Published in final edited form as:

J Neurosci Methods. 2008 September 30; 174(2): 272–280. doi:10.1016/j.jneumeth.2008.07.018.

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, and Michael M. Vanyukov^{a,c,d}

^aCenter for Education and Drug Abuse Research, Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA 15261, USA

^bDepartment of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA 15261, USA

^cDepartment of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15213, USA

^dDepartment of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15261, USA

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 (RBA) 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 [³H] 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*.

Keywords

DRD1; *DRD2*; *DRD3*; *DRD4*; *DRD5*; gene expression; endophenotypes; intermediate trait

*Corresponding author: University of Pittsburgh, Pittsburgh, PA 15261, USA; Tel.: +1 412 648 9909, E-mail address: galinag@pitt.edu (G.P. Kirillova).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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., 2002). 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 Ackenhil, 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).

The goals of this study were to optimize the binding assay conditions for human 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: [³H]SCH23390, 69 Ci/mmol (Amersham); (+)-Butaclamol, pepstatin A, leupeptin hydrochloride, PMFS (phenylmethanesulfonyl fluoride), EDTA, HEPES, Tris-HCl, Histopaque-1077, PEI (polyethylenimine), 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+), five 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 (PBMC) isolation

PBMC were isolated from 50–100 ml of blood drawn into EDTA vacutainers. The blood was diluted two-fold with NaCl physiological solution (Ricci and Amenta, 1994), or HBSS/HEPES buffer (136 mM NaCl, 4.16 mM NaHCO₃, 0.34 mM Na₂HPO₄, 5.36 mM KCl, 0.44 mM KH₂PO₄, 1.25 mM CaCl₂, 0.81 mM MgSO₄, 22.3 mM HEPES, 1 mM EDTA, pH 7.4) (Bondy et al., 1990), or PBS (phosphate buffered saline, 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% CO₂ 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 Hystopaque-1077 in 50 ml tubes. After centrifugation as described above, cells were washed three times, and the final cell pellets (300–400×10⁶ 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 (300–400×10⁶ cells per tube) were reconstituted in 10 ml of the ice-cold hypotonic Tris-HCl buffer (25 mM Tris-HCl, 6 mM MgCl₂, 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 sec by TissueTearor homogenizer (Biospec Products, Inc) at 30,000 rpm with 30-second 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 3 times for 15 sec, 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 1×10⁶ cells from PBMC sample and for isolated membranes from the same amount of cells was approximately 40–50 μg 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 [^3H] SCH23390 (0.6–0.8 nM) was used to characterize the dynamics of the ligand binding at 4°C, 23°C and 37°C. A minimum of 12 concentrations of [^3H]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 nonspecific 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;
2. Buffer 2 (50 mM Tris-HCl): 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , pH 7.4;
3. Buffer 3 (HBSS/HEPES): 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.

Experimental conditions, such as the cell number, buffer composition, incubation temperature and duration of binding reaction, varied in accordance with the experimental task (see figure legends). At the end of the assay the unbound fraction of the ligand was separated by vacuum filtration or by brief centrifugation followed by aspiration of the supernatant.

The separation of the unbound ligand by filtration was conducted by a vacuum Millipore system at room temperature. The GF/B filters (25 mm in diameter) were prewashed with the assay buffer. The unbound ligand was washed out 3 times from the filters with 2 ml of ice-cold assay buffer. Slightly air-dried filters were placed into scintillation vials with 5 ml of Ready-Safe cocktail (Beckman), shaken for 16 h and counted by a Beckman LS 5500 beta-counter for 5 min. Alternatively, the binding reaction was terminated by the brief centrifugation of reaction tubes at $14,000\times g$ at 23°C. The supernatant was aspirated without disturbing the cell spot. The pellet was resuspended in 300 μl of 1% SDS, transferred into a scintillation vial with 5 ml of the scintillation cocktail, and radioactivity was counted for 5 minutes.

2.5.2. PBMC membrane fraction—Membranes (50 μl) were incubated with [^3H] SCH23390 in a buffer containing 120 mM NaCl, 20 mM Tris-HCl, 2 mM MgCl_2 , 1.4 mM ascorbic acid, 10 mg/ml of BSA, pH 7.4 in 750 μl of total volume of reaction mixture. The temperature and time-dependent dynamics of ligand binding with the membranes was determined at 4°C, 23°C and 30°C with a single concentration of the radioligand. Concentration-dependent binding was conducted with increasing ligand concentrations (0.062–30 nM) for 30 min at 23°C. Non-specific binding was determined in the presence of 10 μM (+)-butaclamol. The reaction was started with addition of the membranes and was conducted at low speed shaking. The unbound fraction of the ligand was separated by filtration through GF/B filters presoaked in 0.5 % PEA (pH 7.4) for 1 h prior the filtration step. The filters were washed and counted as described for PBMC.

2.6. Calculation and analysis of binding assay data

Specific binding (SB) of [^3H]SCH23390 was estimated by subtraction of the amount of ligand bound to PBMC or membranes in the presence of (+)-butaclamol (nonspecific binding, NB) from the amount of the ligand bound in the absence of butaclamol (total binding, TB). K_d values were determined from the specific binding curve by nonlinear regression analysis using GraphPad Prism software (version 4).

2.7. Quantitative real-time PCR assay

Total RNA was extracted from isolated PBMC with Trizol-LS reagent according to the manufacturer's protocol, and was purified with DNA-free Kit and RNEasy Mini Kit. Commercial human total brain RNA was used as calibrator, and *GAPDH* was used as the reference housekeeping gene in RT-PCR assays. Total RNA (400 ng in 100 μl) was first reverse-transcribed into first-strand cDNA using Superscript II reverse transcriptase and random hexamers as primers. Amplification of the DR target gene and *GAPDH* was conducted with 20 ng of cDNA in 25 μl of the reaction mixture by gene-specific primers using fluorogenic probes and 7300 TaqMan instrument (ABI). PCR cycling parameters were: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 sec, and 60°C for 1 min.

