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Reduced expression of haloperidol conditioned catalepsy in rats by the dopamine D3 receptor antagonists nafadotride and NGB 2904

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Motivation

Abstract

Haloperidol, a dopamine (DA) D2 receptor-preferring antagonist, produces catalepsy whereby animals maintain awkward posture for a period of time. Sub-threshold doses of haloperidol fail to produce catalepsy initially, however, when the drug is given repeatedly in the same test environment, gradual day-to-day increases in catalepsy are observed. More importantly, if sensitized rats are injected with saline instead of haloperidol they continue to be cataleptic in the test environment suggesting that environment-drug associations may play a role. DA D3 receptors have been implicated in a number of conditioned behaviors. We were interested if DA D3 receptors contribute to catalepsy sensitization and conditioning in rats. We tested this hypothesis using the DA D3 receptor-selective antagonist NGB 2904 (0.5, 1.8 mg/kg) and the DA D3 receptor-preferring antagonist nafadotride (0.1, 0.5 mg/kg). For 10 consecutive conditioning days rats were treated with one of the D3 receptor antagonists alone or in combination with haloperidol (0.25 mg/kg) and tested for catalepsy, quantified by the time a rat remained with its forepaws on a horizontal bar. On test day (day 11), rats were injected with saline or the D3 receptor antagonist and tested for conditioned catalepsy in the previously drug-paired environment. Rats treated with NGB 2904 or nafadotride alone did not develop catalepsy. Rats treated with haloperidol or haloperidol plus NGB 2904 or nafadotride developed catalepsy sensitization with repeated conditioning. When injected with saline they continued to exhibit catalepsy in the test environment — now conditioned. On the other hand, NGB 2904 (1.8 mg/kg) or nafadotride (0.5 mg/kg) given on the test day (after sensitization to haloperidol) significantly attenuated the expression of conditioned catalepsy. Our data suggest that the D3 receptor antagonist NGB 2904 (1.8 mg/kg) and nafadotride (0.5 mg/kg) significantly attenuate conditioned

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catalepsy in rats when given in test but not when given during sensitization. Results implicate DA D3 receptors in regulating the expression of conditioned catalepsy.

1. Introduction

Alterations in dopamine (DA) neurotransmission observed in a number of neuropsychiatric conditions such as schizophrenia, Parkinson's disease (PD) and substance abuse have been associated with changes in DA D3 receptor function. Initially, postmortem studies found elevated D3 receptor levels in the ventral striatum in drug-free schizophrenia patients, but not in patients on medication at the time of death (Guillin et al., 2001; Gurevich et al., 1997). Recent studies using positron emission tomography (PET) found an increase in D3 receptor binding using [11C]-(+)-PHNO in the globus pallidus, substantia nigra and a decrease in the ventral striatum after initiation of repeated antipsychotic treatment in previously drug-naïve schizophrenia patients (Mizrahi et al., 2011). On the other hand, PET studies in drug-naïve PD patients found a decrease in D3 receptor binding in the globus pallidus and ventral striatum (Boileau et al., 2009). Postmortem studies in people who overdosed on cocaine found increased D3 receptor binding and mRNA in the ventral striatum when compared to age-matched cocaine-free subjects (Segal et al., 1997; Staley and Mash, 1996).

Changes in D3 receptor mRNA and/or expression levels in the ventral striatum have been identified in experimental animals treated with drugs that directly or indirectly activate the DA system such as levodopa (Bezard et al., 2003; Bordet et al., 1997), amphetamine (Chiang et al., 2003), cocaine (Le Foll et al., 2002; Neisewander et al., 2004), nicotine (Le Foll et al., 2003) and morphine (Liang et al., 2011). The animals that showed changes in D3 receptor levels also exhibited behavioral changes associated with the repeated drug treatment such as sensitization (Bordet et al., 1997; Chiang et al., 2003; Le Foll et al., 2003) and drug-environment conditioning (Le Foll et al., 2002; Liang et al., 2011; Neisewander et al., 2004). Further studies using pharmacological manipulations have shown that D3 receptor antagonists block the expression of cocaine (Banasikowski et al., 2010; Le Foll et al., 2002) and nicotine (Pak et al., 2006) conditioned activity as well as the expression of conditioned place preference (CPP) based on cocaine (Vorel et al., 2002) and nicotine (Le Foll et al., 2005).

