DOI: https://doi.org/10.53350/pjmhs22169592

ORIGINAL ARTICLE

Applications of the Dopaminergic System Expressed by Peripheral Blood Lymphocytes in Neuropsychiatric Disorders

AMBAR SHUAIB[1], MUAZZAM AHMAD[2], AMBER ASFANDYAR[3], ZAKIA SUBHAN[4], ABDUR RAZAQ[5], SUDHAIR ABBAS BANGASH[6], MUHAMMAD KHURRAM[7], AROOB IDREES[8], ZAKIR ULLAH[9]

[1]*Department of Physiology, Pak International Medical College, Peshawar, Pakistan.*
[2]*Department of Pharmacology & Therapeutics Khyber Medical College Peshawar.*
[3]*Department of Physiology, CMH Medical College Kharian*
[4]*Department of pharmacology, Institute of Pharmaceutical Sciences, Khyber Medical University Peshawar.*
[5]*Department of Pharmacology, Institute of Pharmaceutical Sciences, Khyber Medical University Peshawar*
[6]*Faculty of Life Science, Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar*
[7]*Department of Life Sciences, School of Science, University of Management and Technology Lahore, Pakistan*
[8]*Khyber Medical College, Peshawar*
[9]*Lecturer in DPT, Kohat university of science and technology*
Corresponding authors: Muazzam Ahmad, Email: muazzamabbasi1988@gmail.com & Amber Asfandyar Email: Amberasfandyar@gmail.com

ABSTRACT

Introduction: Dopamine (DA) is essential for the central nervous system (CNS) regulation of neurological, psychological, behavioral, and hormonal processes.

Objective: In order to establish the viability of peripheral blood lymphocytes (PBL) as a molecular tool for examining DA dysregulation in neurological diseases, this research sets out to establish current characterization of the DA system in PBL.

Methods: This study was carried out at Khyber teaching hospital from Jan 2022 to April 2022. Twenty-three (23) patients suffering with Parkinson's disease (PD) participated in that research (average age 56 years, mean disorder length 28 months). Nine of these participants received treatment with direct dopamine agonist medications, whereas the other 14 patients were drug-naive (levodopa-naive group).

Results: Norpramin, a noradrenaline uptake blocker, established its efficacy at dosages numerous times elevated than blockers of dopamine uptake, but the serotonin uptake inhibitor Prozac was inactive. Dopamine transporter immunoreactivity levels varied significantly across groups, according to the ANOVA analysis (KW=27, P=0.001).

Conclusion: PBL may function as a biological probe to identify DA transmission disruption in neuropsychiatric illnesses and to track the efficacy of pharmaceutical therapies

Keywords: dopamine, dopaminergic system, peripheral blood lymphocytes, neuropsychiatric disorders

INTRODUCTION

Dopamine is essential for the central nervous system (CNS) regulation of neurological, psychological, behavioral, and hormonal processes [1]. In the CNS, dopamine cell bodies are situated in the substantia nigra pars compacta (SNc) and the tegmental region of the midbrain [2]. The stimulation of DA receptor sites regulates the impact of DA on the CNS. Every subtype of DA receptor's sequence of amino acids corresponds for one of the seven plasma membrane spanning segments identified in G protein coupled receptor sites [3]. Adenylate cyclase is strongly affected by two different categories of DA binding sites which are just now been discovered. Unlike the D2-like receptor sites (D2, D3, and D4), the D1-like ligands (D1 and D5 ligands) are joined to a G protein that starts adenylate cyclase [4]. However, the multiple DA receptor variants contain unique topographic segregations all across the CNS. As a result, D1 ligands are widely distributed throughout the nucleus, basal ganglia, accumbens, and cerebral cortex; D2 ligands are concentrated in the anterior pituitary and lower ganglia; D3 receptors sites are identified in the striatum and D4 receptors binding sites are most prevalent in the hippocampus [5].

