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Asenapine alters the activity of monoaminergic systems following its subacute and long-term administration: An *in vivo* electrophysiological characterization

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Abstract
Asenapine is a tetracyclic atypical antipsychotic used for treatment of schizophrenia and mania. Previous *in vivo* electrophysiological studies demonstrated antagonistic action of asenapine at dopamine D_2, serotonin $(5\text{-HT})_{2A}$, and α_2-adrenergic receptors. Here, we assessed monoamine system activities after two-day and 21-day asenapine administration at a dosage (0.1 mg/kg/day) resulting in clinically relevant plasma levels. In the ventral tegmental area (VTA), asenapine increased the number of spontaneously active dopamine neurons, while firing parameters remained unchanged. Asenapine partially prevented the D_2 autoreceptor-mediated inhibitory response to apomorphine after two days of administration. This effect was lost after 21 days of administration, suggesting adaptive changes leading to D_2 receptor sensitization. Asenapine increased the firing activity of noradrenergic neurons in the locus coeruleus (LC) after 21, but not two days of administration. Furthermore, it potently blocked 5-HT_{2A} receptors while α_2-adrenergic receptors were unaffected by this drug regimen. Both acute and long-term asenapine administration partially blocked α_2-adrenergic receptors in the CA3 region of the hippocampus, and noradrenergic tone on α_1- and α_2-adrenoceptors remained unchanged. In the dorsal raphe nucleus, asenapine increased the firing rate of 5-HT neurons after two, but not 21 days of administration. In addition, responsiveness of 5-HT_{1A} autoreceptors was unaltered by asenapine. In the hippocampus, 21-day asenapine administration increased serotonergic tone by partial agonistic action on postsynaptic 5-HT_{1A} and terminal 5-HT_{1B} receptors. Taken together, asenapine had profound effects on both catecholamine systems, potently blocked

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5-HT_{2A} receptors, and enhanced 5-HT tone, effects that could be important in treatment of mood disorders and schizophrenia.

1. Introduction

Asenapine (ORG-5222) is indicated for treatment of schizophrenia and acute manic or mixed episodes associated with bipolar 1 disorder as monotherapy or in combination with lithium or sodium valproate. It has a similar affinity for dopamine (DA) D_2 receptors as the prototypical antipsychotic haloperidol, and a higher affinity for several other receptors compared to D_2 receptor affinity. These are, in descending order, serotonin $(5\text{-HT})_{2C}$, 5-HT_{2A}, 5-HT_7, 5-HT_{2B}, 5-HT_6, α_{2B}-adrenoceptors, D_3, H_1, D_4, α_2, α_{1A} and α_{2C} receptors (Shahid et al., 2009). In accordance with negligible action of asenapine on cholinergic receptors, asenapine has little metabolic side-effects in the clinic (Schoemaker et al., 2012) – in contrast to the effects of clozapine, olanzapine and risperidone (Kinon et al., 2001; Potkin et al., 2007). Furthermore, the risk for development of dopaminergic side effects (extrapyramidal symptoms) commonly associated with D_2 receptor antagonism is relatively benign for asenapine, possibly due to its abovementioned activities at a variety of non-dopaminergic receptors acting within the striatonigral pathway (Kane et al., 2010; Tarazi and Stahl, 2012). In this context, stronger antagonistic action on 5-HT_{2A} compared to D_2 receptors is thought to be an important pharmacological feature, which is shared by all atypical antipsychotics (Meltzer et al., 2003).

