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Nitric Oxide Levels in Disruptive Behavioral Disorder

Fatma Varol Tas[a] Taner Guvenir[a] Gultekin Tas[c] Burcu Cakaloz[d]
Murat Ormen[b]

Departments of [a]Child and Adolescent Psychiatry and [b]Biochemistry, Faculty of Medicine, University of Dokuz Eylul, and [c]Department of Biochemistry, Kent Hospital, Izmir, Turkey, and [d]Department of Child and Adolescent Psychiatry, Faculty of Medicine, University of Pamukkale, Denizli, Turkey

Key Words
Attention deficit and hyperactivity disorders · Disruptive behavioral disorder · Nitric oxide

Abstract
There are various evidences of the role of nitric oxide (NO) in several neuropsychiatric disorders. However, there is no clinical study which investigated the role of NO in disruptive behavioral disorders (DBD). The aim of this study is to investigate the relation between NO levels and DBD. NO levels were measured in serum from 45 patients diagnosed as having DBD (30 patients with a diagnosis of attention deficit and hyperactivity disorder [ADHD] and 15 with ADHD + oppositional defiant disorder [ODD]) and 51 healthy control subjects. It is statistically significant that the pure ADHD group's blood NO levels are lower than those of both the ADHD + ODD and control groups. There was no significant difference between the ADHD + ODD group and the controls. The difference of the NO levels in DBD may indicate the effect of NO in the etiology of this disorder spectrum.

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Introduction

The interest in the role of nitric oxide (NO) in psychiatric disorders is currently increasing. Today, the role of NO in the etiology of psychiatric disorders such as schizophrenia, affective disorders, substance abuse and autism is investigated extensively [1–5].

NO is a free oxygen radical found in soluble gas form [6]. It exists in peripheral tissues and nerve cells. NO is synthesized from *L*-arginine by NO synthase (NOS) enzyme in nicotinamide adenine dinucleotide phosphate [6].

In a watery ambient, NO transforms into nitrite and nitrate as a result of a rapid oxidation. In biological systems, NO's half-time is between 3 and 30 s. Nitrite and nitrate are more stable compared to NO. Therefore, the nitrite and nitrate rates in blood are used as an indication of endogenous NO synthesis. It is also stated that peripheral NO metabolites may be used as a marker for NO changes in the central nervous system [7, 8].

NO passes from one nerve cell to another through diffusion and it finds itself a candidate receptor [9]. This shows that to a great extent, NO meets criteria for being a neurotransmitter [10].

In the central nervous system, NO functions as a major messenger molecule regulating neurotransmission and

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Fatma Varol Tas
Dokuz Eylul Universitesi Tıp Fakultesi
Cocuk ve Ergen Ruh Saglıgı ve Hastalıkları AD
TR–35340 Balcova/Izmir (Turkey)
Tel. +90 232 412 3563, Fax +90 232 259 9723, E-Mail fvaroltas@hotmail.com

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vasodilatation [1]. In the brain it regulates noradrenaline (NA) and dopamine (DA) release, memory, learning, awareness, smelling, food and liquid intake [1, 11].

In addition to regulating releases, NO interacts with NA and DA and changes their regulating influences in synaptic transmission. There are studies which report that NO facilitates calcium-induced DA flow, especially in basal ganglions. It is demonstrated that in rat striatum, there is a relation between NO-releasing neurons and DA-releasing neurons [12]. Also, it is stated that NO changes serotonin (5-hydroxytryptophane; 5-HT) into its inactive form, and this has an influence on neuromodulation [13]. Similarly NA has also been reported to react with NO [14].

Disruptive behavioral disorders (DBD), which include attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD) and conduct disorder, are some of the most common disorders encountered by child and adult psychiatrists. The frequency of the disorder increased the attention in this field. Despite intensive studies on the etiology of ADHD, the etiological reasons cannot be defined clearly yet [15].

The studies on the role of NO in ADHD are limited to animal experiments. It is claimed that NO has an influence on attention and activity [16, 17]. Also, NO has a role in regulating aggression in female and male rats [18–20]. Some of the studies demonstrate that NOS enzyme inhibitors provide an attention increase as much as methylphenidate used in ADHD treatment and that they decrease overactivity [21].