The amplification of the PCR product was expressed as the number of amplification cycles at which this particular product was first detected above the background (C_T). The analysis of relative gene expression was performed by the comparative $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001). The average C_T from four replicates was calculated for both target and *GAPDH* genes. The ΔC_T ($C_{T, \text{DR}} - C_{T, \text{GAPDH}}$) was determined to normalize for amounts of RNA used in reverse transcription reactions from PBMC and brain samples. The $\Delta\Delta C_T$ ($\Delta C_{T, \text{PBMC}} - 10 \Delta C_{T, \text{brain}}$) was determined to calculate target gene expression in the PBMC sample relative to the target gene expression in the brain sample. Therefore, the data are presented as fold change ($2^{-\Delta\Delta C_T} \pm \text{SD}$) in PBMC gene expression relative to the brain.

3. Results

3.1. Characterization of isolated PBMC samples used for binding assay

The recovery of lymphocytes isolated from the blood of different donors varied from 0.75×10^6 cells/ml to 1.6×10^6 cells/ml. The total number of PBMC isolated from 60 ml of six specimens of buffy coat varied from 350×10^6 to 800×10^6 cells. All techniques utilizing blood samples met the criteria recommended for PBMC isolation to conserve the integrity of the cell membrane (Bondy et al., 1990), and did not exceed 2 hr. Preparation of PBMC from the buffy coat was more time-consuming (2.5–3 hr) due to the larger sample volume.

The final PBMC suspension contained less than 10% of erythrocytes, and the platelet/lymphocyte ratio was on average 8:1. Viability of the isolated cells was 96–98% regardless of whether NaCl, PBS, or HBSS buffers were used for the isolation procedure. However, 1 hr incubation of isolated cells at 37°C in 5% CO_2 , conducted to remove the adherent monocytes (Ricci et al., 1994), caused about 10% death of lymphocytes in NaCl solution, but only 2% in HBSS buffer. The recovery of nonadherent cells after incubation depended upon the labware used for monocyte removal: 60–70% of the original cell count when regular plastic Petri dishes were used, and 45–50% when culture flasks or vacuum gas plasma treated Petri dishes were used instead. There was no change in binding after the removal of monocytes from PBMC sample. At the end of 1 h of the binding procedure there was about 3% cell death in the reaction mixture at both 23°C and 37°C.

3.2. The influence of assay conditions on [³H]SCH23390 binding with intact PBMC

Using a single ligand concentration, we evaluated binding of [³H]SCH23390 to PBMC under assay conditions previously described in other studies (Ricci and Amenta, 1994; Bondy et al., 1990). The methods of separation of the unbound ligand (centrifugation or filtration procedures), the buffer composition, and the temperature and duration of the binding steps were among the tested conditions.

3.2.1. Specific and nonspecific binding—Figure 1 demonstrates the [³H]SCH23390 binding with PBMC in 170 mM Tris-HCl buffer (assay buffer used in Ricci et al., 1994) when 0.8 nM of radioligand was used for binding, and centrifugation was applied to separate unbound fraction. Under these conditions, relatively low specific binding (15–20% of total binding in different experiments) and a high level of non-specific association of [³H]SCH23390 were estimated in the presence of 1 μM (+)-butaclamol. To ensure that specific binding was not an artifact, a parallel set of tubes with PBMC was preincubated in the boiling waterbath prior to the binding assay (100% of cells were dead after 8 minutes of incubation). The total radioligand binding to the dead cells was still high, approximately 70% of that with intact cells, and specific binding was abolished, as indicated by the absence of the displacing effect of 1 μM (+)-butaclamol in these samples.

3.2.2. Influence of washing procedure—To simulate the washing procedure that is routinely used in protocols with filtration to remove traces of the unbound ligand, the PBMC pellets were rinsed with fresh ice-cold assay buffer (without pellet distortion), followed by brief centrifugation. This procedure displaced almost 70% of the ligand from the pellet after the first washing step (Fig. 2). Repeated washing decreased total binding to the background level (the level of radioactivity absorbed by the tubes incubated with the ligand in the absence of cells).

As these experiments show, a relatively low proportion of radioligand specifically bound to the PBMC, in combination with weak association of the ligand with these sites, could impede the detection of the radioactive signal related to specific binding. The washing procedure can render this signal undetectable.

3.2.3. Influence of buffer composition—The assay buffer composition significantly influenced binding of [³H]SCH23390 with PBMC. The presence of Tris-HCl noticeably suppressed total as well as specific binding of the 0.8 nM radioligand with cells (Fig. 3a). As shown in Fig. 3b, approximately 9% of the added radioligand was bound in HBSS/HEPES (buffer 3; used in the protocols of Bondy et al., 1990). The bound fraction decreased approximately 1.5- and 2.3-fold respectively in the buffers containing 50 mM (buffer 2) and 170 mM Tris-HCl (buffer 1; as used by Ricci et al., 1994).

The specific binding was also the highest in the HBSS/HEPES buffer, and decreased 1.4 and 3.5 times, respectively, when 50 mM and 170 mM Tris-HCl were used instead. However, the fractions of ligand bound to the cells specifically (SB/TB) were similar in HBSS/HEPES and 50 mM Tris-HCl solutions (35 and 37% of the total bound ligand), whereas this fraction was decreased to 21% in the presence of 170 mM Tris-HCl. The latter observation indicates that the presence of 50 mM Tris-HCl affected both specific and nonspecific binding sites to a similar degree, while in the presence of 170 mM Tris-HCl specific binding sites were affected to a greater degree.

