Neuroscience 290 (2015) 165–174

REELIN INFLUENCES THE EXPRESSION AND FUNCTION OF DOPAMINE D_2 AND SEROTONIN 5-HT_{2A} RECEPTORS: A COMPARATIVE STUDY

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Abstract—Reelin is an extracellular matrix protein that plays a critical role in neuronal guidance during brain neurodevelopment and in synaptic plasticity in adults and has been associated with schizophrenia. Reelin mRNA and protein levels are reduced in various structures of post-mortem schizophrenic brains, in a similar way to those found in heterozygous reeler mice (HRM). Reelin is involved in protein expression in dendritic spines that are the major location where synaptic connections are established. Thus, we hypothesized that a genetic deficit in reelin would affect the expression and function of dopamine D_2 and serotonin 5-HT_{2A} receptors that are associated with the action of current antipsychotic drugs. In this study, D_2 and 5-HT_{2A} receptor expression and function were quantitated by using radioligand binding studies in the frontal cortex and striatum of HRM and wild-type mice (WTM). We observed increased expression ($p < 0.05$) in striatum membranes and decreased expression ($p < 0.05$) in frontal cortex membranes for both dopamine D_2 and serotonin 5-HT_{2A} receptors from HRM compared to WTM. Our results show parallel alterations of D_2 and 5-HT_{2A} receptors that are compatible with a possible hetero-oligomeric nature of these receptors. These changes are similar to changes described in schizophrenic patients and provide further support for the suitability of using HRM as a model for studying this disease and the effects of antipsychotic drugs.

Key words: reelin, heterozygous reeler mice, striatum, frontal cortex, GPCRs, schizophrenia.

INTRODUCTION

Schizophrenia is a chronic and devastating disease and has high prevalence; schizophrenia involves a complex set of disturbances in thinking, perception, and behavior. Despite the immense human and economic impact of this disease, recent investigations have made little progress in the development of new and more effective treatments. From a genetic point of view, schizophrenia is considered to be a heterogeneous polygenic disease with multiple common genetic polymorphisms, each of which contributes to disease susceptibility (Tandon et al., 2008).

Among the potential candidate proteins, several schizophrenia genetic association studies place reelin as a top candidate gene associated with schizophrenia (Jia et al., 2010; Kim and Webster, 2010). The downregulation of the reelin gene has been detected in several brain regions, including the hippocampus, prefrontal and temporal cortices, cerebellum and caudate nucleus, of subjects with schizophrenia (Impagnatiello et al., 1998; Fatemi et al., 2000; Guidotti et al., 2000; Costa et al., 2001; Maloku et al., 2010). Allelic variations of the reelin gene also correlate with working memory, memory and executive functioning in nuclear families where one of the members suffers from schizophrenia (Wedenoja et al., 2008).

Reelin plays a role in regulating neural migration during brain development and synaptic plasticity in the adult brain (see as review Förster et al., 2010). Reelin also induces the clustering of its receptors (Strasser et al., 2004), increases the translation of selective mRNAs involved in dendritic spine morphology (Dong et al., 2003), and augments the number of intramembranous particles (i.e., transmembrane proteins) in synaptosomal membranes (Caruncho et al., 2004). Therefore, abnormalities in this protein may contribute to several aspects of the pathophysiology of this disease, including disrupted connectivity and neuronal migration, synaptic anomalies and altered GABAergic, glutamatergic and dopaminergic neurotransmission.

The heterozygous reeler mouse (HRM) has a single reelin allele disruption that results in the expression of ≈50% of the amount of reelin mRNA and protein that are usually present in the brain of a wild-type mouse

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Abbreviations: ANOVA, analysis of variance; DOI, (±)-2,5-dimethoxy-4-iodoamphetamine; DTT, DL-dithiothreitol; EGTA, ethylene glycol tetraacetic acid; GDP, guanosine 5′-diphosphate; [^{35}S]GTPγS, [^{35}S]guanosine 5′-O-(gamma-thio)triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HRM, heterozygous reeler mice; [^{3}H]LSD, [^{3}H]-lysergic acid diethylamide; PCR, polymerase chain reaction; WTM, wild-type mice.

http://dx.doi.org/10.1016/j.neuroscience.2015.01.031
0306-4522/

(Tueting et al., 1999; Costa et al., 2001). This mutation displays autosomic recessive transmission and is characterized by a deletion of 150 kb that removes a large portion of the *Reln* gene (D'Arcangelo et al., 1995; Bar et al., 1995). HRM present subtle behavioral and anatomic abnormalities reminiscent of those found in schizophrenic patients who are reported to show a 50% reelin expression decrease. Previous studies have shown a decreased neuropil and dendritic spine density and behavioral changes, including impulsivity and impaired executive function (Costa et al., 2001; Liu et al., 2001; Tueting et al., 2006). A recent report showed that HRM are more vulnerable to the depressogenic effects of repeated corticosterone treatment. The authors proposed that a combination of genetic and stress effects on hippocampal reelin expression may underlie the onset of depressive symptoms during the prodromal stage of schizophrenia (Lussier et al., 2011). There is increasing evidence that HRM and wild-type mice (WTM) differ in behavior and underlying neurobiology, suggesting the suitability of the HRM as a model for psychosis (Tueting et al., 2008).