The studies about the effects of NO in ADHD are essential for the unknowns in the etiology and for the treatment alternatives.

The purpose of the study is to evaluate the NO levels in patients with a diagnosis of DBD and a healthy control group matched in terms of age and sex. Hypothetically, we assume that NO levels in normal controls differ from those of the DBD group.

Materials and Method

Subjects

The study was carried out with 6- to 11-year-old boys who were diagnosed as having DBD in the Child and Adolescent Psychiatry Department of Dokuz Eylul University Medicine Faculty (DEUMF). Forty-five children and families, out of 65 meeting inclusion criteria, accepted to participate in the study.

Seventy-six children were randomly sampled from a primary school which has socioeconomic properties similar to the patient group. These children were also of similar age and sex; they neither had physical/neurological/psychological disorders or clinical mental retardation nor did they use psychotropic medication. Fifty-seven of these boys accepted to participate in the study. Two boys quitted upon request of their families, and 4 children who did not complete the scales were disqualified. Fifty-one boys meeting the study criteria composed the control group of the study.

First of all, the DBD diagnosis was evaluated in a semistructured interview with a clinician according to DSM-IV diagnosis criteria [22]; after that, it was reevaluated by another clinician in order to reach a consensus on diagnosis. Also, the Scanning and Evaluation Scale based on DSM-IV for attention deficit and DBD completed by the parents and the teachers as well as Conners' Teacher Rating Scale short form (CTRS 28) completed by teachers were used as supplementary tools for diagnostic purposes.

Children who were not on medication and those who were on a fixed dose (1 mg/kg/g) of methylphenidate for DBD treatment were included in the study. Those who were on medication other than methylphenidate were not admitted to the study. Written informed consent of the parents and verbal informed consent of the children were obtained for participation in the study. The study was also approved by the DEUMF ethics committee.

The existence of another DSM-IV axis diagnosis, clinical mental retardation, a history of seizure, chronic systemic disorder/inflammatory disease or severe head trauma, methylphenidate treatment shorter than 2 months, use of psychotropic medication other than methylphenidate, and alcohol and substance use in the last few months were determined as exclusion criteria.

Sample Collection and Preparation

A 10-ml blood sample was taken from every child who participated in the study. These samples were taken in the morning after 12 h of overnight fasting at 8.00–9.00 h from the cubital vein and transferred into a flat tube without any protective and anticoagulant. The samples were centrifuged for 10 min at 3,000 rpm to obtain serums. These serums were separated into Eppendorf tubes, each with 0.5 ml. Separated serums were stored at –70°C until they were used.

Measurement of NO Levels

NO measurement was made with the modified manual kinetic cadmium reduction method, used by Najwa and Nabil [23]. By this method all nitrate molecules were transformed into nitrite using cadmium granules. Finally, the NO level was determined by making a total nitrite measurement. The results were given in micromoles per liter.

Statistical Analysis

SPSS Windows 11.0 software was used to evaluate the data obtained in the study. Categorical variables were evaluated with the χ^2 square, quantitative variables with the Mann-Whitney U and Kruskal-Wallis tests. Also, the Pearson correlation test was used to evaluate the data. In all analyses, p values smaller than 0.05 were deemed to be statistically significant.

Results

All the children who participated in the study were in the 7- to 11-year age range. The average age of the study group was 9.4 ± 1.1 years, whereas the average age of the

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control group was 9.2 ± 1.2 years. The difference between the two groups in terms of average age was not significant ($p > 0.05$).

Thirty children out of 45 in the study group were diagnosed as having ADHD (31.3%), and 15 were diagnosed as having ADHD + ODD (15.6%). Twenty-three children out of 45 (51.1%) in the study group were under treatment with a fixed dose of methylphenidate.

The NO levels found were 30.3 ± 6.7 μmol/l in the ADHD group, 36.4 ± 9.8 μmol/l in the ADHD + ODD group and 34.1 ± 8.6 μmol/l in the control group. Statistically, a significant difference was found between the groups ($p = 0.037$). In advanced statistical evaluation, the lowest NO level is in the ADHD group and the highest in the ADHD + ODD group. From a statistical point of view, the NO level in the ADHD group is significantly lower compared to those of the ADHD + ODD and control groups ($p < 0.05$). There is no significant difference between the ADHD + ODD and control groups ($p > 0.05$; fig. 1).