These data demonstrate that the influence of Tris-HCl on the interaction of [³H]SCH23390 with specific binding sites on human PBMC could be essential for the characterization of this sites and the detecting capacity of the method.

3.2.4. Influence of incubation temperature and assay duration—To evaluate the optimal temperature and the duration of the binding assay, the dynamics of [³H]SCH23390 binding to PBMC was tested at 4°C, 23°C and 37°C at different time intervals. Ligand binding in HBSS/HEPES was temperature- and time-dependent and was the highest at 37°C after 60 min of incubation. At all tested temperatures, however, binding was unstable and was followed by relatively rapid dissociation (Fig. 4).

3.2.5. Concentration-dependent binding—Concentration-dependent binding assays were conducted at 37°C. The displaceable binding curve generated with increasing ligand concentrations in the HBSS/HEPES buffer had saturable and nonsaturable components. Saturated specific binding (albeit not prominent) was reproducible when centrifugation was used for the separation of the unbound ligand (a representative experiment is shown on Fig. 5). No saturation was observed when the unbound ligand was separated by filtration, or when the 170 mM Tris-HCl buffer was used for assay. The average K_d ($\pm$ SD) determined from three experiments (assayed in triplicates) was 5.2 ± 1.6 nM.

The data presented demonstrate that the choice of assay buffer as well as centrifugation or filtration steps may be crucial for the detection of high affinity receptor binding sites on intact PBMC.

3.3. Relative expression of DR mRNAs isolated from PBMC

Low DR protein expression in lymphocytes may be a factor explaining the difficulties and variations in the detection of DR in PBMC samples. To address this possibility, we estimated the relative levels of mRNA for the five DR in 38 CEDAR PBMC samples, using human brain RNA as reference. In the brain samples utilized in our study, mRNA for all five DR was present. The lowest level of expression was observed for the *DRD3* mRNA. The expression of the mRNA for the *DRD1*, *DRD2*, *DRD4* and *DRD5*, relative to the *DRD3* mRNA, was 151.9 ± 19.53 ; 37.9 ± 12.02 ; 1.3 ± 0.53 and 48.6 ± 14.22 respectively (mean folds $\pm$ SD calculated using the $2^{-\Delta\Delta CT}$ method). In the PBMC samples, unlike the brain, the *DRD1* mRNA was not detected (Table 1; only the total sample data are shown as no group differences were detected). The levels of *DRD3* and *DRD4* mRNA in all samples were comparable to those in the brain. The levels of the *DRD5* were 10–200 times lower than those in the brain. The *DRD2* mRNA was detected in 31 sample out of 38, and was 100–1000 lower than that in the brain.

Assuming correlation between the levels of DR mRNA and receptor protein, these results suggest that the low and variable expression of the DR may explain the inconsistent results of binding assays with isolated PBMC, as well as the negative results with PBMC membranes (Fleminger et al., 1982; Rao et al., 1990).

3.4. [³H]SCH23390 binding with PBMC membranes

Due to the low receptor expression, the sensitivity of the binding method may be insufficient when applied to PBMC, because the amount of receptor protein in the cell material used for assay (generally 1.5×10^6 cells/reaction, corresponding to ~ 15 μ g of total protein) is relatively small compared to that in the brain studies, where the same method is successfully applied (Seeman et al., 1984; Hill et al., 1996; Sweet et al., 2001a,b; Hurley et al., 2001). Using the PBMC membrane fraction, which contains a higher level of total protein (130–260 μ g/750 μ l of reaction mixture), we were able to demonstrate that the specific binding of [³H]SCH23390 was time- and temperature-dependent (Fig. 6). Binding reached its maximum in 30 minutes after the beginning of incubation at all tested temperatures, and was the highest at 30°C (binding at 37°C was not tested because of rapid ligand dissociation, as shown above).

Because of rapid dissociation of the ligand from the membranes at 30°C, saturation binding assays were conducted at room temperature. The data from a representative experiment are shown on Figure 7. The concentration-dependent specific-binding curve contained saturable and non-saturable components. The saturable component had two transient plateaus and was followed by the non-saturable component up to 30 nM of the radioligand (high concentrations are not shown). These results were reproduced on three different membrane preparations (in triplicates). Two K_d values were determined from these experiments, indicating two distinct binding sites. The lower K_d value, 0.61 ± 0.22 nM, was close to those reported for D5 DR from the binding studies with intact lymphocytes (Ricci and Amenta, 1994; Ricci et al., 1999). The higher K_d value, 4.3 ± 2.2 nM, was similar to the values determined from our experiments with whole PBMC (Fig. 5).

These data suggest that, because of low expression, the amount of receptor protein in PBMC preparations (cells or membranes) used in binding assays is a critical factor for the detection of high affinity binding sites related to DR.

4. Discussion

Peripheral indicators of dopaminergic and other neurobiological activity could be of great benefit in the validation of results of genetic studies of behavior and psychopathology, including addictions. The potential for estimating expression of dopamine receptors on human lymphocytes and the relatively facile availability of these cells have triggered interest to the DR binding assay. Whereas the results of prior binding studies related to DR in isolated human lymphocytes have been contradictory, we attempted to adopt the methodologies that have been consistently reported as resulting in reliable evaluation of DR expression in these cells (Bondy et al., 1990; Ricci and Amenta, 1994; Ricci et al., 1999).

Various conditions were reported in different studies to be essential for the success of binding assays. These conditions include integrity of the outer membrane of isolated lymphocytes and the presence of ascorbic acid in the assay buffer (Shaskan et al., 1984), presence of EDTA in blood collecting tubes and the concentrations of butaclamol in reaction mixture (Bondy et al., 1990), and the pretreatment of filters with PEI solution prior filtration step (Gundlach et al., 1984; Wodarz et al., 1992). Despite some deviations from the original procedure (Bondy et al., 1990), very consistent data for DR expression in lymphocytes were reported for binding assays conducted with highly selective ligands for D1-like (Ricci & Amenta, 1994, 1999) and D2-like receptors (Ricci et al., 1995, 1997, 1998; Amenta et al., 1999). We did not, however, observe saturated specific binding and were unable to detect high-affinity binding sites for the dopamine D5 receptor in human PBMC in conditions described by these authors. Consequently, we attempted to apply the assay conditions as originally described by Bondy et al. (1990).

