Association of Plasma Dopamine and Norepinephrine Levels and Cannabis Use among Male Cannabis Smokers

Olasore HSA, Osuntoki AA, Magbagbeola OA

Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria.

Corresponding Author
HSA Olasore
Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria.
Email: holashore@unilag.edu.ng; Tel.: +2348036953281

ABSTRACT

Background: Many studies have employed both direct and indirect methods of measuring the brain dopaminergic activity in relation to drug dependence, internet addiction and other disorders in which dopamine signalling has been implicated. However, only a few scientific reports have found a relationship between plasma dopamine and/or norepinephrine levels and addiction.

Objective: The present work was aimed at determining the association between plasma dopamine level and consumption of cannabis as the most widely used illicit drug in the world.

Methods: One hundred and six participants with cannabis use disorder based on International Classification of Diseases version 10 (ICD-10) were recruited for the study. Screening for current cannabis use disorder was done using Cannabis Use Disorder Identification Test (CUDIT). Cannabis dependence was assessed using the Severity of Dependence Scale (SDS). Venous blood samples were taken from the subjects to assay for the plasma dopamine by enzyme-linked immunosorbent assay (ELISA) method.

Results: There was a high correlation between plasma dopamine and SDS scores with plasma dopamine accounting for more than 31% of the variance in SDS scores. However, the relationship between plasma dopamine and current cannabis use disorder was not strong as it accounts for just more than 9% of the variance in CUDIT scores. There was a relationship found between plasma norepinephrine and SDS scores. Plasma norepinephrine only accounts for about 5% of the variance in SDS score while there was virtually no association between plasma norepinephrine and CUDIT scores.

Conclusion: Higher plasma dopamine level was found to be significantly associated with cannabis use and dependence as measured by SDS but less associated with current cannabis use disorder as measured by CUDIT.

Keywords: Plasma dopamine, cannabis, SDS, CUDIT.

INTRODUCTION

The role of central dopaminergic signalling in the brain reward system has been a subject of discussion for decades. Dopaminergic system has been linked to substance use and sensation seeking behaviours (1). In the central nervous system (CNS), the activity of dopamine as a neurotransmitter is localized in the dopaminergic pathways as dopamine is unable to cross the blood brain barrier (2, 3). For these reasons, studies have employed indirect methods of assessing dopaminergic activity in the brain. These methods include measurements of dopamine metabolites in the blood and urine (4) and using brain scanning such as positron-emission tomography (PET) and magnetic resonance imaging (MRI) to study the activity of the brain dopaminergic pathways during certain reward activating activities. Other studies have correlated the effect of genetic variation in the genes involved in dopamine signal pathways and with central dopaminergic activities (5, 6).

Studies have also shown that norepinephrine too plays a key role in the brain reward system (7). Many regions of the mesolimbic dopaminergic system including the nucleus accumbens (NAc), ventral tegmental area (VTA), amygdala and the bed nucleus of stria terminalis have been reported to receive noradrenergic input (8, 9). Lesion of noradrenergic neurons in the locus ceruleus (LC) decreases dopamine release in the NAc (10) and conversely, activation of the LC’s noradrenergic neuron increases the activity of dopaminergic neurons in the VTA (11, 12). It has been suggested that individuals with low noradrenergic activity will tend to have high levels reward dependence (13).

The most accessible pools of dopamine and norepinephrine with a minimal invasiveness are the plasma pools. However, due to the earlier mentioned blood brain barrier, it would be reasonable to think that activities that affect the CNS catecholamine neurotransmitters’ levels may have no effect on the plasma levels of the catecholamines. This position may however be challenged considering the fact that the enzyme responsible for the metabolism of central dopamine are also found in the peripheral dopamine metabolism. It might be justifiable to suggest that the peripheral dopamine levels may mirror the central dopamine levels. This is supported by some
studies (14, 15) which reported a significant association between internet addiction, cocaine use and plasma dopamine levels.

There has been a rise in the abuse of substances such as cannabis, which now tops the list of the illicit drugs worldwide (16). Increased abuse of pharmaceutical products such as tramadol and codeine among Nigerian youth has led to the recent ban on these products. Finding correlation between plasma dopamine and perhaps norepinephrine levels and behaviours associated with breakdown in the brain reward system can help to better understand the role and influence of peripheral dopamine on substance use or sensation-seeking behaviour. It can also help predict the possibility of developing addiction to such behaviours, and to determine the prognosis of disorders such as substance dependence. A strong correlation between plasma levels of the two catecholamines under study and substance use disorder may be useful in predicting which patients will benefit from a particular treatment intervention.

