European Neuropsychopharmacology (2009) **19**, 177–187

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Electrophysiological characterization of the effects of asenapine at 5-HT_{1A}, 5-HT_{2A}, α_2-adrenergic and D_2 receptors in the rat brain

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Received 8 August 2008; received in revised form 23 September 2008; accepted 4 November 2008

KEYWORDS
Asenapine;
Schizophrenia;
Dopamine;
Norepinephrine;
Serotonin;
Electrophysiology

Abstract

Asenapine is a psychopharmacologic agent being developed for schizophrenia and bipolar disorder. This study electrophysiologically characterized the *in vivo* effects of asenapine at dorsal raphe nucleus (DRN) and hippocampus serotonin-1A (5-HT_{1A}), ventral tegmental area D_2, locus coeruleus 5-$HT_{2A,}$ and α_2-adrenergic receptors in anesthetized rats. Asenapine displayed potent antagonistic activity at α_2-adrenoceptors (ED_{50}, 85 ± 2 μg/kg), 5-HT_{2A} (ED_{50}, 75 ± 2 μg/kg) and D_2 receptors (ED_{50}, 40 ± 2 μg/kg) as evidenced by its reversal of clonidine-, DOI-, and apomorphine-induced inhibition of norepinephrine and dopamine neurons. In contrast, asenapine acted as a partial agonist at 5-HT_{1A} receptors in DRN and hippocampus, as indicated by blockade of its inhibitory effect on neuronal firing by the 5-HT_{1A} antagonist WAY 100635 and the partial inhibition of the suppressant action of 5-HT when co-applied by microiontophoresis. These results confirm that asenapine displays potent antagonistic activity at 5-HT_{2A}, D_2, α_2-adrenergic receptors and provide evidence to support its 5-HT_{1A} partial agonistic activity.

1. Introduction

Schizophrenia is a complex disorder characterized by positive and negative signs as well as cognitive impairment. Conventional antipsychotic medications have been the backbone of the pharmacotherapy of this debilitating disease for decades (Kapur and Mamo, 2003). Despite the fact that classical dopamine (DA) D_2 receptor antagonists, such as haloperidol, dampen the symptoms of schizophrenia, their clinical use is associated with major drawbacks such as

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); DRN, dorsal raphe nucleus; LC, locus coeruleus; NE, norepinephrine; SSRI, selective serotonin reuptake inhibitor; VTA, ventral tegmental area; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin; WAY100635, N-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride; DOI, ([1-(2,5-dimethoxy-4-iodophenyl-2-aminopropane hydrochloride].

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doi:10.1016/j.euroneuro.2008.11.001

extrapyramidal symptoms (EPS), tardive dyskinesia, and low efficacy against negative symptoms (Meltzer, 1995). The new generation of atypical antipsychotics has a combination of pharmacological activities at DA, serotonin (5-HT), and noradrenergic receptors (Newman-Tancredi et al., 1996; Schotte et al., 1996; Shahid et al., 2008). Indeed, the pharmacological features of atypical antipsychotics produce advantages over the typical agents in managing schizophrenia (Kane et al., 1988; Davis et al., 2003). Thus, the atypical antipsychotics have become the first-line treatment of schizophrenia (Conley and Kelly, 2002; Kane et al., 2003).

A unique characteristic of atypical antipsychotics is their superior antagonistic effect at 5-HT_{2A} than at D_2 receptors. The antagonistic effect of these drugs at 5-HT_{2A} receptors is also important in the treatment of patients with unipolar and bipolar depression (Blier, 2005). This pharmacological feature leads to the reversal of the inhibitory action of selective serotonin reuptake inhibitors (SSRIs) on locus coeruleus (LC) norepinephrine (NE) firing (Szabo et al., 1999; Dremencov et al., 2007a) and release of NE (Hatanaka et al., 2000; Kawahara et al., 2007). Indeed, the selective 5-HT_{2A} receptor antagonist MDL 100907 significantly increases NE release in the rat forebrain in the presence of 5-HT reuptake inhibition (Hatanaka et al., 2000). Another important pharmacological characteristic of certain atypical antipsychotics such as clozapine is 5-HT_{1A} receptor agonism (Newman-Tancredi et al., 1996, 1998), which enhances DA neurotransmission. Consistent with this view, administration of 5-HT_{1A} agonists enhanced DA and acetylcholine release in rat medial prefrontal cortex (mPFC) (Ichikawa et al., 2001, 2002; Diaz-Mataix et al., 2005), whereas the 5-HT_{1A} antagonist WAY 100635 abolished the enhanced cortical DA release of 5-HT_{1A} agonists (Ichikawa et al., 2001) and that of clozapine (Rollema et al., 1997).

