Neuroscience 225 (2012) 130–139

REDUCED STRIATAL DOPAMINE D1–D2 RECEPTOR HETEROMER EXPRESSION AND BEHAVIOURAL SUBSENSITIVITY IN JUVENILE RATS

M. L. PERREAULT,[a,b1] A. HASBI,[a,b1] M. ALIJANIARAM,[a,b] B. F. O'DOWD[a,b] AND S. R. GEORGE[a,b,c]*

[a] *Centre for Addiction and Mental Health, Toronto, Ontario, Canada*

[b] *Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada*

[c] *Department of Medicine, University of Toronto, Toronto, Ontario, Canada*

Abstract—In adult rat striatum the dopamine D1–D2 receptor heteromer is expressed selectively in a subset of medium spiny neurons (MSNs) that coexpress the dopamine D1 and D2 receptors (D1R and D2R) as well as dynorphin (DYN) and enkephalin (ENK), with higher coexpression in nucleus accumbens (NAc) and much lower in the caudate putamen (CP). In the present study we showed that in neonatal striatal cultured neurons >90% exhibited the D1R/D2R–DYN/ENK phenotype. Similarly, in the striatum of juvenile rats (age 26–28 days) coexpression of D1R and D2R was also coincident with the expression of both DYN and ENK. Quantification of the number of striatal MSNs exhibiting coexpression of D1R and D2R in juvenile rats revealed significantly lower coexpression in NAc shell, but not core, and CP than in adult rats. However, within MSNs that coexpressed D1R and D2R, the propensity to form the D1–D2 receptor heteromer did not differ between age groups. Consistent with reduced coexpression of the D1R and D2R, juvenile rats exhibited subsensitivity to D1–D2 receptor heteromer-induced grooming following activation by SKF 83959. Given the proposed role of D1R/D2R-coexpressing MSNs in the regulation of thalamic output, and the recent discovery that these MSNs exhibit both inhibitory and excitatory capabilities, these findings suggest that the functional regulation of neurotransmission by the dopamine D1–D2 receptor heteromer within the juvenile striatum may be significantly different than in the adult. **© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: dopamine D1–D2 receptor heteromer, juvenile, dynorphin, enkephalin, nucleus accumbens, grooming.

INTRODUCTION

Dopaminergic signaling within the basal ganglia has classically been thought to occur within two distinct neuronal pathways; the direct striatonigral pathway which contains the dopamine D1 receptor (D1R) and the neuropeptides dynorphin (DYN) and substance P, and the indirect striatopallidal pathway which expresses the dopamine D2 receptor (D2R) and enkephalin (ENK). A number of studies have also shown, however, that D1R and D2R can co-exist within a certain fraction of medium spiny neurons (MSN) (Bertran-Gonzalez et al., 2008; Valjent et al., 2009; Perreault et al., 2010; Lim et al., 2012). Emerging evidence additionally indicates that these D1R/D2R-coexpressing neurons, which also express DYN and ENK (Perreault et al., 2010) as well as the neurotransmitters GABA and glutamate (Perreault et al., 2012), may comprise a distinct neuronal network, as evidenced by a recent report showing D1R and D2R coexpression in the cell bodies and presynaptic terminals of regions of both the striatonigral and striatopallidal projections of the basal ganglia (Perreault et al., 2010). Furthermore, within these coexpressing neurons it has been shown that the D1R and D2R can form a novel and pharmacologically distinct receptor complex, the dopamine D1–D2 receptor heteromer. The D1–D2 heteromer has been shown to exhibit cell signaling properties distinct from its constituent receptors (Lee et al., 2004; Rashid et al., 2007; Hasbi et al., 2009; So et al., 2009; Verma et al., 2010), emphasizing the functionally unique role for these coexpressing MSNs in the brain.

