ORIGINAL ARTICLES

The Detection of Dopamine Gene Receptors (DRD_1-DRD_5) Expression on Human Peripheral Blood Lymphocytes by Real Time PCR

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ABSTRACT

There is interrelationship between the immune and nervous systems that is accomplished by the molecular mediators. Dopamine is one of the most important neurotransmitters. Five different dopamine receptor genes (DRD1, DRD2, DRD3, DRD4, and DRD5) have been recognized and cloned. The expression of the dopamine receptors is well characterized in the brain but little work has been done to examine their expression in other organ tissues. In certain diseases of the immune and nervous systems, alterations in dopamine receptors gene expression in different cells have been reported. This suggests that dopamine and its receptors have important role in pathophysiology of above-mentioned diseases.

In the present study, using Real Time Polymerase Chain Reaction (PCR) technique, we investigated dopamine receptors genes expression in PBMC of normal individuals. The PBMC was separated from normal whole blood by Ficoll-hypaque; the total cellular RNA was then extracted and the cDNA was synthesized. This process followed by real time-PCR using primer pairs specific for five dopamine receptors mRNAs and β-actin as internal control. The results showed the presence of all types of dopamine receptors in lymphocytes of normal individuals. The specificities of the obtained PCR products for the respective dopamine receptors fragments were confirmed by sequenced analysis capillary system. In conclusion, the present study has shown that human lymphocytes express five dopamine receptors DR1-DR5. However, the conclusive evidence on the possible function of these receptors in lymphocytes remains unknown. Because lymphocytes express all of the five neuronal dopamine receptors, it is quite reasonable to consider them as a model of dopaminergic neuron.

Keywords: Dopamine Receptors; Gene Expression; Lymphocytes; Polymerase Chain Reaction

INTRODUCTION

Dopamine, together with other catecholamine such as norepinephrine, is also a critical transmitter in sympatoadrenergic terminals. Such terminals lie in close contact with immune cells in lymphoid organs and there is increasing evidence which points to the ability of dopamine to affect immune cell function.[1] Dopamine receptors are integral membrane proteins

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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.[2] The human genome is known to contain five genes encoding the functional dopamine receptors, DRD1, DRD2, DRD3, DRD4, DRD5 and two genes highly homologous to the DRD5 encoding the pseudogenes.[3] The expression of the dopamine receptors is well characterized in the brain but little work has been done to examine the expression in other tissue organs.

Human peripheral blood lymphocytes (PBL) express dopamine receptors and dopamine transporters and synthesize endogenous dopamine and the related catecholamine norepinephrine and epinephrine through tyrosine hydroxylase dependent pathway.[4] Interestingly, dysfunction of dopaminergic pathways in PBL have been reported in neurological disorders characterized by dysfunctional central dopaminergic neurotransmission such as peripheral dopaminergic.[5]

Although it is yet unclear whether they simply mirror dysfunctional dopaminergic mechanisms or primarily reflect a dynamic interaction between the central nervous system and circulating immune cells.

It has been proposed that neurotransmitter expression in peripheral immune cells reflects expression of these receptors in the brain. We conducted this study to examine whether all dopamine receptors are expressed on PBL "peripheral biomarker hypothesis"?

MATERIALS AND METHODS

Thirty individuals (18 male, 12 female; age 20-35 years) took part in this study. Any past or current major neurological, psychiatric, cardiovascular or endocrinoloical as well as any current infectious or inflammatory diseases and any current medication were regarded as exclusion criteria. Written informed consent was obtained from each individual. Peripheral blood samples (4ml) were obtained from the cubital vein and collected in cell preparation tubes containing an anticoagulant. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density centrifugation (Pharmacia. Uppsala, Sweden). The lymphocyte layer was collected and washed three times in Phosphate buffer saline (PBS). The total mRNA was isolated from lymphocytes by RNA blood minikit (Roach, Germany), and the amount and purity of RNA were determined by spectrophotometery.[6] Dopamine receptor mRNA expression was determined by the 3' 5' fluorogenic Taqman approach (Roach, Germany).

