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REGULATION OF C-FOS EXPRESSION BY THE DOPAMINE D1-D2 RECEPTOR HETEROMER

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Abstract—The dopamine D1 and D2 receptors form the D1-D2 receptor heteromer in a subset of neurons and couple to the Gq protein to regulate intracellular calcium signaling. In the present study the effect of D1-D2 heteromer activation and disruption on neuronal activation in the rat brain was mapped. This was accomplished using the dopamine agonist SKF 83959 to activate the D1-D2 heteromer in combination with a TAT-D1 disrupting peptide we developed, and which has been shown to disrupt the D1/D2 receptor interaction and antagonize D1-D2 heteromer-induced cell signaling and behavior. Acute SKF 83959 administration to rats induced significant c-fos expression in the nucleus accumbens that was significantly inhibited by TAT-D1 pretreatment. No effects of SKF 83959 were seen in caudate putamen. D1-D2 heteromer disruption by TAT-D1 did not have any effects in any striatal subregions, but induced significant c-fos immunoreactivity in a number of cortical regions including the orbitofrontal cortex, prelimbic and infralimbic cortices and piriform cortex. The induction of c-fos by TAT-D1 was also evident in the anterior olfactory nucleus, as well as the lateral habenula and thalamic nuclei. These findings show for the first time that the D1-D2 heteromer can differentially regulate c-fos expression in a region-dependent manner either through its activation or through tonic inhibition of neuronal activity.

Key words: dopamine D1-D2 heteromer, c-fos, nucleus accumbens, cortex, lateral habenula.

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Abbreviations: ANOVA, analysis of variance; AOP, anterior olfactory nucleus; AP, anterior–posterior; BDNF, brain-derived neurotrophic factor; CP, caudate putamen; DV, dorsoventral; GSK-3β, glycogen synthase kinase-3β; IL, infralimbic cortex; LHb, lateral habenula; MHb, medial habenula; ML, mediolateral; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; OFC, orbitofrontal cortex; PFC, prefrontal cortex; PiC, piriform cortex; PL, prelimbic cortex; PVP, paraventricular thalamic nucleus; TAT-Sc, TAT-scrambled peptide control; TBS-T, Tris-buffered saline-Tween.

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INTRODUCTION

The dopamine D1 and D2 receptors (D1R and D2R) can form a heteromeric receptor complex, the D1-D2 receptor heteromer, that exhibits pharmacological and functional properties distinct from its constituent receptors (Lee et al., 2004; Rashid et al., 2007; Hasbi et al., 2009). The distribution of the dopamine D1-D2 receptor heteromer has only been partially characterized thus far, with the major focus being directed to the striatal subregions. In rodents and non-human primates an abundance of neuroanatomical evidence now suggests that the D1R and D2R are coexpressed in a subset of striatal medium spiny neurons (Meador-Woodruff et al., 1991; Surmeier et al., 1992, 1996; Lester et al., 1993; Aubert et al., 2000; Lee et al., 2004; Deng et al., 2006; Bertran-Gonzalez et al., 2008; Hasbi et al., 2009; Matamales et al., 2009; Perreault et al., 2010; Gangarossa et al., 2013), with low receptor coexpression (~4–6% of neurons) in caudate putamen (CP) and higher coexpression levels in the nucleus accumbens (NAc) (~17–30% of neurons) (Bertran-Gonzalez et al., 2008; Perreault et al., 2010; Gangarossa et al., 2013). Approximately 90% of coexpressing neurons in NAc expressed the D1-D2 heteromer with only about 25% in CP (Perreault et al., 2010). While D1-D2 heteromer expression in other brain regions has for the most part not been determined, it is expressed in globus pallidus (Perreault et al., 2011), in the medial prefrontal cortex (mPFC) (Pei et al., 2010). The dopamine D1-D2 receptor heteromer has been linked to Gq-mediated phospholipase C activation and intracellular calcium signaling (Lee et al., 2004; Rashid et al., 2007; Hasbi et al., 2009), the activation of calcium calmodulin kinase II (Rashid et al., 2007; Ng et al., 2010), the expression and release of brain-derived neurotrophic factor (BDNF) in NAc (Hasbi et al., 2009; Perreault et al., 2012) and reduced activation of glycogen synthase kinase-3β (GSK-3β) in the prefrontal cortex (PFC) (Perreault et al., 2013). Furthermore, activation of the D1-D2 heteromer in NAc shell was shown to regulate the expression of protein markers of GABA and glutamate in ventral tegmental area and substantia nigra (Perreault et al., 2012) suggesting that activation of the D1-D2 heteromer may exert local effects as well as have farther reaching effects through efferent projections.

