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5-HT_{2A} receptor or α_1-adrenoceptor activation induces excitatory postsynaptic currents in layer V pyramidal cells of the medial prefrontal cortex

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Abstract

We compared 5-hydroxytryptamine (5-HT), norepinephrine and dopamine for their efficacy at increasing excitatory postsynaptic current frequency in layer V pyramidal cells from rat medial prefrontal cortical slices. 5-HT, norepinephrine and dopamine increased the excitatory postsynaptic current frequency by 15.9-, 4.5- and 1.7-fold, respectively. Similar to previous results with 5-HT-induced excitatory postsynaptic currents, blockade of μ-opioid receptors, of α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptors and fast Na^+ channels suppressed the norepinephrine-induced excitatory postsynaptic currents. The norepinephrine-induced, and in most cases, the dopamine-induced increase in excitatory postsynaptic current frequency was blocked by the α_1-adrenoceptor antagonist prazosin while the α_2-adrenoceptor antagonist yohimbine did not block either the norepinephrine- or the 5-HT-induced increase in excitatory postsynaptic currents frequency. The potency of three 5-HT_2 receptor antagonists with varying selectivity for 5-$HT_{2A/2B/2C}$ receptors tested against the 5-HT-induced increase in excitatory postsynaptic current frequency are in agreement with the affinity of these drugs for the 5-HT_{2A} receptor. These findings suggest that 5-HT_{2A} receptor or α_1-adrenoceptor activation enhance neurotransmitter release from a similar subset of glutamate terminals that innervate apical dendrites of layer V pyramidal cells.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Norepinephrine; Dopamine; Glutamate; Neocortex

1. Introduction

We have shown recently that 5-HT_{2A} receptor activation increases excitatory postsynaptic current frequency in apical dendrites of neocortical and medial prefrontal cortical layer V pyramidal cells, in part, by increasing glutamate release from presynaptic terminals (Aghajanian and Marek, 1997). Furthermore, activation of presynaptic inhibitory metabotropic glutamate receptors (Aghajanian and Marek, 1997) and μ-opioid receptors (Marek and Aghajanian, 1998) suppresses 5-hydroxytryptamine (5-HT)-induced increases in excitatory postsynaptic current frequency. The restricted but highly similar laminar distribution of both 5-HT_{2A} receptors and μ-opioid receptors in the rat neocortex and medial prefrontal cortex, together with the observation that 5-HT generally decreases glutamate release evoked by electrical stimulation in the neocortex and medial prefrontal cortex (Tanaka and North, 1993; Read et al., 1994; Aghajanian and Marek, 1997) suggests that the 5-HT-mediated induction of glutamate release involves only a subset of the afferents to the apical dendrites of layer V neocortical and medial prefrontal cortical pyramidal cells. The regional and laminar distribution of α_1-adrenoceptors (Palacios et al., 1987) in the rat neocortex and medial prefrontal cortex is highly similar to the regional and laminar distribution of 5-HT_{2A} and μ-opioid receptors. The aims of the present studies are to compare the catecholamines norepinephrine and dopamine to 5-HT in the induction of an increase in excitatory postsynaptic current frequency in the medial prefrontal cortex and to further characterize the receptor subtype(s) mediating the responses.

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2. Materials and methods

2.1. Slice preparation

Brain slices were prepared from male Sprague–Dawley rats (120–200 g, Camm, Wayne, NJ) as described previously (Aghajanian and Rasmussen, 1989). Briefly, rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.), in adherence to protocols approved by the Yale University Animal Care and Use Committee. Following decapitation, the brain was removed rapidly and placed in an ice-cold modified artificial cerebrospinal fluid (ACSF) in which sucrose (252 mM) was substituted for NaCl. A block of the neocortex was dissected free and coronal slices (500 μm) were cut with an oscillating-blade tissue slicer (FHC) at a level corresponding to approximately 2.5 mm anterior to bregma (Paxinos and Watson, 1986). A slice containing the medial prefrontal cortex was then transferred to the stage of a fluid–gas interface chamber which had a constant flow of humidified 95% O_2, 5% CO_2. The chamber was heated slowly from room temperature to 34°C. The slices were perfused with normal ACSF which consisted of (in mM) NaCl 126; KCl 3; $CaCl_2$ 2; $MgSO_4$ 2; $NaHCO_3$ 26; NaH_2PO_4 1.25; D-glucose 10. The ACSF flow rate was 1–1.5 ml/min. There was a 2 h recovery period prior to beginning experiments.

2.2. Electrophysiological recording

Intracellular recording and single-electrode voltage clamping were conducted in medial prefrontal cortex layer V pyramidal cells using an Axoclamp 2-A (Axon Instruments, Burlingame, CA). Stubby electrodes (≅8 mm, shank to tip) with relatively low capacitance and resistance (30–60 MΩ) were pulled from filament-containing capillary tubing (1.5 μm) with a Brown–Flaming electrode puller (Sutter Instruments) and filled with 1 M potassium acetate. Under voltage clamp, electrodes prepared in this manner had rapid settling times (50–75 μs), allowing switching frequencies of 4–6 kHz and a loop gain of 10 nA/mV (30% duty cycle). Phase lag was used to prevent oscillations; false clamping was avoided by utilizing optimal capacitance neutralization and by allowing settling to a horizontal baseline, as verified by monitoring input voltage continuously. Pyramidal cells were identified according to criteria as previously described (Sheldon and Aghajanian, 1990). Only cells having a resting potential > -60 mV, an apparent input resistance of > 20 MΩ and a spike amplitude of > 75 mV were used for experiments. In the present study, pyramidal cells were voltage-clamped at -70 to -75 mV. The voltage-clamp signals were low-pass filtered (1000 Hz) and data were acquired with a pCLAMP/Digidata 1200 system (Axon Instruments). Excitatory postsynaptic current frequencies were obtained from 10 successive episodes (1 s duration) during the baseline and drug treatment periods by means of Axograph software using a peak detect program.

