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Regulation of rat cortical 5-hydroxytryptamine_{2A}-receptor mediated electrophysiological responses by repeated daily treatment with electroconvulsive shock or imipramine

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Abstract

Down-regulation of 5-hydroxytryptamine_{2A} (5-HT_{2A}) receptors has been a consistent effect induced by most antidepressant drugs. In contrast, electroconvulsive shock (ECS) up-regulates the number of 5-HT_{2A} receptor binding sites. However, the effects of antidepressants on 5-HT_{2A} receptor-mediated responses on identified cells of the cerebral cortex has not been examined. The purpose of the present study was to compare the effects of the tricyclic antidepressant imipramine and ECS on 5-HT_{2A} receptor-mediated electrophysiological responses involving glutamatergic and GABAergic neurotransmission in the rat medial prefrontal cortex (mPFC) and piriform cortex, respectively. The electrophysiological effects of activating 5-HT_{2A} receptors was consistent with 5-HT_{2A} receptor binding regulation for imipramine and ECS except for the mPFC where chronic ECS decreased the potency of 5-HT at a 5-HT_{2A} receptor-mediated response. These findings are consistent with the general hypothesis that chronic antidepressant treatments shift the balance of serotonergic neurotransmission towards inhibitory effects in the cortex.

Keywords

antidepressant drugs; excitatory postsynaptic currents; pyramidal cells; interneurons; prefrontal cortex; piriform cortex

1. Introduction

Blockade of the serotonin transporter (SERT) by the tricyclic antidepressants (TCA) clomipramine and imipramine, selective serotonin reuptake inhibitors (SSRIs), or serotonin/norepinephrine reuptake inhibitors (SNRIs) appears related to the therapeutic effects of these drugs in treating depressive and anxiety disorders (Delgado et al., 1990; Delgado et al., 1999; Little et al., 1999; Richelson, 2001). SERT inhibition results in wide-spread elevations of 5-hydroxytryptamine (5-HT) throughout the brain, with subsequent activation of all fifteen 5-HT receptor subtypes both acutely and after the return of serotonergic neuron firing rates during chronic antidepressant drug administration (Chaput et al., 1986; Rueter et al., 1998). The actual 5-HT receptors involved in mediating the antidepressant effects of most antidepressant drugs has not been clarified, although postsynaptic 5-HT_{1A} receptors have been suggested to be a principal target (Haddjeri et al., 1998). Given the complexity of the serotonin

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receptor families, it would seem surprising that optimal antidepressant action would occur by 5-HT acting on all 5-HT receptors with the corresponding corollary that blockade of one or more 5-HT receptors would enhance the therapeutic action of SSRIs and other antidepressants.

Activation of 5-HT_{2A} receptors may be counterproductive to the optimal efficacy of SSRIs and SNRIs. Activation of 5-HT_{2A} receptors in rodents appears to increase certain forms of impulsivity and interfere with arousal and attention (Carli et al., 2004; Koskinen et al., 2000; Winstanley et al., 2003; Winstanley et al., 2004). Activation of 5-HT_{2A} receptors in humans also appears to result in a disturbance of attentional processes (Umbricht et al., 2003; Vollenweider et al., 1998). Blockade of 5-HT_{2A} receptors was found to enhance the antidepressant-like action of the SSRI fluoxetine in a rodent behavioral screen for antidepressant drugs involving suppression of “inappropriate” or impulsive responding (Marek et al., 2005). At a clinical level, addition of drugs which block 5-HT_{2A} receptors (mirtazapine, mianserin, olanzapine, quetiapine) to ongoing treatment with SSRIs appears to result in enhanced antidepressant efficacy (Calabrese et al., 2005; Marek et al., 2003; Tohen et al., 2003). An increased density of 5-HT_{2A} receptors has been associated in many, but not all, studies of suicide victims with either depression or schizophrenia or alcoholism (Laruelle et al., 1993; Pandey et al., 2002; Stanley and Mann, 1983; Stockmeier et al., 1997; Underwood et al., 2004). A polymorphism of 5-HT_{2A} receptor has been associated with poor medication tolerability in elderly depressed patients treated with a high dose of paroxetine (40 mg qd) but not the 5-HT_{2A/2C} antagonist mirtazapine (Murphy et al., 2003). Furthermore, a different polymorphism of the 5-HT_{2A} receptor has also been associated with a good treatment response to an SSRI in the STAR-D depression trial (McMahon et al., 2006). Finally, most antidepressant drugs either acutely block 5-HT_{2A} receptors (mirtazapine, mianserin, trazodone, nefazodone, amitriptyline, nortriptyline, imipramine) or decrease the density of 5-HT_{2A} receptors following chronic drug administration (TCAs; monoamine oxidase inhibitors, MAOIs; atypical antidepressants such as trazodone, mianserin and mirtazapine) (Blackshear and Sanders-Bush, 1982; Conn and Sanders-Bush, 1986; Peroutka and Snyder, 1980; Riblet and Taylor, 1981; Richelson, 2001; Sanders-Bush et al., 1989). Human PET imaging studies have found that chronic treatment with the TCA desipramine decreases 5-HT_{2A} receptor binding (Yatham et al., 1999). Thus, a wide range of studies at both the preclinical and clinical level lend support to the notion that modulation of 5-HT_{2A} receptors may be related to depression, suicide and therapeutic responses to antidepressant drugs.