Since the quality of isolated lymphocytes may affect binding results, it was evaluated with different assay buffers and isolation protocols. The viability of cells could not influence binding results because it was consistently high in all PBMC preparations, and did not differ regardless of the buffer composition used for cell isolation. The depletion of monocytes from PBMC samples, resulting in an increased proportion of non-adherent lymphocytes in the PBMC fraction, did not improve the results of the binding assays either. Therefore, variations in the lymphocyte preparation procedure did not affect the results of the binding assays in our study.

The choice of conditions for the binding reaction was more critical. Using buffers of different composition, we demonstrated that Tris-HCl affected the association of [3 H]SCH23390 with PBMC and significantly decreased both total and specific binding. We were unable to replicate saturated specific binding in the assay buffer containing 170 mM Tris-HCl – the conditions

under which high affinity binding sites for D₅ receptor were reported by Ricci et al. (1994, 1999). However, when the HBSS/HEPES buffer was used for assay, as in Bondy et al. (1990), the displaceable binding curve had both saturated and non-saturated components. The K_d estimated, however, was not consistent with that expected for the D₅ DR and exceeded ~10-fold those previously reported for D₅ receptors (Ricci and Amenta, 1994; Ricci et al., 1999), indicating either a decreased affinity of ligand to the D₅ binding sites, or that binding sites of a different kind were detected in our study. The presence of Tris-HCl may affect the interaction of [³H]SCH23390 with PBMC either by decreasing the ligand affinity or by preventing its access to specific binding sites.

In line with prior research with ligands for D₂-like receptors (Maloteaux et al., 1983; Bondy et al., 1990), our data show that methods used for the termination of the binding reaction and for separation of the unbound radioligand (centrifugation, filtration and washing) can also affect results of binding assays with [³H]SCH23390. Due to the weak association of this ligand with PBMC, the residual radioactivity shared between specific and non-specific binding sites could be at the edge of the method's detecting limits. In addition, a significant amount of total protein (up to 30%) and a comparable percentage of receptors could be lost during washing steps (Seeman et al., 1984). A combination of these factors could partly explain failures in prior research (Maloteaux et al., 1982, 1983; Fleminger et al., 1982) and in our binding studies. Although pretreatment of the filters with PEI was suggested as beneficial to the binding assays (Gundlach et al., 1984; Wodarz et al., 1992), no improvement was observed in our experiments with PBMC.

The methodological factors we tested could be especially important for PBMC samples with small numbers of DR, and the wide range of inter-individual variability could be a possible cause of discrepant reports. Significant variability of D₂-like receptor expression was observed in lymphocyte samples isolated from different subjects (Rotstein et al., 1983; Griffiths et al., 1992; McKenna et al., 2002). A very wide range of receptor density (B_{max}), was also reported for the D₅ receptor by Ricci and colleagues: 11.2 fmol/5×10⁶ cells (Ricci & Amenta, 1994), 223 pmol/10⁶ cells (Ricci et al., 1995), and 231 fmol/10⁵ cells (Ricci et al., 1999). Since the cell composition of isolated PBMC preparations is heterogeneous, and DR are mainly expressed on T- and B-lymphocytes (Santambrogio et al., 1993; McKenna et al., 2002), the proportion of these cells and the variability of the levels of DR expressed on them may be critical for detection by the binding method.

D₂-like DR expression estimated from the levels of mRNA isolated from lymphocytes was shown to be too low to produce a sufficient amount of the protein detectable with binding assays (Vile and Strange, 1996). Our data from RT-PCR study show that the range of expression of *DRD2*, *DRD3*, *DRD4* (as well as *DRD5*) mRNA in PBMC varies widely. Our data also show that expression of the *DRD3* and *DRD4* mRNA in PBMC is comparable to that in the brain, whereas expression of the *DRD2* and *DRD5* mRNA in PBMC is significantly lower. Assuming that the expression of proteins and related mRNA for DR are correlated, the amount of lymphocyte material used for binding assays may be critical for assay performance. A low content of the membrane protein may partially explain the unsuccessful attempts to detect the D₂-like DR specific binding on membranes preparations from the lymphocytes (Maloteaux et al., 1983; Fleminger et al., 1982). Although attempts to increase the amounts of lymphocyte membranes in binding assays were made, negative results were reported (Rao et al., 1990), which may be due to conditions chosen for binding reaction: high membrane content (110 mg) in relatively small volume (250 µl) of reaction mixture, and absence of protease inhibitors in the membrane isolation medium. We were able to demonstrate time-, temperature-, and concentration-dependent binding when similar amounts of membranes isolated with protease inhibitors were incubated in a larger volume of reaction mixture (750 µl).

A novel finding of this study was the demonstration of two binding sites for [³H]SCH23390 with membranes isolated from PBMC. The displaceable binding curve in our experiments had saturated and non-saturated components. The saturated component of the specific binding curve was biphasic, indicating the presence of two binding sites, one of which is consistent with characteristics (K_d) of the D5 receptors reported for lymphocytes by Ricci et al. (1994; 1999). The second binding site was characterized by higher K_d values. Whereas its nature is unclear, it is tempting to speculate about the serotonergic origin of this site. Serotonin (5-HT) receptors have been shown to be expressed in human T-lymphocytes (Aune et al., 1993; Grimaldi et al., 1998; Marazziti et al., 2001), and monocytes (Fiebich et al., 2004; Frank et al., 2001). Despite the reportedly high selectivity of [³H]SCH23390 for the D1-like DR (Hyttel, 1983; Bourne, 2001), it also was shown to have a relatively high affinity to serotonin receptors. In experiments with platelets (De Keyser et al., 1989) and cloned human D1-like and 5-HT_{2C} type receptors (Millan et al., 2001), binding characteristics of [³H]SCH23390 were similar to those observed in this study.

