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Molecular mechanisms of serotonergic action of the HIV-1 antiretroviral efavirenz

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ABSTRACT

Efavirenz is highly effective at suppressing HIV-1, and the WHO guidelines list it as a component of the first-line antiretroviral (ARV) therapies for treatment-naïve patients. Though the pharmacological basis is unclear, efavirenz is commonly associated with a risk for neuropsychiatric adverse events (NPAEs) when taken at the prescribed dose. In many patients these NPAEs appear to subside after several weeks of treatment, though long-term studies show that in some patients the NPAEs persist. In a recent study focusing on the abuse potential of efavirenz, its receptor psychopharmacology was reported to include interactions with a number of established molecular targets for known drugs of abuse, and it displayed a prevailing behavioral profile in rodents resembling an LSD-like activity. In this report, we discovered interactions with additional serotonergic targets that may be associated with efavirenz-induced NPAEs. The most robust interactions were with 5-HT_{3A} and 5-HT_6 receptors, with more modest interactions noted for the 5-HT_{2B} receptor and monoamine oxidase A. From a molecular mechanistic perspective, efavirenz acts as a 5-HT_6 receptor inverse agonist of G_s-signaling, 5-HT_{2A} and 5-HT_{2C} antagonist of G_q-signaling, and a blocker of the 5-HT_{3A} receptor currents. Efavirenz also completely or partially blocks agonist stimulation of the M_1 and M_3 muscarinic receptors, respectively. Schild analysis suggests that efavirenz competes for the same site on the 5-HT_{2A} receptor as two known hallucinogenic partial agonists (±)-DOI and LSD. Prolonged exposure to efavirenz reduces 5-HT_{2A} receptor density and responsiveness to 5-HT. Other ARVs such as zidovudine, nevirapine and emtricitabine did not share the same complex pharmacological profile as efavirenz, though some of them weakly interact with the 5-HT_6 receptor or modestly block $GABA_A$ currents.

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1. Introduction

Efavirenz [(4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one] is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) and one of the preferred components of highly active antiretroviral therapy (HAART) [1,2]. Though very effective at suppressing replication of the virus that causes AIDS, a standard dose of efavirenz is known to carry a significant risk for CNS-mediated NPAEs [3–8]. However, little is known from a mechanistic perspective as to why these NPAEs occur and which CNS off-targets might be involved. The findings of a previous report [9] led us to examine the molecular

Abbreviations: NPAEs, neuropsychiatric adverse events; ARV, antiretroviral; HAART, highly active antiretroviral therapy; ZDV, zidovudine; FTC, emtricitabine; NVP, nevirapine; CNS, central nervous system.

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mechanisms of efavirenz across a broader range of serotonergic targets.

Though efavirenz is considered to have good CNS penetration [10] consistent with its physicochemical properties, only low levels are detected in cerebral spinal fluid (CSF) with a mean concentration across studies of approximately 44 nM that corresponds to CSF levels 0.52% of plasma concentrations [11–13]. Efavirenz has a very high tendency to bind proteins (e.g, approximately 99.8% is bound to plasma proteins) [14,15], and healthy CSF contains very little protein compared to that in blood plasma (<1%). Since the concentration of free efavirenz in the aqueous fraction is expected to be low and it binds to protein-rich brain tissue [16], one might expect that 44 nM in CSF, if it accounted for approximately 0.2% (100–99.8%) of the efavirenz dose, would correspond to an estimated 22 μM (44 nM ÷ 0.002) concentration of efavirenz in brain tissue. A study in rats suggests that efavirenz readily accumulates in the brain to levels that exceed 4.6 times the plasma levels within 1 h of an *i.p.* dose of 15 mg/kg [16]. Physiological effects have been reported in rodent studies at efavirenz doses in the same range: 10–30 mg/kg depending upon the behavioral measure examined [9,17]. High plasma levels of efavirenz (1–4 μg/mL) are needed to keep the HIV-1 virus suppressed to clinically meaningful levels [18], but efavirenz also has a narrow therapeutic window [3]: plasma levels less than 1 μg/mL result in virologic failure while those greater than 2.74 μg/mL increase the risk of NPAEs [3,18]. Assuming a similar level of brain accumulation occurs in humans as in rats, then efavirenz plasma levels >2.74 μg/mL would correspond to brain concentrations >40 μM (2.74×10^{-3} g/L × 1/315.7 g/mole × 4.6). Based upon these types of assumptions and calculations, it would appear that efavirenz can rapidly accumulate in the brain to concentrations in the range of tens of micromolars. Rapid accumulation of relatively high concentrations of efavirenz in the brain and evidence of CNS behavioral effects in animals and humans suggest that this reverse transcriptase inhibitor has CNS off-targets.