Electroconvulsive shock is an exception to the pattern of most antidepressant drugs in that an up-regulation of cortical 5-HT_{2A} receptors are found following chronic ECS treatment in rodents (Biegon and Israeli, 1987; Butler et al., 1993; Goodwin et al., 1984; Kellar and Bergstrom, 1983; Kellar et al., 1981; Pandey et al., 1992; Stockmeier and Kellar, 1986; Vetulani et al., 1981). The only robust widely replicated indirect change in 5-HT_{2A} receptor function following repeated daily administration of ECS is an enhancement of head shakes or head twitches induced by 5-HT_{2A} receptor agonists (Godfrey et al., 1988; Goodwin et al., 1984; Lebrecht and Nowak, 1980; Moorman et al., 1996). This head shake response induced by 5-HT_{2A} receptor agonists does appear to involve local 5-HT_{2A} receptors in the mPFC (Granhoff et al., 1992; Willins et al., 1997). However, since head shakes/twitches induced by activation of 5-HT_{2A} receptors are altered by activation or blockade of many other transmitter receptor subtypes (5-HT_{1A} receptors, 5-HT_{2C} receptors, α_1 -adrenoceptors, α_2 -adrenoceptors, β_2 -adrenoceptors [G. Marek, unpublished observations]; dopamine D1 receptors, μ -opioid receptors, mGlu2 receptors, NMDA receptors, AMPA receptors (Zhang and Marek, in press), adenosine A1 receptors [G. Marek, unpublished observations], melatonin receptors, CB1 receptors and others), it would seem premature to assume that the up-regulation of 5-HT_{2A} receptor binding by ECS is invariably related to an increased functional activation of 5-HT_{2A} receptors (Cheer et al., 1999; Dall’Olio et al., 1999; Eison et al., 1995; Gewirtz and Marek, 2000; Handley and Brown, 1982; Handley and Singh, 1986; Marek, 2003; Schreiber

et al., 1995; Vickers et al., 2001). While there is generally a good relationship between 5-HT_{2A} receptor regulation in the primate and rodent, a primate study found that 5-HT_{2A} receptors were not up-regulated following repeated ECS treatments. Since cortical 5-HT_{2A} receptors modulate both glutamatergic (Aghajanian and Marek, 1997; Marek et al., 2001) and GABAergic transmission (Marek and Aghajanian, 1994; Sheldon and Aghajanian, 1990), investigation into the effects of 5-HT_{2A} receptor activation on neurotransmission mediated by both excitatory and inhibitory amino acids is warranted.

Therefore, the purpose of the present experiments was to examine the regulation of 5-HT_{2A} responses on glutamatergic thalamocortical afferents in the mPFC (Lambe and Aghajanian, 2001; Marek et al., 2001; Scruggs et al., 2000) and on GABAergic interneurons in the piriform cortex in the rat (Marek and Aghajanian, 1994; Sheldon and Aghajanian, 1991; Sheldon and Aghajanian, 1990). The tricyclic antidepressant imipramine and ECS were chosen in order to compare the regulation of these electrophysiological responses by these different antidepressant treatments since rodent receptor binding studies suggest that either a down-regulation or an up-regulation occurs for cortical 5-HT_{2A} receptors after chronic treatment with these two different somatic antidepressant treatments, respectively. TCAs and ECS were also chosen as they may both represent two of the most efficacious treatments for major depression as well. The results from the present experiments are consistent with the general hypothesis that a balance of activity away from 5-HT_{2A} receptor activation and toward activation of other 5-HT receptor subtypes in the cerebral cortex might result in optimal antidepressant activity.

