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The dopamine D1–D2 receptor heteromer exerts a tonic inhibitory effect on the expression of amphetamine-induced locomotor sensitization

Maurice Y.F. Shen [a,b], Melissa L. Perreault [a,b], Theresa Fan [a,b], Susan R. George [a,b,c,*]

[a] Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, University of Toronto, Toronto, Ontario, Canada
[b] Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada
[c] Department of Medicine, University of Toronto, Toronto, Ontario, Canada

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ABSTRACT

A role for the dopamine D1–D2 receptor heteromer in the regulation of reward and addiction-related processes has been previously implicated. In the present study, we examined the effects of D1–D2 heteromer stimulation by the agonist SKF 83959 and its disruption by a selective TAT-D1 peptide on amphetamine-induced locomotor sensitization, a behavioral model widely used to study the neuroadaptations associated with psychostimulant addiction. D1–D2 heteromer activation by SKF 83959 did not alter the acute locomotor effects of amphetamine but significantly inhibited amphetamine-induced locomotor responding across the 5 day treatment regimen. In addition, a single injection of SKF 83959 was sufficient to abolish the expression of locomotor sensitization induced by a priming injection of amphetamine after a 72-hour withdrawal. Conversely, inhibition of D1–D2 heteromer activity by the TAT-D1 peptide enhanced subchronic amphetamine-induced locomotion and the expression of amphetamine locomotor sensitization. Treatment solely with the TAT-D1 disrupting peptide during the initial 5 day treatment phase was sufficient to induce a sensitized locomotor phenotype in response to the priming injection of amphetamine. Together these findings demonstrate that the dopamine D1–D2 receptor heteromer exerts a tonic inhibitory control on neurobiological processes involved in sensitization to amphetamine, indicating that the dopamine D1–D2 receptor heteromer may be a novel molecular substrate in addiction processes involving psychostimulants.

1. Introduction

The dopamine D1–D2 receptor heteromer is a G protein-coupled receptor (GPCR) complex that couples to the Gαq protein to elicit a phospholipase C (PLC)-dependent calcium signal upon its activation (Rashid et al., 2007). It has been reported that a significant proportion of D1 receptor (D1R)-expressing medium spiny neurons (MSNs) in the nucleus accumbens (NAc) co-express the dopamine D1 and D2 receptors (17–25%) (Bertran-Gonzalez et al., 2008; Matamales et al., 2009; Perreault et al., 2010; Gangarossa et al., 2013) and approximately 90% of these MSNs express the D1–D2 heteromer (Hasbi et al., 2009; Perreault et al., 2010). In contrast, only 2–6% of D1R-expressing MSNs in the caudate putamen co-express the D1R and D2R, of which only 25% of the neurons exhibit D1–D2 heteromer formation (Perreault et al., 2010). The D1–D2 co-expressing neurons in the NAc extend efferent projections which directly or indirectly influence the ventral tegmental area (VTA) (Perreault et al., 2012a), a region widely known for its role in mediating addiction-like behaviors and reward through the regulation of mesolimbic dopamine activity (reviewed: Chen et al., 2010; Koob and Volkow, 2010).

We have previously shown that activation of the D1–D2 receptor heteromer modulated the expression of proteins involved in drug addiction (Hasbi et al., 2009; Ng et al., 2010; Perreault et al., 2010, 2012a), such as brain-derived neurotrophic factor (BDNF) and calcium-calmodulin kinase II (CaMKII) in the NAc and VTA, and D1–D2 heteromer activation in NAc shell enhanced production of the inhibitory neurotransmitter GABA in VTA (Perreault et al., 2012a). These findings thus suggest a potential role for the D1–D2 heteromer in the regulation of neuronal activity in the VTA and possibly as a regulator of brain reward processes.

Since repeated amphetamine treatment was previously shown to enhance the functional activity of the D1–D2 heteromer in rat striatum (Perreault et al., 2010), in this study we aimed to further examine the potential involvement of the D1–D2 heteromer in processes linked with addiction using the amphetamine-induced locomotor sensitization model in rats. Psychostimulant-induced locomotor sensitization was proposed to be an animal model for drug craving, and is characterized by a context-dependent, progressive augmentation of locomotor responsiveness following repeated non-contingent administration of psychostimulants such as cocaine and amphetamine (Robinson and

* Corresponding author at: Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, University of Toronto, Toronto, Ontario, Canada.
E-mail address: s.george@utoronto.ca (S.R. George).

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Becker, 1986; Kalivas and Stewart, 1991; Robinson and Berridge, 1993; Anagnostaras and Robinson, 1996). Amphetamine-induced locomotor sensitization is associated with neuroadaptations of the mesolimbic dopamine system that may enhance the reinforcing properties of cocaine and amphetamine, as animals that were previously sensitized with repeated amphetamine treatment showed increased acquisition of drug self-administration (Mendrek et al., 1998; Suto et al., 2002; Vezina et al., 2002).

Once established, amphetamine locomotor sensitization has been reported to persist for over a year (Paulson and Robinson, 1991), which may be a reflection of some of the long-term neurobiological adaptations that accompany the persistent drug-seeking behaviors typically seen in addicted patients (Robinson and Berridge, 2000). Similarly, animals that exhibited the reinstatement of cocaine-seeking behavior induced by a single exposure to amphetamine also expressed locomotor sensitization (De Vries, 1998), suggesting that changes in the mesolimbic dopamine system that accompany the expression of amphetamine locomotor sensitization may also contribute to relapse of drug seeking.