Evidence suggests that striatal coexpression of the D1R and D2R, and the expression of the D1–D2 receptor heteromer, may be age-dependent thus implicating age as a critical factor in the contribution of these neurons to the regulation of striatal neurotransmission. For example, while it has been shown that the majority of rat neonatal striatal neurons may coexpress D1R and D2R (Aizman et al., 2000; Falk et al., 2006; Iwatsubo et al., 2007; Hasbi et al., 2009) and form D1–D2 receptor heteromers (Hasbi et al., 2009), receptor coexpression is greatly reduced and anatomically circumscribed in adults with approximately 17–35% of nucleus accumbens (NAc) MSNs and 5–6% of caudate putamen (CP) MSNs expressing both receptors (Bertran-Gonzalez et al., 2008; Valjent et al., 2009; Perreault et al., 2010). In addition, increased D1–D2 receptor heteromer-induced signaling was more robust in mid-life compared to younger adults (Rashid et al., 2007), an effect that may correspond to

*Correspondence to: S. R. George, Department of Pharmacology, Medical Sciences Building, Room 4358, University of Toronto, 1 King's College Circle, Toronto, ON, Canada M5S 1A8. Tel: +1-416-978-3367; fax: +1-416-971-2868.
E-mail address: s.george@utoronto.ca (S. R. George).

[1] These authors contributed equally to the preparation of this manuscript.

Abbreviations: CP, caudate putamen; D1R, D1 receptor; D2R, D2 receptor; DYN, dynorphin; ENK, enkephalin; HBSS, Hanks' balanced salt solution; MSN, medium spiny neurons; NAc, nucleus accumbens; pFRET, processed FRET.

0306-4522/12 $36.00 © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuroscience.2012.08.042

increased D1R and D2R coexpression and/or increased D1–D2 receptor heteromer densities.

There have been many reports detailing the differences in behavioral responding to dopamine drugs in immature versus mature rats, such as enhanced responses to pychostimulant-induced reward (Shahbazi et al., 2008; Zakharova et al., 2009a,b; Anker and Carroll, 2010), but reduced responsiveness to the locomotor activating effects of the drugs (Bolanos et al., 1998; Banerjee et al., 2009; Zakharova et al., 2009a), findings indicative of age-dependent differences in dopamine neurotransmission. Thus, given the evidence of time-dependent changes in striatal D1–D2 receptor heteromer expression, in the present study we sought to compare the expression levels of the D1–D2 heteromer in NAc and CP in juvenile and adult rats, and additionally, to evaluate their grooming responses, a behavior previously shown to be mediated by the D1–D2 heteromer (Perreault et al., 2012), following activation of the receptor complex. We showed that juvenile animals exhibited reduced coexpression of D1R and D2R in NAc shell and CP, but not in NAc core, suggestive of lower D1–D2 receptor heteromer densities and region-specific decreases in D1–D2 heteromer-induced neurotransmission. Accordingly, these changes in heteromer expression were associated with reduced responsiveness to the induction of grooming induced by activation of the receptor complex.

EXPERIMENTAL PROCEDURES

Neuronal cultures

Neonatal rat striata (1 day of age) were trypsinized in Hanks' balanced salt solution (HBSS) with 0.25% trypsin and 0.05% DNase (Sigma) at 37 °C, and cells were washed three times in HBSS with 12 mM MgSO4. Cells were dissociated in DMEM with 2 mM glutamine and 10% FBS and plated at 2×10^5 cells per poly-L-lysine-coated well (Sigma; 50 g/mL). The next day, media were changed to Neurobasal medium with 50X B27 Supplement and 2 mM glutamine (Invitrogen). On day 3 of culture, 5 μM of cytosine arabinoside was added to inhibit glial cell proliferation. Half of the medium was changed every 3 days. Neurons were fixed on day 4, 11 or 25 of culture for use in fluorescence immunocytochemistry.

Animals

Thirty-four experimentally naïve juvenile (aged 26–28 days) or adult male Sprague–Dawley rats (Charles River, Canada), weighing 50–70 g or 400–450 g at the start of the experiment were used. Rats were pair-housed in polyethylene cages in a colony room maintained on a 12-h light–dark cycle with free access to food and water. Following arrival, rats were handled for 2 min daily for 3 days before the start of experiments. 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 Use of Experimental Animals (Canadian Council on Animal Care, 1993).