The amount of neurotransmitter accessible to connect with DA sites controls how much DA is sent in the CNS. The quantity of DA produced by axonal firing, the rate of metabolic activities, and the rate of re-consumption of the transmitter are all factors that influence this. The monoamineoxidase and catechol-O-methyl-transferase pathways, which are independent but somewhat interconnected, are crucial for DA metabolic activities [6]. Norepinephrine, serotonin, GABA, and glycine carriers are really members of the vast Na+/Cl-dependent transporter family, which also includes the DA transporter (DAT), an 70 kD glycoprotein, is crucial for DA re-uptake under physiological circumstances [7]. Vesicular monoamine carriers that condense DA into presynaptic vesicles, control internal DA values that are accessible for synaptic activity. Several neurological or psychiatric diseases share the primary characteristic of neurobiological or physiological impairment of the DA transmitter in the central nervous system (CNS) [8].

In order to establish the viability of PBL as a molecular tool for examining DA dysregulation in neurological diseases, this research sets out to establish current characterization of the DA system in PBL.

MATERIALS AND METHODS

This study was carried out at Khyber teaching hospital from Jan 2022 to April 2022. Twenty-three (23) patients suffering with Parkinson's disease (PD) participated in that research (average age 56 years, mean disorder length 28 months). On the Hoehn and Yahr scale, each patient was in stage I or stage II (1967). Nine of these participants received treatment with direct dopamine agonist medications, whereas the other 14 patients were drug-naive (levodopa-naive group). Following the blood sample's collection, a positive response to the levodopa test was shown in all individuals. 15 healthy individuals with a mean age of 54 were selected as the control group. Prior to enrollment, each participant provided signed consent permission for research. All included participants' primary biochemical and hematological indicators fell inside the acceptable range of the lab. Additionally, none of the subjects were taking any additional medication that would have affected their immunological or central neurological systems.

PBL was produced from venous blood (10ml) collected into test tubes carrying Ethylene diamine tetra acetic acid (EDTA) from healthy and sick volunteers. Using a Ficoll gradient centrifugation, PBL were isolated, then the erythrocytes were hypotonically lysed, marked on slides, and preserved for 45 minutes at room temperature in PBS with paraformaldehyde 5% and glutaraldehyde 3.5%. PBL had been cleaned in PBS and then sterlized for 15 minutes with PBS 0.1% H_2O_2. Before being treated with a rat monoclonal antibody and Calbiochem concentrated 1:50 in PBS for one hour at room temperature, PBL were once more rinsed in PBS. The used dilution was the greatest that could be used without saturating the immunocytochemical signal in healthy patients' PBL. This was established in preliminary studies employing the antibody diluted scalarly in the PBL of normal people. PBL were next treated with anti-rat Ig biotinylated for 30 minutes. H_2O_2/diaminobenzidine

in PBS (20 ml/6 mg in 15 ml) was used for 40 minutes to demonstrate the immunological response.

Procedure checks were performed by substituting mouse non-immune Ig for the anti-dopamine transporter antibody lying on PBL from each patient and healthy donor. Positive controls included the commercially available LAN5 (patient neuroblastoma) cell lines mounted on slides. White light microscopy was used to analyses the slides after they had been rinsed in PBS, insert in glycerin, and subjected to an image processing programme. At least 100 cells out of each person had been examined, and the average optical density of dopamine carrier immune reactivity was determined. The statistical examination employed the Kruskal-Wallis test and the Dunn's multiple comparisons test.