Whole cell recordings were performed using low-resistance patch pipettes (2.5–3 MΩ) containing (in mM): K gluconate 120, HEPES 10, 1,2-bis(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA K_4) 5, sucrose 20, $CaCl_2$ 2.38, $MgCl_2$ 1, K_2ATP 1 and GTP 0.1 (pH = 7.35). Following giga-seal formation induced by gentle suction, additional suction was applied to attain whole cell mode. Series resistance was monitored throughout the experiment and was usually 4–8 MΩ; cells were only used if the series resistance could be maintained below 10 MΩ. As previously reported, when series resistance was kept in this low range, the input resistance of cells recorded by whole cell recording were not different from values obtained with intracellular recording provided that adequate time is allowed for formation of a stable seal after impalement (Pineda et al., 1996). Postsynaptic currents were studied in continuous single-electrode voltage-clamp mode (3 kHz low-pass filter cutoff frequency); cell were usually clamped near their resting potential to minimize holding currents.

2.3. Data analyses

Excitatory postsynaptic current frequency and amplitude were determined with Axograph peak detect software; signals < 10 pA were excluded from the measurements. Experiments employing whole cell recording excluded signals < 2 pA due to decreased noise compared to intracellular recording. Statistical comparisons of within-cell responses were made using two-tailed paired *t*-tests requiring $P < 0.05$ for statistical significance.

The determination of pA_2 values for the blockade of 5-HT-induced increases in excitatory postsynaptic current frequency was performed by Schild analysis as previously described (Marek and Aghajanian, 1994). In the present studies, we tested relatively low concentrations of 5-$HT_{2B/2C}$ receptor antagonists to complement previous studies examining relatively low concentrations of 5-HT_{2A} receptor antagonists. In brief, concentration–response determinations were made by perfusing increasing concentrations of 5-HT ranging from near threshold (3–10 μM) through near maximal (100–300 μM) for 1 min and then testing the next highest dose after allowing 4–5 min for washout of the 5-HT response. The shift in the EC_{50} for 5-HT-induced excitatory postsynaptic currents was determined in the presence of the selective 5-HT_{2C} receptor antagonist SB 242,084 (30 nM), the 5-$HT_{2B/2C}$ receptor antagonist SB 206,553 (100, 300 or 1000 nM) and the 5-HT_{2A} receptor antagonist ketanserin (30 nM). The 5-HT receptor antagonists were applied for 20–25 min prior to the second EC_{50} determination and the antagonist continued to be perfused onto the slice during the washout period from 5-HT. EC_{50} values for 5-HT were calculated by non-linear curve fitting (Delta Graph).

2.4. Drugs

The drugs used in this study were obtained from the following suppliers: Sigma (St. Louis, MO, USA: 5-HT creatinine sulfate, norepinephrine bitartrate, prazosin); RBI (Natick, MA, USA: clonidine HCl, dopamine, [D-Ala^2, *N*-Me-Phe^4,Gly-ol^5]enkephalin; DAMGO, ketanserin tartrate and yohimbine HCl); Eli Lilly (Indianapolis, IN generously donated ((3*S*,4*aR*,6*R*,8*aR*)-6-[2-(2)*H*-tetrazole-5-yl)ethyl]decahydroisoquinoline-3 carboxylic acid; LY293558); SmithKline Beechum Pharmaceuticals (Harlow, Essex, UK generously donated 5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolol[2,3-*f*]indole; SB 206,553 and 6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomoyl] indoline; SB 242084); and Alomone Labs (Jerusalem, Israel: tetrodotoxin).

3. Results

3.1. Electrophysiological characteristics of mPF layer V pyramidal cells

Layer V pyramidal cells were recorded from the medial prefrontal cortex in a zone ca. 1/2–2/3 the distance between the pial surface and the subcortical white matter. The pyramidal cells in the present study had the following characteristics: resting potential, -69.8 ± 1.0 mV; action potential amplitude, 83.0 ± 1.7 mV; action potential duration (at half amplitude), 0.73 ± 0.04 ms; input resistance (-0.4 nA test pulse), 34.9 ± 2.3 MΩ ($n = 40$). Almost all of the cells in the present series had the previously reported characteristics (McCormick et al., 1985; Connors and Gutnick, 1990) of regularly spiking pyramidal cells