Drugs

SKF 83959 hydrobromide (Tocris Bioscience) was dissolved in physiological saline containing 5% DMSO and administered subcutaneously. SKF 83959 has been previously shown to selectively activate the Gq-coupled D1–D2 receptor heteromer (Rashid et al., 2007). For non-drug injections, an equivalent volume of saline was administered. All injections were administered at a volume of 1.0 ml/kg.

Fluorescence immunochemistry

Immunochemistry was performed on paraformaldehyde-fixed striatal neurons or floating coronal sections from perfused rat brains that were incubated for 24 or 60 h at 4 °C with primary antibodies (1:500 or 1:200; D1R, Sigma–Aldrich; D2R, ENK, Chemicon; DYN, Neuromics) as previously described (Lee et al., 2004; Hasbi et al., 2009). Specificity of the dopamine receptor antibodies for the D1R and D2R have been previously tested and reported (Lee et al., 2004; Perreault et al., 2010) and were tested using the five dopamine receptors (D1–D5) individually expressed in HEK293 cells. The D1R antibody was also shown to have no reactivity in striatal tissue of D1R gene-deleted mice, nor did the D2R antibody exhibit reactivity in striatal tissue of D2R gene-deleted mice. Additional controls were performed in the absence of the primary or secondary antibodies. Antibody dilutions were also used to identify the optimal working concentrations. To minimize background and prevent cross-excitation of the secondary antibody-linked fluorophores, only three primary antibodies were used on the tissue at any given time. Images were obtained using an Olympus Fluoview 1000 confocal microscope with a 60×/1.4 NA objective. Lower magnification images (40×) were obtained for the purpose of cell counting, which was performed using 300 μm^2 regions.

Confocal microscopy FRET and data processing

Confocal microscopy FRET was performed as previously described (Hasbi et al., 2009). Paraformaldehyde-fixed striatal neurons or floating sections from rat brain were incubated with primary antibodies to D1R and D2R, and the species-specific secondary antibodies conjugated to Alexa Fluors. The images were acquired with an Olympus Fluoview FV 1000 laser scanning confocal microscope with a 60×/1.4 NA objective. Anti-D2-Alexa 350 was used as the donor dipole, while anti-D1-Alexa 488 was used as the acceptor dipole. The donor was excited with a krypton laser at 405 nm, while the acceptor was excited with an argon laser at 488 nm. The emissions were collected at 430/20 and 530/20 nm LP filter. Other FRET pairs (Alexa 488–Alexa 568 and Alexa 568–Alexa 647) were tested and showed comparable results. Eleven images were acquired for each FRET analysis in accordance with an algorithm (Chen et al., 2005), designed to remove both the donor and acceptor spectral bleed-through (SBT) signals and to correct for variation in fluorophore expression level (FEL) associated with FRET imaging. The processed FRET (pFRET) images were generated using a software developed based on the described algorithm (Chen et al., 2005), in which:

$$\text{pFRET} = \text{UFRET} - \text{ASBT} - \text{DSBT},$$

where UFRET is uncorrected FRET, ASBT and DSBT are the acceptor and the donor spectral bleed-through signals. The rate of energy transfer efficiency (E) and the distance (r) between the donor (D) and the acceptor (A) molecules were estimated by selecting small regions of interest (ROIs) using the same images and software, based on the following equation:

$$\text{Efficiency}: E = 1 - I_{DA}/[I_{DA} + \text{pFRET} * ((\psi_{dd}/\psi_{aa}) * (Q_d/Q_a))],$$

where I_{DA} is the donor image in the presence of acceptor, ψ_{dd} and ψ_{aa} are collection efficiency of the donor and acceptor channels, Q_d and Q_a are the quantum yields of the donor and the acceptor. E is proportional to the 6th power of the distance separating the FRET pair. Thus,

Download English Version:

https://daneshyari.com/en/article/4338244

Download Persian Version:

https://daneshyari.com/article/4338244

Daneshyari.com