Noradrenergic Augmentation of Escitalopram Response by Risperidone: Electrophysiologic Studies in the Rat Brain

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Background: Atypical antipsychotic drugs have been used in depressed patients not responding adequately to the selective serotonin reuptake inhibitors (SSRIs). The aim of the current study was to investigate putative mechanisms of the beneficial effect of atypical antipsychotic drugs during their co-administration with SSRIs. In previous electrophysiological studies, it was found that SSRIs decrease, while atypical antipsychotics increase, norepinephrine neuronal firing. Thus, the resistance to SSRIs could be explained, at least in part, by the SSRI-induced decrease of norepinephrine neuronal firing activity, and the beneficial effect of atypical antipsychotic drugs could be explained by the reversal of the above-mentioned suppression of firing.

Methods: Rats were administered the SSRI escitalopram and the atypical antipsychotic drug risperidone. Norepinephrine neuronal activity was determined using in vivo electrophysiology.

Results: Subacute and long-term escitalopram decreased, while risperidone co-administered with escitalopram increased, norepinephrine neuronal firing. Attempts at reversing the escitalopram-induced decrease of firing with various selective antagonists revealed that the serotonin-2A receptor antagonistic property of risperidone may mediate the pronoradrenergic action of atypical antipsychotics in the presence of serotonin reuptake inhibition.

Conclusions: Risperidone reverses escitalopram-induced inhibition of norepinephrine neuronal activity by a mechanism involving serotonin-2A receptors. This reversal may explain the beneficial effect of atypical antipsychotics in treatment-resistant depression.

Key Words: Escitalopram, locus coeruleus, risperidone, selective serotonin reuptake inhibitors, serotonin, serotonin-2A receptor

Selective serotonin reuptake inhibitors (SSRIs) have been successfully used in the treatment of depressive and anxiety disorders for more than 20 years. However, many depressed patients do not respond to SSRIs and various augmentation strategies have been used to obtain optimal therapeutic effects (Blier 2005; Hirschfeld et al 2002; Kennedy and Lam 2003). Recently, atypical antipsychotic drugs have been reported to be effective in SSRI-resistant depression (Kennedy and Lam 2003; Nemeroff 2005; Shelton and Stahl 2004; Tohen et al 2003). However, the mechanism enabling atypical antipsychotic drugs to potentiate the antidepressant response in such patients is not well understood.

The family of atypical antipsychotics is represented by aripiprazole, clozapine, olanzapine, risperidone, quetiapine, and ziprazidone. All these medications act as dopamine-2 (D_2) and serotonin (5-HT) 2A receptor ($5\text{-}HT_{2A}$) antagonists (Bymaster et al 1996a, 1996b; Hirose and Kikuchi 2005; Janssen et al 1988; Uzun et al 2005). Classical antipsychotics, such as haloperidol, block only D_2 receptors and show no benefit as an augmentation strategy, unless psychotic symptoms are present. Therefore, it is unlikely that the augmentation effect of atypical antipsychotic drugs is mediated by D_2 receptor antagonism. Given that all atypical antipsychotics are effective in treatment-resistant depression (Nemeroff 2005; Papakostas et al 2005; Simon and Nemeroff 2005; Tohen et al 2003) but that only clozapine, risperidone, and quetiapine are potent α_2-adrenoceptor blockers (Schotte et al 1996), their beneficial effect may not be explained by antagonism of this receptor. Thus, it appears that $5\text{-}HT_{2A}$ receptor antagonism could account for their clinical benefits in mood disorders, because all atypical antipsychotics share this property. Therefore, it was hypothesized that the potentiating effect of atypical antipsychotic drugs in SSRI-resistant depressed patients is mediated via their effect on $5\text{-}HT_{2A}$ receptors (Szabo and Blier 2002).

In previous electrophysiological studies, it was demonstrated that $5\text{-}HT_{2A}$ receptors play an important role in the interaction between 5-HT and norephinephrine (NE) systems in the brain (Szabo and Blier 2001a). Serotonin 2A receptors are expressed on γ-aminobutyric acid (GABA) cells innervating NE neurons in the locus coeruleus (LC). It was suggested that the elevation in synaptic availability of 5-HT results in activation of GABA interneurons followed by inhibition of NE neuronal activity (Szabo and Blier 2001b). It is thus conceivable that the inhibition of NE neuronal firing, caused by 5-HT reuptake inhibition (Szabo et al 2000), could explain, in some patients, the lack of optimal response to SSRIs.

In contrast to SSRIs, which induce an inhibition of NE neuronal firing, long-term risperidone (Nasif et al 2000) and acute olanzapine (Dawe et al 2001) increase NE neuronal firing. Risperidone and the SSRI escitalopram were thus administered to laboratory rats, alone and in combination, and electrophysiological recordings of NE neurons were then obtained. To identify the receptor(s) involved in the alteration of NE neuronal firing by atypical antipsychotics in the presence of escitalopram, the effect of serotonin 2C ($5\text{-}HT_{2C}$), $5\text{-}HT_{2A}$, D_2, and α_2-adrenergic receptor antagonists were examined alone and in combination with escitalopram.