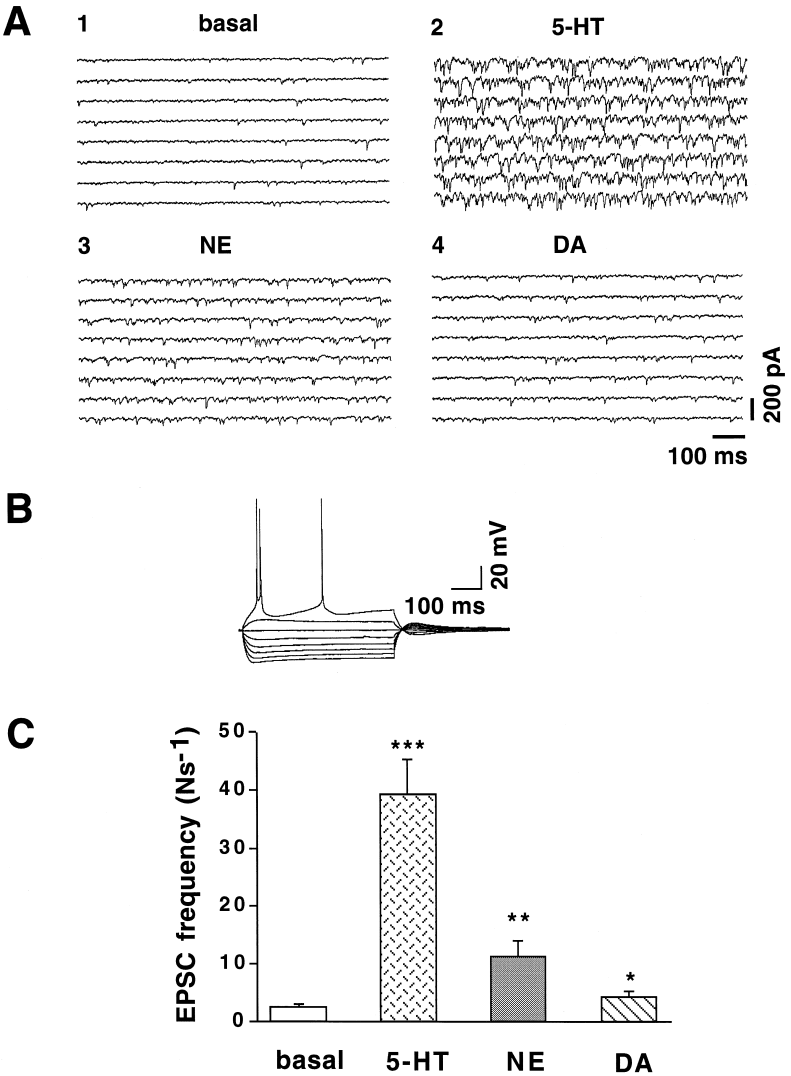

Fig. 1. 5-HT, norepinephrine and dopamine increase the frequency of excitatory postsynaptic currents in medial prefrontal cortical layer V pyramidal cells. (A) A whole cell recording in voltage clamp mode of the effect of bath-applied 5-HT (100 μM), norepinephrine (NE; 100 μm) and dopamine (DA; 100 μM) in the same cell. All traces display eight consecutive 1-s episodes. (B) A current clamp recording of the same cell in response to +0.4 nA current pulse with superimposed successive current steps of −0.2 nA. (C) A plot of the excitatory postsynaptic current frequency for all twelve cells in which 5-HT, norepinephrine and dopamine were tested. $* P < 0.05$, $** P < 0.01$, $*** P < 0.001$.

while only a single cell was an intrinsically bursting pyramidal cell.

3.2. 5-HT, norepinephrine and dopamine increase excitatory postsynaptic current frequency

The effects of 5-HT, norepinephrine and dopamine were each tested in a random sample of 12 cells. Whole cell recording was used to record from two of the cells and representative data is shown from one of these cells in Fig. 1; intracellular recording was used in the remainder of cells. Since the results from whole cell and intracellular recordings were similar, the results for all 12 cells were pooled. Near maximal concentrations of 5-HT, norepinephrine and dopamine (100 μM each) increased the excitatory postsynaptic current frequency by 15.9-fold ($P < 0.001$), 4.5-fold ($P < 0.01$) and 1.7-fold ($P < 0.05$), respectively (Fig. 1). The rank order of efficacy was 5-HT > norepinephrine > dopamine except in one cell where dopamine was slightly more effective than norepinephrine. 5-HT increased the excitatory postsynaptic current frequency by at least 4-fold in all twelve cells. In contrast, norepinephrine and dopamine increased the excitatory postsynaptic current frequency by at least 2-fold in only eight and four cells, respectively.

5-HT and norepinephrine exerted variable effects on the membrane holding currents that were not correlated to the presence or frequency of the excitatory postsynaptic currents in response to the monoamines. 5-HT (100 μM) induced small 50–200 pA inward currents in 3 of 12 cells and 125–130 pA outward currents in 2 of 12 cells (presumably via 5-HT_{2A} and 5-HT_{1A} receptors, respectively) and no obvious effect in the other cells. These results are consistent with previous reports (Araneda and Andrade, 1991; Tanaka and North, 1993; Ashby et al., 1994; Aghajanian and Marek, 1997). Norepinephrine (100 μM) induced 100–250 pA inward currents in 4 of the 12 cells that were not blocked by 100 nM prazosin, consistent with a previous report that β-adrenoceptor activation depolarizes cat layer V pyramidal cells (Foehring et al., 1989). Norepinephrine also induced a 60 pA outward current in a single cell.

Table 1
Blockade of norepinephrine (NE)-induced excitatory postsynaptic currents by LY293558, tetrodotoxin and DAMGO (all values expressed as means ± S.E.M, N s^{-1}; number/s)

Drug	Basal	NE alone	Drug alone	Drug + NE
LY293558 ($n = 6$)	5.7 ± 1.5	16.5 ± 3.1[a]	2.5 ± 0.8[a]	2.5 ± 0.6[b]
Tetrodotoxin ($n = 7$)	3.8 ± 0.9	16.5 ± 4.5[a]	1.6 ± 0.8	1.5 ± 0.5[b]
DAMGO ($n = 5$)	4.6 ± 1.4	12.3 ± 1.7[a]	3.6 ± 1.1	4.3 ± 1.3[b]

[a]Significantly different from basal, $P < 0.05$.
[b]Significantly different from norepinephrine alone, $P < 0.05$.

