ORIGINAL ARTICLE

Increased dopamine DRD4 receptor mRNA expression in lymphocytes of musicians and autistic individuals: bridging the music-autism connection

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Abstract

BACKGROUND: People with autistic spectrum disorder (ASD) are affected by a long-life disabling condition, characterized by communication deficits, severe impairments in social functioning, and stereotyped behaviors. Although ASD individuals display several problems in interactions, it has been reported that they may show a peculiar interest in music. Previous studies have suggested a pivotal role for the dopaminergic system in the psychobiology of reward, including the pleasure of music.

DESIGN: In the present study, we sought to investigate dopamine DRD3 and DRD4 receptor expression in peripheral blood lymphocytes of adult healthy musicians and age- and gender-matched patients with ASD against the background hypothesis that the dopaminergic system may contribute a biological cause to the reward dimensions of the musical experience in both healthy and autistic individuals.

RESULTS: ANOVA showed significant differences in DRD4 mRNA expression between the groups ($P = 0.008$). *Post-hoc* analysis showed significant differences between the control group and both musicians ($P < 0.05$) and ASD individuals ($P < 0.05$). No differences were found for DRD3 mRNA expression between the groups.

CONCLUSION: Our current results provide intriguing preliminary evidence for a possible molecular link between dopamine DRD4 receptor, music and autism, possibly via mechanisms involving the reward system and the appraisal of emotions.

Introduction

People with autistic spectrum disorder (ASD) are affected by a long-life disabling condition, characterized by communication deficits, severe impairments in social functioning, and stereotyped behaviours (Wing, 1997). Although ASD individuals display several problems in interactions, it has been reported, that they may show a peculiar interest in music, occasionally accompanied by an unexpectedly extraordinary talent (Treffert & Wallace, 2002). Accordingly, the intrinsic interactive potential of music has been successfully used to promote social interactions, communicative behaviour and emotional responsiveness in autistic subjects. Notably, previous studies in the literature have shown improvements of the behaviour profile in

ASD individuals following music therapy (Gold *et al* 2006; Boso *et al* 2007).

The biological basis whereby people with ASD are often engaged by music remains to be elucidated. It could be hypothesized, however, that the musical experience may exert a rewarding role in this patient group. Since decades, experimental approaches and clinical experience have suggested a pivotal role for the dopaminergic system in the psychobiology of reward (Ikemoto, 2007; Schultz, 2007), including the pleasure of music. Accordingly, it has been demonstrated that dopamine is released from the ventral striatum and in the ventral tegmental area during listening to pleasant music (Menon & Levitin, 2005). Dopamine receptors are integral membrane proteins interacting with G proteins; thus far, five different dopamine receptors have been described, namely DRD1, DRD2, DRD3, DRD4, DRD5 (Hoenicka *et al* 2007). They are classified into DRD1-like (the DRD1 and DRD5) or DRD2-like (the DRD3, DRD4 and DRD5) receptor subtypes according to their capacity of stimulating or inhibiting adenylate cyclase and their pharmacological peculiarities (Emilien *et al* 1999). Dopamine receptors are expressed not only in the brain, but also in human peripheral blood lymphocytes (PBL). In this regard, it has been suggested that dopamine receptor expression in peripheral blood lymphocytes can reflect, at least in part, brain dopamine status (Czermak *et al* 2004a; 2004b).

In the present study, we sought to investigate dopamine DRD3 and DRD4 receptor expression in peripheral blood lymphocytes of adult musicians and age- and gender-matched patients with ASD against the background hypothesis that the dopaminergic system may contribute a biological cause to the reward dimensions of the musical experience in both healthy and autistic individuals. We focused on the dopamine DRD3 and DRD4 receptors since they are expressed in PBL and thus might serve as a potential peripheral marker of central dopaminergic activity. Moreover, these receptors are known to be particularly involved in the neurobiology of pleasant experiences.