2. Experimental Procedures

2.1. Animals

Male Sprague-Dawley rats (n=35; Camm, Wayne, NJ) were 120–200 g at the beginning of chronic antidepressant treatment. All subjects were allowed a 7 day adaptation period following arrival from the supplier. They were housed in suspended stainless steel wire cages (18 × 36 × 20 cm) with two rats occupying each cage. The colony room was maintained at 20°C and relative humidity (60%). The room was illuminated 12 h/day (0700–1900 hr). All rats had free access to laboratory chow (Teklad 4% Rat Diet) and water. The principles of laboratory animal care (NIH publication No. 80–23, revised 1996) were followed. All procedures were approved by the Yale University Animal Care and Use Committee.

2.2. Antidepressant drug and ECS treatment

Imipramine hydrochloride (15 mg/kg for 21 days, i.p.; Sigma, St. Louis, MO) was injected once daily in a volume of 1 ml/kg. Electroconvulsive shock was administered daily for 10 consecutive days through ear clip electrodes (50 mA, 0.3 s) and full tonic/clonic seizures were induced once daily for ≥ 30 s. Control animals underwent sham ECS treatment or vehicle injections. The drug treated animals were killed 24 h after the last drug treatment or 16–18 h after the last ECS treatment. The ECS equipment and procedures were the same as previously found to alter 5-HT_{2A} receptor binding and mRNA levels (Butler et al., 1993).

2.3. Brain slice preparation

Brain slices were prepared as described previously (Marek et al., 2000). Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and decapitated. Coronal slices (500 μM) were cut with an oscillating-blade tissue slicer at a level corresponding to approximately 2.5 mm anterior to bregma for recording from the medial prefrontal cortex (mPFC) or to approximately 2.2 mm anterior to bregma for recording from the anterior piriform cortex. A slice containing the mPFC or piriform cortex was then transferred to the stage of a fluid-gas interface chamber, which had a constant flow of humidified 95% O₂, 5% CO₂. The slices were perfused in a chamber heated to 34 °C with normal ACSF, which consisted of 126 mM NaCl,

3 mM KCl, 2 mM CaCl₂, 2 mM MgSO₄, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, and 10 mM D-glucose.

2.4. Electrophysiological recording

Intracellular recording and single-electrode voltage clamping were conducted in prefrontal cortical layer V pyramidal cells by using an Axoclamp-2A (Axon Instruments, Inc., Foster City, CA) as previously described (Aghajanian and Marek, 1997). Stubby electrodes (~8 mm, shank to tip) with relatively low capacitance and resistance (30–60 MOhms) were filled with 1 M potassium acetate. The cells were voltage clamped at –70 mV. 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. Layer V pyramidal cells were recorded in a zone one-half to two-thirds the distance between the pial surface and the white matter in the mPFC (anterior cingulate and prelimbic area; Cg1, Cg2, Cg3). The excitatory postsynaptic currents (EPSCs) recorded under these conditions do not appear to be contaminated by reversed inhibitory postsynaptic currents as previously discussed (Aghajanian and Marek, 1997). The voltage-clamp signals were low-pass filtered (1000 Hz) and data were acquired with a pCLAMP/Digidata 1200 system (Axon Instruments, Inc., Foster City, CA).

Extracellular recordings were conducted using an Axoclamp-2A (Axon Instruments, Burlingame, CA, USA). Placement of the recording electrodes was guided by visualizing the three layers of the piriform cortex at low magnification under reflected light. Extracellular recordings from interneurons located in layer III of the piriform cortex were made using glass microelectrodes filled with 2 M NaCl (5–10 MOhms) as described previously (Sheldon and Aghajanian, 1990). Cells were found by searching while the slice was perfused with 30 μ M 5-HT in order to activate quiescent cells. 5-HT was applied for no longer than 15 min and was turned off for an equivalent period if necessary to search for an additional cell. Cells identified as interneurons had the ability to sustain rapid firing rates as previously characterized by both intracellular and extracellular recording (Sheldon and Aghajanian, 1991; Sheldon and Aghajanian, 1990). Once an interneuron was located, the solution was switched from 30 μ M 5-HT back to control ACSF and the 5-HT activated cells gradually slowed and, in almost all cases, ceased firing. Then the effects of 5-HT creatine sulfate (Sigma Chemical Co., St. Louis, MO, USA) and α -amino-3-hydroxy-5-methyl-isoxazolepropionate (AMPA; RBI, Natick, MA, USA) were tested. It should be noted that continuous 5-HT application has not been found to produce significant tachyphylaxis (>10–15%) of the 5-HT-induced firing rate in the piriform cortex either when measured during continuous application or when measuring the steady-state response to 30 μ M 5-HT with 15 min durations between consecutive applications (not shown).

