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EXPRESSION OF THE D3 DOPAMINE RECEPTOR GENE AND A NOVEL VARIANT TRANSCRIPT GENERATED BY ALTERNATIVE SPLICING IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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The sequential reverse transcription and polymerase chain reaction amplified the mRNA coding for the D3 dopamine receptor (D3R) in human peripheral blood lymphocytes. Pharmacological analysis confirmed the binding of the D3R specific ligand to the lymphocytes. In addition, there was a novel shorter variant transcript of the D3R gene generated by alternative splicing in lymphocytes and brain. This variant, termed D3(TM4-del), has a 143 bp deletion and encodes a 138 amino acid protein containing the first three transmembrane domains of the native D3R.

The dopaminergic transmission plays a central role in the control of motor, affective and cognitive functions, and its disturbance can cause neuropsychiatric disorders, including Parkinson's disease and schizophrenia. The dopamine receptors are the primary targets for the drugs used to treat these disorders. Since five cDNAs encoding D1-D5 dopamine receptors were cloned [1-8], each subtype has been studied extensively using molecular techniques. Because of the limited availability of test specimens, however, human dopamine receptors have not been well characterized and their presence in organ tissues outside brain has been controversial. To circumvent the limited tissue availability, it is desirable to amplify gene transcripts

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Abbreviations: reverse transcription and polymerase chain reaction (RT-PCR), D5 dopamine receptor (D5R), D3 dopamine receptor (D3R), 7-[^{3}H]hydroxy-N,N-di-n-propyl-2-aminitetralin ([^{3}H]7-OH-DPAT), the fourth transmembrane domain (TM4).

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from easily accessible cells. Using the sequential reverse transcription and polymerase chain reaction (RT-PCR) technique, we, thus identified the expression of the D5 receptor (D5R) mRNA in human peripheral blood lymphocytes in the past [9]. In this report, we show that human peripheral blood lymphocytes also express the D3 dopamine receptor (D3R) and demonstrate the presence of a novel shorter variant transcript generated by alternative splicing.

MATERIALS AND METHODS

Preparation of blood lymphocytes Human peripheral blood lymphocytes were isolated from heparinized fresh venous blood obtained from healthy adult volunteers by Ficoll-Paque (Pharmacia LKB) gradient centrifugation for 30 min at 1500 x g and hypotonic lysis of erythrocytes.

Extraction of RNA Total RNAs were extracted from human lymphocytes and other tissues including various brain areas, muscle, liver and kidney using the acid guanidinium thiocyanate-phenol-chloroform method [10] and treated with RQ1 RNase-Free DNase (Promega).

Reverse transcription and polymerase chain reaction (RT-PCR) analysis Two μg of total RNA from human lymphocytes or whole brain tissues were reverse transcribed into first-strand cDNA using random hexanucleotide primers and 2 U M-MLV reverse transcriptase as suggested by supplier (Pharmacia LKB). One fifteenth of the cDNA products was amplified by PCR using 1 U Taq DNA polymerase (Perkin-Elmer-Cetus) and 750 nM oligonucleotide primers in a 25 μl final volume. The primers used for PCR were chosen to correspond to the two deletion sites in the previously reported D3R variants [11,12], so that mRNAs coding for these variants might not be amplified (Table 1). PCR was performed in DNA Thermal Cycler PJ2000 (Perkin-Elmer-Cetus) for 40 cycles (each for 1 min at 94℃, 1 min at 60℃ and 1 min at 72 ℃) after first denaturation for 3 min at 94℃, and final extension for 5 min at 74℃. Five μl of the RT-PCR products were resolved in a 1.5 % agarose gel, stained with ethidium bromide. The RT-PCR products transferred onto nylon membrane Hybond-N+ (Amersham) were hybridized with the D3R cDNA probe radiolabeled with [α-^{32}P]dCTP at 42℃ and washed with 0.2 x SSC at 65 ℃. The membrane was exposed to a X-ray films (Kodak) at -80℃ for 2 days. The products were directly sequenced by the dideoxy termination method using 373A DNA Autosequencer (ABI).

Tissue distribution analysis of D3R mRNA by RT-PCR 100 ng of total RNA from various human tissues including lymphocytes were reverse transcribed into first-strand cDNA and amplified in a 10 μl final volume using 0.5 U Taq DNA polymerase and 150 nM D3R primers radiolabeled with [γ-^{32}P]ATP. PCR was performed for 30, 35 and 40 cycles. The RT-PCR products were resolved in a 6 % polyacrylamide gel and autoradiographed for 6 hours.