Subjects and methods

Subjects

A total of 58 subjects were investigated. Participants were divided in three groups. Group 1 consisted of 20 ASD patients (age range: 15-42 years, 18 males and 2 females) recruited from a single farm community center specifically designed for individuals with autism (Cascina Rossago, Ponte Nizza, Pavia, Italy). The diagnosis of ASD was confirmed in all participants jointly by two independent psychiatrists specialized in autism spectrum disorders who made the diagnosis according to the guidelines of the Structured Clinical Interview for Axis I DSM-IV Disorders, Patient Version. All patients in this study scored more than 30 on the Childhood Autism Rating Scale (CARS) (Schopler *et al* 1980), the standard threshold used to distinguish autism. Group 2 consisted of 19 adult professional musicians (age range, 20-44 years, 15 males and 4 females) with at least seven years of daily musical practice. A total of 19 age- and gender-matched individuals (age range 18-41 years, 15 males and 4 females) with no interest in playing or listening to music served as controls (Group 3). All subjects in Group 2 and 3 had no past or present history of any psychiatric disorder and none of them had ever taken medications for psychiatric conditions. Additionally, subjects with axis-I diagnosis of first-degree relatives were not included in this group. All subjects described here were Caucasians of Italian descent. In this report, the criterion of Italian descent was met when an individual's parents and four grandparents originated from Italy. The investigation was performed in accordance with the ethical requirements defined in the Helsinki Declaration. All the participants and/or legal guardians provided their full informed consent to participate in the study according to our internal review board guidelines.

Methods

Peripheral blood samples (8 ml) were obtained from the antecubital vein and collected in cell plastic tubes containing sodium-heparin solution (120 UI/ml) as anticoagulant. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density centrifugation (GE Healthcare Bio-Sciences Corp; Piscataway, NJ, USA). The lymphocyte layer was collected and washed three times in Phosphate buffer saline (PBS, Sigma-Aldrich; St. Louis, MO, USA). The total mRNA was isolated from lymphocytes by RNA blood mini kit (QIAGEN, Hilden, Germany), and the amount and purity of RNA were determined by spectrophotometery. Dopamine receptor mRNA expression was determined by the 3'-5' fluorogenic Taqman approach (Applera, Monza, Italy). 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 30 μl. Primers for DRD3, DRD4 and the housekeeping gene β-actin were previously described (Czermak *et al* 2004b). The specificity of the obtained PCR products for the respective dopamine receptor fragments was confirmed by sequence analysis. All experiments were performed in triplicate and the results were averaged. The sample target quantity was corrected by the respective value of β-actin and adjusted to the corresponding value of a calibrator that was used to obtain comparable sample values between different plates. DRD3 and DRD4 mRNA expression values were obtained in arbitrary units.

Statistics

Data normality was checked by Kolmogorov–Smirnov tests. All data were normally distributed and only parametric tests were used. Intergroup comparisons of con-

Table 1. DRD3 and DRD4 mRNA expression in the three study groups.

	Control subjects (n =19)	Healthy musicians (n = 19)	ASD patients (n = 20)
DRD4 receptor	1.33 ± 1.29	2.50 ± 1.70*	2.58 ± 1.99*
DRD3 receptor	1.00 ± 0.81	0.95 ± 0.74	1.10 ± 0.47

* $P < 0.05$ versus control subjects

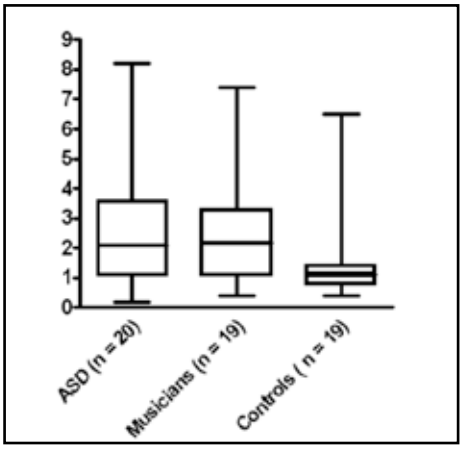

Figure 1. Expression of DRD4 receptor mRNA in PBL of ASD patients, healthy musicians and control subjects. The box surrounding the median shows the 25th (lower) and 75th (higher) percentiles. Results are expressed in arbitrary units.

tinuous variables were assessed by one-way ANOVA followed by *post-hoc* Newman-Keuls test. Chi-square analysis was used for categorical data. Adjustment for gender and age was performed by analysis of covariance (ANCOVA). Statistical analysis was performed using the SPSS software, version 11.0 (SPSS Inc., Chicago, IL, USA). *P* values < 0.05 (two-tailed) were considered statistically significant.

