Journal of Neuroscience Methods 174 (2008) 272–280

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Dopamine receptors in human lymphocytes: Radioligand binding and quantitative RT-PCR assays

Galina P. Kirillova[a,*], Rebecca J. Hrutkay[a], Michael R. Shurin[b], Galina V. Shurin[b], Irina L. Tourkova[b], Michael M. Vanyukov[a,c,d]

[a] Center for Education and Drug Abuse Research, Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA 15261, USA
[b] Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA 15261, USA
[c] Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15213, USA
[d] Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15261, USA

ARTICLE INFO

Article history:
Received 25 June 2008
Received in revised form 22 July 2008
Accepted 22 July 2008

Keywords:
DRD1
DRD2
DRD3
DRD4
DRD5
Gene expression
Endophenotypes
Intermediate trait

ABSTRACT

Analysis of dopamine receptors (DR) in lymphocytes of the human peripheral blood mononuclear cell (PBMC) fraction is an attractive tool for evaluation of functional properties of dopaminergic function underlying variation in complex psychological/psychopathological traits. Receptor binding assays (RBAs) with selective radioligands, which are widely used in CNS studies, have not produced consistent results when applied to isolated PBMC. We tested the assay conditions that could be essential for detection of DR in human PBMC and their membrane preparations. Using [^{3}H]SCH23390, a dopamine D1-like receptor antagonist, we demonstrated the presence of two binding sites in PBMC-derived membrane fraction. One of them is characterized by the K_d value consistent with that reported for D5 dopamine receptors in human lymphocytes, whereas the other K_d value possibly corresponds to serotonin receptor(s). Although D5 receptor binding sites in PBMC membranes could be characterized by binding assays, the low protein expression and the large volume of blood needed for membrane preparation render the binding method impracticable for individual phenotyping. In contrast, real-time RT-PCR may be used for this purpose, contingent on the relationship between DR expression in the brain and in lymphocytes. The expression of the *DRD2–DRD5* genes, as detected by this method, varied widely among samples, whereas the *DRD1* expression was not detected. The expression levels were comparable with those in the brain for *DRD3* and *DRD4*, and were significantly lower for *DRD2* and *DRD5*.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Dysfunction of dopamine neurotransmission in the central nervous system (CNS) is implicated in the development and progression of behavioral dysregulation and drug addiction (Koob and Nestler, 1997; Wise, 1998; Tarter et al., 1999; Vallone et al., 2000; Maher et al., 2002a,b). Dopamine receptors (DR) are one of the key elements of the dopaminergic system. Five types of DR, D1 through D5, are coexpressed at different levels and in various combinations in the CNS and peripheral tissues.

Studying DR in the human CNS has obvious limitations. Determining peripheral sources of DR, accessible in a minimally invasive way, would be advantageous for research and clinical applications. Human peripheral blood lymphocytes have attracted attention as a convenient candidate for evaluation of the functional properties of DR. Molecular biological studies have detected D3, D4 and D5 receptors mRNA in human lymphocytes (Takahashi et al., 1992; Nagai et al., 1993; Bondy et al., 1996). However, DR detection in lymphocytes by the radioligand binding method (Bondy et al., 1990) produced controversial results (Fleminger et al., 1982; Maloteaux et al., 1982, 1983; Rao et al., 1990; Vile and Strange, 1995, 1996). Nevertheless, one group has consistently reported quantitative detection of D3, D4 and D5, but not D1 or D2 receptors on human lymphocytes (Ricci and Amenta, 1994; Ricci et al., 1995, 1997, 1999; Amenta et al., 1999). Despite controversy in the results of binding studies with lymphocytes, there have been attempts to apply the receptor binding assay (RBA) in clinical studies of schizophrenia, migraine, Alzheimer's disease and Parkinson's disease (Le Fur et al., 1980, 1983; Rotstein et al., 1983; Bondy et al., 1985; Bondy and Ackenheil, 1987; Halbach and Henning, 1989; Barbanti et al., 1999, 2000a, b). Some of these RBA findings were supported by RT-PCR results (Nagai et al., 1996; Ilani et al., 2001; Kwak et al., 2001).