Total RNA of 1500 ng was reverse transcribed into first-strand cDNA by using random hexamers and 2.5 units of multiscribe (recombinant moloney murine leukemia virus) Reverse transcriptase in a final volume of 40 μl.[7] Primers for DR1, DR2, DR3, DR4, DR5 and housekeeping gene β-actin were designed using primer express software to exclude amplification of genomic DNA and pseudo genes. Primers sequences were used

DRD1F; 5'-AAACCCACAAGCCCCTCTGA-3',
DRD1R; 5'-GATGAATTAGCCCACCCAAAC-3',
DRD2F; 5'-GCGGACAGACCCCACTACAA-3',
DRD2R; 5'-AAGGGCACGTAGAAGGAGA-3',
DRD3F; 5'-CCGCATTTGCTGTGATGTT-3',
DRD4F; 5'-CCTGCGGCTCCAACTGTGC-3',
DRD4R; 5'-GGAAGCCCCGACCACCAC-3',
DRD5F; 5'-AACCTGTGCGTCATCAGCG-3',
DRD5R; 5'-CAGATCCATGAGGGGGTTT-3'.

cDNA of 75 ng was used for Polymerase Chain Reaction (PCR) amplification in a final volume of 25 μl with 1 unit of Taq DNA polymerase (Roach, Germany).

PCR was carried out in a Real Time-PCR (Roach, Germany) with a Cyber green fluorogenic neuclotide to monitor cDNA amplification by the increase in Fluorescence intensity. Each PCR product of dopamine receptor (DRD1-DRD5) was sequenced by DNA sequencer ABI 3700 capillary system (Appliyed Biosystem, USA).

RESULTS

The aim of this study was to determine whether dopamine gene receptors are expressed on peripheral blood lymphocytes? In order to test this hypothesis, we examined detection of the mRNA expressions of dopamine receptors (DRD1 and DRD2 like) in peripheral blood lymphocytes with highly sensitive methods. We focused on the all subtypes of dopamine receptors (DRD1-DRD5). The experiments were performed using peripheral blood lymphocytes. Expression of the different dopamine receptors gene segments was studied by analyzing total RNA extracted from the samples.

Figure 1. The RNA extracted from PBL on agarose gel electrophoresis (18S and 28S band).

Figure 1 shows the RNA agarose gel electrophoresis. In order to detect dopamine gene receptors expression on RNA level Real Time PCR was performed for the regions of different dopamine receptors.

Figure 2 shows agarose gel electrophoresis of PCR products. Dopamine receptors gene-specific amplification products were different in size. Results revealed that all dopamine receptors gene subtypes expressed on peripheral blood lymphocytes. Next step we carried out multiplex PCR with β-actin as internal control.

Figure 3 shows agarose gel electrophoresis of multiplex PCR product of dopamine gene receptors with β-actine as internal control. All PCR products amplified β-actin as internal control with the same size (447 bp) and dopamine gene receptor with different size in agarose gel electrophoresis.

Dopamine gene receptors in peripheral blood lymphocytes were expressed on thirty normal individuals. All dopamine receptors on females and males were expressed on PBL. The results are presented in table 1.

Our results presented here provide direct evidence that human lymphocytes express dopamine D1 and D2 like receptors belonging to DRD1 - DRD5 receptors subtypes respectively. The taqman assays were tested by blasting against the entire human genome (NCBI-national Center for Biotechnology Information 2005) to exclude sequencing at unwarranted sites.[8] The specificities of the obtained PCR products for the respective dopamine receptors fragments were confirmed by capillary sequenced analysis ABI 3700 machine.

Table 1. All dopamine receptors (DRD1, DRD2, DRD3, DRD4 and DRD5) in females and males were expressed on PBL.