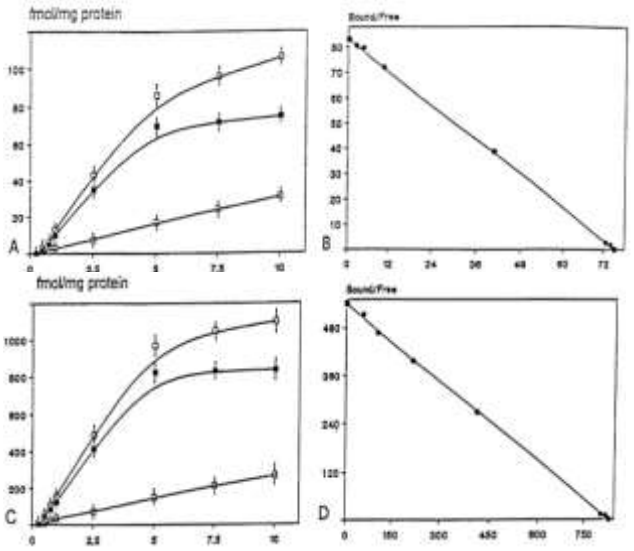
RESULTS

Human PBL particularly absorbed dopamine in relation to concentrations. Temperature and time both had a role in the uptake, which peaked at 37 °C. The dopamine uptake inhibitors Mazanor and Benzatropine constituted the majority potent blocker of H dopamine (Table 1). At doses greater than those of Mazanor or Benzatropine, the un-marked DAT transporter receptor GBR-12909 prevented the absorption of dopamine. Norpramin, a noradrenaline uptake blocker, established its efficacy at dosages numerous times elevated than blockers of dopamine uptake, but the serotonin uptake inhibitor Prozac was inactive.

Table 1: Impact of different inhibitors on dopamine uptake x to human PBL

Compound	Inhibitory constant (in nanomoles)
Benzatropine	95±5.7
Norpramin	3917±259
Prozac	≤10,000
GBR-12909	868±71
Mazanor	39±2.4

Figure 1 demonstrates that dopamine transporter was selectively linked to the stiatum and peripheral blood lymphocyte membranes of humans, but not to the cerebellum. In the membranes of lymphocytes and the striatum, linking belonged to a particular class of specific receptor regions that were concentration dependent. In peripheral lymphocytes and the striatum, the dissociation constant Kd value averaged 2.75nM and 2.68 nM, respectively. For lymphocytes, the greatest density of adhesion points was 75 fmol/mg protein, whereas in the striatum, it was 836 fmol/mg protein. Saturation curves in lymphocytes and the striatum both reached a plateau at 5 nM of radioligand. The specific binding was greater than 70% at this concentration.



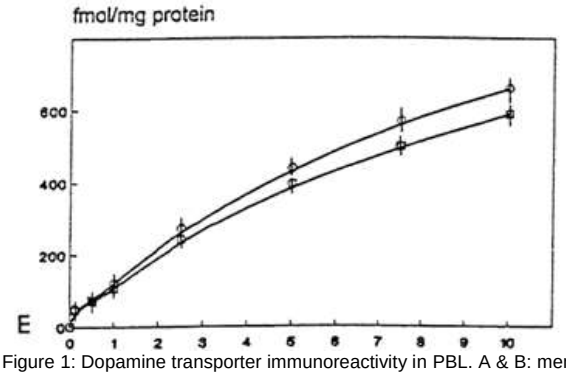


Figure 1: Dopamine transporter immunoreactivity in PBL. A & B: membranes of human peripheral blood lymphocytes, C & D: membranes ofstiatum, E: membranes of cerebellum.

Dopamine transporter immunoreactivity levels varied significantly across groups, according to the ANOVA analysis (KW=27, P=0.001). In PBL of drug-naive (0.21±0.08 SD) and agonist- addressed with dopamine (0.19±0.04 SD) PD participants, the intensity of the immune response was substantially less active than in the normal population (0.70±0.11 SD), according to post hoc analysis (Dunn's multiple assessment analysis). Between the two PD groups, there was no discernible change in dopamine transporter immunoreactivity. The SD of all tested participants' single optical density measurements was 10%. Additionally, age and DAT immunoreactivity did not correlate in the findings in healthy young people.

DISCUSSION

Finding biomarkers for the early detection of neurological illnesses is of growing interest. The decline of dopaminergic nerve terminals in the striatum may be seen in participants with PD in particular using single photon emission tomography or positron emission tomography imaging of dopamine transporter binding (Schwarz et al., 2000). These methods might also track the development of the condition and spot a decline in striatal dopaminergic innervation that hasn't yet manifested as parkinsonian signs a study conducted by Marek et al., (1996). According to latest research by Amenta et al., 2021, PBL might be a biologically important marker to detect dopamine system impairment in PD.

