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Human peripheral blood lymphocytes express D5 dopamine receptor gene and transcribe the two pseudogenes

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Sequential reverse transcription and polymerase chain reaction (RT-PCR) of the mRNA were used to investigate the expression of dopamine receptors in human peripheral blood lymphocytes. The RT-PCR products contained three types of sequences, each corresponding to those of the D5 dopamine receptor gene and the two related pseudogenes. The lymphocyte cDNA library also contained the clones encoding parts of the three genes. Binding profiles of dopaminergic ligands to the lymphocytes were similar to those for the native neuronal membranes.

Dopamine receptor; Lymphocyte; Reverse transcription; Polymerase chain reaction

1. INTRODUCTION

Dopamine receptors are integral membrane proteins that interact with G proteins to transduce dopamine stimulation into intracellular responses. Dopaminergic neurons in the human central nervous system are involved in the control of motor activity and in emotional and cognitive processes. Disturbances in dopaminergic transmission may cause psychomotor disorders, including Parkinson's disease and schizophrenia, and the receptors are primary targets for drugs used to treat the disorders. The human genome is known to contain 5 genes encoding the functional dopamine receptors, DRD1, DRD2, DRD3, DRD4 and DRD5, and 2 genes highly homologous to the DRD5 encoding the pseudogenes [1–9]. Each of the 2 pseudogenes has 5 identical defects in the sequence, 2 stop codons and 3 frame shifts, that preclude encoding of the functional receptor [8,9]. The expression of the dopamine receptors is well characterized in the brain but little work has been made to examine the expression in other organ tissues.

In this study, dopamine receptor mRNA in human peripheral lymphocytes was investigated using the reverse transcription-polymerase chain reaction (RT-PCR) and by a ligand binding assay. We report on the expression of the D5 dopamine receptor gene (DRD5) and on the transcription of the 2 pseudogenes, DRD5-ψ1 and DRD5-ψ2, in lymphocytes.

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Abbreviations: RT, reverse transcription; PCR, polymerase chain reaction; DR, dopamine receptor gene; bp, base pairs.

2. MATERIALS AND METHODS

2.1. *Preparation of blood lymphocytes*

Human peripheral lymphocytes were isolated from venous blood obtained from healthy adult volunteers by Ficoll/Hypaque (Pharmacia LKB) gradient centrifugation and hypotonic lysis of erythrocytes.

2.2. *Reverse transcription and polymerase chain reaction*

Total RNA isolated from lymphocytes using the guanidium thiocyanate/phenol/chloroform method [10] was treated with RQ1 RNase-free DNase (Promega) and then converted to first-strand cDNA copies by random priming of 1–5 μg of total RNA with Moloney murine leukemia virus reverse transcriptase as suggested by the supplier (Pharmacia LKB). Oligoprimers with corresponding sequences of DRD1, DRD2, DRD3, DRD4, DRD5 and β-actin gene were synthesized for RT-PCR reaction. The primer sequences used in the RT-PCR for the DRD5 gene are F1; 5'-TCCAGCCTGAATCGAACCTAC-3', R1; 5'-ACTGCAGAAAGGGACCATGCA-3', F2; 5'-CAATGAGCTCATCTCCTACAAC-3', R2; 5'-TAATGGAATCCATTCGGGGTGAA-3' and for β-actin gene F: 5'-AAGAGAGGCATCCTCACCCT-3', R; 5'-TACATGGCTGGGGTGTTGAA-3' [7,13,14]. The one-twentieth of cDNA synthesis reaction volume was combined in a 20 μl final volume for PCR amplification containing each primer and 0.2 unit Taq polymerase (Perkin-Elmer-Cetus). PCR was performed for 35 cycles, each cycle consisting of denaturation at 94°C (1 min), annealing at 57°C (1 min) and elongation at 72°C (1 min). The PCR-amplified products were analyzed by restriction fragment length polymorphism (RFLP) (*Bgl*II, *Bbh*II, *Sma*I) and sequence analysis by the dideoxy termination method [11].

2.3. *Preparation of DRD5 probe*

Full length of the DRD5 was amplified from lymphocyte genomic DNA, and cloned into M13 vectors. After the coding sequence was confirmed to be identical to that of the DRD5, insertion DNA was radiolabeled with [α-^{32}P]dCTP using a random priming procedure [12].