The average NO level of the group on medication (n = 23) was 32.9 ± 9.9 μmol/l whereas the average NO level of the group which did not use medication (n = 22) was 33.3 ± 8.0 μmol/l. There is no significant difference between NO levels of the groups using medication and not using medication ($p > 0.05$).

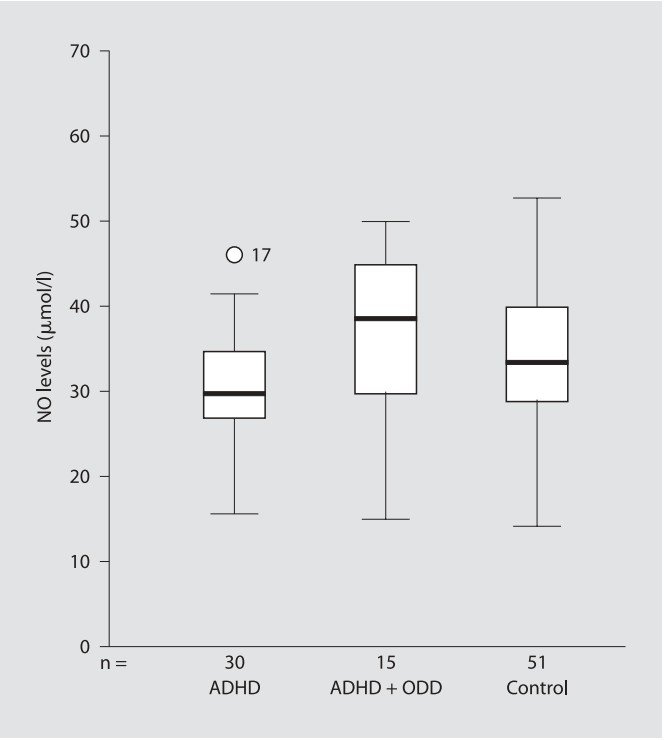


Fig. 1. Groups according to NO levels.

Discussion

There are only a few studies in the literature investigating NO levels and influence mechanisms in ADHD and aggression. The existing ones are limited to animal studies.

In our study, NO levels in the ADHD group were found to be significantly lower compared to both ADHD + ODD and control groups ($p < 0.05$). NO levels in children with ADHD accompanied with ODD were the highest of all three groups. The difference was statistically significant for the ADHD group, whereas it was not for the control.

Grammatikopoulos et al. [16] investigated the influence of NO on nonselective attention, using 7-nitroindazole (7-NI) – which is a selective NOS inhibitor – in a genetic animal model of ADHD. 7-NI is selective on neuronal NOS (nNOS). In this study, it was determined that when an NOS inhibitor is used in genetic animal models, attention improves and hyperactivity decreases. The researchers state that selective inhibition of NO release on the neuronal level may decrease neurotransmission of DA in the midbrain and forebrain sites.

In another study made with the NOS inhibitor *L*-nitroarginine-methylester (*L*-NAME), the role of NO on attention processes of rats, which are ADHD genetic animal models, was investigated. As a result of *L*-NAME application, it was determined that attention increased significantly and hyperactivity decreased significantly. Based on these findings, the researchers claimed that NO had an influence on attention [17].

In another study, the influence of NOS inhibitors was compared to the therapeutic effect of methylphenidate used in ADHD treatment [21]. Rats were administered intraperitoneal methylphenidate or the NOS inhibitor *L*-NAME every day for 2 weeks. As a result, NOS inhibitors were found to increase attention and decrease overactivity as much as methylphenidate. The researchers stated that NOS inhibitors may be used as a new treatment option for ADHD [21].

The findings of these studies suggest a complicated relation between ADHD and NO.

The results of our study are different from the findings of the above-mentioned studies. Increase in attention and decrease in activity by NOS inhibitors suggest that these medicines demonstrate a therapeutic effect by decreasing

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NO levels. However, in our study, NO levels are low in children with ADHD.