A role for the dopamine D1-D2 receptor heteromer in the regulation of neuronal activity has not been explored but can be examined through the expression of immediate early genes, such as c-fos, which have often been used as a measure of neuronal activation within

circuits (Perez-Cadahia et al., 2011). The dopamine agonist SKF 83959, which is a partial agonist for the D1-D2 heteromer (Rashid et al., 2007), has been shown to induce dorsal striatal Fos expression at high doses (Wirtshafter and Osborn, 2005). SKF 83959 has often been used to activate the D1-D2 heteromer with dopamine receptor knockout mice (D1R−/− and D5R−/−) used to validate selectivity as SKF 83959 also activates the PLC-coupled D5R (Sahu et al., 2009; Perreault et al., 2013). However recent reports indicate that this compound also exhibits affinity at other receptors such as the serotonin 5HT-2c receptor (Chun et al., 2013), may act as an allosteric modulator at the sigma-1 receptor (Guo et al., 2013), and 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 (Hasbi et al., 2014). Therefore in the present study, using SKF 83959 together with TAT-D1, we sought to address the involvement of the D1-D2 heteromer in regulating neuronal activation as indexed by the induction of c-fos expression.

EXPERIMENTAL PROCEDURES

Animals

Sixty-eight 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), 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 accordance with the guidelines described in the Guide to the Care and the Use of Experimental Animals (Canadian Council on Animal Care, 1993), and were approved by the Animal Care Ethics Committee of the University of Toronto.

Drugs and peptides

SKF 83959 hydrobromide (Tocris Bioscience, distributed by Cedarlane Corp, Burlington, ON, Canada) was dissolved in physiological saline containing 5% dimethyl sulfoxide (DMSO), and was administered subcutaneously (0.4, 2.5 mg/kg, s.c.). Haloperidol (0.5 mg/kg, Sigma Aldrich, Oakville, ON, Canada) was used as a positive control for c-fos immunochemistry in striatum, dissolved in a 0.3% tartaric acid in water, and administered intraperitoneally (i.p.). For non-drug injections, an equivalent volume of vehicle was administered and all injections were given at a volume of 1.0 ml/kg. The TAT-D1 disrupting peptide, or TAT-scrambled peptide control (TAT-Sc) (Hasbi et al., 2014), was dissolved in saline containing a protease inhibitor cocktail (1:1000) and administered into the intracerebroventricular space (300 pmol/4 μL, i.c.v.) 15 min prior to vehicle or SKF 83959. The dose of TAT-D1 was chosen based on a previous study which showed an the loss of SKF 83959-induced activation of the calcium signaling pathway mediated through the D1-D2 receptor heteromers as well as the physical interaction between the D1 and D2 receptor could be disrupted, as shown by coimmunoprecipitation and BRET analysis (Hasbi et al., 2014). The study further showed that 300 pmol of TAT-D1 did not affect the function of closely related oligomers such as the D1-D1 and D2-D2 homomers or the D5-D2 heteromer.

Surgery

Rats were anesthetized with isoflurane, administered analgesic ketoprofen (5 mg/kg) and secured in a stereotaxic frame. A cannula was placed unilaterally into the intracerebroventricular space close to the midline according to the following stereotaxic anterior–posterior (AP), mediolateral (ML), and dorsoventral (DV) coordinates: AP −0.8 mm, ML +1.3 mm, DV −3.7 mm. AP and ML coordinates were taken from bregma, DV coordinate from skull surface. All animals underwent surgery and were allowed to recover in their home cage for a minimum of 5 days before the experiments were performed.

Grooming

Grooming activity was monitored for 30 min immediately following SKF 83959 (0.4 mg/kg) injection. Animals were placed in clear cages containing no bedding ($20 \times 20 \times 45$ cm^3). The measurement of grooming behavior followed a previously described protocol (Culver et al., 2000). The animal's grooming was scored for 30 s intervals, for a total of 4 min (2 min sampled from the first 15 min of testing and 2 min sampled from the last 15 min of testing). Ventilated polyethylene lids were used to cover the cages to prevent animals from escaping.

Immunochemistry

Ninety minutes following SKF 83959 (0.4, 2.5 mg/kg) or vehicle injection, brains were rapidly removed and frozen in isopentane (−60 °C) and stored at −80 °C until cryostat sectioning. Serial sections through the PFC (Bregma 3.2 mm), CP/NAc (Bregma 1.6 mm), ventral pallidum (Bregma 0.2), globus pallidus (Bregma −0.8 mm), lateral hypothalamus (Bregma −1.8), habenula/hippocampus/thalamus/amygdala (Bregma −3.6), substantia nigra/ventral tegmental area (Bregma −5.6 mm) and rostromedial tegmental nucleus (Bregma −6.8 mm) were cut coronally at 16-μm thickness and mounted onto gelatin-coated glass slides. Sections were air dried and stored at −35 °C until use. Immunochemistry for c-fos was performed as previously described (Sundquist and Nisenbaum, 2005) with the following changes. Slides were brought to room temperature, immersed in 4% paraformaldehyde and