The present study was carried out to find the possible relationship between plasma dopamine and norepinephrine levels and cannabis use among Nigerian youths.

MATERIALS AND METHODS
Ethical Considerations and Subjects
<br>After obtaining the approval of the Human Research Ethics Committee of the Federal Neuropsychiatric Hospital, Yaba, Lagos State (FNPHY/ERC/09/001), a total of 106 subjects seeking treatment for cannabis use disorder at the Drug Unit of the aforementioned hospital were recruited for the study. Informed consent was obtained from all individual participants included in the study. Venous blood was taken in the morning after an overnight fast for plasma dopamine and norepinephrine assays. The blood samples were taken after one hour of the patients remaining in resting position.

Screening for Current Cannabis Use Disorders
<br>The Cannabis Use Disorders Identification Test (CUDIT) (16) was used to screen for current cannabis use disorders according to ICD-10. This instrument is a 10-item questionnaire that provides a score indicating current cannabis use disorders (abuse or dependence) according to DSM-IV. Each of the 10 items is scored on a 4-point scale (0–3). The total score is obtained through the addition of the 10-item ratings.

Assessment of Cannabis Dependence
<br>Diagnosis of cannabis dependence was done based on the ICD-10 criteria. Individuals who met at least three of the six listed criteria were taken as cannabis dependent. Assessment of cannabis dependence was done using the Severity of Dependence Scale (SDS) (18) to measure the degree of psychological dependence specifically related to the individuals’ feeling of impaired control over cannabis and preoccupation and anxiety towards cannabis taking. The SDS is a 5-item questionnaire that provides a score indicating the severity of drug dependence. Each of the five items is scored on a 5-point scale (0–4). The total score is obtained through the addition of the 5-item ratings.

Plasma Dopamine and Norepinephrine Assays
<br>Assays for the quantitative determination of plasma dopamine, norepinephrine and epinephrine were carried out by enzyme-linked immunosorbent assay (ELISA) method using TriÇat ELISA kit (Cat number: RE59395, IBL International, Hamburg, Germany). This kit is designed to detect the three catecholamines - dopamine, norepinephrine and epinephrine in human plasma and urine samples using solid phase ELISA based on the sandwich principle. It consists of wells coated with a goat anti rabbit antibody. The added liquid antibody, directed towards an epitope of an antigen molecule (in the sample) binds to the plate within the incubation time; this was followed by series of washing. The samples were then incubated in the coated well with enzyme conjugated second antibody (E-Ab), directed towards a different region of the antigen molecule. After the substrate reaction, the intensity of the developed colour was read at 405 nm proportional to the amount of the antigen. Standards were also run along with the samples to produce a standard curve. Results of the samples were determined directly using the standard curve obtained. The detailed procedure is available in the manufacturer’s manual.

Statistical Analysis
<br>The results were analysed using SPSS version 17. The association between the plasma catecholamine levels were measured using the regression analysis with the $R^2$ values showing the contributions of the catecholamines to the variance in the SDS and CUDIT scores.

RESULTS
<br>Figure 1 shows the relationship between plasma dopamine and SDS. There is an inverse relationship between plasma dopamine and SDS. More than 31% of the variance in the SDS score was due to plasma dopamine with individuals with higher plasma dopamine having lower SDS score. In Figure 2, an inverse relationship is also found between mean plasma dopamine and CUDIT. The observed 9% variance in CUDIT score ($R^2 = 0.091$) is accounted for by plasma dopamine, less than the effect found in the case of SDS.

In Figure 3, increase in plasma norepinephrine level is correlated with a decrease in the SDS score as a measure of...
cannabis dependence but the plasma norepinephrine level only contributes very little (about 5%) to the variance in SDS score ($R^2 = 0.049$). As shown in Figure 4, the relationship between plasma norepinephrine and CUDIT score as measure of cannabis use disorder was also inverse but it was not significant.