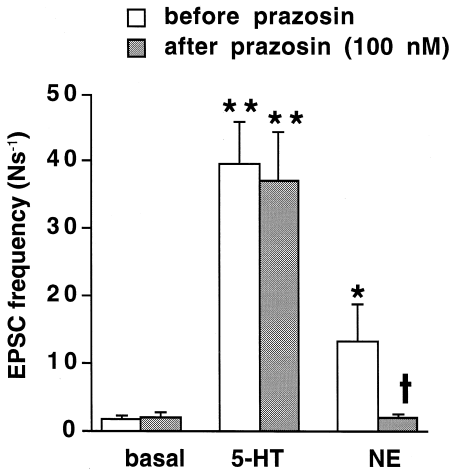

Fig. 2. Prazosin selectively blocks the norepinephrine-induced, but not 5-HT-induced increase in excitatory postsynaptic current frequency. The histogram shows a summary of six cells in which both norepinephrine and 5-HT were tested both before and after prazosin (100 nM; 15 min). * $P < 0.05$ and ** $P < 0.01$ compared to basal excitatory postsynaptic currents. † $P < 0.05$ compared to norepinephrine before prazosin.

3.3. Pharmacological characterization of catecholamine-induced excitatory postsynaptic currents

At the holding potentials used (i.e., −70 to −75 mV), the norepinephrine-induced excitatory postsynaptic currents were shown to be mediated by activation of α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptors since the AMPA receptor antagonist LY293558 (3 μM, 5 min) almost completely blocked the norepinephrine-induced increase in excitatory postsynaptic current frequency (Table 1; $P < 0.05$). Similar to previous

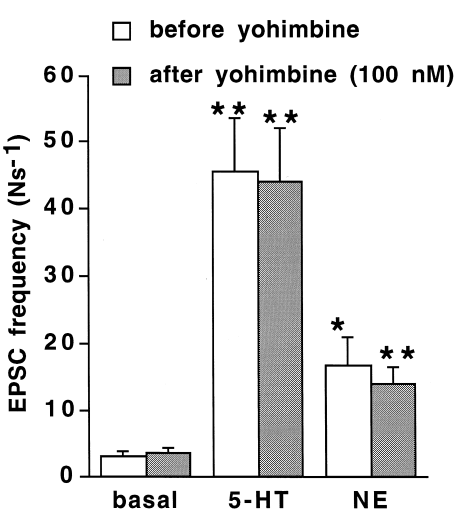

Fig. 3. Yohimbine does not decrease either norepinephrine-induced or 5-HT-induced excitatory postsynaptic currents. The histogram shows a summary of six cells in which both norepinephrine and 5-HT were tested both before and after yohimbine. * $P < 0.05$ and ** $P < 0.01$ compared to basal excitatory postsynaptic current frequency.

results (Aghajanian and Marek, 1997) LY293558 alone decreased the basal excitatory postsynaptic current frequency (Table 1; $P < 0.05$). As in the case of 5-HT-induced excitatory postsynaptic currents (Aghajanian and Marek, 1997), the norepinephrine-induced excitatory postsynaptic currents were suppressed totally by the fast sodium channel blocker tetrodotoxin (2 μM; Table 1; $P < 0.05$). Previously, activation of μ-opioid receptors, which share a similar laminar distribution in the medial prefrontal cortex and neocortex with 5-HT_{2A} receptors and α_1-adrenoceptors (Palacios et al., 1987; Tempel and Zukin, 1987; Blue et al., 1988), suppressed release of glutamate by 5-HT from a subset of nerve terminals innervating layer V pyramidal cells (Marek and Aghajanian, 1998). Therefore, we tested the selective μ-opioid receptor agonist DAMGO against the norepinephrine-induced increase in excitatory postsynaptic current frequency. As in the case of 5-HT-induced excitatory postsynaptic currents (Marek and Aghajanian, 1998), DAMGO (1 μM) suppressed the norepinephrine-induced increase in excitatory postsynaptic current frequency (Table 1; $P < 0.01$). DAMGO induced an outward shift of the holding potential in only one of the five cells tested (80 pA) similar to previous reports (Tanaka and North, 1994; Marek and Aghajanian, 1998).