* Corresponding author at: Center for Education and Drug Abuse Research, Department of Pharmaceutical Sciences, University of Pittsburgh, 513B Salk Hall, Pittsburgh, PA 15261, USA. Tel.: +1 412 648 9909.
E-mail address: galinag@pitt.edu (G.P. Kirillova).

0165-0270/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2008.07.018

The goals of this study were to optimize the binding assay conditions for human peripheral blood mononuclear cell (PBMC) preparations as well as examine real-time RT-PCR technique, to use these methods in the peripheral measurement of DR expression for possible application in substance use disorder (SUD) research and other behavior-related studies. We have previously obtained pilot data indicating association of the *DRD5* gene with the risk for SUD (Vanyukov et al., 1998, 2001) and antisociality (Vanyukov et al., 2000), and thus aimed at exploring the possibility of relating these results to the properties of the dopamine D5 receptor.

2. Materials and methods

2.1. Reagents

The following reagents were used: [^{3}H]SCH23390, 69 Ci/mmol (Amersham); (+)-butaclamol, pepstatin A, leupeptin hydrochloride, phenylmethanesulfonyl fluoride (PMFS), EDTA, HEPES, Tris–HCl, Histopaque-1077, polyethylenimine (PEI), ascorbic acid, and NaCl physiological solution (Sigma); HBSS and PBS buffers, and Trizol-LS reagent (Invitrogen); Ready-Safe scintillation cocktail (Beckman); BCA protein assay reagent kit (Pierce); RNEasy Mini Kit (Qiagen); Superscript II (Gibco BRL); RNAsin (Promega); DNA-free Kit and human brain total RNA (Ambion); dNTP set (Gene Choice). Primers and fluorescence-labeled probes (assays-on-demand) for D1 (ref: Hs00265245_s1), D2 (ref: Hs00241436_m1), D3 (ref: Hs00168045_m1), D4 (ref: Hs00609526_m1), D5 (ref: Hs00361234_s1) receptors and human *GAPDH* control reagents (with included forward and reverse primers and probes) were ordered from Applied Biosystems.

2.2. Specimen collection

Blood for PBMC isolation and optimization of binding assays was drawn from healthy volunteers. Lymphocyte-enriched buffy coat product received from a local blood bank was used for PBMC membrane fraction preparation. Blood for extraction of total RNA for real-time PCR experiments was drawn from 38 adult male participants in a study of substance use disorder etiology [Center for Education and Drug Abuse Research, CEDAR (Tarter and Vanyukov, 2001)]. In this group, 14 individuals were diagnosed with a DSM-III-R substance use disorder related to an illicit drug (SUD+), 5 with alcohol use disorder only, 3 with another psychiatric disorder, and 16 had no psychiatric disorder (SUD−). An expanded version of the Structured Clinical Interview for DSM-III-R-outpatient version (SCID-OP) (Spitzer et al., 1987) was administered by experienced research associates to obtain psychiatric diagnoses. The expanded SCID evaluates current episode (past 6 months) and worst past (before the past 6 months) episode of psychopathology. Informed consent was obtained from all participants. The study was approved by the University of Pittsburgh Institutional Review Board.

