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A two-hit model of suicide-trait-related behaviors in the context of a schizophrenia-like phenotype: Distinct effects of lithium chloride and clozapine

Jessica Deslauriers [a], Karine Belleville [a], Nicolas Beaudet [a], Philippe Sarret [a], Sylvain Grignon [a,b,*]

[a] Department of Physiology and Biophysics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, 12e avenue Nord, Sherbrooke, QC J1H 5N4, Canada
[b] Department of Psychiatry, Centre Hospitalier Universitaire de Sherbrooke, 580 Bowen Sud, Sherbrooke, QC J1G 2E8, Canada

HIGHLIGHTS

- The mechanisms involved in suicidal behaviors in schizophrenia are unknown.
- A double-hit model, using prenatal polyI:C and social isolation, was developed.
- Lithium chloride and clozapine had distinct effects in the double-hit model.

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ABSTRACT

Schizophrenia patients show a high rate of premature mortality due to suicide. The pathophysiological mechanisms of these suicidal behaviors in schizophrenia do not appear to involve serotonergic neurotransmission as found in the general population. Our aim was to develop an *in vivo* model of schizophrenia presenting suicide-trait-related behaviors such as aggressiveness, impulsivity, anxiety and helplessness. We opted for a two-hit model: C57BL/6 dams were injected with polyI:C on gestational day 12. The pups were submitted to social isolation for 4 weeks after weaning. During the last week of social isolation and 30 min before behavioral testing, the mice received vehicle, lithium chloride or clozapine. Lithium chloride is well known for its suicide preventive effects in the non-schizophrenic population, while clozapine is the antipsychotic with the best-established suicide preventive effect. The two-hit model induced several schizophrenia-related and suicide-trait-related behaviors in male, but not female, mice. Additionally, lithium chloride improved prepulse inhibition, aggressiveness, impulsivity and anxiety-like behavior in socially isolated mice only, whereas clozapine prevented behavioral abnormalities mainly in mice prenatally exposed to polyI:C and submitted to isolated rearing. The distinct effects of lithium chloride and clozapine suggested that mice prenatally exposed to polyI:C and submitted to social isolation presented a distinct phenotype from that of mice submitted to social isolation only. Because diagnosing suicidal risk in patients is a challenge for psychiatrists given the lack of specific clinical predictors, our *in vivo* model could help in gaining a better understanding of the mechanisms underlying suicidal behavior in the context of schizophrenia.

1. Introduction

Suicide is the leading cause of premature death among schizophrenic patients. Compared to the lifetime suicide risk of 1% in the general population [1–3], estimates of attempted suicide among patients with schizophrenia range from 20 to 40%, with a stronger risk for completed suicide in male patients [4]. In the general population, disturbances of the serotonergic system, mainly lower 5-hydroxyindoleacetic acid (5-HIAA) levels in the cerebrospinal fluid (CSF) and low serotonin levels in the postmortem brains of suicide victims, have been associated with suicidal behaviors [5–9]. Conversely, other studies have failed to demonstrate a significant difference in CSF 5-HIAA levels between schizophrenic patients who attempted suicide and non-attempters [2,10]. These findings and others [11,12], suggest that the pathophysiology of suicidal behaviors in schizophrenia does not involve serotonergic neurotransmission. The current evidence suggests that clozapine has prominent suicide prevention effects compared to other antipsychotic drugs, which is not fully explained by its superior

* Corresponding author at: CHUS Hôtel-Dieu, Department of Psychiatry, 580 rue Bowen Sud, Sherbrooke, QC J1G 2E8, Canada.
E-mail address: Sylvain.Grignon@USherbrooke.ca (S. Grignon).

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antipsychotic efficacy [4,13–15]. However, the mechanisms underlying the suicide-preventing effect observed with clozapine are poorly understood. Therefore, an animal model of suicide-related behaviors in the context of schizophrenia must be developed to help increase understanding of the mechanisms implicated in this phenomenon.

Because there are no "required characteristics" to obtain a diagnosis of suicidal ideation in schizophrenia patients, research groups have commonly focused on risk factors in humans and *in vivo* to understand the mechanisms implicated in suicidal risk and to reduce suicide attempts among schizophrenia patients [16]. As a general strategy to study suicidal behaviors and to develop preventive treatments, many research groups have suggested the use of personality traits as endophenotypes [6,8,17,18]. Personality traits that have been associated with suicidal behaviors include impulsivity, aggressiveness, introversion and anxiety (neuroticism) [18]. Unlike suicidal behavior, which cannot be directly assessed in animals, some of these complex behaviors can be modeled in rodents [19]. Furthermore, post-weaning social isolation (SI) has been demonstrated to induce aggressive behaviors, helplessness and anxiety-like behaviors in rodents [20].

The emerging double-hit theory of schizophrenia proposes that genetic vulnerability or an early environmental factor, a "first hit", acts early to disrupt central nervous system (CNS) development, producing long-term vulnerability to a "second hit" [21]. In agreement with this hypothesis, we and others have reported the synergistic effects of two environmental factors [22,23] or as a result of gene × environment interactions [24,25]. Epidemiological studies have shown that viral infection during pregnancy and social isolation are risk factors for schizophrenia [26–28]. Prenatal administration of polyinosinic:polycytidylic acid (polyI:C), a Toll-like receptor 3 (TLR3) agonist mimicking a viral infection [29,30], and post-weaning social isolation (SI) [31] have shown to induce schizophrenia-relevant behavioral abnormalities, such as PPI deficits.

