}$-mediated reduction of AMPA receptor currents, which provides a potential mechanism for the inhibitory role of 5-HT$_{1A}$ receptors on PFC pyramidal neuron activity (1). Our previous studies show that 5-HT$_2$ and 5-HT$_4$ receptors are linked to the regulation of γ-aminobutyric acid, type A receptor function in PFC via a PKC- and PKA-mediated mechanism, respectively (18, 49). The specific coupling of these receptors to various signaling pathways provides serotonin with a precise and flexible mechanism to regulate different ion channels. By doing so, serotonin can simultaneously remodel excitability in a functionally appropriate manner.

Multiple signaling pathways have been found with the activation of 5-HT$_1$ receptors, including the inhibition of adenylyl cyclase and stimulation of the mitogen-activated protein kinase (50). Our results with PKA activators and inhibitors suggest that the 5-HT$_{1A}$-mediated reduction of AMPA receptor cur-

rents is dependent on PKA inhibition. Previous studies have shown that PKA phosphorylation of GluR1 subunit at Ser^{845} enhances AMPA currents (35), giving the possibility that the 5-HT$_{1A}$ reduction of AMPA currents is directly due to the decreased GluR1 phosphorylation by PKA. However, the blockade of 5-HT$_{1A}$ effects on AMPA currents by PP1 inhibitors suggests that this modulation requires the activation of PP1.

The activity of PP1 is controlled by PKA through the regulatory protein I-1. I-1, upon phosphorylation by PKA at Thr^{35}, becomes a potent inhibitor of PP1 (36). Inhibition of PKA activity will decrease the rate of I-1 phosphorylation, leading to PP1 activation due to a net dephosphorylation of phospho-I-1. *In vitro* biochemical studies have shown that PP2B and PP2A can effectively dephosphorylate Thr^{34}(PKA site)-phosphorylated DARPP-32, a dopamine- and cAMP-regulated phosphoprotein with a molecular mass of 32 kDa (37, 51). Based on sequence homology and function similarity between DARPP-32 and I-1 (52), it is likely that PP2A, as well as PP2B, can also effectively dephosphorylate Thr^{35}-phosphorylated I-1. Our electrophysiological experiments show that the calcineurin/PP2B autoinhibitory peptide did not prevent 5-HT$_{1A}$/PP1-mediated reduction of AMPA currents, but okadaic acid (a PP1/2A inhibitor) did (Fig. 5, *A* and *B*), suggesting that constitutively active PP2A may provide a way of dephosphorylating phospho-I-1 in these neurons. Therefore, okadaic acid exerts dual actions in the blockade of 5-HT$_{1A}$ modulation of AMPA currents. One is via the direct inhibition of PP1 activity. The other is via the prevention of PP2A dephosphorylation of phospho-I-1, which will keep PP1 inhibited. PP1 is localized to postsynaptic densities (53) and plays a key role in regulating synaptic plasticity by modifying AMPA receptors (26, 54). The 5-HT$_{1A}$-induced activation of PP1 could act on AMPA receptors by reducing CaMKII autophosphorylation at Thr^{286} and its consequent Ca^{2+}-independent activity (40, 41), therefore decreasing phosphorylation of GluR1 subunit on the CaMKII site Ser^{831} (24, 39). This model was confirmed by the electrophysiological results showing that the 5-HT$_{1A}$ effect on AMPA currents was occluded by CaMKII inhibitors and blocked by CaMKII activation.

CaMKII is primarily expressed in non-GABAergic neurons that use excitatory amino acid transmitters (55) and highly concentrated in the postsynaptic density (PSD), a cytoplasmic structure attached to the postsynaptic membrane at glutamatergic synapses (56, 57). This ideal position allows the multifunctional enzyme to play a central role in transmitting postsynaptic signals required for long term potentiation, a form of synaptic plasticity thought to be involved in learning and memory (21). CaMKII is autophosphorylated when the enzyme is activated in the presence of Ca^{2+}/calmodulin, leading to the appearance of a sustained Ca^{2+}-independent activity (40). It has been shown that the persistent autophosphorylation and activation of CaMKII is required for long term potentiation induction (58, 59). Dephosphorylation of CaMKII occurs specifically by PP1 in synaptic junctions (60), and PP1 is held in PSD by scaffolding proteins (26, 61, 62). Therefore, PSD provides an isolated biochemical compartment within which phosphatase saturation occurs and CaMKII can stay highly phosphorylated (63). In agreement with these results, our biochemical experiments demonstrated that the basal Thr^{286} phosphorylation level of CaMKII was high at resting [Ca^{2+}] in PFC slices, and 5-HT$_{1A}$ receptors did reduce the CaMKII autophosphorylation through activation of PP1. We also notice that activation of 5-HT$_2$ receptors by 5-HT application did not cause an increase of CaMKII autophosphorylation in PFC slices. One intriguing possibility is that 5-HT$_2$-induced elevation of cytosolic Ca^{2+} from intracellular stores is not enough to reach CaMKII that is enriched in PSD. CaMKII is more likely to be activated by Ca^{2+} entering through NMDA receptors that are also concentrated in PSD. In response to 5-HT, phosphorylated CaMKII is more likely to be dephosphorylated by 5-HT$_{1A}$-activated PP1 in PSD.

One mechanism for activated CaMKII to potentiate synaptic transmission is by phosphorylating GluR1 subunits of AMPA receptors and enhancing their conductance (25, 27). Correlating with the 5-HT$_{1A}$ reduction of AMPA currents, our biochemical data show that 5-HT$_{1A}$ receptor activation also reduced GluR1 phosphorylation at the CaMKII site. It suggests that changes in the CaMKII phosphorylation of GluR1 subunit may underlie the 5-HT$_{1A}$ effect on AMPA currents. However, recent studies show that CaMKII can enhance transmission by delivering new AMPA receptors to the synapse through a mechanism that does not require GluR1 phosphorylation but requires the association between GluR1 and a PDZ domain protein (64). Based on the binding interactions between PSD proteins, it has been proposed that the CaMKII/PP1 switch potentiates transmission by organizing an AMPA receptor anchoring assembly: the autophosphorylated CaMKII binds tightly to the NMDA receptor and forms additional sites for anchoring AMPA receptors at synapses through a supramolecular linkage (65). This model provides an alternative potential mechanism for 5-HT$_{1A}$ reduction of AMPA currents: the decreased CaMKII autophosphorylation causes the dissociation of AMPA receptors from the anchoring complex.

Taken together, this study provides evidence showing that serotonin, by activating 5-HT$_{1A}$ receptors, can suppress glutamatergic signaling in PFC through the inhibition of CaMKII. The enhanced-anxiety phenotype of 5-HT$_{1A}$ knockout mice (46, 47) may result from the loss of this suppression of excitatory transmission due to the absence of forebrain postsynaptic 5-HT$_{1A}$ receptors and inactivation of the associated signaling cascade. Consistent with this, decreased anxiety has been found in mice deficient in the α-isoform of CaMKII (66). The 5-HT$_{1A}$ reduction of AMPA receptor function also provides a potential mechanism for the 5-HT$_{1A}$ inhibition of long term potentiation (67, 68), a synaptic model of memory that requires CaMKII for its induction (69).

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