Recently it has been shown that repeated dopamine receptor antagonism (haloperidol) can produce catalepsy sensitization in a context-dependent manner (Klein and Schmidt, 2003; Lanis and Schmidt, 2001; Riedinger et al., 2011; Wiecki et al., 2009) and conditioned catalepsy (Amtage and Schmidt, 2003; Banasikowski and Beninger, 2012; Schmidt and Beninger, 2006) when tested drug-free in the drug-paired environment. Similarly to sensitization and conditioning with drugs that increase DA concentrations, haloperidol catalepsy sensitization and conditioning is dependent on the environmental stimuli present during conditioning and test (Schmidt and Beninger, 2006).

As D3 receptors play a significant role in the expression of conditioned behaviors based on drugs that activate the DA system (i.e., conditioned activity, CPP), it is important to test whether D3 receptors are involved in conditioning to drugs that reduce DA activity (i.e., conditioned catalepsy). Our studies examined the role of D3 receptors in haloperidol catalepsy sensitization, and in acquisition and expression of conditioned catalepsy. We tested the hypothesis that the D3 receptor-preferring antagonist nafadotride and the D3 receptor-selective antagonist NGB 2904 will attenuate the expression of haloperidol conditioned catalepsy at doses that will fail to attenuate its acquisition.

2. Experimental procedures

2.1. Subjects

Experimentally naïve male albino Wistar rats (N = 135, Charles River Canada, St. Constant QC) weighing 320–340 g upon the start of experiments were housed in pairs or threes in clear Plexiglas cages (45.0 × 25.0 × 22.0 cm). Average temperature in the colony was 21 °C, humidity 70% with reversed light–dark cycle (lights off from 0700 to 1900 h). Rats were maintained with food (LabDiet 5001, PMI Nutrition International, Brentwood, MO, USA) and water continuously available. Treatment of rats was in accordance with the guidelines of the Animals for Research Act, the Canadian Council on Animal Care, and was approved by the Queen's University Animal Care Committee.

2.2. Drugs

Haloperidol, (4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one (Sigma, St. Louis, MO, USA) was prepared in a 0.3% distilled water solution of tartaric acid. Nafadotride, *N*-[(1-Butyl-2-pyrrolidinyl)methyl]-4-cyano-1-methoxy-2-naphthalenecarboxamide and NGB 2904, *N*-[4-[4-(2,3-Dichlorophenyl)-1-piperazinyl] butyl]-9*H*-fluorene-2-carboxamide (Tocris, Oakville, ON) was prepared in a 10% distilled water solution of 2-hydroxypropyl-β-cyclodextrin. Injections were administered intraperitoneally (i.p.) in a volume of 1 ml/kg.

2.3. Behavioral testing

Rats were randomly assigned to treatment (paired) and control groups (unpaired). Animals were removed from the colony room and injected i.p. with drug or saline and put back into their cages in a hallway lit by 34-watt fluorescent tubes. Thirty minutes [nafadotride (NAF), NGB 2904 (NGB)] or 60 min [haloperidol (hal)] later, they were tested, in a small testing room also illuminated by two 34-watt fluorescent tubes, on a horizontal bar (1.6 cm diam. threaded or smooth rod with end bolts attached to Plexiglas supports, 10 cm above the surface), by gently placing both forepaws on the bar. Descent latency was measured as the time span from placing the animal on the bar until the first active paw movement (i.e., one forepaw left the bar or the hindlegs left the floor to climb onto the bar). A cut-off time of 180 s was used, i.e., the trial was terminated when the animal did not make an active paw movement within that time.