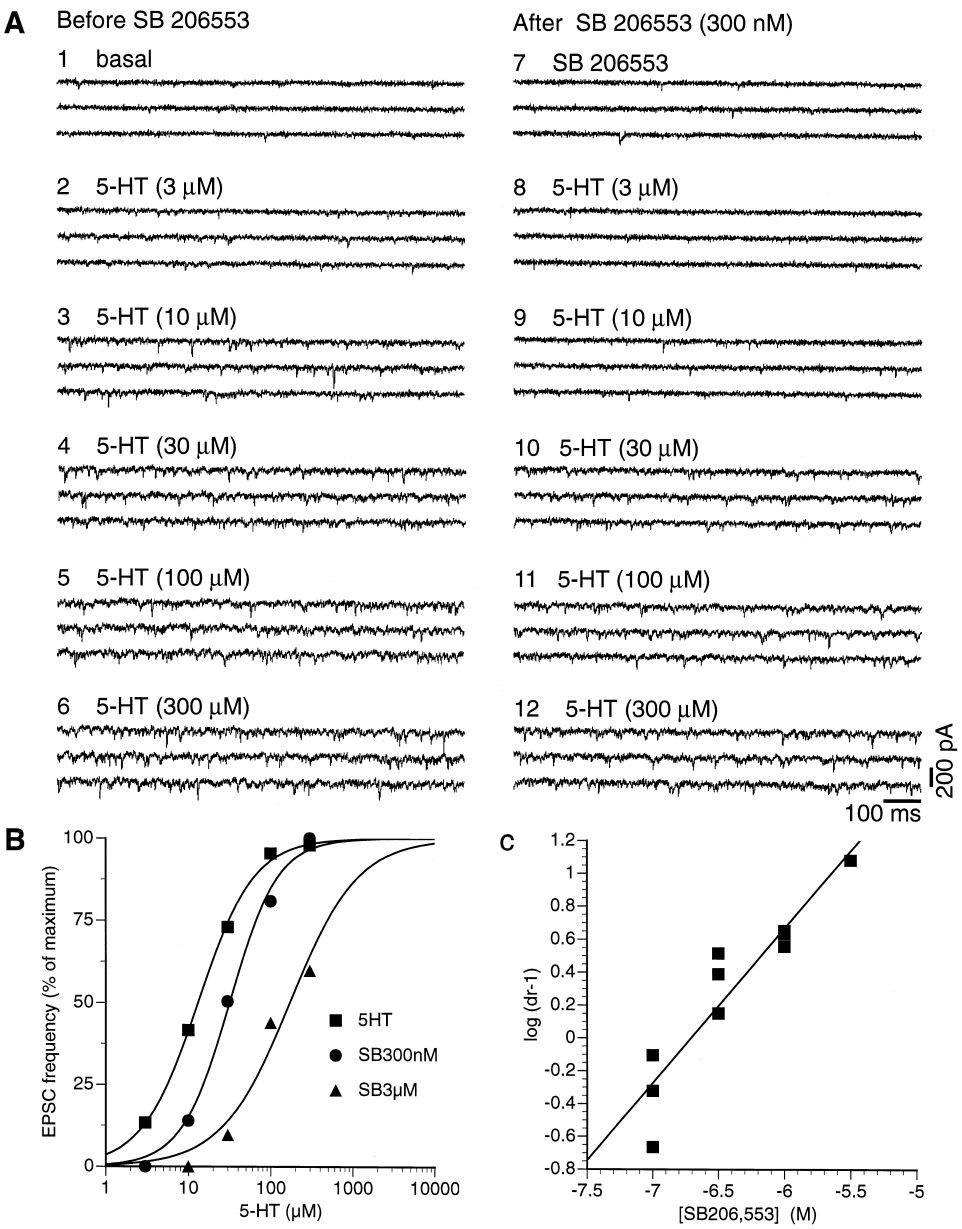

Fig. 4. Blockade by SB 206553 on 5-HT-induced excitatory postsynaptic currents. (A) Shows an example of a concentration–response curve for 5-HT-induced excitatory postsynaptic currents both before and after a 20 min application of SB 206553 (300 nM). (B) Shows a graphic representation of the same data shown in (A) in addition to a 5-HT concentration–response curve following a further 20 min application of SB 206553 (3 μM). (C) Shows a Schild plot for a number of similar experiments where $dr = EC_{50}$ for 5-HT in the presence of the antagonists/EC_{50} for 5-HT in the absence of the antagonist. The pA_2 (and putative pK_B) calculated as the y-axis intercept was 6.7 and the slope of the regression line was 0.94.

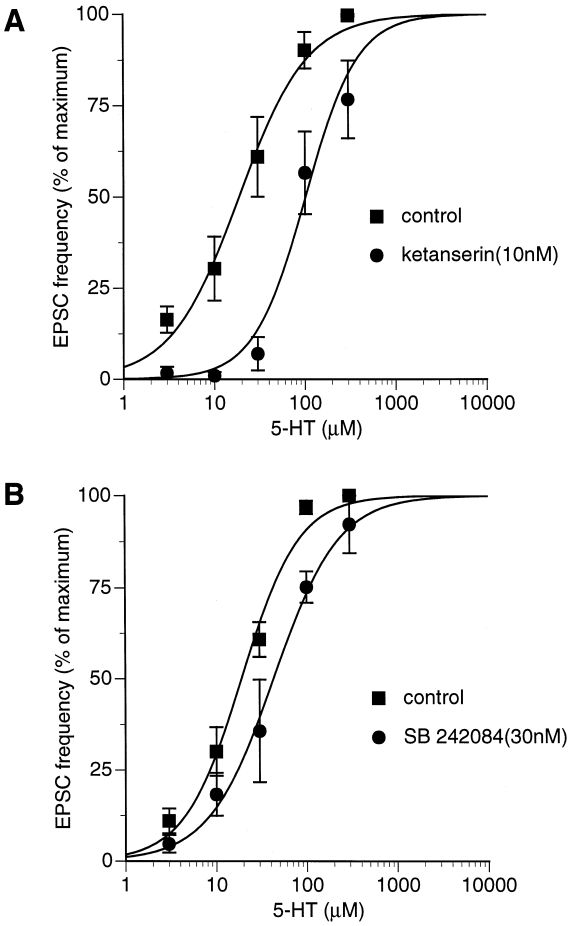

Fig. 5. Blockade by ketanserin and SB 242084 against 5-HT-induced excitatory postsynaptic currents. (A) Shows the summary of three separate experiments with ketanserin (10 nM) resulting in a pA_2 of 8.70. (B) Shows the summary of three separate experiments with SB 242084 (30 nM) resulting in a pA_2 of 7.70.