Results

There were no age and gender differences in the three study groups. Covariance analysis revealed no influence of age and sex on dopamine receptor mRNA expression. Correlation between the DRD3 and DRD4 receptor mRNA expression was not significant (r = 0.071, P = 0.554). Expression of β-actin in PBL did not differ between the groups (data not shown). One-way ANOVA showed significant differences in DRD4 mRNA expression between the groups (P = 0.008, ***Table 1***). *Post-hoc* analysis showed significant differences between the control group and both musicians ($P < 0.05$) and ASD individuals ($P < 0.05$, ***Figure 1***). No differences were found for DRD3 mRNA expression between the groups.

Discussion

This study provides evidence for the first time that both musicians and ASD patients exhibit an increase in DRD4 receptor mRNA expression in PBL compared to controls. On the other hand, no significant differences with regard to DRD3 receptor mRNA levels were found across the three study groups. Since activation of the dopaminergic system has been suggested to play a crucial role in the rewarding effects of musical pleasure (Menon & Levitin, 2005), our current data provide intriguing preliminary evidence that the altered expression of dopamine DRD4 receptor may play role in mediating the remarkable interest in music showed by several ASD subjects.

The involvement of the DRD4 receptor in human rewarding system has been hypothesized from the observation that this receptor may play a role in some human "reward deficit conditions" including pathological gambling and addiction (Koob & Le Moal, 2008). Interestingly, it has been reported that clozapine, which has a high affinity with the DRD4 receptor, may be particularly suitable for treating autistic disorders, especially in the presence of aggressiveness (Gobbi & Pulvirenti, 2001).

Previous neuroimaging studies have shown that listening to music strongly modulates activity in a network of mesolimbic structures involved in reward processing including the nucleus accumbens (NAc) and the ventral tegmental area (VTA) as well as the hypothalamus and insula (Menon & Levitin, 2005). Notably, studies on expression of DRD4 receptor proteins have shown selective localization of DRD4 receptors in mesolimbic and mesocortical areas (Gardner & Ashby Jr, 2000; Broderick & Piercey, 1998).

On the other hand, no differences in DRD3 mRNA expression in PBL were found in ASD, musicians and controls not interested in music. Although the dopamine DRD3 receptor has been suggested to be involved in mediating rewarding effects in humans (Schultz, 2007), our present data does not seem to suggest a primary role of the DRD3 receptor in ASD subjects and musicians, compared with controls, at least at a transcriptional level.

In interpreting our findings, important limitations must be considered. Firstly, our results are limited by the small sample size of each group, and additional investigations with a larger number of subjects would be useful to confirm the importance of the DRD4 recep-

tor in mediating musical pleasure in health and disease. Secondly, it has been previously reported that expression of human DRD4 receptor displays a remarkable degree of individual variability probably due to genetic factors (Livak *et al* 1995). Since in this study we did not perform genotyping of the DRD4 locus, further studies are needed to shed more light on this issue. Thirdly, in this study we used peripheral markers of dopamine status. Nonetheless, it is generally accepted that DRD3 and DRD4 receptor mRNA expression in peripheral blood lymphocytes correlates well with dopaminergic brain status (Czermak *et al* 2004a; 2004b). Finally, we are aware that one disadvantage of RT-PCR is the difficulty in estimating the amount of template amplified.

These limitations notwithstanding, we believe that our results provide intriguing preliminary evidence for a possible molecular link between dopamine DRD4 receptor, music and autism, possibly via mechanisms involving the reward system and the appraisal of emotions. Further study is warranted to shed more light on this fascinating topic.

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