Thus, to gain a better understanding of suicidal risk in the schizophrenic population, our aim was to develop an *in vivo* animal model of schizophrenia mimicking some suicide-related behaviors (aggressiveness, impulsivity, anxiety and helplessness). We opted for a two-hit model involving prenatal immune challenge with polyI:C, a well-known neurodevelopmental animal model of schizophrenia [29,30], followed by post-weaning SI, which has been reported to induce some of these suicide-related behaviors [20], in the offspring. The effects of lithium chloride (LiCl), a GSK-3β inhibitor [32], and clozapine, mainly an agonist of the 5-HT1A receptor and a 5-HT2A receptor antagonist [33], were tested because they are recognized, respectively, for their suicide preventive effects in the general and schizophrenic populations [34,35].

2. Material and methods

2.1. Animals

Male and female C57BL/6 mice were obtained from Charles River Laboratories (QC, Canada) at an age of eight to ten weeks. The animals were kept at 20 °C, on a 10-h light/14-h dark cycle (lights on at 8:00 AM and off at 6:00 PM). They had *ad libitum* access to food and water. The experimental protocol was approved by the institutional Animal Research Ethics Review Board of the Université de Sherbrooke, in compliance with the policies of the Canadian Council on Animal Care. Mating was performed on site. Timed pregnant mice were injected intraperitoneally (IP) (0.1 mL) with saline or 20 mg/kg polyI:C (Sigma-Aldrich, ON, Canada) on gestational day 12 (G12) [22,36]. We chose to perform prenatal immune activation on gestational day 12 because, during the fetal period, the development of a dopaminergic phenotype begins at gestational days 11 to 15 in the mouse, with the expression of dopaminergic markers, such as tyrosine hydroxylase (TH) and dopamine transporter (DAT) [37–39]. Accordingly, we previously showed increased levels of dopamine D2 receptor in offspring for polyI:C-exposed dams [22]. On postnatal day 21 (PN21), the pups were weaned and submitted to group housing (4 per cage) and social isolation (SI). Behavioral testing began 4 weeks after weaning at PN50, as previously described [20]. During the SI procedure, cage changing occurred once per week to minimize handling.

2.2. Drug administration and groups

Vehicle (DMSO:saline 1:6), LiCl (200 mg/kg) (Sigma-Aldrich) or clozapine (3 mg/kg) (Sigma-Aldrich) was administered daily by IP injections during the last week of group housing or SI (7 days). The doses were chosen based on previous studies reporting clozapine-induced PPI enhancement [40] and antidepressant effects of lithium chloride [41] in C57BL/6 mice. On testing days, the drugs were injected 30 min before behavioral testing. LiCl and clozapine were dissolved in a DMSO:saline mix (1:6). The pups from 24 saline-treated dams (mean: 7.7 per litter; $n = 185$) and 34 polyI:C-treated dams (mean: 5.4 per litter; $n = 185$) (see Supplemental Fig. 1) were randomized into twelve groups: (1) saline with vehicle (control mice) ($n = 30$); (2) saline with clozapine ($n = 31$); (3) saline with LiCl ($n = 30$); (4) saline + SI with vehicle ($n = 32$); (5) saline + SI with clozapine ($n = 32$); (6) saline + SI with LiCl ($n = 30$); (7) polyI:C with vehicle ($n = 31$); (8) polyI:C with clozapine ($n = 30$); (9) polyI:C with LiCl ($n = 30$); (10) polyI:C + SI with vehicle ($n = 32$); (11) polyI:C + SI with clozapine ($n = 30$); and (12) polyI:C + SI with LiCl ($n = 32$). Additionally, we used a minimum number of 20 and 28 litters for the saline and polyI:C groups, respectively, and pups from each litter were assigned equally to each group for group housing and SI. The protocol was divided into two sets of experiments to minimize the effects of experimental stress: each animal was used once for either the first set (prepulse inhibition of acoustic startle reflex/exploratory behavior/resident–intruder test) or the second set (elevated plus maze/forced swim test), reaching $n = 14$–15 per group ($n = 7$–8 per sex in each group) (Fig. 1). The order of testing and the one-day break were established according to previous studies, and we conducted the least disruptive tests first [20,42,43]. Behavioral observations took place between 10:00 AM and 5:00 PM (light cycle).

2.3. Prepulse inhibition of acoustic startle reflex

Deficits in sensorimotor gating, assessed by the measurement of PPI, constitute a well-known schizophrenia phenotype [44]. Prepulse inhibition (PPI) of acoustic startle was evaluated using the SR-LAB apparatus (San Diego Instruments, CA, USA), as previously described [22,45]. Testing began with a 5 min acclimatization period with 71 dB of background noise, followed by 4 blocks. The first block consisted of 6 pulse trials (120 dB; 40 ms). The second block included 5 pulse trials (120 dB; 40 ms), five null trials (no stimulation) and five prepulse + pulse trials at four different intensities (75, 79, 83 or 87 dB; 20 ms), presented in randomized order. The interstimulus trial was set at 100 ms. Then, a 60 s break preceded the third block, which consisted again of 5 pulse trials (120 dB; 40 ms), five null trials and five prepulse + pulse trials at the four different intensities presented in randomized order. Finally, the last block included 6 pulse trials (120 dB; 40 ms). The inter-trial interval averaged 15 s (10–20 s). PPI was evaluated as PPI %, calculated as (1 − [startle amplitude on prepulse + pulse trial / mean startle amplitude on pulse alone trials]) × 100.

Startle responses did not differ between the prepulse (75, 79, 83 and 87 dB) and "no stimulation" conditions (Supplemental Fig. 2). No component of the two-hit model had an effect on the startle response. However, in males, treatment with clozapine or LiCl tended to affect the startle response (Supplemental Fig. 3A). The startle response was added as a covariate to the analysis of PPI% and, as a covariate, the startle response did not attain statistical significance either in males or females, confirming that the variations in startle response did not confound the interpretation of the PPI%.