Norepinephrine (100 μM) was tested after a 30 min treatment with a concentration of the α_1-adrenoceptor antagonist prazosin (100 nM) which completely blocks the excitatory effect of an α_{1B}-adrenoceptor-mediated response to 100 μM norepinephrine in the piriform cortex (Marek and Aghajanian, 1996, unpublished observations). Prazosin completely blocked the norepinephrine-induced, but not the 5-HT-induced, increase in excitatory postsynaptic currents in all six cells tested ($P < 0.05$; Fig. 2). In contrast to the blockade by prazosin, the α_2-adrenoceptor antagonist yohimbine did not block either the norepinephrine-induced or the 5-HT-induced increase in excitatory postsynaptic current frequency (Fig. 3). The α_2-adrenoceptor agonist clonidine (400 nM, 3 min) did not change the baseline excitatory postsynaptic current frequency (baseline, 3.5 ± 1.0 N s^{-1}; clonidine, 3.8 ± 0.5 N s^{-1}; $n = 5$, not shown; values are means ± S.E.M.; number/s). Clonidine also did not decrease the 5-HT-induced increase in excitatory postsynaptic current frequency (5-HT alone, 33.7 ± 4.8 Ns^{-1}; 5-HT + clonidine, 35.5 ± 4.7 Ns^{-1}; $n = 5$, not shown).

The dopamine-induced (100 μM) increase in excitatory postsynaptic current frequency was blocked in three out of four cells tested following prazosin (not shown), suggesting that dopamine was activating α_1-adrenoceptors. The reliable small response to dopamine is consistent with the ~20-fold lower affinity of dopamine compared to norepinephrine for the ^{3}[H]prazosin binding site (Hornung et al., 1979; Miach et al., 1980). The dopamine D_1/D_5 receptor agonist SKF 38393 (20 μM, $n = 2$) and the dopamine $D_2/D_3/D_4$ receptor agonist quinpirole (20 μM; $n = 2$) did not increase or decrease the excitatory postsynaptic current frequency (not shown).

3.4. Pharmacological characterization of 5-HT-induced excitatory postsynaptic currents

To further pharmacologically characterize the 5-HT-induced excitatory postsynaptic current response, antagonists 100-fold selective for the 5-$HT_{2B/2C}$ vs. the 5-HT_{2A} recep-

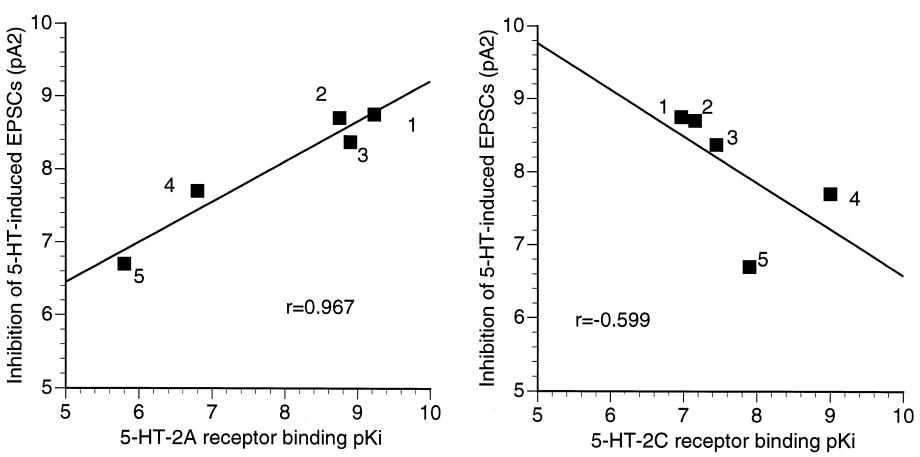

Fig. 6. Blockade of 5-HT-induced excitatory postsynaptic currents is positively correlated to affinity of 5-HT antagonists for the 5-HT_{2A} receptor. A series of drugs blockade of 5-HT-induced excitatory postsynaptic currents was positively correlated to the affinity of the drugs for the 5-HT_{2A} receptor ($r = 0.967$, $p < 0.01$) and negatively correlated to the affinity of the drugs for the 5-HT_{2C} receptor ($r = -0.599$, $p > 0.05$). (1) MDL 100,907; (2) ketanserin; (3) SR 46349B; (4) SB 242084; (5) SB 206553.

tor (SB 206,553), 100-fold selective for the 5-HT_{2C} vs. the 5-HT_{2A} receptor (SB 242,084) and 30-fold selective for the 5-HT_{2A} vs. the 5-HT_{2C} receptor (ketanserin) were tested to determine if 5-$HT_{2B/2C}$ receptor activation could contribute to the 5-HT-induced excitatory postsynaptic current response. The 5-$HT_{2B/2C}$ receptor antagonist SB 206,553 (100 nM) minimally inhibited the 5-HT response (not shown), but did result in a parallel rightward shift of the 5-HT concentration–response curve (3–300 μM). SB 206,553 (300 nM) resulted in a parallel rightward shift of the 5-HT-induced concentration–response curve (3–300 μM), but the maximal response to 5-HT was not altered (Fig. 4A,B). The pK_B determined by the Schild plot (Fig. 4C) for SB 206,553 (100 nM–3 μM) was 6.7 and the slope of the linear regression line-of-best fit was 0.94. The selective 5-HT_{2C} receptor antagonist SB 242,084 (30 nM, 30 min) caused a parallel rightward shift of the 5-HT concentration–response curve resulting in a calculated pA_2 of 7.70 ± 0.10 (Fig. 5B). Ketanserin (10 nM, 30 min) caused a parallel rightward shift of the 5-HT concentration–response curve (3–300 μM) resulting in pA_2 value of 8.70 ± 0.01 (Fig. 5A). A comparison of the pA_2 values of ketanserin, SB 242,084 and SB 206,553 and previously published values for M100,907 (formerly MDL 100,907) and SR 46349B (Aghajanian and Marek, 1997) with the published pK_i values (Schreiber et al., 1995; Kennett et al., 1996; Kennett et al., 1997) of these drugs for the 5-HT_{2A} receptor resulted in a significant positive correlation ($r = 0.967$, $P < 0.01$, Fig. 6). In contrast, a negative correlation was observed for a similar comparison of the pA_2 values with the published pK_i values of these drugs at the 5-HT_{2C} receptor ($r = -0.599$, $P > 0.05$).