It is the mechanism by which toxins can enter dopaminergic neurons (Amenta et al., 2021). The in-vivo investigation of brain DAT made possible by PET or SPECT imaging (Kugaya et al., 2000) is a costly and intrusive process that is mostly used for research and in a limited set of specialist facilities. Given the significance of DAT for dopaminergic neurotransmission, finding a trustworthy systemic biomarker of this system may help us utilise it to investigate a related brain system. Three of the five dopamine receptor subtypes that have been discovered thus far are expressed by peripheral blood cells, which also manufacture dopamine (Amenta et al., 1999; Amenta et al., 2021).The research analysed by Buttarelli et al. (2011) helped to describe the DA in PBL and to make clear the physiologic influence of DA on PBL activity. This indicates that all subgroups of the individual PBL have a relatively dispersed dopaminergic system. Our investigation demonstrated the uniqueness of the dopaminergic system's physiology and its prospective applicability to neuropsychiatric diseases in PBL.

CONCLUSION

PBL in the initial clinical phases of PD exhibits decreased dopamine transporter immunoreactivity. Since populations of people with PDand normal individuals did not intersect, these findings are especially pertinent. They suggest that in the early phases of clinical PD, the loss in dopamine transporter immunoreactivity may operate as a reliable substitute measure of

the patient's condition. We now know more about how dopamine markers in PBL in PD are changed as a result of these discoveries. It is proposed that more research be done in order to connect the degree of PBL and central nervous system damage as the disease progresses.The results imply that PBL may function as a biological probe to identify DA transmission disruption in neuropsychiatric illnesses and to track the efficacy of pharmaceutical therapies.

REFERENCES

1. Kandel, E.R.; Schwartz, J.H.; Jessel, T.M. Principles of Neural Science; McGraw-Hill: New York, 2000.
2. Medina, L., Reiner, A. Neurotransmitter organization and connectivity of the basal ganglia in vertebrates: implications for the evolution of basal ganglia. Brain Behav. Evol., 1995, 46, 253-258
3. Cave, J.W., Baker, H. Dopamine systems in the forebrain. Adv. Exp. Med. Biol., 2009, 651, 15-35.
4. Sibley, D.R., Monsma, F.J. Molecular biology of dopamine receptors. Trends Pharm. Sci., 1992, 13, 61-69.
5. Strange, P.G. New insight into dopamine receptors in the central nervous system. Neurochem. Int., 1993, 22, 223-236.
6. Nagatsua, T., Sawadab, M. L-Dopa therapy for Parkinson's disease: past, present, and future. Parkinsonism Relat. Disord., 2009, 15 (suppl. 1), S3-S8
7. Giros, B., El Mestikawy, S., Bertrand, L., Caron, M.G. Cloning and functional characterization of a cocaine-sensitive dopamine transporter. FEBS Lett., 1991, 295, 149-154
8. Masson, J., Sagnè, C., Hamon, M, El Mestikawy, S. Neurotransmitter transporters in the central nervous system. Pharmacol. Rev., 1999, 51, 439-464
9. Schwarz J, Linke R, Kerner M, Mozley D, Trenkwalder C, Gasser T, Tatsch K (2000) Striatal dopamine transporter binding assessed by [I-123]IPT and single photon emission computed tomography in patients with early Parkinson's disease: implications for a preclinical diagnosis. Arch Neurol 57: 205–208
10. Marek KL, Seibyl JP, Zoghbi SS, Zea-Ponce Y, Baldwin RM, Fussell B, Charney DS, van Dyck C, Hoffer PB, Innis RP (1996) [123I]-CIT SPECT imaging demonstrates bilateral loss of dopamine transporters in hemi-Parkinson's disease. Neurology 46: 231– 237
11. Amenta et al., 2021. Identification of dopamine plasma membrane and vesicular transporters in human peripheral blood lymphocytes. Journal of Neuroimmunology 117 2001 133–142.
12. Kugaya, A., Fujita, M., Innis, R.B., 2000. Applications of SPECT imaging of dopaminergic neurotransmission in neuropsychiatric disorders. Ann. Nucl. Med. 14, 1–9.