In summary, our data with intact PBMC show limitations of the binding assay in the identification and characterization of dopamine D5 receptor binding sites. Although binding assays could be applied to PBMC membranes, their preparations require large volumes of blood. Because of that and due to the lack of assay precision, this approach is not suitable for individual phenotyping. An alternative methodology that could be used, for instance, for functional studies to support findings of genetic associations with complex traits is the real-time RT-PCR approach. Although we did not detect, using this method, group differences between SUD+ and SUD− individuals, this may in part be due to the lack of power: the sample was small while the effect sizes are expected to be low. It is also possible that DR expression in lymphocytes is not reflective of that in the brain and thus may not be informative for behavioral traits. For instance, as our data show, the relative expression of DR in lymphocytes does not parallel that in the brain. Nevertheless, studies suggest that lymphocytes remain an attractive potential window to DR expression relevant to mental disorders (Biermann et al., 2007; Boneberg et al., 2006; Zvara et al., 2005). The utility of lymphocytes as the peripheral site of DR expression will be further explored in our ongoing genetic research.

Acknowledgments

The authors thank Drs. Robert E. Ferrell and Robert A. Sweet and their assistants Liane Fairfull and Ruth Henteleff for helpful discussion and practical assistance, and the CEDAR faculty and staff for their help and support. This research was supported by grants P50DA005605, R01DA019157, and K02DA018701 from the National Institute on Drug Abuse.

References

- Amenta F, Bronzetti E, Felici L, Ricci A, Tayebati SK. Dopamine D2-like receptors on human peripheral blood lymphocytes: a radioligand binding assay and immunocytochemical study. *J Auton Pharmacol* 1999;19:151–159. [PubMed: 10511471]
- Aune TM, McGrath KM, Sarr T, Bombara MP, Kelley KA. Expression of 5HT_{1a} receptors on activated human T cells. Regulation of cyclic AMP levels and T cell proliferation by 5-hydroxytryptamine. *J Immunol* 1993;151:1175–1183. [PubMed: 8393041]
- Barbanti P, Fabbrini G, Ricci A, Bruno G, Cerbo R, Bronzetti E, Amenta F, Luigi Lenzi G. Reduced density of dopamine D2-like receptors on peripheral blood lymphocytes in Alzheimer's disease. *Mech Ageing Dev* 2000a;120:65–75. [PubMed: 11087905]
- Barbanti P, Fabbrini G, Ricci A, Cerbo R, Bronzetti E, Caronti B, Calderaro C, Felici L, Stocchi F, Meco G, et al. Increased expression of dopamine receptors on lymphocytes in Parkinson's disease. *Mov Disord* 1999;14:764–771. [PubMed: 10495037]

- Barbanti P, Fabbri G, Ricci A, Pascali MP, Bronzetti E, Amenta F, Lenzi GL, Cerbo R. Migraine patients show an increased density of dopamine D3 and D4 receptors on lymphocytes. *Cephalalgia* 2000b;20:15–19. [PubMed: 10817442]
- Biermann T, Bonsch D, Reulbach U, Kornhuber J, Bleich S. Dopamine and N-methyl-D-aspartate receptor expression in peripheral blood of patients undergoing alcohol withdrawal. *J Neural Transm* 2007;114:1081–1084. [PubMed: 17370105]
- Bondy B, Ackenheil M. 3H-spiperone binding sites in lymphocytes as possible vulnerability marker in schizophrenia. *J Psychiatr Res* 1987;21:521–529. [PubMed: 3481637]
- Bondy B, Ackenheil M, Elbers R, Frohler M. Binding of 3H-spiperone to human lymphocytes: a biological marker in schizophrenia? *Psychiatry Res* 1985;15:41–48. [PubMed: 2409568]
- Bondy B, Ackenheil M, Engel RR. Methodology of 3H-spiperone binding to lymphocytes. *J Psychiatr Res* 1990;24:83–92. [PubMed: 1694898]
- Bondy B, de Jonge S, Pander S, Primbs J, Ackenheil M. Identification of dopamine D4 receptor mRNA in circulating human lymphocytes using nested polymerase chain reaction. *J Neuroimmunol* 1996;71:139–144. [PubMed: 8982113]
- Boneberg EM, von Seydlitz E, Propster K, Watzl H, Rockstroh B, Illges H. D3 dopamine receptor mRNA is elevated in T cells of schizophrenic patients whereas D4 dopamine receptor mRNA is reduced in CD4+ -T cells. *J Neuroimmunol* 2006;173:180–187. [PubMed: 16376996]
- Bourne JA. SCH 23390: the first selective dopamine D1-like receptor antagonist. *CNS Drug Rev* 2001;7:399–414. [PubMed: 11830757]
- Chiu S, Ferris JA, Johnson R, Mishra RK. CNS putative L-prolyl-L-leucyl-glycinamide (PLG) receptors, brain and lymphocyte dopamine receptors. *Prog Neuropsychopharmacol Biol Psychiatry* 1982;6:365–368. [PubMed: 6298884]
- De Keyser J, Walraevens H, Convents A, Ebinger G, Vauquelin G. [3H]SCH 23390 labels a novel 5-hydroxytryptamine binding site in human blood platelet membranes. *Eur J Pharmacol* 1989;162:437–445. [PubMed: 2568263]
- Fiebich BL, Akundi RS, Seidel M, Geyer V, Haus U, Muller W, Stratz T, Candelario-Jalil E. Expression of 5-HT3A receptors in cells of the immune system. *Scand J Rheumatol Suppl* 2004;9:11. [PubMed: 15515405]
- Fleminger S, Jenner P, Marsden CD. Are dopamine receptors present on human lymphocytes? *J Pharm Pharmacol* 1982;34:658–663. [PubMed: 6128389]
- Frank MG, Johnson DR, Hendricks SE, Frank JL. Monocyte 5-HT1A receptors mediate pindobind suppression of natural killer cell activity: modulation by catalase. *Int Immunopharmacol* 2001;1:247–253. [PubMed: 11360926]
- Griffiths RS, Chung-a-on KO, Griffiths KD, Payne JW, Davies JI. The sequestration of. *J Psychiatr Res* 1992;26:77–84. [PubMed: 1560411]
- Grimaldi B, Sibella-Arguelles C, Bonnin A, Fillion MP, Massot O, Rousselle JC, Seznec JC, Fillion G. Functional properties of 5-HT-module in the immune system: a model for central nervous system investigation. *Ann N Y Acad Sci* 1998;861:249–250. [PubMed: 9928272]
- Gundlach AL, Largent BL, Snyder SH. 125I-Spiperone: a novel ligand for D2 dopamine receptors. *Life Sci* 1984;35:1981–1988. [PubMed: 6149442]
- Halbach M, Henning U. Abnormal glucocorticoid dependent increase of spiperone binding sites on lymphocytes from schizophrenics in vitro. *Pharmacopsychiatry* 1989;22:169–173. [PubMed: 2813506]
- Hill P, Lai Y, Hnilo J, Lin CC, Karla M, Bounds S, Herz J, Mitchell R. Cloning, expression, and comparison of the binding characteristics of the known human dopamine receptors. *Adv Neurol* 1996;69:41–52. [PubMed: 8615159]
- Hurley MJ, Mash DC, Jenner P. Dopamine D(1) receptor expression in human basal ganglia and changes in Parkinson's disease. *Brain Res Mol Brain Res* 2001;87:271–279. [PubMed: 11245931]
- Hyttel J. SCH 23390 - the first selective dopamine D-1 antagonist. *Eur J Pharmacol* 1983;91:153–154. [PubMed: 6225651]
- Ilani T, Ben-Shachar D, Strous RD, Mazor M, Sheinkman A, Kotler M, Fuchs S. A peripheral marker for schizophrenia: Increased levels of D3 dopamine receptor mRNA in blood lymphocytes. *Proc Natl Acad Sci U S A* 2001;98:625–628. [PubMed: 11149951]