**DISCUSSION**

Dopamine has been established to be central to pleasurable and rewarding activities (5). Dysfunction of dopaminergic signalling has therefore been regarded as a major factor in the development of addiction (19, 20). Much attention has not been given to plasma dopamine and norepinephrine with a possibility of finding correlation between the levels of these catecholamines in the plasma and forms of addiction. In the present study, a significant association was found between plasma dopamine and norepinephrine levels among cannabis smokers and severity of cannabis dependence. Cannabis use disorder was also found to be well correlated with plasma levels of these catecholamines. However, the correlation with plasma norepinephrine was not statistically significant. These relationships between SDS and CUDIT and plasma catecholamines were found to be inverse in all cases. This study is the first, to the best of our knowledge, to find a correlation between plasma levels of these catecholamines and cannabis use. However, as reported earlier, a study has found an association between plasma dopamine and cocaine use (15).

As noted earlier, addiction and substance abuse are often linked to breakdown in the brain reward system. This is often associated with hypodopaminergic states (21). This state may be consequent upon reduced dopamine release and/or increased elimination. For example, functional polymorphisms of tyrosine hydroxylase, an enzyme catalysing the rate limiting step in catecholamine synthesis, has been reported to influence the level of dopamine at the dopaminergic neurons (22). Polymorphisms resulting in low activity of this enzyme has been linked to nicotine dependence (23). Dopaminergic receptor gene variants associated with reduced signalling have also been linked to increased food and substance craving (24), as well as increase in sensation seeking behaviours (25). Thirdly, high activity variants of enzymes that eliminate dopamine have been reported to predispose individuals to addictive and sensation seeking behaviours (26, 7). Since all the somatic cells within the body possess identical genetic makeup, these receptors and enzymes wherever they are expressed within the body are expected to be similar and may modulate the peripheral dopamine levels much the same way they do in the CNS.

Peripheral dopamine has been shown to influence food intake (27), while increased dopamine release reportedly inhibited insulin (28). Discontinuation of administration of dopamine agonist in DRD2 knockout rats has led to increased food consumption and weight gain suggesting the role of peripheral dopamine in appetite (29). Conversely, sulpiride (a dopamine receptor antagonist) which crosses the blood brain barrier very poorly, was found to increase food intake (30), and further substantiate the influence of peripheral dopamine separate from the CNS dopamine on appetite. Behavioural aspect of obesity with increased food craving may be viewed as an addiction-like syndrome. Addictive behaviours may be influenced by peripheral dopaminergic activity which may involve some indirect mechanisms. A link between plasma dopamine and internet addiction (14), suggests that addictive behaviours may have an association with factors outside the brain reward circuit. Whether the dopaminergic activity seen...
peripherally in the above cases only mirrors the CNS activity or if there are other peripheral events involved in this kind of addictive behaviour need to be further studied.

Cannabinoid receptors have been reported to mediate the effect of Δ⁴-tetrahydrocannabinol (THC) which is the main psychoactive component of cannabis on the CNS dopaminergic system (31). These receptors, especially the CB2 have been shown to activate dopaminergic neurons and increase dopamine release (32). Cannabinoid receptors, especially CB2, have been found also to exist peripherally (33). Binding of THC to these receptors may be responsible for the inverse relationship between the plasma dopamine and cannabis dependence (measured by SDS) observed in this study.

Aside from activation of the dopaminergic system, certain drugs such as cocaine have been shown to inhibit the norepinephrine transporter, thus increasing synaptic norepinephrine (34). This contributes to the addictive properties of the drugs and also underscores the role of norepinephrine in substance dependence. In this study, an inverse relationship was found between SDS scores and plasma norepinephrine. However, the observed inverse relationship between plasma norepinephrine and measures of dependence (SDS) may be due to the relationship between dopamine and norepinephrine. Since norepinephrine is produced from dopamine in one step reaction catalysed by dopamine β hydroxylase, increased dopamine production may also increase norepinephrine production. On the other hand, the relationship between norepinephrine and SDS may be due to other molecular events not yet fully understood. An inverse relationship was also observed between norepinephrine and CUDIT in this study; however, this was statistically insignificant.

CONCLUSION

There is increasing evidence to support that the dopaminergic system not only functions within the brain reward circuits to influence substance craving, sensation seeking, addiction, but also acts peripherally to influence these behaviours. The finding from this study is the first, as much as we know, to show a link between plasma dopamine and norepinephrine and cannabis dependence. Establishing a link between these plasma catecholamines and cannabis use may be useful in predicting the possibility of developing cannabis dependence. To consider the plasma levels of these catecholamines for diagnostic or prognostic purpose in the case of cannabis use disorder and dependence, more studies will be needed.

Conflict of Interest

Authors declare no conflict of interest.

REFERENCES