2.5. Data analysis

For the intracellular recordings, EPSC frequencies were obtained from 10 consecutive episodes (1 s duration) during the baseline and drug treatment periods. EPSC frequencies were determined with Axograph peak detect software; signals <10 pA were excluded from the measurements. The determination of EC₅₀ values for the suppression of 5-HT-induced increases in EPSC frequency or of evoked EPSCs were calculated by nonlinear curve fitting (Graphpad Prism; www.Graphpad.com). For the extracellular recordings, the total number of spikes for each concentration of 5-HT (10, 30, 100 and 300 μ M) was recorded. Where multiple cells from an individual slice preparation were used, the mean of the multiple cells was used for data analysis. A two-factor (5-HT concentration and drug treatment) repeated measures ANOVA was performed. Post-hoc followup of significant results utilized the Newman-Keuls test. Comparisons for treatment effects on AMPA-mediated responses utilized a paired t-test. Significance levels was set at $p < 0.05$.

3. Results

3.1. Chronic ECS: mPFC

5-HT induced a substantial increase in the frequency of spontaneous EPSCs when recording from layer V pyramidal cells in a slice preparation of the medial prefrontal cortex from both sham ECS- and ECS-treated rats (Fig. 1), consistent with past results in treatment-naïve rats. A significant concentration-related increase in the frequency of 5-HT-induced EPSCs was observed ($F(1,12)=32.84$, $p<0.001$; Fig. 2). The frequency of 5-HT-induced EPSCs was significantly less in the ECS-treated group ($n=7$ with 13 cells sampled; 10 consecutive ECS daily sessions) compared to the sham-treated group ($n=7$ with 10 cells sampled; $F(1,12)=5.08$, $p<0.05$; Fig. 2). The interaction between these two main effects failed to reach significance ($F(1,12)=0.22$, $p>0.1$). The ECS-treated group had a significantly lower response than the sham-treated group at the 10 μM ($p<0.05$, Newman-Keuls test) and the 100 μM 5-HT concentration ($p<0.01$). The basal EPSC frequency for the ECS-treated group (3.95 ± 1.05 N/s; $n=7$) was not significantly different from the sham-treated group (1.62 ± 0.31 N/s; $n=7$). The frequency of AMPA (5 μM)-induced EPSCs was not different between the two treatment groups ($t(10)=-0.67$, $p>0.1$; ECS, $n=6$, 7 cells, 45.83 ± 7.99 N/s; sham, $n=6$, 6 cells, 56.5 ± 13.65 N/s; not shown). Similarly, the amplitude of the steady state inward current induced by AMPA (5 μM) was not different between the two treatment groups ($t(9)=0.26$, $p>0.1$; ECS group, $n=6$; $1,212 \pm 312$ nA; sham group, $n=5$; $1,110 \pm 202$ nA).

3.2. Chronic ECS: piriform cortex

A significant 5-HT concentration-dependent increase in the firing rate of piriform cortical GABAergic interneurons was found ($F(1,12)=39.55$, $p<0.001$). The 5-HT-induced firing rate of piriform cortical interneurons in the ECS-treated animals ($n=4$; 10 cells sampled) was significantly greater than for the sham-treated animals ($n=4$; 7 cells sampled; $F(1,6)=5.40$, $p<0.05$). The interaction between the 5-HT concentration and the group treatment was not significant ($F(2,12)=2.39$, $p>0.1$). Post-hoc testing found that the ECS-treated group had a significantly higher firing rate than the sham-treated group at the 100 μM 5-HT concentrations ($p<0.01$, Newman-Keuls test). Only a trend was present for the difference between the two groups at 30 μM 5-HT ($p=0.077$). In a subset of 3 cells, the 300 μM 5-HT response in cells from subjects treated with ECS was 105% of the 100 μM response of the same ECS-treated cells and 132% of the response to the 300 μM 5-HT sham-treated group in a subset of 3 cells tested. In contrast, the firing rate of cells treated with AMPA (5 μM) was similar for the same in ECS-treated (14.60 ± 0.86 Hz) compared to the sham-treated (10.87 ± 1.2 Hz) subjects ($t(6)=0.13$, $p>0.1$).