2.3. Peripheral blood mononuclear cell isolation

PBMC were isolated from 50 to 100 ml of blood drawn into EDTA vacutainers. The blood was diluted twofold with NaCl physiological solution (Ricci and Amenta, 1994), or HBSS/HEPES buffer (136 mM NaCl, 4.16 mM $NaHCO_3$, 0.34 mM Na_2HPO_4, 5.36 mM KCl, 0.44 mM KH_2PO_4, 1.25 mM $CaCl_2$, 0.81 mM $MgSO_4$, 22.3 mM HEPES, 1 mM EDTA, pH 7.4) (Bondy et al., 1990), or phosphate buffered saline (PBS, pH 7.4). The diluted blood (15 ml) was layered on Histopaque-1077 reagent (15 ml) in 50 ml polypropylene centrifuge tubes and centrifuged at 22–23 °C for 40 min at 400 × *g*. Collected PBMC were washed in 30 ml of isolation solution by three consequent centrifugation steps (15 min at 300 × *g*), every time gently resuspended with a pipette tip precut to protect cells integrity. Viability of cells was tested by Trypan blue exclusion. The cell suspension was used for the assay within 20–30 min after preparation. To test the influence of the presence of monocytes in the PBMC fraction, the binding step was performed with and without monocytes in the samples. Monocytes were removed by incubation of cell suspension at 37 °C and 5% CO_2 for an additional 60 min (Ricci and Amenta, 1994), and the non-adherent cells were used for binding assay.

To isolate PBMC from the buffy coat product, the contents of the pack (~60 ml) were diluted 3-fold with the HBSS isolation buffer, and 10 ml of the suspension were layered on 15 ml of Histopaque-1077 in 50 ml tubes. After centrifugation as described above, cells were washed three times, and the final cell pellets (300–400 × 10^6 cells per tube) were frozen at −80 °C and used later for the membrane preparation.

2.4. Membrane preparation from PBMC isolated from buffy coat

The membrane preparation procedure used in this study was modified from protocols described elsewhere (Chiu et al., 1982; Seeman et al., 1984; Zhou et al., 1990; Hill et al., 1996). Cells defrosted on ice (300–400 × 10^6 cells per tube) were reconstituted in 10 ml of the ice-cold hypotonic Tris–HCl buffer (25 mM Tris–HCl, 6 mM $MgCl_2$, 1 mM EDTA, pH 7.4) that contained 10 μM PMFS, 1 μM pepstatin A, 1 μM leupeptin. Cells were homogenized on ice three times for 15 s by TissueTearor homogenizer (Biospec Products, Inc) at 30,000 rpm with 30-s intervals between the homogenization steps. The homogenate was diluted with an additional 10 ml of the ice-cold isolation buffer and centrifuged for 30 min at 4 °C at 24,000 × *g*. The residue was resuspended in 10 ml of the isolation buffer, homogenized three times for 15 s, and centrifuged for 30 min at 39,000 × *g* at 4 °C. The final pellet was resuspended in 1–2 ml of the isolation buffer to contain about 100–260 μg of membrane protein in 50 μl. Protein concentration was measured at the different steps of the membrane preparation. The protein content estimated for 1 × 10^6 cells from PBMC sample and for isolated membranes from the same amount of cells was approximately 40–50 and 13–17 μg, respectively. The membranes were kept on ice and used for binding assay within 20–30 min after preparation.

2.5. Binding procedure

2.5.1. Isolated PBMC

Binding assays were conducted in glass tubes in experiments where the unbound fraction of the ligand was separated by filtration, or in Eppendorf microcentrifuge tubes when centrifugation was used to terminate binding. A single concentration of [^{3}H]SCH23390 (0.6–0.8 nM) was used to characterize the dynamics of the ligand binding at 4, 23 and 37 °C. A minimum of 12 concentrations of [^{3}H]SCH23390 in the range of 0.062–8 nM were used to generate the concentration-dependent binding curve with triplicated PBMC probes. Whereas this compound is a highly potent antagonist for both D1 and D5 receptors (Hyttel, 1983; Bourne, 2001), we used it as a specific D5 receptor antagonist because the D1 receptor had not been detected in human lymphocytes (Ricci et al., 1999). (+)-Butaclamol (1 μM) was used as the displacer to determine non-specific binding. The reaction mixture contained 250 μl of buffer with radioligand, 250 μl of buffer with butaclamol (or without butaclamol) and 250 μl of the lymphocyte suspension.

Buffers of the following composition were used:

(1) Buffer 1 (170 mM Tris–HCl): 170 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 1 mM EDTA, pH 7.4;

Download English Version:

https://daneshyari.com/en/article/4335959

Download Persian Version:

https://daneshyari.com/article/4335959

Daneshyari.com