Individual	Number	DRD1	DRD2	DRD3	DRD4	DRD5
Men	18	+	+	+	+	+
Women	12	+	+	+	+	+
Total	30					

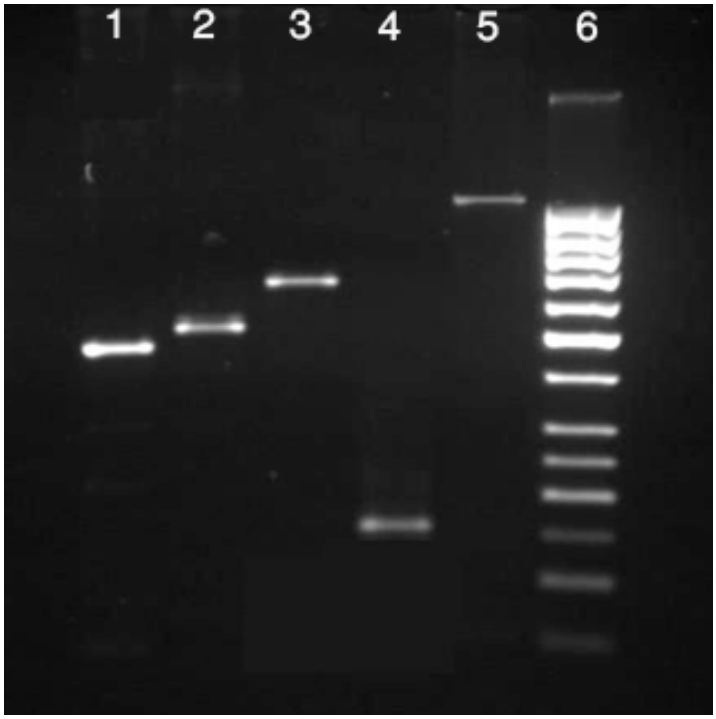

Figure 2. Agarose gel electrophoresis of PCR product. Dopamine receptors gene-specific amplification products were different in size. From left to right lane 1 consists of dopamine receptor DRD1 (size 471 bp), Lane 2; DRD2 (size 521 bp), Lane 3; DRD3 (size 670 bp), Lane4; DRD4 (size 153), Lane5; DRD5 (size 1078) and Lane 6 is Molecular Weight Marker.

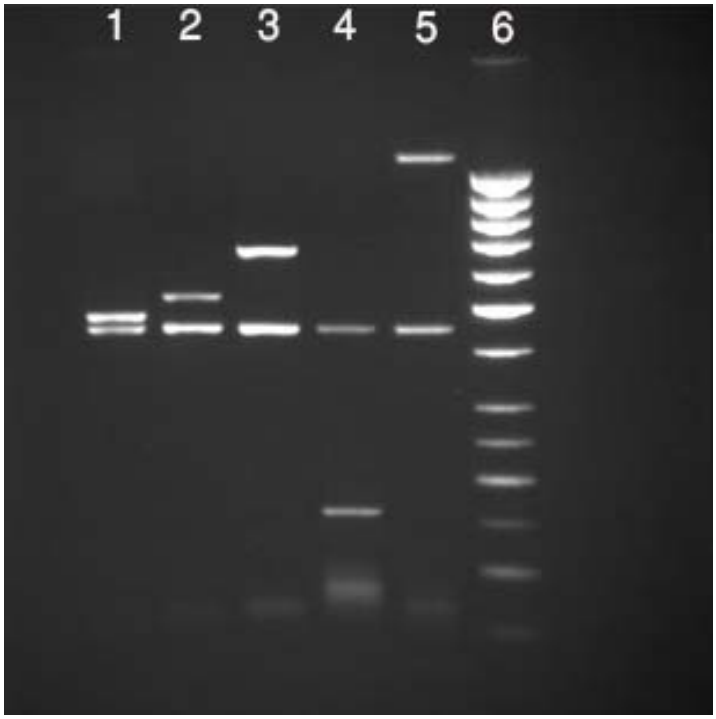

Figure 3. Agarose gel electrophoresis of multiplex PCR product of dopamine gene receptors with β-actine as internal control. Lane 1 consists of dopamine receptor DRD1 (size 471 bp), Lane 2; DRD2 (size 521 bp), Lane 3; DRD3 (size 670 bp), Lane4; DRD4 (size 153), Lane5; DRD5 (size 1078) and Lane 6 is Molecular Marker. All PCR product amplified β-actin as internal control (size 447 bp).