It has been almost two decades since efavirenz was approved by the FDA and to date, there is only one study that has sought to investigate the receptor neuropharmacology underpinning efavirenz's CNS targets and this was in the context of its reported recreational use [9]. In that study, a rationalized mechanistic approach was utilized in that receptor targets known to interact with drugs of abuse were selected to narrow the receptor profiling effort leading to a number of CNS receptors being identified as possible targets for efavirenz. At the receptor level, efavirenz was shown to interact with the 5-HT_{2A}, 5-HT_{2C} and $GABA_A$ receptors, as well as DAT, SERT, and $VMAT_2$ transporters [9]. Mechanistically, efavirenz potentiated GABA-mediated chloride currents at the $GABA_A$ receptor, and functioned as a DAT and SERT blocker [9]. However, *in vivo*, efavirenz failed to strongly substitute in tests of discrimination for drugs known to have these same functional properties on DAT, SERT, $VMAT_2$ transporters, or the $GABA_A$ receptor [9]. In contrast, when efavirenz was used as the discriminative stimulus, LSD partially substituted for efavirenz. Moreover, in rats trained to discriminate LSD from saline, efavirenz partially substituted for LSD and this substitution was blocked by pre-treatment with the 5-HT_{2A} receptor selective antagonist MDL100,907 [9]. In a rodent head-twitch assay, efavirenz produced head-twitch responses in *wild type* but not 5-HT_{2A}-KO mice; though, the response was far weaker than for LSD and initiated much more rapidly [9]. There is a strong positive correlation between compounds that are hallucinogens in humans and those that induce a head-twitch response in rodents [19–21]. Efavirenz also dose-dependently depressed novel open field locomotor activity, similar to LSD in the same strain of mice [9]. For these reasons, it was concluded that efavirenz's predominate behavioral profile in rodents is most consistent with an LSD-like effect mediated by the 5-HT_{2A} receptor [9].

In this study we report for the first time that efavirenz interacts with the 5-HT_3, 5-HT_6 receptors and the enzyme MAO-A, and elucidate its molecular mechanisms of action at these targets as well as at the 5-HT_{2A} and 5-HT_{2C} receptors. Within a similar concentration range, efavirenz also blocks agonist responses at the M_1 and M_3 muscarinic receptors. Schild shift analysis suggests efavirenz acts at the same binding site on the 5-HT_{2A} receptor as the partial agonists LSD and (±)-DOI, but it is unique in that it does not activate the G_q-signaling pathway. Chronic exposure of the 5-HT_{2A} receptor to efavirenz reduces receptor density and responsiveness to 5-HT. We also demonstrate here that efavirenz's mechanism of action across a range of CNS targets is distinct from other prominent HIV-1 medications like emtricitabine (FTC), nevirapine (NVP) and zidovudine (ZDV).

2. Materials and methods

2.1. Chemicals

Radiochemicals were from Perkin Elmer (Saint Louis, MO): 4-(2″-Methoxy)-phenyl-1-[2″-(*N*-2-pyridinyl)-*p*-fluorobenzamido] ethyl-piperazine ([3H]MPPF, NET-1109, 80Ci/mmol); [3H]methylspiperone ([3H]MSP, NET-856, 84Ci/mmol); [3H]Lysergic acid diethylamide ([3H]LSD, NET638, 70Ci/mmol); [3H]Mesulergine (NET1148, 80Ci/mmol); [3H]BRL-43694 (granisetron) (NET1030, 65Ci/mmol); [3H]GR113808 (NET1152, 85Ci/mmol). Efavirenz, [(4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one], and the other antiretroviral drugs (emtricitabine (FTC), zidovudine (ZDV) and nevirapine (NVP)) were purchased from Sequoia Research Products Limited (Pangbourne, UK). MDL100,907 was synthesized and kindly provided by Dr. Kenner C. Rice (NIDA/NIAAA). Unless otherwise noted, all other drugs and reagents were purchased from Tocris Biosciences (via R&D Systems, Inc. in Minneapolis, MN) or Sigma-Aldrich (St. Louis, MO). All ARV drugs were solubilized in DMSO at concentrations ranging from 10 to 100 mM and diluted at least 1:1000 v/v in the final assay solution.

2.2. Profiling serotonin receptors by radioligand binding

The interaction of efavirenz (10 μM) with serotonin receptors was measured by its ability to displace specifically bound radioligands from the metabotropic 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_4, 5-HT_{5A}, 5-HT_6 and 5-HT_7 receptors, and an ionotropic 5-HT_{3A} receptor using the conditions outlined in Table 1. With the exception of the 5-HT_4 receptor, which was sourced from Duncan Hartley guinea pig striatal tissue, all other serotonin receptors were cloned receptors heterologously expressed in mammalian cell lines lacking the receptor subtypes of interest. Radioligand, purified membranes expressing individual serotonin receptors and a fixed concentration of 10 μM efavirenz in a total volume of 1 mL binding buffer were allowed to equilibrate. Receptors were then isolated by rapid filtration through glass fiber filters pretreated for 10 min with 0.5% polyethyleneimine (Sigma-Aldrich) and three rapid washes with 3 mL of ice-cold wash buffer (50 mM Tris, pH 7.4 at 0–2 °C). GF/C filters were used for membranes derived from cell cultures and GF/B filters for membranes derived from brain tissue (Brandel, Gaithersburg, MD). Dried filters were cut into individual scintillation vials, filled with 3.5 mL of scintillation fluid, mixed, and the radioactivity bound to the filters was quantified via scintillation spectroscopy. Membrane protein concentrations varied from 0.02-0.06 mg/mL. All data for each experiment was measured in triplicate. Each experiment was then repeated two or three times and the mean values of these experiments were reported with their associated SEM. A one-way ANOVA followed by a Bonferroni *post-*