2.4. *PCR amplification of lymphocyte cDNA library and Southern blotting*

Aliquots of DNA purified from a lymphocyte cDNA library (Clontech) were subjected to PCR amplification with DRD5-specific prim-

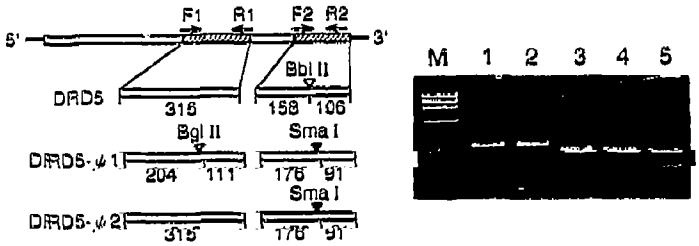

Fig. 1. (Left) Restriction map of the DRD5 and 2 pseudogenes based on the published sequences [7–9]. (Right) 315-bp fragment flanked by F1 × R1 (lane 1) was digested with *Bgl*II, resulting in the appearance of additional 204 and 111-bp fragments (lane 2). *Bbi*II digestion of 264, 267-bp fragments flanked by F2 × R2 (lane 3) produced two extra bands (158 and 106 bp) (lane 4), and *Sma*I digestion produced two extra bands (176 and 91 bp) (lane 5). Lane M: $\phi\chi$174/*Hae*III.

ers. The amplified products were electrophoresed on 2.5% agarose gels, transferred to nylon membranes (Hybond N, Amersham) and hybridized with α-^{32}P-labeled DRD5 probe. The membrane was then washed at 65°C with 0.1 × SSC and autoradiographed.

2.5. *Dopamine binding to the lymphocytes*

The lymphocyte preparation was diluted with sufficient Hank's BSS so that 100 μl contained 1.0×10^6 cells. For saturation experiments, 100 μl aliquots of the lymphocyte suspension were incubated in triplicate with increasing concentrations of [^{3}H]SCH-23390 (77 Ci/mmol), 0.05–10 nM) for 120 min at 22°C. For competition binding experiments, assays were initiated by addition of 100 μl aliquots of the lymphocyte suspension and incubation in triplicate with increasing concentration of dopamine and [^{3}H]SCH-23390 (5 nM) for 120 min at 22°C. Assays were terminated by rapid filtration through a cell harvester. The filters were washed with 50 mM Tris-HCl buffer (pH 7.4), and the radioactivity was measured in a scintillation counter.

3. RESULTS AND DISCUSSION

Fig. 1 (left) shows the restriction sites for *Bgl*II, *Bbi*II and *Sma*I in the regions amplified from the lymphocyte mRNA primed by the oligonucleotide primers F1 and R1 (F1 × R1) and by F2 × R2. We used this restriction site difference to locate the cDNA encoding the DRD5 and the pseudogenes. When a 315-bp fragment flanked by F1 × R1 was digested with *Bgl*II and subjected to electrophoresis analysis, three bands were observed (Fig. 1, lanes 1,2). Based on the restriction map, it was assumed that the lower two bands represented the

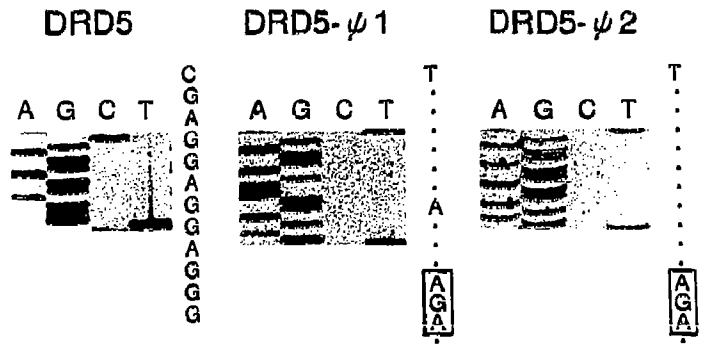

Fig. 2. RT-PCR products contained sequences corresponding to those of DRD5, DRD5-ψ1 and DRD5-ψ2. Boxed sequences indicate an insertion relative to the DRD5 sequence.

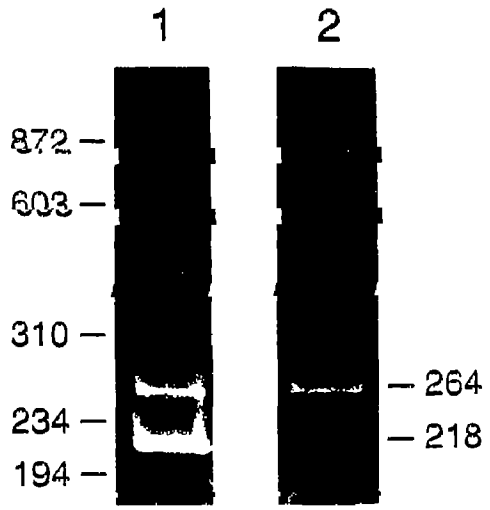

Fig. 3. Co-reverse transcription-amplification of the lymphocyte mRNA with the 2 primer sets, F2 × R2, F × R (lane 1), and with F2 × R2 (lane 2). The lengths of amplified products were 218 bp for β-actin cDNA and 264 bp for the DRD5 cDNA.

