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Increased levels of serotonin$_{2A}$ receptors and serotonin transporter in the CNS of neuregulin 1 hypomorphic/mutant mice

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Abstract

Changes in neuregulin 1 expression have been reported in the CNS from subjects with schizophrenia. As neuregulin 1 is important in cortical development we postulated that changes in neuregulin 1 expression may contribute towards changes in cholinergic, glutamatergic and serotonergic markers that are well documented in the CNS of subjects with that disorder. To begin to test this hypothesis, we used *in situ* radioligand binding to measure levels of muscarinic M1/M4 receptors, the kainate receptor, the NMDA receptor, the serotonin 2A receptor, the serotonin 1A receptor and the serotonin transporter in the CNS from heterozygous transmembrane domain neuregulin 1 mutant mice. The major outcomes from these studies was the demonstration of an overall increase in levels of the serotonin 2A receptor ($F=11.3$, d.f.$=3,1,72$, $p=0.0012$) and serotonin transporter ($F=5.00$, d.f.$=1,3,72$, $p<0.05$) in the mutant mice. Levels of the other receptors did not vary in the mutant mice compared to their wild type-like litter mates. These data are the first evidence to suggest that *NRG1* gene expression may be involved in regulating the development of the serotonergic system in the mammalian CNS.

Keywords: Neuregulin; Schizophrenia; Serotonin receptors; Serotonin transporter; Muscarinic receptors; Glutamate receptors

1. Introduction

Numerous genetic studies support the hypothesis that variation in the neuregulin 1 (*NRG1*) gene is associated with an increased susceptibility for schizophrenia (Stefansson et al., 2002; Stefansson et al., 2003; Williams et al., 2003; Bakker et al., 2004; Hall et al., 2004;Yang et al., 2003; Tang et al., 2004; Li et al., 2004; Zhao et al., 2004). This posit is further supported by the finding that the expression of type 1 *NRG1* is increased in dorsolateral prefrontal cortex (Hashimoto et al., 2004) and hippocampus (Law et al., 2006) from subjects with the disorder. Up to 15 isoforms NRG1 exist as a result of

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alternative splicing and multiple promoters (Harrison and Law, 2006) with postmortem CNS studies failing to show changes in mRNA for either type II / type III (Hashimoto et al., 2004) or type II to IV (Law et al., 2006) in schizophrenia. It has also been reported that cortical levels of total NRG1 protein are either not changed (Hahn et al., 2006) or decreased (Bertram et al., 2007) in subjects with schizophrenia. Hence it appears that CNS region and transcript-specific changes in *NRG1*, which may not always manifest as a change total NRG1 protein, may contribute to the pathology of schizophrenia.

It has long been accepted that abnormal cortical functioning is present in subjects with schizophrenia (Weinberger, 1988). This is particularly relevant to *NRG1*, which plays a role in cortical development (Lopez-Bendito et al., 2006). Extending this argument, there have been replicated findings of a changed cortical molecular cytoarchitecture in subjects with schizophrenia; these changes include important components of the glutamatergic, cholinergic and serotonergic pathways (Meltzer, 1987; Dean, 2000; Raedler et al., 2007). Given the role of NRG1 in cortical development, it seems possible that changes in *NRG1* expression in the CNS from subjects with schizophrenia could be contributing to the abnormalities in the glutamatergic, cholinergic and serotonergic pathways that have been observed in the CNS of subjects with the disorder. To begin to address this issue, we have determined whether there are changes in important components of the glutamatergic, cholinergic and serotonergic systems in the CNS of heterozygous transmembrane domain neuregulin 1 mutant (*Nrg1*$^{+/-}$) mice.

2. Materials and methods

2.1. Materials

[^{3}H]pirenzepine, [^{3}H]kainate, [^{3}H]MK-801, [^{3}H]ketanserin and [^{3}H]citalopram were obtained from New England Nuclear. [^{3}H]8OH-DPAT and [^{3}H]microscales were obtained from Amersham. Paroxetine was kindly donated by GlaxoSmithKline. All other chemicals were obtained from Sigma.

2.2. Tissue collection

Frozen CNS from 5-6 months old male mutant mice *Nrg1*$^{+/-}$mice on a C57BL/6j background (17th backcross generation- heterozygous breeding) and wild type-like (WT) littermates were obtained from the colony maintained at the biological testing facility of the Garvan Institute of Medical Research under enriched environmental conditions (Karl et al., 2007).

For each radioligand binding measured; five frozen sections (3 total binding; 2 non-specific binding [NSB]) were cut from CNS of 10 *Nrg1*$^{+/-}$and 10 WT mice, approximately 1.5 mm anterior to bregma.

The binding of [^{3}H]pirenzepine to muscarinic M1/M4 receptors (Dean et al., 1996a), [^{3}H]kainate to kainate receptors (Scarr et al., 2005), [^{3}H]MK-801 to NMDA receptors (Scarr et al., 2005), [^{3}H]ketanserin to serotonin (5HT) 2A receptors (Dean and Hayes, 1996), [^{3}H]8OH-DPAT to 5HT1AR (Dean et al., 1999b) and [^{3}H] citalopram to the 5HT transporter (SERT) (Dean et al., 1999b) in mouse CNS was measured as described previously. A more comprehensive methodology is supplied as supplementary material. Importantly, the levels of radioligand binding on the resulting autoradiographs, which were in the form of phosphoimages, could be measured by comparison to the intensity of the blocks of radioactivity on the [^{3}H]microscales that were opposed to the same phosphoimage. These comparisons were achieved using the AIS image analysis software, with results being expressed as dpm mg−1 estimated wet weight tissue equivalents (ETE) and then converted to fmol mg−1 ETE. In this way, radioligand binding was measured using a single point saturation analysis, which gives a good approximation of the density of radioligand binding sites in tissue sections.

Statistically significant variation in radioligand binding was identified by two-way ANOVA with *Nrg1* status (WT vs. *Nrg1*$^{+/-}$mice) and CNS regions as variables. For [^{3}H]ketanserin binding, each layer of cortical radioligand binding was treated as a discrete CNS region. Bonferroni posthoc tests were used to identify the specific differences in radioligand binding that contributed to any global variance in binding. Differences were regarded as statistically significant if $p<.05$.

3. Results

Analyses of [^{3}H]pirenzepine binding across the cortex and striatum showed no significant variation in levels of binding of this radioligand (Fig. 1A) and therefore an integrated measure was taken across the entire cortex and striatum. By contrast, whilst the binding of [^{3}H]kainate, [^{3}H]MK-801, [^{3}H]ketanserin, ^{3}H]citalopram and [^{3}H]8OH-DPAT (Fig. 1B-F) was homogeneous across the striatum, and was therefore taken as an integrated measure across that region, all these radioligands showed layered binding in the cortex. For [^{3}H]kainate and [^{3}H]8OH-DPAT, a comparison with cresyl violet stained sections showed that the distinct outer