- Koob GF, Nestler EJ. The neurobiology of drug addiction. *J Neuropsychiatry Clin Neurosci* 1997;9:482–497. [PubMed: 9276849]
- Kwak YT, Koo MS, Choi CH, Sunwoo I. Change of dopamine receptor mRNA expression in lymphocyte of schizophrenic patients. *BMC Med Genet* 2001;2:3. [PubMed: 11252158]
- Le Fur G, Meininger V, Phan T, Gerard A, Baulac M, Uzan A. Decrease in lymphocyte spiroperidol binding sites in Parkinsonism. *Life Sci* 1980;27:1587–1591. [PubMed: 7442460]
- Le Fur G, Zarifian E, Phan T, Cuhe H, Flamier A, Bouchami F, Burgevin MC, Loo H, Gerard A, Uzan A. [3H]spiroperidol binding on lymphocytes: changes in two different groups of schizophrenic patients and effect of neuroleptic treatment. *Life Sci* 1983;32:249–255. [PubMed: 6130459]
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001;25:402–408. [PubMed: 11846609]
- Maher BS, Marazita ML, Moss HB, Vanyukov MM. Segregation analysis of attention deficit hyperactivity disorder. *Am J Med Genet* 1999;88:71–78. [PubMed: 10050971]
- Maloteaux JM, Gossuin A, Waterkeyn C, Laduron PM. Trapping of labelled ligands in intact cells: a pitfall in binding studies. *Biochem Pharmacol* 1983;32:2543–2548. [PubMed: 6615549]
- Maloteaux JM, Waterkeyn C, Laduron PM. Absence of dopamine and muscarinic receptors on human lymphocytes. *Arch Int Pharmacodyn Ther* 1982;258:174–176. [PubMed: 7138143]
- Marazziti D, Ori M, Nardini M, Rossi A, Nardi I, Cassano GB. mRNA expression of serotonin receptors of type 2C and 5A in human resting lymphocytes. *Neuropsychobiology* 2001;43:123–126. [PubMed: 11287788]
- McKenna F, McLaughlin PJ, Lewis BJ, Sibbring GC, Cummerson JA, Bowen-Jones D, Moots RJ. Dopamine receptor expression on human T- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. *J Neuroimmunol* 2002;132:34–40. [PubMed: 12417431]
- Millan MJ, Newman-Tancredi A, Quentric Y, Cussac D. The "selective" dopamine D1 receptor antagonist, SCH23390, is a potent and high efficacy agonist at cloned human serotonin_{2C} receptors. *Psychopharmacology (Berl)* 2001;156:58–62. [PubMed: 11465634]
- Nagai Y, Ueno S, Saeki Y, Soga F, Hirano M, Yanagihara T. Decrease of the D3 dopamine receptor mRNA expression in lymphocytes from patients with Parkinson's disease. *Neurology* 1996;46:791–795. [PubMed: 8618685]
- Nagai Y, Ueno S, Saeki Y, Soga F, Yanagihara T. Expression of the D3 dopamine receptor gene and a novel variant transcript generated by alternative splicing in human peripheral blood lymphocytes. *Biochem Biophys Res Commun* 1993;194:368–374. [PubMed: 7916609]
- Rao ML, Deister A, Roth A. Lymphocytes of healthy subjects and schizophrenic patients possess no high-affinity binding sites for spiroperidol. *Pharmacopsychiatry* 1990;23:176–181. [PubMed: 2381986]
- Ricci A, Amenta F. Dopamine D5 receptors in human peripheral blood lymphocytes: a radioligand binding study. *J Neuroimmunol* 1994;53:1–7. [PubMed: 8051291]
- Ricci A, Bronzetti E, Felici L, Greco S, Amenta F. Labeling of dopamine D3 and D4 receptor subtypes in human peripheral blood lymphocytes with [3H]7-OH-DPAT: a combined radioligand binding assay and immunochemical study. *J Neuroimmunol* 1998;92:191–195. [PubMed: 9916894]
- Ricci A, Bronzetti E, Felici L, Tayebati SK, Amenta F. Dopamine D4 receptor in human peripheral blood lymphocytes: a radioligand binding assay study. *Neurosci Lett* 1997;229:130–134. [PubMed: 9223608]
- Ricci A, Bronzetti E, Mignini F, Tayebati SK, Zaccheo D, Amenta F. Dopamine D1-like receptor subtypes in human peripheral blood lymphocytes. *J Neuroimmunol* 1999;96:234–240. [PubMed: 10337922]
- Ricci A, Veglio F, Amenta F. Radioligand binding characterization of putative dopamine D3 receptor in human peripheral blood lymphocytes with. *J Neuroimmunol* 1995;58:139–144. [PubMed: 7759603]
- Rotstein E, Mishra RK, Singal DP, Barone D. Lymphocyte 3H-spiroperidol binding in schizophrenia: preliminary findings. *Prog Neuropsychopharmacol Biol Psychiatry* 1983;7:729–732. [PubMed: 6686701]
- Santambrogio L, Lipartiti M, Bruni A, Dal Toso R. Dopamine receptors on human T- and B-lymphocytes. *J Neuroimmunol* 1993;45:113–119. [PubMed: 8392516]