Binding assay Human lymphocytes were resuspended with Hanks' balanced salt solution and their viability was determined by exclusion of trypan blue dye. 1x10^6 cells were incubated in duplicate in a 100 μl total volume with

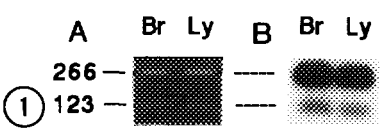
Table 1. Oligonucleotide primers used for PCR amplification of the D3R cDNA

Primer	Sequence	Position (exon)
F (sens)	5'-TTCAGCCGAATTCGCTGTGATGTT-3'	292 → 315 (ex.2)
R (antisens)	5'-GGGTTGGAGATGGATCCGACAGT-3'	557 → 535 (ex.4)

increasing concentration of the D3R specific ligand, 7-[^{3}H]hydroxy-N,N-di-n-propyl-2-aminitetralin ([^{3}H]7-OH-DPAT) (Amersham) [13], in the absence or the presence of 50 μM dopamine to define the specific binding. After incubation for 60 min at room temperature, the cells were separated from free radioligands using a semiautomatic cell harvester (Skatron) by rapid filtration through glass fiber filters. The radioactivity trapped on the filters was counted by 1205 Betaplate liquid scintillation counter (Pharmacia LKB).

RESULTS AND DISCUSSION

The RT-PCR of RNAs from human lymphocytes and brain tissue resulted in amplification of the expected 266 bp bands and additional 123 bp bands, both of which were hybridized with the D3R cDNA probe (Figure 1). The sequence analysis confirmed that the 266 bp DNA fragment had the sequence identical to a part of the D3R cDNA. We excluded the possibility of artificial amplification of contaminating genomic DNA using the primer pairs which were selected from the two separated exons. Northern blot analysis failed to detect the D3R mRNA expressed in lymphocytes. The relative abundance of the D3R mRNA from various tissues was estimated as follows; olfactory tubercle> hypothalamus> lymphocyte> cortex. The D3R mRNA was not detectable in the pituitary gland, muscle, liver and kidney (Figure 2). The relatively high abundance of the D3R mRNA in lymphocytes could rule out the possibility that the transcription was due to the basal transcription of tissue specific genes in any cells which was called "illegitimate transcription" [14]. To ascertain the presence of the full-length D3R mRNA in lymphocytes, RT-PCR was performed using primers flanking the entire coding region. The expected



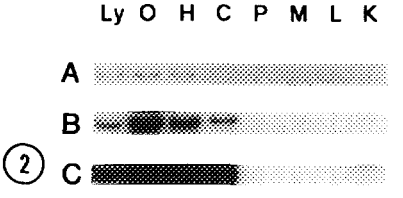


Figure 1. Agarose gel electrophoresis of the amplified D3R mRNA from lymphocytes and brain. A) Ethidium bromide staining of the gel. B) Southern blot hybridization of the RT-PCR products with radiolabeled D3R cDNA probe. Br:Whole Brain, Ly:Lymphocytes.

Figure 2. RT-PCR amplification of D3R mRNA from various human tissues. A:PCR for 30 cycles, B:for 35, C:for 40. Ly:Lymphocytes, O:Olfactory tubercle, H:Hypothalamus, C:Cortex, P:Pituitary, M:Muscle, L:Liver, K:Kidney.

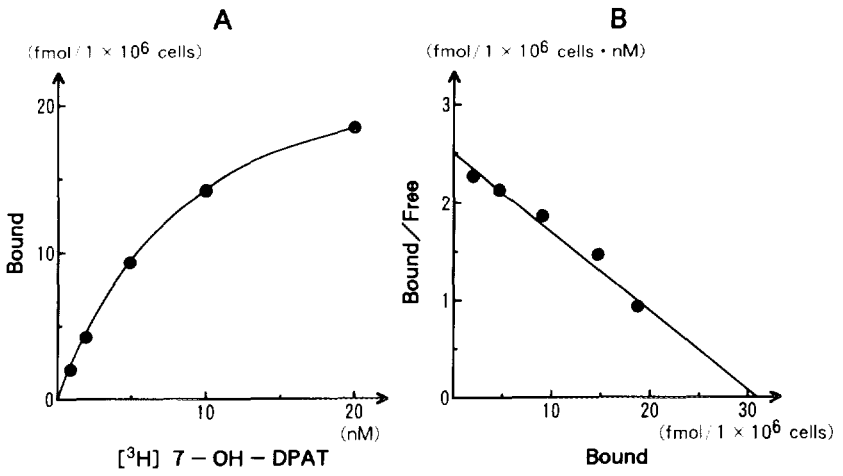


Figure 3. Binding assay of [^{3}H]7-OH-DPAT to human lymphocytes. A) Saturation curve of [^{3}H]7-OH-DPAT binding to lymphocytes. B) Scatchard plot of the same data.

1260 bp fragment was amplified and shown to contain the sequence identical to the published D3R cDNA [6].