Some atypical antipsychotics, such as clozapine, risperidone and quetiapine, are also potent α_2-adrenergic receptor antagonists (Schotte et al., 1996). This feature may have clinical relevance since antagonism of α_2-adrenergic autoreceptors enhances LC NE tone. The α_2-adrenergic autoreceptors, located on the cell body and terminals of NE neurons, exert an inhibitory role on the firing rate of NE neurons and release of NE, respectively. Indeed, the selective α_2-adrenoreceptor antagonist idazoxan attenuates the inhibitory effect of these autoreceptors, resulting in an increase in the firing rate of LC NE neurons and NE release in postsynaptic areas (Freedman and Aghajanian, 1984). Interestingly, these observations supported the finding that clozapine increases plasma NE in patients with schizophrenia (Pickar et al., 1992). Moreover, Litman and colleagues showed that addition of idazoxan to the typical antipsychotic fluphenazine significantly improved the positive and negative signs of schizophrenic treatment-resistant patients (Litman et al., 1996).

Atypical antipsychotics in current use have significant limitations in regard to efficacy as well as serious side effects, such as prolactin elevation, increased blood lipids, weight gain, and sedation or activation which complicate their clinical utility and may be a strong driver for high rate treatment discontinuation. Thus, new therapeutic agents with improved efficacy and side effect profiles are still desired. Asenapine is a psychopharmacologic agent being developed for the treatment of schizophrenia (Potkin et al., 2007) and bipolar disorder (McIntyre et al., 2008). Its distinctive receptor binding profile shows a unique combination of very high affinity for 5-HT, DA and adrenergic receptors (Shahid et al., 2008). The affinity of asenapine for most of these receptors is in the subnanomolar range, suggesting that it may be endowed with effectiveness in schizophrenia and mood disorders. To date there are limited data from *in vivo* models on the functional effects of asenapine on specific receptor subtypes. The present experiments were thus undertaken to explore the *in vivo* electrophysiological effects of asenapine at 5-HT_{1A}, 5-HT_{2A}, α_2-adrenergic and D_2 receptors in the rat brain.

2. Experimental procedures

2.1. Animals

The electrophysiological experiments were carried out in male Sprague-Dawley rats (Charles River, St. Constant, QC, Canada) weighing between 250 and 350 g. The rats were kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water *ad libitum*). Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). Supplemental doses (100 mg/kg, i.p.) were given to maintain constant anesthesia and to prevent any nociceptive reaction to pinching of the hind paws. Body temperature was maintained at 37 °C throughout the experiment utilizing a thermistor-controlled heating pad. Prior to the electrophysiological recordings, a catheter was inserted in a lateral tail vein for systemic i.v. injection of pharmacologic agents. All the experiments were approved by the local Animal Care Committee and conducted in accordance with the Canadian Council on Animal Care, for the care and use of laboratory animals.

2.2. Unitary extracellular recording from dorsal raphe nucleus (DRN) 5-HT, LC NE and ventral tegmental area (VTA) DA neurons

The extracellular recordings of 5-HT, NE and DA neurons were obtained using single-barrel glass micropipettes. The tips of the electrodes were broken back to 1–3 μm and filled with 2 M NaCl solution. The impedance of the electrodes was between 4 and 7 MΩ.

2.3. Recording of DRN 5-HT neurons

Single-barreled microelectrodes were positioned 1 mm anterior to lambda on the midline and lowered into the DRN. The DRN 5-HT neurons were encountered over a distance of 1 mm immediately below the ventral border of the Sylvius aqueduct, and identified by their slow (0.5–2.5 Hz), regular firing rate and long duration (0.8–1.2 ms) positive action potential (Aghajanian, 1978).

2.4. Recording of LC NE neurons

LC NE neurons were recorded with single-barreled glass micropipettes positioned at 1.1–1.2 mm posterior to lambda and 1.1–1.3 mm to the midline suture. These neurons were encountered at a depth of 4.5 to 6.0 mm from the surface of brain. The NE neurons were identified by their regular firing rate (0.5–5 Hz), a biphasic action potential of long duration (0.8–1.2 ms), and a characteristic burst discharge followed by a quiescent period in response to a nociceptive pinch of the contralateral hind paw (Aghajanian and Vandermaelen, 1982).

2.5. Recording of VTA DA neurons

The VTA DA neurons were recorded with single-barreled glass micropipettes lowered at 3.0–3.6 mm anterior to lambda and 0.6–0.8 mm to the midline suture. These neurons were encountered at the depth of 6.0 to 8.5 mm from the surface of brain. The DA neurons