- Seeman P, Ulpian C, Wreggett KA, Wells JW. Dopamine receptor parameters detected by [³H]spiperone depend on tissue concentration: analysis and examples. *J Neurochem* 1984;43:221–235. [PubMed: 6726248]
- Shaskan EG, Ballow M, Lederman M, Margoles SL, Melchreit R. Spiroperidol binding sites on mouse lymphoid cells. Effects of ascorbic acid and psychotropic drugs. *J Neuroimmunol* 1984;6:59–66. [PubMed: 6200497]
- Spitzer, RL.; Williams, JB.; Gibbon, M. Instruction Manual for the Structured Clinical Interview for DSM-III-R. New York: Biometrics Research Department, New York State Psychiatric Institute; 1987.
- Sweet RA, Hamilton RL, Healy MT, Wisniewski SR, Hentleff R, Pollock BG, Lewis DA, DeKosky ST. Alterations of striatal dopamine receptor binding in Alzheimer disease are associated with Lewy body pathology and antemortem psychosis. *Arch Neurol* 2001a;58:466–472. [PubMed: 11255451]
- Sweet RA, Hentleff RA, Meinert KA, DeMichele MA, Kirshner MA, Sorisio DA, Pollock BG. Antipsychotic radioreceptor assay: a modification identifying selective receptor effects. *Ther Drug Monit* 2001b;23:421–426. [PubMed: 11477327]
- Takahashi N, Nagai Y, Ueno S, Saeki Y, Yanagihara T. Human peripheral blood lymphocytes express D5 dopamine receptor gene and transcribe the two pseudogenes. *FEBS Lett* 1992;314:23–25. [PubMed: 1451800]
- Tarter RE, Vanyukov MM. Theoretical and operational framework for research into the etiology of substance use disorder. *J Child Adol Subst Abuse* 2001;10:1–12.
- Tarter R, Vanyukov M, Giancola P, Dawes M, Blackson T, Mezzich A, Clark DB. Etiology of early age onset substance use disorder: a maturational perspective. *Dev Psychopathol* 1999;11:657–683. [PubMed: 10624720]
- Vallone D, Picetti R, Borrelli E. Structure and function of dopamine receptors. *Neurosci Biobehav Rev* 2000;24:125–132. [PubMed: 10654668]
- Vanyukov MM, Maher BS, Ferrell RE, Devlin B, Marazita ML, Kirillova GP, Tarter RE. Association between the dopamine receptor D5 gene and the liability to substance dependence in males: A replication. *J Child Adol Subst Abuse* 2001;10:55–63.
- Vanyukov MM, Moss HB, Gioio AE, Hughes HB, Kaplan BB, Tarter RE. An association between a microsatellite polymorphism at the *DRD5* gene and the liability to substance abuse: pilot study. *Behav Genet* 1998;28:75–82. [PubMed: 9583233]
- Vanyukov MM, Moss HB, Kaplan BB, Kirillova GP, Tarter RE. Antisociality, substance dependence, and the *DRD5* gene: a preliminary study. *Am J Med Genet* 2000;96:654–658. [PubMed: 11054773]
- Vile JM, Strange PG. High-affinity binding sites for neuroleptic drugs in human peripheral blood lymphocytes and their relation to dopamine receptors. A long-standing controversy. *Biochem Pharmacol* 1995;49:747–753. [PubMed: 7702632]
- Vile JM, Strange PG. D2-like dopamine receptors are not detectable on human peripheral blood lymphocytes. *Biol Psychiatry* 1996;40:881–885. [PubMed: 8896774]
- Wise RA. Drug-activation of brain reward pathways. *Drug Alcohol Depend* 1998;51:13–22. [PubMed: 9716927]
- Wodarz N, Fritze J, Kornhuber J, Riederer P. 3H-spiroperidol binding to human peripheral mononuclear cells: methodological aspects. *Biol Psychiatry* 1992;31:291–303. [PubMed: 1532133]
- Zhou QY, Grandy DK, Thambi L, Kushner JA, Van Tol HH, Cone R, Pribnow D, Salon J, Bunzow JR, Civelli O. Cloning and expression of human and rat D1 dopamine receptors. *Nature* 1990;347:76–80. [PubMed: 2168520]
- Zvara A, Szekeres G, Janka Z, Kelemen JZ, Cimmer C, Santha M, Puskas LG. Over-expression of dopamine D2 receptor and inwardly rectifying potassium channel genes in drug-naïve schizophrenic peripheral blood lymphocytes as potential diagnostic markers. *Dis Markers* 2005;21:61–69. [PubMed: 15920292]

Figure 1.

