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Research report

Antipsychotic-like effects of a neurotensin receptor type 1 agonist

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HIGHLIGHTS

- PD149163 treatment inhibits amphetamine-induced hyperactivity in C57BL/6J mice.
- PD149163 reduces amphetamine-mediated disruption of prepulse inhibition.
- PD149163 inhibits GSK-3 in the nucleus accumbens and medial prefrontal cortex.

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ABSTRACT

Although neurotensin (NT) analogs are known to produce antipsychotic-like effects, the therapeutic possibility of a brain penetrant NTS1 agonist in treating psychiatric disorders has not been well studied. Here, we examined whether PD149163, a brain-penetrant NTS1-specific agonist, displays antipsychotic-like effects in C57BL/6J mice by investigating the effect of PD149163 on amphetamine-mediated hyperactivity and amphetamine-induced disruption of prepulse inhibition. In addition, we assessed the effect of PD149163 on glycogen synthase kinase-3 (GSK-3) activity, a downstream molecular target of antipsychotics and mood stabilizers, using phospho-specific antibodies. PD149163 (0.1 and 0.5 mg/kg) inhibited amphetamine-induced hyperactivity in mice, indicating that NTS1 activation inhibits psychomotor agitation. PD149163 (0.5 mg/kg) also increased prepulse inhibition, suggesting that NTS1 activation reduces prepulse inhibition deficits which often co-occur with psychosis in humans. Interestingly, PD149163 increased the inhibitory serine phosphorylation on both GSK-3α and GSK-3β in a dose- and time-dependent manner in the nucleus accumbens and medial prefrontal cortex of the mice. Moreover, PD149163 inhibited GSK-3 activity in the nucleus accumbens and medial prefrontal cortex in the presence of amphetamine. Thus, like most current antipsychotics and mood stabilizers, PD149163 inhibited GSK-3 activity in cortico-striatal circuitry. Together, our findings indicate that PD149163 may be a novel antipsychotic.

1. Introduction

Schizophrenia and mania share phenotypes and pathology. Psychosis, the hallmark of schizophrenia, may also occur during severe manic episodes. Both disorders could present with hyperactivity, and psychosis. Shared phenotypes may result from a common dysregulation in neurotransmitter systems and genetics. For example, elevated dopamine signaling has been proposed to lead to symptoms present in mania and schizophrenia [1,2]. Furthermore, atypical antipsychotics, which typically inhibit dopamine D2 receptors (D2R), along with other receptor subtypes, are approved for the treatment of both schizophrenia and mania. Interestingly, genetic studies indicate that there are shared genetic factors that may increase the risk of development of bipolar disorder or schizophrenia [3]. Although a number of pharmacological agents are approved for the treatment of mania and schizophrenia, including antipsychotics, most current medications exhibit suboptimal efficacy, delayed onset of therapeutic action, and produce numerous side effects [4–6].

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Increasing evidence suggests that neurotensin receptor type 1 (NTS1) is a promising therapeutic target for mania and schizophrenia. Patients with affective disorders, schizoaffective disorder, or schizophrenia were reported to exhibit reduced neurotensin (NT) receptor binding in the entorhinal cortex and decreased NT levels in the cerebrospinal fluid (CSF) [7–9]. It is possible that decreased NT signaling may lead to some of the symptoms in schizophrenia and mania. Moreover, increased NT signaling may underlie the therapeutic effects of antipsychotics [10]. Both acute and chronic antipsychotic administration increased NT levels in rodent brains [10,11]. Consistently, several clinical studies showed that antipsychotic treatment increased NT levels in the CSF [9,12]. The antipsychotic-induced increase in NT signaling may be involved in the therapeutic effects of antipsychotics. NT receptor antagonists were demonstrated to inhibit the therapeutic-like effects of antipsychotics on disruptions in prepulse inhibition (PPI) and amphetamine-induced hyperlocomotion in rodents [13,14]. Furthermore, NT analogs have been shown to suppress psychomotor agitation and disruptions in PPI without causing extrapyramidal side effects, indicating that the effects of NT analogs more closely resemble that of atypical antipsychotics [15,16]. Genetic and pharmacological manipulations suggest that NTS1 may mediate the antipsychotic-like effects of NT [17–19]. PD149163 is a brain permeable, NTS1 selective agonist. Thus, PD149163 has potential as a novel compound to specifically target hyperactivity, agitation, and psychosis in patients with schizophrenia or mania. PD149163 exhibits antipsychotic-like effects in rats, but whether PD149163 dose-dependently inhibits psychomotor agitation in mice is unclear [16,18]. It has been suggested that the NT system in mice, as opposed to rats, more closely resembles the human NT system [20]. Furthermore, given the greater availability of techniques to manipulate the mouse genome, it is important to establish the effects of NTS1 activation in mice, which could pave the way for future circuitry-specific studies.