3.3. Chronic imipramine: mPFC

In accordance with previous results (Aghajanian & Marek, 1997; Marek & Aghajanian, 1999), 5-HT induced a concentration-related increase in the frequency of excitatory postsynaptic currents (EPSCs) with an EC_{50} of 18.8 μM and a near-maximal response at 100 μM (Fig. 3; $n=3$; 6 cells sampled) when recording from slices obtained from rats treated daily with a vehicle injection for 21 days. A 21-day daily treatment regimen with the TCA imipramine did not alter the EC_{50} for 5-HT-induced EPSCs but did decrease the maximal response to 5-HT by ~63% ($n=4$; 8 cells sampled). The frequency of basal EPSCs was similar for mPFC slices prepared from vehicle-treated (2.7 ± 1.1 N/s) and the imipramine-treated rats (1.7 ± 0.4 N/s, $t(5)=0.95$, $p>0.1$). A repeated measures ANOVA revealed a significant effects of 5-HT ($F(4,20)=27.12$, $p<0.001$), chronic drug treatment with imipramine or vehicle ($F(1,5)=7.60$, $p<0.05$), and 5-HT $\times$ chronic drug treatment interaction ($F(4,20)=5.65$, $p<0.01$). The Newman-Keuls test revealed that a significantly lower frequency of 5-HT-induced EPSCs were recorded from the cells from imipramine-treated rats compared to the vehicle-treated rats at the 30 ($p<0.05$), 100 and 300 μM 5-HT concentrations ($p<0.001$).

Since an alteration of 5-HT-induced EPSCs could be mediated either by a direct effect on 5-HT_{2A} receptors or on AMPA receptors, the effects of chronic imipramine treatment on AMPA-mediated responses in the mPFC was determined in a subset of cells. A 21-day treatment with imipramine (15 mg/kg, ip, n=4; 5 cells sampled) tended to decrease AMPA(5 μ M)-induced EPSCs in the mPFC ($t(5)=2.39$, $p=0.063$; 24.8 ± 8.9 N/s) compared to cells from slices of rats treated with chronic saline (n=3; 3 cells sampled; 62.5 ± 14.2 N/s). Chronic imipramine (n=4; 5 cells sampled) tended to decrease the amplitude of the steady-state inward current induced by AMPA (5 μ M; $t(5)=2.35$, $p=0.066$; 385 ± 51 nA) compared to pyramidal cells from the mPFC of vehicle-treated rats (n=3; 3 cells sampled; 833 ± 216 nA). However, chronic imipramine (n=4; 8 cells sampled) did not alter the frequency of basal EPSCs measured in the absence of 5-HT ($t(5)=0.95$, $p>0.1$; 1.7 ± 0.4 N/s) compared to the chronic vehicle group (n=3; 6 cells sampled; 2.7 ± 1.1 N/s).

3.4. Chronic imipramine: Piriform Cortex

In accordance with previous results, 5-HT induced a concentration-related increase in the firing rate of presumed GABAergic interneurons located near the border of layers II and III of the piriform cortex that was consistent with a near maximal response at 100 μ M (Fig. 3) while the maximal response to 5-HT was decreased slices from the ECS-treated rats. The repeated measures ANOVA revealed a significant effect of 5-HT ($F(4,8)=29.74$, $p<0.001$) and a chronic drug treatment effect ($F(1,4)=50.08$, $p=0.01$). The interaction effect was not significant ($F(2,8)=1.28$, $p>0.1$). In following up the significant imipramine effect, the imipramine-treated group (n=3 with 6 cells sampled, vehicle-treated n=3 with 8 cells sampled) had a significantly lower response for the 100 μ M 5-HT concentrations compared to the chronic vehicle-treated group ($p<0.05$, Newman-Keuls test). There was a trend toward a lower response for the imipramine-treated group at the 30 μ M 5-HT concentration ($p=0.07$). In a subset of cells the effect of 5-HT (300 μ M) was only 57% (13.8 ± 3.3 Hz, n=3) of the vehicle-treated group (22.5 ± 7.8 Hz, n=6). The firing rate of piriform interneurons in response to bath application of AMPA (5 μ M) was not different for the imipramine treated animals (n=3 for 6 sampled cells; 10.76 ± 5.12 N/s) compared to the vehicle-treated animals (n=3 for 8 sampled cells; 15.54 ± 7.07 ; $t(12)=1.38$, $p>0.1$).