Binding of [³H]SCH 23390 with isolated PBMC. Cells (1.5×10^6) were incubated with 0.8 nM of [³H]SCH 23390 for 60 min at 23°C in 170 mM Tris-HCl buffer with 1 μM (+)-butaclamol (nonspecific binding, NB) or without it (total binding, TB). Binding was terminated by centrifugation and aspiration of unbound ligand. Specific binding (SB) was determined as a difference between TB and NB values. Data represent mean ± SEM values of triplicate measurements. Binding is expressed in absolute units (disintegrations per minute, dpm; the left ordinate), and as percent of total binding in intact cells.

Grey bars – intact cells; black bars – cells preincubated in the boiling water bath for 8 min prior binding assay.

Figure 2.

Dissociation of [³H]SCH 23390 from PBMC during washing procedure. Binding was conducted in five parallel sets of reaction tubes, each in triplicate. Reaction mixtures with 1.5×10^6 cells (TB) or without cells (background, BKG) were incubated in the presence of 0.8 nM of [³H]SCH 23390 for 60 min at 23°C in 170 mM Tris-HCl buffer (TB). Binding was terminated by centrifugation and aspiration of unbound ligand. One, two or three washing steps were made by addition of ice-cold assay buffer to the cell pellets, which was removed by centrifugation and aspiration. The scales are the same as in Figure 1. Data represent mean $\pm$ SEM values of triplicate measurement. BKG is the background radioactivity absorbed by the Eppendorf microfuge tubes used for assay.

Figure 3.

Binding of [^3H]SCH 23390 with PBMC in the assay buffers of different composition. Cells (3.8×10^6) were incubated with 0.8 nM of [^3H]SCH 23390 for 60 min at 23°C in Buffer 1 (170 mM Tris-HCl), Buffer 2 (50 mM Tris-HCl), or Buffer 3 (HBSS/HEPES). Binding was terminated by centrifugation and aspiration of unbound ligand. NB was determined in the presence of 1 μM (+)-butaclamol. **A.** Binding data are presented in absolute values (dpm) and relative (%) to the highest TB values in Buffer 3. **B.** Ratios of binding parameters determined in buffers 1, 2 and 3: SB/TB is fraction of the ligand bound to cells specifically, and TB/total ligand is the fraction of the ligand associated with cells at the end of incubation with 0.8 nM of the ligand. Data represent mean $\pm$ SEM values of triplicate measurement.

Figure 4.

Time- and temperature-dependent dynamics of the specific [^3H]SCH23390 binding with PBMC. Isolated cells were incubated in three parallel sets of tubes (1.6×10^6 cells/tube) with HBSS/HEPES buffer (Buffer 3) containing 0.6 nM of [^3H]SCH23390. Binding was terminated by centrifugation followed by ligand aspiration. Specific binding expressed in absolute values (dpm) was determined from TB and NB in the presence of 1 μM (+)-butaclamol. Data represent mean $\pm$ SEM values of triplicate measurements.

Figure 5.

Concentration-dependent association of [³H]SCH23390 with PBMC. Cells were incubated in the presence of increasing concentrations of [³H]SCH23390 in HBSS/HEPES buffer at 37°C for 60 minutes with (NB) or without (TB) 1 μM (+)-butaclamol (the concentration scale is truncated to depict binding at low ligand concentrations). Binding was terminated by centrifugation followed by ligand aspiration. Data are expressed as the amount of ligand (fmol) bound to 2.4×10⁶ cells in reaction tubes. Data represent mean± SEM values of triplicate measurement.

Figure 6.

Time- and temperature-dependent dynamics of specific [^3H]SCH23390 binding with PBMC membranes. Membranes (135 μg) were incubated with 0.6 nM of [^3H]SCH 23390. Reaction was terminated by vacuum filtration. Specific binding was calculated by subtraction of non-specific binding in the presence of 10 μM (+)-butaclamol from total binding.. Data represent mean values ($\pm$ SEM) of triplicate measurements.

Figure 7.

Concentration-dependent binding of [³H]SCH 23390 with PBMC membranes. Membranes (260 µg) were incubated in the presence of increasing concentrations of [³H]SCH23390 for 30 minutes at 23°C (the concentration scale is truncated to depict binding at low ligand concentrations). Reaction was terminated by vacuum filtration. Non-specific binding was determined in the presence of 10 µM (+)-butaclamol. Data are expressed as the amount of ligand (fmol) bound to 260 µg of membrane protein. Data represent mean values (± SEM) of triplicate measurements.

Table I

Relative expression of dopamine receptor genes in peripheral blood mononuclear cells (folds of expression relative to that of the respective gene in the brain)

Expressed gene	Mean	SD	Range
<i>DRD1</i>	undetected		
<i>DRD2</i>	0.005	0.0044	0.0003–0.03
<i>DRD3</i>	1.65	1.142	0.21–6.66
<i>DRD4</i>	1.12	1.339	0.05–16.98
<i>DRD5</i>	0.03	0.019	0.004–0.08