These clinical findings are consistent with results from animal studies. For example, asenapine suppressed escape behavior in the conditioned avoidance response (CAR; a model for antipsychotic action) while it failed to induce catalepsy (a model for extrapyramidal symptoms) when administered in the dose range of 0.05–0.2 mg/kg (Frånberg et al., 2008). Furthermore, asenapine effectively reversed apomorphine-induced prepulse inhibition and amphetamine-induced locomotion at a dose as low as 0.03 mg/kg, suggesting potent antagonistic action at D_2 receptors (Marston et al., 2009). Subchronic asenapine (0.2 mg/kg/day for 5 days) reduced escape behavior in the CAR up to 40 days after drug administration, a result attributable to D_2 receptor sensitization associated with antipsychotic action (Gao and Li, 2013). In addition, four-week asenapine administration restored phenylcyclidine (PCP)-induced impairments in reversal learning at a dose of 0.075 mg/kg/day and restored sucrose consumption in animals subjected to chronic mild stress (CMS) at a dose of 0.6 mg/kg/day, suggesting therapeutic potential of asenapine in hedonic and cognitive domains (Marston et al., 2011; McLean et al., 2010).

Previous *in vivo* electrophysiological studies showed that acute asenapine administration increased the firing activity of DA neurons in the ventral tegmental area (VTA) and reversed the inhibitory effect of the DA agonist apomorphine ($ED_{50}=40\pm2$ μg/kg), demonstrating *in vivo* antagonistic action of asenapine at D_2 autoreceptors (Ghanbari et al., 2009; Frånberg et al., 2009). Similarly, asenapine increased the firing activity of locus coeruleus (LC) norepinephrine (NE) neurons and reversed the inhibitory effect of the α_2-adrenoceptor agonist clonidine on these neurons ($ED_{50}=85\pm2$ μg/kg), putting antagonistic activity at α_2-adrenergic autoreceptors into evidence (Ghanbari et al., 2009; Frånberg et al., 2009). Asenapine also reversed the inhibitory effect of the preferential 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI; $ED_{50}=75\pm2$ μg/kg), indicating antagonistic action of asenapine at this receptor subtype (Ghanbari et al., 2009). In support of this, local perfusion of the prefrontal cortex with asenapine blocked both α_2-adrenergic and 5-HT_{2A} receptors (Frånberg et al., 2012). Although acute systemic administration of asenapine did not alter the firing rate of 5-HT neurons in the dorsal raphe nucleus (DRN; Frånberg et al., 2009; Ghanbari et al., 2009), it acted as a partial 5-HT_{1A} agonist when applied microiontophoretically in both the DRN and hippocampus (Ghanbari et al., 2009). Agonistic activity at 5-HT_{1A} receptors together with D_2 and α_2-adrenergic receptor antagonism provides a mechanism by which asenapine increases prefrontal DA and NE levels (Frånberg et al., 2009), a common effect of acute antipsychotic drug administration (Westerink et al., 1998).

To extend insight in the long-term effects of asenapine on therapeutically relevant pharmacological targets, the present study investigated the effects of two and 21 day asenapine administration (0.1 mg/kg/day) on the discharge activity of VTA DA, LC NE, and DRN 5-HT neuron populations. In these brain regions, the status of D_2, 5-HT_{1A}, 5-HT_{2A}, and α_2-adrenergic receptors was characterized using pharmacological tools. In the CA3 region of hippocampus, a major monoaminergic projection site, serotonergic and noradrenergic neurotransmission was assessed following 21-day asenapine administration.

2. Experimental procedures

2.1. Animals

Experiments were carried out in male Sprague-Dawley rats (Charles River, St. Constant, QC, Canada) weighing 300–450 g housed under standard laboratory conditions (12:12 light-dark cycle with food and water *ad libitum*). *In vivo* extracellular unitary recordings were carried out in chloral hydrate anaesthetized rats (400 mg/kg; i.p.) that were mounted in a stereotaxic apparatus. Body temperature was maintained at 37 °C throughout the experiment utilizing a thermistor-controlled heating pad. If applicable, prior to the electrophysiological recordings a catheter was inserted in a lateral tail vein for systemic intravenous (i.v.) injection of pharmacologic agents. At the end of experiments, animals were euthanized by a lethal dose of chloral hydrate (4% solution, i.p.) and a blood and brain sample was collected. All experiments were carried out in accordance with the Canadian Council on Animal Care and the local Animal Care Committee (University of Ottawa, Institute of Mental Health Research, Ottawa, Ontario, Canada).

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