4. Discussion

The main finding from the present results was that both similarities and one major difference emerged when comparing the functional effects of subchronic ECS or imipramine on cortical electrophysiological responses mediated by 5-HT_{2A} receptor activation to the known literature of 5-HT_{2A} receptor regulation. As expected, imipramine down-regulated both 5-HT-induced EPSCs in the mPFC and the 5-HT-induced excitation of GABAergic interneurons in the piriform cortex consistent with the widely replicated down-regulation of 5-HT_{2A} receptor binding by tricyclic antidepressants responses (Burnet et al., 1994; Eison et al., 1989; Kendall and Nahorski, 1985; Maj et al., 1996; Subhash and Jagadeesh, 1997). This functional correlation is consistent with previous observations that imipramine and other TCAs attenuated the 5-HT-induced increase in phosphatidyl inositol turnover pathway in the cerebral cortex (Kendall and Nahorski, 1985; Subhash and Jagadeesh, 1997) or the increase in head shakes induced by DOI or other 5-HT_{2A} receptor agonists (Eison et al., 1989; Kitamura et al., 2002; Maj et al., 1996).

Also as expected, the enhancement by ECS for 5-HT-mediated excitation of GABAergic interneurons in the piriform cortex is consistent with the repeated observation of cortical 5-HT_{2A} receptor up-regulation following ECS (Burnet et al., 1995; Burnett et al., 1999; Kellar et al., 1981). In contrast, the main mismatch between the functional electrophysiological data and the widely replicated results for 5-HT_{2A} receptor binding occurred with chronic ECS in

the mPFC where the frequency of 5-HT-induced EPSCs was actually decreased following ten consecutive daily ECS treatments.

With respect to the mismatch between the novel results supporting a functional desensitization of 5-HT_{2A} responses in the mPFC following repeated daily ECS in contrast to the previously well-known up-regulation of 5-HT_{2A} receptor binding, there is relatively little data outside of 5-HT_{2A} agonist-induced head shakes or head twitches to support a functional increase in 5-HT_{2A} receptor activity by repeated ECS treatments in the prefrontal cortex and neocortex. Several studies failed to observe a change in the 5-HT-induced increase in phosphoinositol turnover mediated by the 5-HT_{2A} receptor stimulation in the cortical slices (Godfrey et al., 1988; Newman et al., 1987), although another group reported that ECS increased the 5-HT-stimulated inositol phosphate accumulation (Pandey et al., 1992). Furthermore, another study failed to observe a change in the expression of an immediate early gene (Fos) induced by DOI following chronic ECS (Moorman et al., 1996). Thus, these results do not support the hypothesis that 5-HT_{2A} receptor function is generally enhanced in the prefrontal cortex following chronic ECS. The multitude of neurotransmitter systems which modulate head shakes/twitches induced by 5-HT_{2A} receptor activation further questions assuming that this single behavioral readout of 5-HT_{2A} receptor function (see introduction) is always correlated to changes in cortical 5-HT_{2A} receptor binding such as after repeated ECS treatment. Thus, it is far from clear that the up-regulation of 5-HT_{2A} receptor binding by electroconvulsive therapy is a serious challenge (Duman et al., 1997) to the hypothesis that blockade or down-regulation of 5-HT_{2A} receptor function contributes to the therapeutic effects of antidepressant drugs as previously suggested.

Analogous to the 5-HT_{2A} receptor, both ECS and reserpine increase the density of α_{1B} -adrenoceptors, but not α_{1A} -adrenoceptors, in the cerebral cortex (Blendy et al., 1990; Blendy et al., 1991; Hayakawa et al., 1992; Pandey et al., 1992). The sensitivity of the α_{1B} binding site to the clonidine alkylating analogue CEC suggests that this site uses phosphatidyl turnover as a transduction pathway (Michel et al., 1990). Despite the ECS-induced up-regulation of α_{1B} -adrenoceptors and a higher signal-to-noise ratio for this assay compared to the cortical 5-HT-stimulated PI turnover assay, four of five studies failed to observe an up-regulation of the NE-stimulated phosphoinositide hydrolysis (Blendy et al., 1991; Blendy et al., 1988; Newman et al., 1987; Pandey et al., 1992; Pilc et al., 1988). This point raises a key question as to whether a decoupling of 5-HT_{2A} receptors and α_{1B} -adrenoceptors from the downstream phosphatidyl inositol pathway occurs following repeated ECS treatment.