In contrast to the dopamine receptors within brain tissues, it is uncertain whether lymphocytes have dopaminergic binding sites or which subtype of the dopamine receptors are expressed on lymphocyte surfaces [15-17]. In this study, we have clearly shown the expression of the D3R mRNA in human lymphocytes by determining the nucleotide sequence of the transcript. This observation was supported by the presence of high affinity binding of [^{3}H]7-OH-DPAT to lymphocytes which was rapid, saturatable and displacable by dopamine, haloperidol and chlorpromazine (Figure 3). The dissociation constant (Kd) was calculated to be 12.2 nM, and the binding capacity (Bmax) to be 30.8 fmol/1x10^6 cells. This binding profile was similar to that of rat neuronal membranes and CHO cells transfected with the D3R cDNA [13]. All these data combined with other reports showing the effect of the dopamine on lymphocyte proliferation [18] and dopamine-mediated immunoresponses [19] led us to conclude that the D3R was specifically expressed and served some biological functions in peripheral blood lymphocytes.

Unexpectedly, a shorter 123 bp band was amplified from lymphocyte and brain RNAs by the RT-PCR procedure (Figure 1). Sequence analysis showed

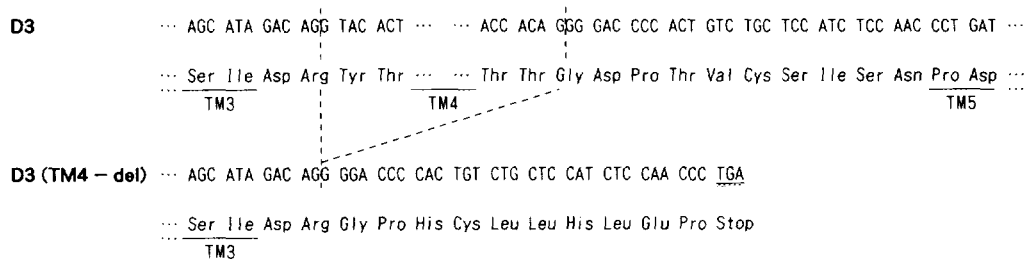


Figure 4. Nucleotide and deduced amino acid sequences of D3R and D3(TM4-del) cDNA. The putative transmembrane domains are underlined and the stop codon double-underlined. The alternative splicing sites are indicated by broken lines.

that these DNA fragments represented a new variant transcript of the D3R gene produced through alternative splicing (Figure 4). The variant has a 143 bp deletion mainly encompassing the fourth transmembrane domain (TM4) of the D3R. Since this deletion alters the reading frame followed by a premature stop codon, the encoded protein, termed D3(TM4-del), is 138 amino acid long and contains only the first three transmembrane domains of the native D3R (Figure 5). It is of interest that this variant was found both in brain and lymphocytes, because it raises the possibility that the same mechanism for the alternative splicing may operate among various organ tissues. D3(TM4-del) presented here is the second D3R variant found in human. The first one, termed D3(TM3-del), also has a frameshift giving rise to a stop codon shortly after a 113 bp deletion and encodes a 109 amino acid protein [11-12]. D3(O2-del), reported only in rat, has a 54 bp deletion without a frameshift and encodes a 428 amino acid protein [11]. These three

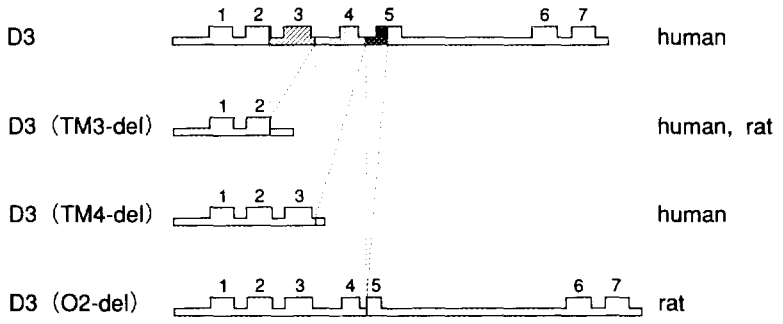


Figure 5. Schematic representation of the products of the D3R and its variant transcripts. Open boxes (1-7) represent the putative transmembrane domains. Oblique lined, dotted and closed boxes represent deleted regions in each variant.

splice variants had no dopaminergic binding activity. It remains to be investigated whether the variant transcripts are formed at random during the biosynthesis of the functionally active D3R, or serve some regulatory or modulatory roles controlling the abundance of the active D3R gene transcript.

In summary, genetic and pharmacological analyses showed the expression of the D3R and a new variant form of the D3R gene transcript in human peripheral blood lymphocytes. We previously reported the expression of the D5R and the transcription of its pseudogenes in human lymphocytes [9]. Relationship between the dopamine receptors of lymphocytes and those of brain in the course of human disease, including Parkinson's disease, is uncertain and is a subject for further studies.

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Note: During preparation of this manuscript, another isoform of the mouse D3R was reported by Fishburn C.S. et al. [20].

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