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Chronic lurasidone treatment normalizes GABAergic marker alterations in the dorsal hippocampus of mice exposed to prenatal immune activation

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Abstract
Prenatal maternal infection represents a risk factor for the development of psychopathologic conditions later in life. Clinical evidence is also supported by animal models in which the vulnerability to develop a schizophrenic-like phenotype likely originates from inflammatory processes as early as in the womb. Prenatal immune challenge, for example, induces a variety of long-term behavioral alterations in mice, such as deficits in recognition and spatial working memory, perseverative behaviors and social impairments, which are relevant to different symptom clusters of schizophrenia. Here, we investigated the modulation of GABAergic markers in the dorsal and ventral hippocampus of adult mice exposed to late prenatal immune challenge with the viral mimetic Poly(I:C) (*polyriboinosinic-polyribocytidilic-acid*) at gestational day 17, and we evaluated the ability of chronic treatment with the multi-receptor antipsychotic lurasidone to modulate the alterations produced by maternal infection. Poly(I:C) mice show a significant reduction of key GABAergic markers, such as GAD67 and parvalbumin, specifically in the dorsal hippocampus, which were normalized by chronic lurasidone administration. Moreover, chronic drug administration increases the expression of the pool of brain derived neurotrophic factor (BDNF) transcripts with the long 3'-UTR as well as the levels of mature BDNF protein in the synaptosomal compartment, selectively in dorsal hippocampus.
All in all, our findings demonstrate that lurasidone is effective in ameliorating molecular abnormalities observed in Poly(I:C) mice, providing further support to the neuroplastic properties of this multi-receptor antipsychotic drug.

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1. Introduction

Schizophrenia is a psychiatric disorder with a neurodevelopmental origin, whose etiology relies on the interaction between genetic and environmental factors. Clinical and preclinical studies have demonstrated that exposure to infection or inflammation during gestation are important environmental risk factors for the susceptibility to neurodevelopmental disorders (Brown et al., 2001; Harvey and Boksa, 2012; Luoni et al., 2015c; Meyer, 2014). Indeed, it is believed that exaggerated levels of cytokines produced by the mother during gestation can interfere with brain development, thus increasing the risk of developing schizophrenia and related disorders later in life (Khandaker et al., 2013; Patterson, 2007). On this basis, several animal models have been established, which have the advantage of stringently controlling the type and timing during which the immune challenge is applied, while excluding confounding effects due to the genetic background. One of the models that has gained great recognition in the last decade is based on the use of polyriboinosinic:polyribocytidilic acid [Poly(I:C)], a synthetic analogue of a double-stranded RNA that produces a rapid inflammatory response, whose functional consequences in the adult offspring depend on the timing of injection to the pregnant dam (reviewed in Meyer et al., 2009). In particular, when the maternal immune response is induced on gestation day (GD) 17, adult offspring show profound behavioral alterations that recapitulate the negative and cognitive symptoms of schizophrenia, such as working memory deficits (Richetto et al., 2013, 2014), as well as alterations of hippocampal-associated behaviour (Giovanoli et al., 2015). Late maternal immune activation is also associated with a number of molecular alterations, largely reproducing changes that have been observed in schizophrenic patients. As an example, we previously observed impairments in the cortical GABAergic transcriptome, including GAD65/67, VGAT and selected alpha-subunits of the $GABA_A$ receptor (Richetto et al., 2013, 2014), which reproduce decreased GABAergic function, one of the most robust changes observed in schizophrenia (Hashimoto et al., 2008; Lewis, 2014; Lewis et al., 2011; Lisman et al., 2008).

Against this background, in the present study we aimed to investigate the impact of maternal immune activation late in gestation on the expression of GABAergic markers in the ventral (VH) and dorsal (DH) hippocampus. Of note, while the DH has a preferential role in mnemonic processes such as working memory and spatial learning, the VH has a preferential role in emotional behavior (Fanselow and Dong, 2010). Interestingly, it has been shown, with the help of many animal models, that altered functional interaction between the hippocampus and the prefrontal cortex contributes to cognitive impairments in schizophrenia (Godsil et al., 2013). As an example, rats exposed to maternal immune activation during the gestational period displayed decreased interactions between the hippocampus and the prefrontal cortex, and this deficit was reversed by the antipsychotic drug clozapine in a dose-dependent manner (Dickerson et al., 2012).

To this purpose we analyzed, in adult male offspring whose mothers were injected with Poly(I:C) at GD17, the modulation of key GABAergic markers, such as the GABA-synthetizing enzyme GAD67, the vesicular GABA transporter VGAT, and a marker of a subpopulation of GABAergic interneurons, namely parvalbumin, whose alterations may provide a molecular substrate for the cognitive impairments observed in this animal model (Canetta et al., 2016; Labouesse et al., 2015; Lewis et al., 2012; Lisman et al., 2008; Lodge et al., 2009; Tse et al., 2015). Furthermore, as an index of the specificity of the alterations for the excitatory versus the inhibitory synapses, we also investigated the modulation of neuroligin-1 and neuroligin-2, which are postsynaptic membrane proteins that are respectively localized in excitatory glutamatergic and inhibitory GABAergic synapses (Kohl et al., 2015; Sudhof, 2008). In addition, we tested the ability of a pharmacological intervention to prevent or normalize at adulthood some of the alterations produced by the gestational manipulation. More specifically, we investigated the multi-receptor antipsychotic drug lurasidone, which is a high affinity antagonist of 5-HT_7 receptors and a partial agonist at 5-HT_{1A} receptors, besides having high affinity for D_2 and 5-HT_{2A} receptors. Interestingly, lurasidone's activity at 5-HT_7 and 5-HT_{1A} receptors may contribute to the antidepressant and pro-cognitive properties of the drug (Ishibashi et al., 2010; Tarazi and Riva, 2013).

2. Experimental procedures

2.1. Animals

C57BL/6 mice were used throughout the study. Female and male breeders were obtained from the in-house specific pathogen free colony of the Physiology and Behavior Laboratory (ETH) at the age of 12–14 weeks. Breeding began after 2 weeks of acclimatization to the new animal holding room, which was at temperature and humidity controlled (21±11 °C, 55±5%) under a reversed light-dark cycle (lights off: 8:00–20:00 h). All animals had *ad libitum* access to water and food (Kliba 3430, Klibamühlen, Kaiseraugst, Switzerland). All procedures described had been previously approved by the Cantonal Veterinarian's Office of Zurich and are in agreement with the principles of laboratory animal in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 86-23, revised 1985).

2.2. Maternal immune activation during pregnancy

For the purpose of the maternal immunological manipulation during pregnancy, 20 female mice were subjected to a timed mating procedure in which groups of 2–3 females were moved to a partitioned cage with one male, allowing olfactory but not physical contact between male and female animals. On the third day of partitioning, the females were brought together with one male and allowed to mate. Successful mating was verified the next morning by the presence of a vaginal plug and that day was referred as gestational day (GD) 0. Pregnant dams on GD17 received either a single injection of vehicle (sterile pyrogen-free 0.9% NaCl, Ctrl group) (N=8) or of the viral mimic polyriboinosinic-polyribocytidilic acid [Poly(I:C)] (potassium salt; Sigma-Aldrich) (N=10) at a dose of 5 mg/kg, that was dissolved in the vehicle to yield a final concentration of 1.0 mg/ml on the day of injection. Injections were made via the intravenous route at the mother's tail vein under mild physical constraint and the volume of injection was 5 ml/kg.

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