The TCA imipramine decreased the maximal efficacy of 5-HT both for 5-HT-induced EPSCs in the mPFC and on the firing rate of GABAergic cells in the piriform cortex. These observations are consistent with a plethora of 5-HT_{2A} receptor binding studies in the rodent prefrontal cortex demonstrating a reduction of 5-HT_{2A} receptor abundance following chronic treatment with TCAs. Since 5-HT-induced EPSCs appear much more prominent than 5-HT-induced IPSCs in the prefrontal cortex and neocortex (Aghajanian and Marek, 1997), these findings might be consistent with the hypothesis that antidepressant drugs generally enhance the inhibitory effects of 5-HT. With respect to the cortical pyramidal cells, the inhibitory effects of 5-HT at non-5-HT_{2A} receptors (Aghajanian and Marek, 1997; Araneda and Andrade, 1991; Read et al., 1994; Tanaka and North, 1993) would be expected to outweigh the excitatory effects of 5-HT mediated by 5-HT_{2A} receptor activation. The decreased effects of 5-HT-induced EPSCs recorded from layer V pyramidal cells would also be consistent with previous observations that the burst frequency of field potentials recorded in layers II and III of the rat frontal cortex was decreased by imipramine, and that the attenuation of the effects of locally applied DOI was also present (Bobula et al., 2003; Bobula et al., 2003). In contrast, these same studies also found that daily treatment with imipramine also enhanced the inhibitory effects of 5-HT_{1A} receptor activation. These findings together emphasize that daily treatment with

imipramine would appear to decrease the excitatory effects of glutamate in the neocortex, and these effects may be due in part to an attenuation of 5-HT_{2A} receptor activity concomitant with an enhancement of the inhibitory effects of 5-HT on non-5-HT_{2A} receptors.

Chronic ECS appeared to decrease the potency of serotonin at 5-HT_{2A} receptors and decreased the efficacy of a near-maximal concentration of 5-HT by ~24% in the mPFC. These findings are remarkable in light of the increase in both 5-HT_{2A} receptor mRNA and protein that occurs with chronic ECS. One hypothesis to explain this mismatch is that the increased 5-HT_{2A} receptor protein does not reflect 5-HT_{2A} receptor function due to desensitized receptors as discussed above. An alternate hypothesis is that the majority of the 5-HT_{2A} receptor binding in the prefrontal cortex does not reflect 5-HT_{2A} receptor protein and function for the cellular compartment controlling 5-HT-induced EPSCs. This latter hypothesis would be consistent with the previous demonstration for a dissociation of 5-HT_{2A} receptor binding and 5-HT-induced EPSCs by thalamic lesions. Fiber-sparing medial thalamic lesions increased 5-HT_{2A} receptor agonist binding in the mPFC while the frequency of 5-HT-induced EPSCs was decreased (Marek et al., 2001). This latter hypothesis is interesting as the changes in 5-HT_{2A} receptor binding and function in the mPFC and piriform cortex are only dissociated for the 5-HT-induced EPSCs recorded from layer V pyramidal cells after daily ECS treatment as opposed to the effects of ECS on piriform cortical interneurons or the effects of repeated treatment with imipramine in either the mPFC or piriform cortex.

A further implication of the dissociation between 5-HT_{2A} receptor function in the PFC compared to the piriform cortex following ECS suggests that the cellular phenotype may play an important role in the mode of 5-HT_{2A} receptor regulation. For example, 5-HT_{2A} receptors transfected into different cell types demonstrated distinctly different patterns of receptor regulation to agonists and antagonists (Gray et al., 2001; Grotewiel and Sanders-Bush, 1994). Other recent evidence with mice overexpressing wild-type G_{αq} proteins and RGS insensitive mutant G_{αq} also suggests cell specific regulation of 5-HT_{2A} receptor transduction pathways (Shi et al., 2006).

These differences in the regulation by electroconvulsive shock of the effects of 5-HT_{2A} receptor action on excitatory synaptic currents compared to firing of piriform cortical interneurons could also reflect known differences in 5-HT_{2A} receptor regulation in different cellular compartments. For example, interactions with arrestins may sequester 5-HT_{2A} receptors in the cell bodies of pyramidal neurons (Roth & Xia, 2004). In contrast, interactions of the 5-HT_{2A} receptor with microtubule-associated protein-1A (MAP-1A) may sequester inactivated 5-HT_{2A} receptors inside the dendritic compartment (Roth and Xia, 2004). The response of the piriform cortical interneurons is likely mediated by somatodendritic 5-HT_{2A} receptors. Mechanisms controlling regulation of 5-HT_{2A} receptors in axonal compartments such as the mesocortical dopamine pathway originating from the ventral tegmental area (Bortolozzi et al., 2005; Nocjar et al., 2002; Pehek et al., 2001; Pehek et al., 2006) have not been delineated.