4. Discussion

The main finding from the present studies is that the rank order of efficacy for the monoamines at inducing an increase in excitatory postsynaptic current frequency in layer V medial prefrontal cortex is 5-HT > norepinephrine > dopamine. This increase in the frequency of excitatory postsynaptic currents implies that both 5-HT and norepinephrine exert a presynaptic influence relative to the layer V pyramidal cells. As previously shown for 5-HT (Aghajanian and Marek, 1997), the AMPA receptor antagonist LY293558 (Schoepp et al., 1995; Rasmussen et al., 1996) completely blocked the norepinephrine-induced increase in excitatory postsynaptic current frequency suggesting that both 5-HT and norepinephrine induce the release of glutamate and/or aspartate. Even more striking, the norepinephrine-induced increase in excitatory postsynaptic current frequency was blocked completely by DAMGO, a selective μ-opioid receptor agonist. Since μ-opioid receptor activation has previously been shown to block completely, the 5-HT-induced increase in excitatory postsynaptic current frequency but not other AMPA-induced effects in medial prefrontal layer V pyramidal cells (Marek and Aghajanian, 1998), by inference, this finding suggests that both 5-HT and norepinephrine release glutamate from the same or a similar population of glutamatergic terminals that synapse onto apical dendrites of layer V pyramidal cells. However, it remains to be determined whether or not 5-HT_{2A} receptors and α_1-adrenoceptors are located on the same subset of glutamatergic terminals in the cortex.

The norepinephrine-induced increase in excitatory postsynaptic current frequency was mediated via α_1-adrenoceptor activation since an α_1-adrenoceptor antagonist, prazosin (Ford et al., 1994), completely and selectively blocked the norepinephrine-induced increase in excitatory postsynaptic current frequency. Although prazosin also potently blocks $\alpha_{2B/2C}$-adrenoceptors, the $\alpha_{2A/2B/2C}$-adrenoceptor antagonist yohimbine (Harrison et al., 1991) did not block the norepinephrine-induced excitatory postsynaptic currents. Conversely, the α_2-adrenoceptor agonist clonidine did not induce a change in excitatory postsynaptic current frequency.

A previous report (Aghajanian and Marek, 1997) found that 5-HT_{2A} receptor activation enhanced the persistent tetrodotoxin-sensitive sodium inward current (I_{NaP}) and this effect was suggested to play a role in the mechanism by which 5-HT induces spontaneous excitatory postsynaptic currents through a tetrodoxtoxin-sensitive focal action in the apical dendritic field involving pre- and/or postsynaptic sites. Similar to 5-HT, norepinephrine was found to increase I_{NaP} in layer V pyramidal cells of the cat neocortex (Foehring et al., 1989). The norepinephrine-induced excitatory postsynaptic currents are sensitive to tetrodotoxin. By analogy with 5-HT, norepinephrine might induce spontaneous excitatory postsynaptic currents through a tetrodotoxin-sensitive focal action in layer V pyramidal cells involving a pre- and/or postsynaptic mechanism.

The suppression by μ-opioid receptor agonists of both 5-HT-induced and norepinephrine-induced increase in the excitatory postsynaptic current frequency suggests that all three neurotransmitter receptors (μ-opioid, 5-HT_{2A} receptors and α_1-adrenoceptors) might be found on the same glutamatergic terminals in the cortex. 5-HT_{2A} receptors have been recently demonstrated to be present in presumed glutamatergic terminals in the neocortex (Jakab and Goldman-Rakic, 1998). We have previously noted that the neocortical localization of μ-opioid receptor binding in superficial layers and layer Va throughout the neocortex and medial prefrontal cortex is remarkable in light of the relative absence of μ-receptor mRNA in these same layers (Delphs et al., 1994; Mansour et al., 1994a,b). The thalamus is a possible source of subcortical afferents which is known to contain both μ-opioid receptor mRNA (Mansour et al., 1994b) and α_1-adrenoceptor mRNA, especially α_{1B}-adrenoceptor mRNA (McCune et al., 1993; Pieribone et al., 1994; Domyancic and Morilak, 1997).

