European Neuropsychopharmacology (2012) 22, 379–386

www.elsevier.com/locate/euroneuro

SHORT COMMUNICATION

Alteration in RGS2 expression level is associated with changes in haloperidol induced extrapyramidal features in a mutant mouse model

Lior Greenbaum [1], Tzuri Lifschytz [1], Polina Zozulinsky, Esther C. Broner, Alexandra Slonimsky, Yoav Kohn, Bernard Lerer*

Biological Psychiatry Laboratory, Department of Psychiatry, Hadassah – Hebrew University Medical Center, Jerusalem, Israel

Received 24 June 2011; received in revised form 10 August 2011; accepted 10 September 2011

KEYWORDS
Schizophrenia;
Extrapyramidal symptoms;
Neuroleptics;
RGS2;
Antipsychotic induced Parkinsonism

Abstract

Antipsychotic induced Parkinsonism (AIP) is a common adverse effect of antipsychotic drug treatment among schizophrenia patients. Two previous studies showed association of the rs4606 SNP in the 3′ untranslated region of the regulator of G protein signaling 2 gene (*RGS2*) with susceptibility to AIP. Since rs4606 reportedly influences expression of RGS2, we applied a translational approach and studied the effect of chronic (24 days) exposure to haloperidol on AIP-like features in mice carrying a mutation that causes lower *Rgs2* gene expression. Haloperidol and vehicle treated male mice heterozygous (HET) or homozygous (HOM) for the mutation, or wild type (WT), were evaluated for open field locomotion, catalepsy duration, pole test performance and rota-rod latency to fall. We showed that in haloperidol treated mice lower *Rgs2* expression is associated with better performance on the open field, catalepsy and rota-rod tests but not the pole test. Results were most consistent for the 0.2 mg/kg/d haloperidol dose. These observations support the possible involvement of *RGS2* in mechanisms underlying susceptibility to AIP.

1. Introduction

The use of antipsychotics drugs, especially typical, first generation antipsychotics, is associated with the development of extrapyramidal symptoms (EPS) (Hansen et al., 1997; Rochon et al., 2005). Antipsychotic induced Parkinsonism (AIP), the most common manifestation of EPS, develops within a few weeks of antipsychotic exposure (Ayd, 1961; Hirose, 2006). Clinically, AIP is similar to idiopathic Parkinson's disease

* Corresponding author at: Hadassah – Hebrew University Medical Center, Department of Psychiatry, Biological Psychiatry Laboratory, Ein Karem, 91120 Jerusalem, Israel. Tel.: +972 2 6777185; fax: +972 2 6439294.
E-mail address: lerer@cc.huji.ac.il (B. Lerer).
[1] These authors contributed equally to this work.

0924-977X/$ - see front matter © 2011 Elsevier B.V. and ECNP. All rights reserved.
doi:10.1016/j.euroneuro.2011.09.006

and characterized by bradykinesia, tremor, rigidity and stooped posture (Hansen et al., 1997; Hassin-Baer et al., 2001). The pathophysiology of AIP is unclear but is directly related to nigrostriatal (NS) pathway dopamine D2 receptor occupancy by antipsychotics (Hirose, 2006). AIP prevalence varies widely, ranging from 15% to more than 50% of antipsychotic treated patients. Well described clinical and demographic risk factors for AIP are antipsychotic drug type (first vs. second generation), high doses of antipsychotics, older age and female gender (Hassin-Baer et al., 2001; Rochon et al., 2005). In addition to the epidemiological risk factors, genetic factors may contribute to inter-individual differences in AIP susceptibility (Greenbaum et al., 2007).

Our group has reported association of the *RGS2* gene with AIP among schizophrenia patients treated with antipsychotics from Israel and the USA (Greenbaum et al., 2007; Greenbaum et al., 2009). Of particular interest was the association of rs4606, a C1114G polymorphism located in the 3′ untranslated region of the gene. Carriers of the rs4606-G allele were significantly protected from development of AIP (in both samples independently). This SNP has functional significance and the AIP protective "G" allele has been reported to be associated with reduced expression of *RGS2* (Semplicini et al., 2006).

RGS2 (regulator of G protein signaling 2) is a GTPase activating protein which enhances the termination of G protein coupled receptor (GPCR) signaling (Bernstein et al., 2004; Han et al., 2006). Following binding of a ligand to its receptor and the initiation of a signal transduction cascade, RGS2 binds to activated Galpha subunits, accelerates their intrinsic GTPase activity and hydrolyzes the bound GTP to GDP (Wang et al., 2004). As a result, the now deactivated alpha units are able to reunite with the beta and gamma G protein subunits, and form an inactive heterodimer (GDP bound) (Hollinger and Hepler, 2002; Ghavami et al., 2004).