The dopamine agonist SKF 83959 is a partial agonist for the D1–D2 heteromer with a number of *in vitro* and *in vivo* studies demonstrating its ability to induce D1–D2 heteromer-mediated calcium signaling (Rashid et al., 2007; Verma et al., 2010), Gq and PLC activation (Rashid et al., 2007), CaMKII phosphorylation (Ng et al., 2010) and BDNF production (Hasbi et al., 2009). Validation of selectivity to the D1–D2 heteromer in these studies employed D1 and D2 antagonists and dopamine receptor knockout mice. Recent studies, however, have indicated that SKF 83959 has affinity for, or activates, a number of other receptors, such as the dopamine D5 receptor, the α-adrenergic receptor 2C, and the serotonin 5HT-2C receptor (Sahu et al., 2009; Perreault et al., 2012b; Chun et al., 2013). In addition, there are conflicting reports as to whether SKF 83959 functions as an antagonist (Downes and Waddington, 1993; Cools et al., 2002; Jin et al., 2003), a partial agonist (Lee et al., 2014), or has no effect (Lee et al., 2004; Rashid et al., 2007) at the D1R. To assist in elucidating the physiological role of the D1–D2 heteromer, we developed a selective D1–D2 heteromer antagonist, the TAT-D1 peptide, which occludes the interaction site between the two receptors (O'Dowd et al., 2012), thus inhibiting D1–D2 heteromer expression and function and abolishing the physiological effects of D1–D2 heteromer activation by SKF 83959 without affecting other receptor oligomers such as D1–D1 homomers or D2–D5 heteromers (Hasbi et al., 2014). In the present study, we assessed the effects of SKF 83959 on the expression of amphetamine locomotor responses and sensitization. We only attributed an effect to be D1–D2 heteromer-specific when the TAT-D1 peptide produced an opposite behavioral output compared to SKF 83959. Our findings showed a novel role for the D1–D2 heteromer in the suppression of amphetamine-induced locomotion and locomotor sensitization.

2. Material and methods

2.1. Animals

Ninety-six adult male Sprague–Dawley rats (Charles River, Canada), weighing 300–350 g at the start of the experiment, were used. Rats were housed in polyethylene cages in a temperature-controlled colony room, maintained on a 12-h light–dark cycle (lights on at 0700 h), with *ad libitum* access to food and water. Rats were handled daily for 5 days before the start of the experiment. All treatments were performed during the light phase of the day-night cycle. Animals were housed and tested in compliance with the guidelines described in the Guide to the Care and the Use of Experimental Animals (Canadian Council on Animal Care), and were approved by the Animal Care Ethics Committee of the University of Toronto.

2.2. Drugs

SKF 83959 hydrobromide (Tocris Bioscience) was dissolved in physiological saline containing 5% DMSO, and was administered subcutaneously (s.c.). Amphetamine hydrochloride (Sigma-Aldrich) was dissolved in physiological saline (0.9% NaCl), and was administered intraperitoneally (i.p.). The TAT-D1 disrupting peptide was dissolved in saline and administered into the intracerebroventricular (i.c.v.) space 15 min prior to vehicle, SKF 83959, or amphetamine injection. For non-drug injections, an equivalent volume of saline or vehicle was administered. All systemic injections were given at a volume of 1.0 ml/kg just prior to behavioral testing.

2.3. Surgery

Rats were anesthetized with isoflurane (5%), administered analgesic ketoprofen (5 mg/kg, s.c.) and secured in a stereotaxic frame. A cannula (22-gauge, Plastics One) was placed unilaterally into the intracerebroventricular space close to the midline according to the following stereotaxic coordinates: AP −0.8 mm, ML +1.3 mm, DV −3.7 mm, and was secured by dental cement anchored with four stainless steel screws (Plastics One) fixed on the dorsal surface of the skull. AP and ML coordinates were taken from bregma, DV coordinate from the dura (Paxinos and Watson, 1998). The animals were allowed to recover in their home cage for a minimum of five days before the experiments were performed. Cannulae placement was visually validated postmortem in brain slices.

2.4. Locomotor activity apparatus

The testing environment was a non-colony room containing 8 empty activity chambers that are 20 cm in height, 25 cm in width, and 40 cm in length. Two arrays of 16 infrared photocells were attached along the longer sides of the polyethylene cages. The activity chambers were interfaced to a computer that provided automated recording of horizontal locomotor activity when both top and bottom infrared photocells were triggered. Ventilated polyethylene lids were used to cover the activity chambers to prevent animals from escaping.

2.5. Locomotor sensitization protocol

The behavioral testing for locomotor sensitization to amphetamine was conducted using a previously described protocol (Hall et al., 2008), which consisted of 3 phases:

Phase I: *Habituation*
Rats were first habituated to the activity chamber for 2 days for 30 min per day.

Phase II: *The sensitizing regimen*
In this phase, we examined the effects of D1–D2 heteromer stimulation and inactivation on locomotion induced by acute and subchronic amphetamine treatment (1.5 mg/kg, i.p.). Animals were administered their designated drug treatment, VEH + SAL, SKF 83959 + SAL, TAT-D1 + SAL, VEH + amphetamine, SKF 83959 + amphetamine, and TAT-D1 + amphetamine, once daily for 5 consecutive days. Immediately following each injection, horizontal locomotor activity was monitored for 60 min. The dose of SKF 83959 (0.4 mg/kg, s.c.) given in this phase was chosen based on our previous study showing that repeated SKF 83959 treatment significantly enhanced locomotor activity and grooming responses without desensitizing the D1–D2 heteromer (Perreault et al., 2010). The dose of the TAT-D1 peptide (300 pmol, i.c.v.) was previously shown to disrupt D1–D2 heteromer formation *in vivo* as indicated by the loss of co-immunoprecipitation of D1R with D2R in rat NAc tissue (Hasbi et al., 2014).