The localization of 5-HT_{2A} receptors mediating the excitatory synaptic currents when recording from prefrontal layer V pyramidal cells is not clear. Recent work has suggested that a retrograde influence of apical dendritic 5-HT_{2A} receptors in pyramidal cells does not lead to glutamate release in the prefrontal cortex (Beique et al., 2007). Other work involving rescue of cerebral cortical 5-HT_{2A} receptors in mice with a global disruption of 5-HT_{2A} receptors has seemingly suggested that cortical 5-HT_{2A} receptors are sufficient to induce 5-HT-induced excitatory synaptic currents (Weisstaub et al., 2006). However, these results do not rule out an afferent source to the prefrontal cortex and the neocortex since the claustrum was a region where 5-HT_{2A} receptors were rescued. The claustrum contains glutamatergic principle cells which projects throughout the cortex to layers I and V. Layers I and Va in the PFC and throughout the neocortex are near the “hot spots” for 5-HT-induced EPSCs. Finally, the

observations that lesions of the midline thalamus result in ~ a 60% decrease in the frequency of 5-HT-induced EPSCs (Lambe and Aghajanian, 2001; Marek et al., 2001) also challenges the hypothesis that the synaptic currents induced by 5-HT_{2A} receptors is simply an intracortical process.

In summary, these results are consistent with the suggestion that a relative enhancement of the inhibitory effects of 5-HT in limbic cortical circuits may, in part, mediate the effects of most classes of antidepressant treatments, including imipramine and ECS (Bobula et al., 2003; Chaput et al., 1986; De Montigny and Aghajanian, 1978; Haddjeri et al., 1998; Rueter et al., 1998). The complexity of the serotonergic receptor family makes it likely that an attenuation of receptors mediating excitatory effects such as the 5-HT_{2A} receptor and an enhancement of non-5-HT_{2A} receptors mediating inhibitory effects play a role in the long-term effects of antidepressant drugs. Furthermore, increasing attention to how these 5-HT receptors modulate glutamatergic and GABAergic neurotransmission will likely be critical to understanding how different monoaminergic receptors mediate the effects of known antidepressant drugs.

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Sham ECS: basal

Sham ECS: 100 μ M 5-HT

Chronic ECS: basal

Chronic ECS: 100 μ M 5-HT

300 pA

200 ms

Figure 1.

Effect of daily repeated electroconvulsive shock (ECS) or sham ECS on 5-HT-induced EPSCs recorded from layer V pyramidal cells of the mPFC. Shown in are three consecutive one second recordings under voltage clamp conditions for a cell from a sham-ECS treated rat under basal conditions (top traces) and 100 μ M 5-HT (second set of traces) as well as recordings for a cell from a rat treated with daily ECS for 10 days under basal conditions (third set of traces) and after 100 mM 5-HT (fourth set of traces).

Fig. 2.

Alteration in electrophysiological prefrontal cortical and piriform cortical electrophysiological responses mediated by 5-HT_{2A} receptors. The top left and top right graphs show the contrasting effects recorded in cells from rats treated daily for 10 days with ECS (circles, dashed lines) vs. sham ECS treatment (squares, solid line) in the mPFC (excitatory synaptic currents) and piriform cortex (increased firing of GABAergic interneurons) respectively. The frequency of EPSCs recorded from layer V pyramidal cells was significantly lower in the ECS-treated at both the 10 and 100 μM 5-HT concentrations. The bottom left and bottom right graphs show the similar effect where daily treatment for 21 days with imipramine (15 mg/kg, ip) decreased responses to 5-HT in both the mPFC and piriform cortex, respectively. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to the sham ECS- or vehicle-treated group.

Fig. 3.

Daily administration of ECS increases the excitation of piriform cortical interneurons in response to 5-HT. The top tracings show a concentration-dependent increase in the firing rate (N/s) of a piriform cortical interneuron in response to 5-HT from a cell from a sham-ECS treated rat. Also shown is the response to the same cell to a 5 μ M AMPA bath perfusion for 60 s. The numbers displayed by each individual peak indicates the maximal amplitude of the firing rate over a 10 s interval. Temporal discontinuity in the tracing is indicated by diagonal lines representing that a longer time interval between drug applications was present than actually shown. The bottom tracings show the 5-HT concentration-response relationship for a piriform cortical interneuron from a rat treated daily with ECS for 10 consecutive days. The brain slices from the animals treated for these two separate experiments were recorded on consecutive days.