To examine whether PD149163 exerts antipsychotic-like effects, we examined the effect of PD149163 on amphetamine-mediated hyperactivity and amphetamine-induced disruption of PPI in mice. Acute amphetamine treatment is commonly used to model certain behavioral domains of mania and schizophrenia, and screen if novel drugs exert antipsychotic-like effects. The acute amphetamine model induces schizophrenia- and mania-like psychomotor agitation in rodents [21,22]. Amphetamine also produces deficits in PPI of startle reactivity, modeling the disruption in PPI observed in humans with schizophrenia, and possibly mania [23–25]. Furthermore, amphetamine increases dopamine output, and dysregulated dopaminergic signaling has been hypothesized to explain some of the symptoms in mania and schizophrenia [1,2]. The model is also commonly used since antipsychotics and other anti-manic drugs typically inhibit amphetamine-induced hyperactivity and disruptions in PPI [26–28].

One of the most well studied molecular targets of current antipsychotics and anti-manic drugs is glycogen synthase kinase-3 (GSK-3). GSK-3 is a ubiquitously expressed serine/threonine kinase with two isoforms, GSK-3α and GSK-3β. Antipsychotics and other anti-manic drugs are known to inhibit GSK-3 activity [29,30]. GSK-3 inhibitors have been shown to produce antipsychotic-like effects, indicating that some of the effects of current medications could be through inhibition of GSK-3 [31,32]. It is not known if activation of the G protein-coupled receptor, NTS1, also inhibits GSK-3 activity in cortico-striatal circuitry by increasing the inhibitory serine phosphorylation on GSK-3 (pGSK-3α Ser21 and pGSK-3β Ser9) like many existing antipsychotics. NT was found to inhibit GSK-3 activity in human colon cancer cells, suggesting that it is possible that NT may inhibit GSK-3 in the brain [33]. Therefore, in this study, we examined whether PD149163 exerts antipsychotic-like effects in mice by examining the effect of PD149163 on amphetamine-induced behaviors and GSK-3 phosphorylation in cortico-striatal circuitry.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (6 weeks old, Jackson Laboratories, Bar Harbor, ME) were group housed (4–5 mice per group) in standard plexiglass cages under a 12 h light/dark cycle with lights on at 6:00 AM. Behavioral experiments were carried out during the light phase. Food and water were provided *ad libitum*. Mice were used for the behavioral studies between 8 and 16 weeks of age. Animal care and handling procedures were approved by the Mayo Clinic Institutional Animal Care and Use Committees in accordance with National Institutes of Health guidelines.

2.2. Drugs

PD149163 tetrahydrochloride hydrate (PD149163) and D-amphetamine hemisulfate salt (amphetamine) were purchased from Sigma-Aldrich (St. Louis, MO). Both drugs were diluted in saline. A dilution of 0.02 mg/mL of PD149163 was used for the 0.5 mg/kg dose. For the 0.05 and 0.1 mg/kg doses, PD149163 was injected at a concentration of 0.01 mg/mL. Amphetamine was administered at a dose of 2 mg/kg (0.2 mg/mL) or 10 mg/kg (1 mg/mL). Mice received intraperitoneal (*i.p.*) injections of amphetamine, PD149163, or an equal volume of saline.

2.3. Behavioral assays

2.3.1. Open-field

Spontaneous locomotor activity was measured during the light phase in open-field chambers (27 × 27 × 20.3 cm) equipped with infrared photobeams to record X-Y ambulatory movements at a 50 ms resolution (Med Associates Inc., St. Albans, VT; Vadnie et al. [34]). The chambers were located in brightly lit (500 lx), sound-attenuating cubicles. All mice were allowed to habituate to the room for 1 h prior to locomotor measurements. Mice were first injected with saline or PD149163 (0.05, 0.1 or 0.5 mg/kg, *i.p.*), then injected with saline or amphetamine (2 mg/kg, *i.p.*) after one hour. After the second injection, mice were immediately placed in the open-field and locomotor activity was recorded for 90 min. Activity was quantified as horizontal distance traveled (cm). Two mice were excluded from the open-field locomotor analysis due to malfunction of the sensors.

2.3.2. Prepulse inhibition (PPI)

One week after the open-field experiment, mice were randomly assigned to experimental groups for prepulse inhibition (PPI) testing. Sound-attenuating chambers were used to examine startle reactivity and PPI (SR-LAB, San Diego Instruments, San Diego, CA). Chambers were equipped with a house light and a loudspeaker. Each chamber contained a cylindrical plexiglass animal enclosure that rested on a platform with a piezoelectric accelerometer mounted below. The piezoelectric accelerometer converted vibrations of the mouse in the enclosure to analog signals that were stored on a computer. At the onset of the startle stimulus, 65 readings were recorded at 1 ms intervals to capture maximum startle amplitude. We used the maximum startle amplitude to determine the startle response.

Each session began with a 5-min acclimation period followed by two successive 120 dB stimulus alone trials. These two initial trials were excluded from the analysis. Four different trial types were then presented randomly: "no stimulus" (background, 65 dB), "startle pulse alone" (120 dB; 40 ms), "prepulse alone" (4, 8 or 16 dB