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Methods and Materials

Animals

The experiments were carried out in male Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) weighing between 300 and 350 g were kept under standard laboratory conditions (12:12 hour light-dark cycle with access to food and water ad libitum). All animal procedures were approved by the Ottawa Health Research Institute Animal Care Committee and were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

Drugs

The SSRIs citalopram and escitalopram (Lundbeck, Copenhagen, Denmark) were dissolved in distilled water and administered via osmotic minipumps (Alza, Palo Alto, California). The pumps were implanted subcutaneously (SC) under isoflurane (Abbot, Montreal, Quebec, Canada) anesthesia. The antagonists of α_2-adrenergic receptors, idazoxan (Sigma, St. Louis, Missouri); of 5-HT_{2C} receptors, 6-chloro-5-methyl-1[(2-[2-methylpyrid-3-yloxy]pyrid-5yl)carbamoyl]indoline (SB 242084) (Sigma); and of 5-HT_{2A} receptors, R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinem ethanol (M100907, formerly MDL 100907) (Marion Merrell Dow, Cincinnati, Ohio) were dissolved in distilled water. The antipsychotic drugs risperidone (Janssen, Titusville, New Jersey) and haloperidol (Sigma) were dissolved in 10% tartaric acid (Sigma) at pH 1 and then in distilled water (1:100) and the pH normalized.

Treatments

Escitalopram and risperidone were administered for 2 and 14 days at daily doses of 10 mg/kg and 1 mg/kg, respectively, alone or in their combination. Citalopram was given at 40 mg/kg/day for 2 days. Control animals were implanted with minipumps containing distilled water. The M100907 and SB 242084 were given SC at .5 mg/kg/day for 2 days alone and in combination with escitalopram. Haloperidol was administered SC (.1 mg/kg daily) for 2 days alone and in combination with escitalopram. Idazoxan was administered subcutaneously (1 mg/kg daily) for 2 days alone and in combination with escitalopram. The last injection was given one hour prior to electrophysiological readings. The doses of escitalopram, risperidone, haloperidol, M100907, and SB 242084 were chosen on the basis of previous studies (Andersson et al 1994; Cremers et al 2004; El Mansari et al 2005; Hatanaka et al 2000; Nilsson et al 2005; Zhang et al 2000).

Inhibition of 5-HT Synthesis

Serotonin synthesis inhibition was performed using intraperitoneal administration of (±)-p-chlorophenylalanine (PCPA) (Sigma). The PCPA was dissolved in 40% solution of (2-hydrosypropil)-β-cyclodextrin (Acros, Geel, Belgium) and given at 300 mg/kg/day for 3 days, as previously described (Chaput et al 1990). To characterize the effect of escitalopram and risperidone on NE neuronal firing during 5-HT synthesis inhibition, these compounds were administered at 10 mg/kg/day and 1 mg/kg/day, respectively, for 2 days starting from the second day of PCPA administration.

Electrophysiological Experiments

Rats were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal) (Sigma) and mounted in a stereotaxic apparatus (David Kopf Instuments, Tujunga, California). Supplemental doses were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37°C throughout the experiments utilizing a thermostat-controlled heating pad (Seabrook Medical Instruments, Cincinnati, Ohio). Extracellular unitary recordings of NE neurons were conducted with single-barreled glass electrodes filled with a 2 mol/L NaCl solution. Their impedance range was between 4 and 6 megohms. A 2-mm burr hole was drilled .5 mm posterior to lambda and 1 mm lateral to midline for NE neurons recordings. Bleeding from disruption of the sagittal sinus was immediately stopped using bone wax. Norepinephrine neurons were recorded with micropipettes lowered at .5 to .7 mm posterior to interaural and .8 to 1.1 lateral to midline. Spontaneously active NE neurons of the LC were identified using the following criteria: regular firing rate (.5–5.0 Hz) and positive action potential of long duration (.8–1.2 milliseconds) exhibiting a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw. First locating the mesencephalic fifth motor nucleus neurons that respond to lower jaw depression and moving medially to record LC NE neurons provided additional indication for the validity of the site of recordings. Norepinephrine neurons were recorded for at least 1 minute following stabilization to establish basal firing rate.

Analysis of Burst Firing

The firing patterns of NE neurons were analyzed by interspike interval burst analysis (Grace and Bunney 1984). The onset of a burst was defined as the concurrence of two spikes with interspike interval shorter than .08 seconds. The termination of burst was defined as an interspike interval of .16 seconds or longer (Dawe et al 2001).

Statistical Analysis

All results were expressed as means (± SEM) of single neuron values. Statistical comparisons between the differences in NE neuron firing were carried out using two-tailed Student t test or two-way analysis of variance (treatment with vehicle or escitalopram versus co-treatment with risperidone, haloperidol, idazoxan, M100907, SB 242084, or no co-treatment). Statistically significant differences were determined using the $p < .05$ criterion.

Results

Effect of 2-Day Escitalopram and Citalopram Administration on NE Neuronal Firing Activity

The firing rate of NE neurons in control rats was 1.53 ± .20 Hz ($n = 137$ neurons from 25 rats). The rate and the pattern of the firing (Figure 1) were typical for NE neurons, as previously described (Dawe et al 2001). A 2-day regimen of 10 mg/kg/day of escitalopram significantly ($n = 11$ and $n = 9$ rats, respectively; $p < .001$) decreased firing rate of NE neurons (Figure 2). In contrast, a regimen of 20 mg/kg/day was without effect in the same paradigm in a prior study (Szabo et al 2000). Therefore, to rule out that potency difference was explaining the robust effect of escitalopram, 40 mg/kg/day of citalopram was tested. This still did not attenuate the firing rate of NE neurons in the LC ($n = 5$ rats, Figure 2). To ascertain whether the decrease was due to an enhancement of synaptic 5-HT, rats were given the 5-HT synthesis inhibitor PCPA prior to the administration of escitalopram. The PCPA pretreatment reversed the escitalopram-induced inhibition of firing rate of NE neurons ($n = 8$ rats, Figure 2).

Effect of 2-Day Risperidone on NE Neuronal Firing Activity in Vehicle-Treated and Escitalopram-Treated Rats

When combined with escitalopram, risperidone increased the firing rate of NE neurons in comparison with that of control