DRD5-ψ1 and the upper band represented either DRD5 or DRD5-ψ2 or both. Another fragment flanked by F2 × R2 could be partly digested with *Bbi*II or *Sma*I, indicating the presence of mRNAs coding for the DRD5, DRD5-ψ1 and DRD5-ψ2 in the lymphocytes (Fig. 1, lanes 3,4,5). Sequence analysis showed that the amplified fragments were parts of the DRD5 and the 2 pseudogenes that corresponded to hD$^5\Psi_1$ and ψDRD5-2 designated by other workers [7–9] (Fig. 2).

The possibility of amplification of contaminating genomic DNA was eliminated in the following way. First, RNA samples treated by DNase were divided into two portions. One was subjected to PCR amplification and the other to reverse transcription followed by PCR

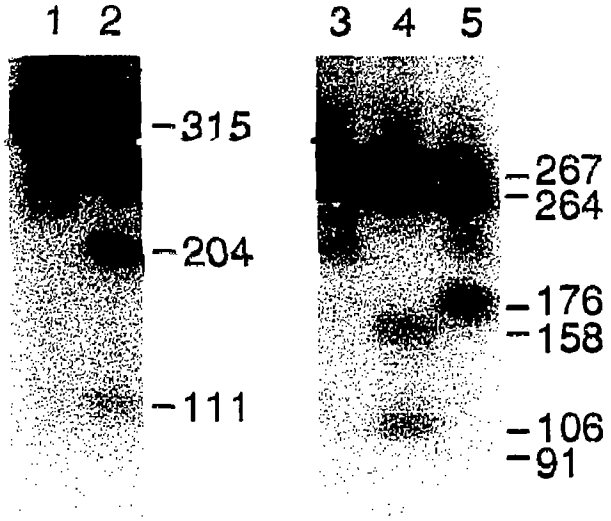

Fig. 4. RFLP and Southern blot analyses of PCR-amplified products from human lymphocyte cDNA library. The product flanked by F1 × R1 (lane 1) was digested with *Bgl*II (lane 2). The product flanked by F2 × R2 (lane 3) was digested with *Bbi*II (lane 4) and with *Sma*I (lane 5). The hybridization pattern was compatible with the RFLP pattern in Fig. 1.

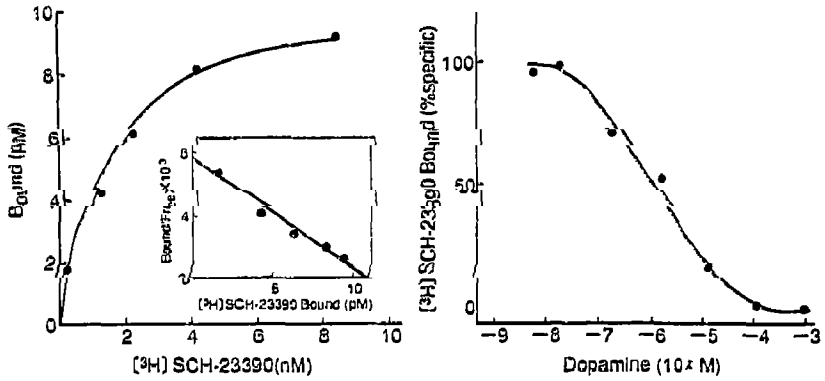

Fig. 5. (Left) Saturation of [^{3}H]SCH-23390 binding to lymphocytes. Insert: Scatchard plot of the same data. (Right) Specific inhibition of [^{3}H]SCH-23390 binding to the lymphocytes with dopamine.

amplification. Only the latter could produce the fragment with the expected length of the D5 dopamine receptor cDNA. Second, the β-actin mRNA was reversely transcribed and amplified in parallel with the same reaction mixture. The β-actin-specific primer pairs were selected from two exons separated by one intronic sequence [13,14]. As shown in Fig. 3, the β-actin cDNA (218 bp) but no gene fragment (659 bp) was observed.

Human lymphocyte cDNA library (Clontech) was selectively amplified using the primer sets, F1 × R1 and F2 × R2. The sequences of the products were shown to be identical to those of the DRD5 and the pseudogenes by RFLP and hybridization analyses (Fig. 4).

Dopaminergic antagonist [^{3}H]SCH-23390 bound to the lymphocytes in a dose-dependent and saturable manner with a dissociation constant (K_d) of 1400 pM, and the binding was inhibited by dopamine with an inhibitory constant (K_i) of 1040 pM (Fig. 5). These pharmacologic profiles were similar to those of human and rat neuronal membranes [15,16]. The binding capacity (B_{max}) of [^{3}H]SCH-23390 to the lymphocytes was 3 fmol per mg protein. This value was approximately 6 times lower than that for the neural membranes of the human brain and 140 times lower than that for the D5 dopamine receptor transiently expressed in Cos-7 cells [7,15].

The results obtained from genetic and pharmacological analyses have indicated the expression of the DRD5 and the transcription of the 2 pseudogenes in peripheral blood lymphocytes. We are concerned with the in vivo interaction of receptors and drugs in circulating blood, and the relation between lymphocytes and brain dopaminergic receptors in the course of Parkinson's disease. These points await further investigation.

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