It has been reported that schizophrenic patients showing a 50% reduction in reelin expression levels had a decrease in dendritic spines where monoamine receptors are abundantly expressed (Costa et al., 2001). There is a large body of evidence for alterations in dopaminergic and serotonergic neurotransmitter systems in schizophrenic patients. Additionally, the tuning of the dopaminergic system by 5-HT_{2A} receptors has been considered to be the principal mechanism of action of atypical antipsychotics. This relationship has been demonstrated in animal paradigms at the postsynaptic density level and in intracellular machinery (for review, see de Bartolomeis et al., 2013). It has been reported that these receptors are expressed as a functional heterooligomer in discrete regions of the ventral and dorsal striata and in HEK293 cells after co-transfection of both receptors (Borroto-Escuela et al., 2010; Lukasiewicz et al., 2010; Albizu et al., 2011; Borroto-Escuela et al., 2014). The heterocomplex formation is involved in conditioning the response of both receptors to hallucinogenic ligands and antipsychotic drugs. Two reports focused on studies of dopaminergic systems in the striatum of HRM. Ballmaier et al. (2002) showed a decrease of D_2 immunoreactivity and mRNA expression in HRM that tends to be more pronounced in the nucleus accumbens and olfactory tubercle. However, van den Buuse et al. (2012) identified no alterations related to the phenotype when studying [^{3}H]YM-09151 binding.

We hypothesize that a deficit in reelin expression may control specific alterations in the expression of D_2 and 5-HT_{2A} receptors in areas characterized by relatively high (cortex) or low (striatum) levels of reelin expression in the adult brain. Therefore, we aimed to compare the expression and function of these receptors in the cortex and striatum of HRM and WTM.

EXPERIMENTAL PROCEDURES

Animals

We used a total of 60 adult mice (36 male and 24 female) in these studies, including 30 wild-type (WTM) and 30 heterozygous reeler mice (HRM) (B6C3Fe-a/a-Reln$^{rl/+}$). The animals were obtained from heterozygous reeler pairs (Jackson Laboratory, Bar Harbor, ME, USA) maintained in our colony at the University of Santiago de Compostela, Spain. These mice have an autosomal recessive mutation that deletes approximately 150 kb in the reelin gene (Andersen et al., 2002). The genotype of each mouse was confirmed by standard polymerase chain reaction (PCR) techniques on tail samples as described previously (D'Arcangelo et al., 1995). The following oligonucleotide primers were used: the forward primer is common to both wild-type and mutant alleles (sequence 5′-TAA TCT GTC CTC ACT CTG CC-3′), one reverse primer is specific for the wild-type allele (sequence 5′-ACA GTT GAC ATA CCT TAA TC-3′), and the other primer is specific for the reeler allele (sequence 5′-TGC ATT AAT GTG CAG TGT TGT-3′). The PCR products were analyzed in a 2% agarose gel. The product from wild-type mouse DNA is 266 bp, and the product from heterozygous reeler mouse DNA is 363 bp. All mice used in this experiment were randomly chosen from a number of litters that were born at approximately the same time.

The animals were distributed in groups of 4–5 per cage and were maintained in a room at constant temperature (22 ± 1 °C) with 12 h of light (08:00–20:00 h). The animals were housed with food and water *ad libitum* throughout the study. All experimental procedures were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), the Declaration of Helsinki, the Spanish Royal Decree of 10 October 2005 (1201/2005/BOE) and the Bioethics Committee of the University of Santiago de Compostela.

Isolation of mice striatum and frontal cortex and membrane preparation

Mice were sacrificed by cervical dislocation and their brains were removed. Then, the striatum and the frontal cortex were isolated for membrane preparation. Samples were homogenized in a 0.32 M solution of sucrose with a Polytron homogenizer. The samples were then centrifuged at 850*g* for 10 min at 4 °C. The resulting supernatant was centrifuged at 37,800*g* for 30 min at 4 °C. The pellet was resuspended in an incubation buffer containing 50 mM HEPES, 2.5 mM $MgCl_2$, 2 mM EGTA, 0.1% ascorbic acid, pH7.5 and was manually homogenized. The homogenate was incubated at 37 °C for 15 min to eliminate endogenous dopamine and serotonin. The homogenate was then centrifuged at 37,800*g* for 39 min at 4 °C. The resulting pellet was manually homogenized in an incubation buffer and stored at −80 °C until saturation studies.

[^{3}H]spiperone saturation studies on mice striatum and frontal cortex

The striatum and/or frontal cortex membranes were incubated with nine different concentrations of [^{3}H]spiperone (0.005–1.5 nM) (68–141 Ci/mmol, Perkin