DISCUSSION

The expression of the dopamine receptors is well characterized in the brain but little work has been done to examine the expression in other tissue organs. In the present study, we focused on mRNA and we did not attempt protein expression of PBL dopamine receptors. The protein receptor expression of dopamine receptor on peripheral blood leukocytes has been investigated by laserflowcytometry.[9] Only DRD2, DRD3, DRD4 and DRD5 were detected. Dopamine receptors DRD3 and DRD5 were found in most Individuals. Whereas DRD2 and DRD4 had more variable expression and DRD1 was never found.[9] Dopamine receptor mRNA in human peripheral lymphocytes was investigated using the reverse transcription- polymerase chain reaction. In accordance with previous studies, β-actin was used as housekeeping gene for determination of dopamine gene receptor mRNA expression in PBL.[10] Dopamine induced cytotoxicity is believed to be induced via binding to its receptor and to be activated via spontaneous as well as metabolism dependent oxidation, leading to oxidative stress-induced apoptotic cell death.[5,11,12] Present study showed that human peripheral blood lymphocytes (PBL) expressed dopamine receptor subtype (DRD1- DRD5). They appear as a specific part of the neuroimmunological circuit in transmitting information from the CNS through sympathetic nerve fibers innervating lymphoid organs.[13] According to the peripheral biomarker hypothesis the expression of neurotransmitter receptors in PBL parallels and reflect their expression in the brain, though, due to methodological limitations; such a correlation has not yet been shown directly. However, this notion appears to be supported by accumulating evidence of altered neurotransmitter receptor expression in PBL of patients who are affected by neuropsychiatric disorders, which are believed to involve respective receptor alterations in the brain.[14-16] Disturbances in dopaminergic transmission may cause psychomotor disorders, including Parkinson's disease and schizophrenia and the receptors are primary targets for the drugs used to treat the disorders.[17,18]

This mechanism plays a critical role in neurodegenerative disorders such as Parkinson's disease and Huntington's disease and possibly even in neurodegeneration after ischemia.[19] Dopamine can also exert neuroprotective effects. The peripheral marker hypothesis does specify a causal relationship between cerebral and PBL dopamine receptor expression. The dopaminergic system has been also implicated in personality traits in healthy individuals.[9] The aim of the present study was therefore to evaluate the peripheral marker hypothesis in healthy individuals by comparing expression of all dopamine receptors in normal individuals. The comparison between our results and others indicate that we were able to show gene expression of all dopamine receptors (DRD1-DRD5) in all normal individuals. The difference between our methods and their methods is sensitivity for extracting RNA and amplification system by Real Time-PCR. In Parkinson's diseases, a reduced mRNA expression of the dopamine receptors DR3 in PBL, correlates with clinical severity.[17] A reduced PBL expression of the DRD3 has been found in patients with Alzheimer's diseases. An increased PBL expression of the DRD3, DRD4 and DRD5 was found in patients suffering from migraine.[20] The results presented here provide direct evidence that human lymphocytes express dopamine D1-like and D2-like receptors belonging to the DRD1- DRD5 receptors subtypes, respectively.

In conclusion, the present study has shown that human lymphocytes express dopamine receptors DRD1, DRD2, DRD3, DRD4 and DRD5. There is no conclusive evidence on the possible immunological role of these receptors in lymphocytes function. The fact that lymphocytes express all of the five neuronal dopamine receptors provides the temptation to consider these receptors on lymphocytes as a model of dopaminergic neuron.

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