Also, conflicting outcomes exist in other psychiatric disorders, where NO levels are evaluated. While there are studies which show significantly higher NO levels in schizophrenia compared to control groups [24, 25], there are also others which show lower levels [26, 27]. Also, different results were obtained in affective disorders [1, 27, 28].

There might be several reasons for these conflicting results in such studies as NO has many effects on the central nervous system. Especially, it affects both the release of neurotransmitters such as DA, NA or 5-HT, claimed to be dysfunctional in ADHD etiology [29], and synaptic transmission [12].

Since DA is an important neurotransmitter in ADHD [30], it is necessary to examine the relation between NO and DA. There are studies which report that NO facilitates calcium-induced DA flow, especially in basal ganglions [12]. It is demonstrated that in rat striatum, there is a relation between NO-releasing and DA-releasing neurons [12]. It is reported that direct stimulations coming from striatal DA neurons may regulate NO release [31].

The complex relationship between NO and DA might be the cause of such results in our study.

In the literature, there are animal studies which investigate the effectiveness of NO in aggression. These studies are focused on comprehending the relation between aggression and NO with genetic applications of nNOS enzyme.

Also, both pharmacological and clinical studies demonstrate that 5-HT has a key role in aggression and impulsiveness. A negative correlation is reported between 5-HT system activity and aggression [32].

As a result of all these studies, when the relation between NO and 5-HT is evaluated in aggression, it is claimed that overaggressive and impulsive orientation in nNOS–/– male rats, is the result of a deficit in 5-HT1A and 1B receptor functions in brain parts providing a decrease in 5-HT turnover and emotional regulation [33, 34].

Fossier et al. [13] reported that the regulatory role of 5-HT in synaptic transmission decreased when an NO donor was given. In this study, it is stated that NO converted 5-HT into its inactive form and this affected neuromodulation.

In animal studies, where aggression and NO relation are examined, the behaviors of nNOS–/– rats were investigated. However, NO may affect neuroendocrine and behavioral processes in one of two ways. It either regulates blood flow influencing neuroendocrine segregations indirectly via endothelial NOS or influences neuronal functions with nNOS activity [18]. Therefore, the increase in aggression in nNOS–/– rats may not be caused by a low level of NO. It is not known how other NOS enzymes contribute to NO generation and what the level of NO is in rats with high aggression.

When we evaluate the findings of the studies, NO levels may be expected to be low in aggressive nNOS–/– rats. There is no clinical study which evaluates NO levels in individuals with aggression and impulsiveness.

The relationship between NO and 5-HT might be the cause of high NO levels in the ADHD + ODD group where we assume the aggression is higher.

ADHD and ODD comorbidity is frequently reported in the literature [35]. In our study, ODD which causes more aggression, shows comorbidity with ADHD. It is stated that the relation between 5-HT and catecholamine metabolism is different in aggressive children with ADHD and those without ADHD [36]. It is not known how NO levels will be in a group, with a diagnosis of only ODD.

No significant difference was found between the NO levels of the group which used medication and that which did not ($p > 0.05$). There are a few studies evaluating the relation between psychotropic medication and NO levels [12, 37]. Itzhak and Martin [37] studied the role of 7-NI – a NOS inhibitor – on overactivity associated with low- and high-dose methylphenidate. While 7-NI blocked the acute response against low-dose methylphenidate, it did not have any influence on the locomotor activity created with high-dose methylphenidate [37]. These findings suggest that there may be no direct relation between methylphenidate use and NO.

There are not enough relevant clinical studies to safely compare the results, which might be considered as a limitation for our study. Additionally, the comorbidity of ODD and ADHD in some of the sample, and the low number of cases, of which some are on medication, are the other limitations.

The results of our study suggest that NO is an important molecule. However, many comprehensive animal and clinical studies are necessary to learn further what kind of an effect it has and what kind of mechanisms create this effect. We believe that these studies on NO will help us to understand the etiology of DBD and the relation between complicated neurotransmitter systems, and they will contribute to formulating appropriate treatment protocols in the future.

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