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Disruption of a dopamine receptor complex amplifies the actions of cocaine

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Abstract
Cocaine-induced increases in dopamine signaling in nucleus accumbens (NAc) play a significant role in cocaine seeking behavior. The majority of cocaine addiction research has focused on neuroanatomically segregated dopamine D1 and D2 receptor-expressing neurons, yet an involvement for those NAc neurons coexpressing D1 and D2 receptors in cocaine addiction has never been explored. *In situ* proximity ligation assay, confocal fluorescence resonance energy transfer and coimmunoprecipitation were used to show native D1 and D2 receptors formed a heteromeric complex in D1/D2 receptor-coexpressing neurons in rat and non-human primate NAc. D1–D2 heteromer expression was lower in NAc of adolescent rats compared to their adult counterparts. Functional disruption of the dopamine D1–D2 receptor heteromer, using a peptide targeting the site of interaction between the D1 and D2 receptor, induced conditioned place preference and increased NAc expression of ΔFosB. D1–D2 heteromer disruption also resulted in the promotion, exacerbation and acceleration of the locomotor activating and incentive motivational effects of cocaine in the self-administration paradigm.

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These findings support a model for tonic inhibition of basal and cocaine-induced reward processes by the D1-D2 heteromer thus highlighting its potential value as a novel target for drug discovery in cocaine addiction. Given that adolescents show increased drug abuse susceptibility, an involvement for reduced D1-D2 heteromer function in the heightened sensitivity to the rewarding effects of cocaine in adolescence is also implicated.

1. Introduction

Cocaine addiction is a chronic disorder associated with uncontrollable craving together with compulsive drug-seeking and drug-taking, which persists despite devastatingly negative health, social and economic consequences. It is well established that increased dopamine signaling and activation of dopamine D1 receptors in nucleus accumbens (NAc) plays a significant role in the development and maintenance of cocaine seeking (Anderson and Pierce, 2005; Nestler, 2005). Yet despite this knowledge, therapeutic targeting of specific neuronal pathways in vivo remains difficult to accomplish and, as such, the mainstay of current addiction therapy remains substitution, which has significant limitations, low success rates and high propensity for relapse. Thus the identification of a specific receptor substrate that regulates addiction processes could completely shift drug discovery paradigms.

In the basal ganglia dopamine D1 and D2 receptors are predominantly segregated to distinct neuronal pathways, the direct striatonigral and indirect striatopallidal pathways respectively, and are thought to be the predominant mediators of dopamine effects in this region. However, a fraction of striatal medium spiny neurons (MSNs) express both receptors with colocalization of D1 and D2 receptors occurring predominantly in NAc (Aubert et al., 2000; Bertran-Gonzalez et al., 2008; Deng et al., 2006; Gangarossa et al., 2013; Perreault et al., 2010). Dopamine signaling in NAc has been widely demonstrated to play a pivotal role in cocaine addiction (Anderson and Pierce, 2005; Lammel et al., 2014), yet the contribution of these D1/D2 receptor-coexpressing MSNs in mediating addiction processes has been ignored. As a therapeutic target with proven efficacy in cocaine addiction has yet to be identified, discerning the physiological relevance of these MSNs is imperative to discover novel avenues for drug discovery.

Up to 17–38% of D1-expressing MSNs also express the D2 receptor in specific NAc subregions of the mouse, rat and monkey (Aubert et al., 2000; Bertran-Gonzalez et al., 2008; Deng et al., 2006; Gangarossa et al., 2013; Perreault et al., 2010) whereas in the rodent caudate nucleus (CN) neuronal cell bodies only 1–6% of such MSNs exhibit D1 and D2 receptor colocalization (Bertran-Gonzalez et al., 2008; Perreault et al., 2010). Within most of the D1/D2 receptor-coexpressing MSNs in NAc the receptors exist within a complex forming the D1-D2 receptor heteromer (Perreault et al., 2010). The D1-D2 heteromer was shown to exhibit distinct pharmacological and functional properties (Lee et al., 2004; Rashid et al., 2007) and to induce the activation or expression of proteins known to be highly involved in addiction such as calcium calmodulin kinase II and brain-derived neurotrophic factor (Hasbi et al., 2009; Ng et al., 2010; Perreault et al., 2012a). The role of the D1-D2 heteromer in mediating the behavioral and biochemical effects of cocaine was evaluated.

2. Experimental procedures

2.1. Animals

Adult male Sprague-Dawley rats (Charles River, Canada) weighing 300–350 g at the start of each experiment and a total of two adult male Macaca fascicularis primates (body weight 3.8–4.5 kg) were used. Homozygous whole body D1 and D2 receptor gene deleted mice were congenic, backcrossed into a C57BL/6J genetic background.

2.2. TAT-D1 and scrambled peptide

The TAT-D1 peptide was generated and its specificity tested as described using different strategies including mutations, truncations, BRET and pharmacological studies (Hasbi et al., 2014). The receptor complexes we tested and found not affected by the TAT-D1 peptide were D1-D1, D2-D2, D5-D5, D1-D3, D1-mu, D2-D5 and D2-5HT2A (Hasbi et al., 2014). The TAT-D1 peptide is composed of 18 amino acids between amino acids 396 and 413 of the D1 receptor C-tail fused in its NH2 terminus to a TAT peptide sequence (GeneScript, Piscataway, NJ, USA) to render it cell permeable. To avoid the nonspecific effects of the TAT sequence or random collisions, a peptide with the same amino acid composition as the TAT-D1 peptide but with a scrambled sequence attached to TAT was used as a control.

2.3. Confocal microscopy fluorescence resonance energy transfer (FRET)

Confocal microscopy FRET analysis and data processing was performed as described previously (Hasbi et al., 2009; Perreault et al., 2010). Alexa Fluor-488 was directly coupled to the D1 receptor antibody (Sigma Aldrich, D2944) and Alexa Fluor- 569 to the D2 receptor antibody (Millipore, AB5084P – formerly Chemicon) using an antibody labeling kit (Invitrogen) to eliminate the necessity of secondary antibodies. The primary antibodies were also previously validated both in cells and in mice gene-deleted for the D1 and D2 receptors (Lee et al., 2004; Perreault et al., 2010).

2.4. Proximity Ligation Assay (PLA)

PLA probes consisted of affinity-purified primary antibodies modified by covalent attachment of 5′ end of various oligonucleotides. For the monkey studies, the anti-D1 receptor antibody (Sigma Aldrich, D2944) was conjugated with a PLUS oligonucleotide (Duolink® In Situ Probemaker PLUS DUO92009, Sigma Chemical

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