RGS2 is a member of the RGS protein family, possess the 120 amino acid RGS domain which mediates direct binding to the Galpha subunit, and is widely expressed in various mouse and human tissues including brain (Hollinger and Hepler, 2002; Schoeber et al., 2006; Nguyen et al., 2009). It is able to inhibit signaling via Galpha-q, Galpha-s and Galpha-i/o subunits of the G protein but is more selective for Gq than for the other subunits (Roy et al., 2006).

In the current study, we sought to better understand the biological mechanism underlying our pharmacogenetic finding of association of *RGS2* with susceptibility to AIP, in particular the possibility that association of *RGS2* with AIP is linked to expression level. Therefore, we used a mouse strain containing a mutation that causes a reduction in *Rgs2* gene expression to assess the influence of *Rgs2* expression level on the development of AIP like manifestations among mice administered haloperidol for 3 weeks. Our results provide support for involvement of *RGS2* in the pathophysiology of AIP.

2. Experimental procedures

2.1. Animals

Animals were derived from a reproduction colony that is maintained in the Reproduction Unit of the Hebrew University Animal Facility, Jerusalem, Israel (AAALAC International Accredited Institute). A breeding litter of C57BL (strain Nu. NIH1291#11639) mice (males and females), heterozygous (HET) for a *Rgs2* gene mutation or wild type (WT), was purchased from the Mutant Mouse Regional Resource Centers Consortium (MMRRC, Univ. of North Carolina, Chapel Hill, USA), and bred in the Hebrew University Animal Facility. The mutation consists of a Lac-Z bacterial cassette inserted into the end of the gene promoter and the first exon. Offspring formed a reproduction colony consisting of three lines of reproduction cages, reproducing mice HET or homozygous (HOM) for the mutation or WT. Expression analysis of the *Rgs2* gene in mice of the three genotype groups established significant differences in expression levels of this gene (Supp. Fig. 1). Mice were weaned and separated according to gender at the age of 21 days. All offspring were genotyped by PCR analysis of DNA isolated from tail tissue. Male mice aged 60–90 days were used for all experiments. Animals had free access to water and food and were housed under a 12 h light/12 h dark cycle; all experiments were performed during the light phase (morning period). Throughout the behavioral tests, experimenters were blind to genotype and treatment. Experiments were conducted according to protocols approved by the Animal Care and Use Committee of the Hebrew University and Hadassah Medical Center.

2.2. Treatment

As a preliminary step to determine the effect of genotype in the absence of haloperidol administration, HOM, HET and WT mice were treated with saline (0.9% NaCl in distilled water administered intraperitoneally [I.P.]) for 24 days. From day 21 of the treatment protocol, animals were subjected to the battery of tests described below to evaluate AIP like manifestations, in the same order (a single test on each consecutive day). Saline injections were given 1 h before each test. In the definitive part of the study, different male mice of the three genotype groups were treated in three subsequent experiments with haloperidol doses of 0.1, 0.2 or 0.5 mg/kg/d I.P. for 24 days. For each haloperidol dose, three treatment groups, consisting of HOM, HET or WT mice were treated in parallel. From day 21 of treatment, mice were subjected to the behavioral test battery to assess AIP like features, in the same order (a single test on each consecutive day). Haloperidol injections were administered 1 h before each test. Last, in a different experiment, WT mice were administered saline or haloperidol 0.5 mg/kg/d I.P. for 21 days, and then dissected for studying the effect of chronic haloperidol administration on *Rgs2* gene expression in cortex and striatum (Supp. Methods).

2.3. Motor tests for assessing AIP like manifestations

a) Open field test: animals were entered into an open field arena 50×50 cm (divided into 10 cm×10 cm squares). Thereafter ambulation was measured as squares crossed in 6 min (Tadaiesky et al., 2006).
b) Catalepsy test: forepaws were placed over a horizontal bar, fixed at a height of 5 cm above the work surface. The time elapsing from the placement of the mouse until the removal of both its forepaws was recorded (Perez and Palmiter, 2005), with a cut-off time of 2 min. Trials were repeated (at 20 minute intervals) 3 times for each animal and means of the results were used for statistical analysis.
c) Pole test: animals were placed head-up on the top of a vertical pole, 50 cm long (1 cm in diameter). The time required to orient downward and the time to descend the entire length of the pole to its base were measured as an indication for the presence or absence of AIP (Fleming et al., 2004) with a cutoff of 2 min. The test consisted of 5 trials (20 minute interval between each) and trial average was used for analysis.
d) Rotarod test: in a single day long test (similar to Yang et al., 2007) animals were placed on the rotarod and the rotation of the device was accelerated from 2.5 rounds per minute (rpm)