The present results with the 5-$HT_{2A/2C}$ receptor antagonists are consistent with previous pharmacological characterization of the 5-HT-response involving 5-HT_{2A} receptor activation (Aghajanian and Marek, 1997). The rank order of potency for the three 5-HT_2 antagonists in blocking the 5-HT-induced excitatory postsynaptic currents was ketanserin > SB 242,084 > SB 206,553 which exactly agrees with the rank order of potency for the three antagonists at the 5-HT_{2A} receptor (Schreiber et al., 1995; Kennett et al., 1996; Kennett et al., 1997). In contrast, the rank order of potency for the three 5-HT_2 antagonists at blocking the 5-HT-induced excitatory postsynaptic currents did not agree with the rank order of potency for the three 5-HT_2 antagonists at the 5-HT_{2B} (SB 206,553 > SB 242,084 > ketanserin, Wainscott et al., 1996) or the 5-HT_{2C} (SB 242,084 > SB 206,553 > ketanserin) receptor. The pA_2 values obtained with ketanserin and SB 206,553 are consistent with the nM affinity of ketanserin and the μM affinity of SB 206,553 for 5-HT_{2A} receptors. In contrast, the pK_B for SB 206,553 against 5-HT-stimulated phosphoinositide turnover in the choroid plexus (a 5-HT_{2C} response) is 9.0 (Kennett et al., 1996). 5-HT_{2C} receptor protein and mRNA are both present in the medial prefrontal cortex/neocortex and until recent years, relatively few agonists or antagonists could differentiate 5-HT_{2C} from 5-HT_{2A} receptors. The presence of 5-HT_{2B} receptors in neocortical nerve fibers has been suggested recently (Duxon et al., 1996). However, the relatively low pK_B and pA_2 values obtained in the present study for 5-HT-induced excitatory postsynaptic currents with SB 206,553 and SB 242,084 suggest that 5-$HT_{2B/2C}$ activation does not participate appreciably in the 5-HT-induced increase in excitatory postsynaptic current frequency in neocortical pyramidal cells.

5-HT_{2A} receptors and α_1-adrenoceptors, both of which are known to be coupled to phospholipase C and phosphoinositol turnover, often are present within the same cellular elements in different tissues. Within the neocortex, 5-HT_{2A} and α_1-adrenoceptors share a striking similarity in their regional distribution, laminar distribution (with the heaviest concentration in layer Va) (Palacios et al., 1987; Blue et al., 1988) and in their laminar mRNA distribution (McCune et al., 1993; Pieribone et al., 1994; Pompeiano et al., 1994; Wright et al., 1995). 5-HT_{2A} receptors and α_1-adrenoceptors appear to modulate both cortical pyramidal cells and interneurons in a parallel manner (Marek and Aghajanian, 1994; Marek and Aghajanian, 1996). On a behavioral level, activation of 5-HT_{2A} receptors (Jarvik and Chorover, 1960) and α_1-adrenoceptors (Arnsten and Jentsch, 1997) impairs delayed response tasks dependent upon the medial prefrontal cortex in primates. On a clinical level, the prefrontal cortex has increasingly been implicated in the pathophysiology of schizophrenia. 5-HT_{2A} receptors may play a role in either the pathophysiology (Burnet et al., 1996; Williams et al., 1996; Gurevich and Joyce, 1997) or treatment (Altar et al., 1986; Rasmussen and Aghajanian, 1988; Meltzer et al., 1989; Gellman and Aghajanian, 1994) of schizophrenia. Similarly, others have noted the potential importance of norepinephrine and/or α_1-adrenoceptors in mediating therapeutic effects of antipsychotic drugs (Cohen and Lipinski, 1986; Breier et al., 1998). Thus, it is of interest that activation of 5-HT_{2A} receptors and α_1-adrenoceptors appears to have similar effects on medial prefrontal cortical function.

In contrast to both 5-HT and norepinephrine, dopamine failed to induce even a 2-fold increase in excitatory postsynaptic current frequency. In three of four cells, prazosin decreased the dopamine-mediated increase in excitatory postsynaptic current frequency, suggesting that these minimal effects of dopamine may have been mediated in part via α_1-adrenoceptor stimulation. A dopamine $D_{1/5}$ receptor agonist failed to increase the excitatory postsynaptic current frequency. The relative lack of effect of dopamine at increasing spontaneous excitatory postsynaptic current frequency stands in contrast to a recent report that dopamine D_1 receptor stimulation alters the excitability of medial prefrontal layer V pyramidal cells and also alters Ca^{2+} currents in the apical dendrites in a manner that may amplify inputs from the distal branches (Yang and Seamans, 1996). While we cannot completely exclude a minor role for dopamine, these findings suggests that both 5-HT and norepinephrine, unlike dopamine, have a presynaptic influence on the same or similar inputs to the apical dendrites of layer V pyramidal cells in the neocortex.

Neuroanatomical considerations suggest that the prefrontal cortex has a special relationship to the 5-HT and noradrenergic systems. While 5-HT-containing cells in the dorsal and median raphe and norepinephrine-containing cells in the locus coeruleus project throughout the neocortex, *direct* connections from the cortex to the raphe nuclei and locus coeruleus appear to be restricted to the prefrontal and insular cortex (Aghajanian and Wang, 1977; Cedarbaum and Aghajanian, 1978; Arnsten and Goldman-Rakic, 1984; Luppi et al., 1995). The layer V pyramidal cells, in which the 5-HT- and norepinephrine-induced excitatory postsynaptic currents are observed, are the principal output cells for the neocortex with respect to corticobulbar projections (Branchereau et al., 1996; DeFelipe and Farinas, 1992).

In summary, the order of efficacy for the monoamines was 5-HT > norepinephrine > dopamine at increasing excitatory postsynaptic current frequency in the apical dendrites of medial prefrontal cortex layer V pyramidal cells. Pharmacological analyses of these responses further confirmed that the 5-HT-induced increase in excitatory postsynaptic current frequency was mediated by 5-HT_{2A} receptor activation while the norepinephrine-induced increase in excitatory postsynaptic current frequency was mediated by α_1-adrenoceptor activation. Thus, both 5-HT and norepinephrine appear to play similar roles with respect to this particular subset(s) of glutamatergic terminals that innervate the apical dendrites of layer V pyramidal cells.

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