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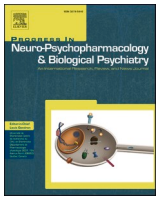

Effects of the PAM of mGluR2, JNJ-46356479, on brain apoptotic protein levels in a mouse model of schizophrenia

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ABSTRACT

Current treatment for schizophrenia (SZ) ameliorates the positive symptoms, but is inefficient in treating the negative and cognitive symptoms. The SZ glutamatergic dysfunction hypothesis has opened new avenues in the development of novel drugs targeting the glutamate storm, an inducer of progressive neuropathological changes. Positive allosteric modulators of metabotropic glutamate receptor 2 (mGluR2), such as JNJ-46356479 (JNJ), reduce the presynaptic release of glutamate, which has previously been demonstrated to attenuate glutamate- and dopamine-induced apoptosis in human neuroblastoma cell cultures. We hypothesised that JNJ treatment would modify the brain levels of apoptotic proteins in a mouse model of ketamine (KET)-induced schizophrenia. We analysed the levels of proapoptotic (caspase-3 and Bax) and antiapoptotic (Bcl-2) proteins by western blot in the prefrontal cortex and hippocampus of JNJ-treated mice. JNJ attenuated apoptosis in the brain by partially restoring the levels of the antiapoptotic Bcl-2 protein, which is significantly reduced in animals exposed to KET. Additionally, a significant inverse correlation was observed between proapoptotic protein levels and behavioural deficits in the mice. Our findings suggest that JNJ may attenuate brain apoptosis in vivo, as previously described in cell cultures, providing a link between neuropathological deficits and SZ symptomatology.

1. Introduction

Schizophrenia (SZ) is a heterogenous psychiatric disorder characterised by its chronicity, severity, and complexity. SZ symptomatology consists of a pool of symptoms that can be divided into positive symptoms (delusions, hallucinations, and disorganised speech), negative symptoms (diminished emotional expression or avolition), and cognitive deficits (impairments in working memory, problem solving or social cognition) (Keefe, 2008; Tandon et al., 2013). The aetiopathogenesis of the disease is poorly understood and different theories have been proposed, such as alterations in neurotransmitter systems including dopaminergic (Howes and Kapur, 2009), serotoninergic (Remington, 2008) or glutamatergic ones (Uno and Coyle, 2019). The glutamate (GLU) hypothesis arises from the fact that *N*-methyl-D-aspartate (NMDA) GLU receptor antagonists, such as ketamine (KET) and phencyclidine (PCP), can induce SZ-like symptomatology or even exacerbate it in patients with the disease (Dienel et al., 2022). According to this theory, during the early stages of SZ, NMDA receptor alterations leading to receptor hypofunction in the gamma-aminobutyric acid (GABAergic) inhibitory interneurons expressing parvalbumin (PV+) reduce GABA

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levels, resulting in increased GLU release due to the disinhibition of the glutamatergic neurons, thereby generating a GLU storm (Uno and Coyle, 2019). Excessive levels of GLU promote excitotoxic damage in the brain, leading to cognitive impairments, impulsive behaviour, anxiety, or psychotic symptoms (Yuede et al., 2010). Neurotoxicity can be triggered through different cellular pathways, such as increased apoptosis that can lead to increased synaptic pruning of the dendritic spines in critical brain areas (Parellada and Gassó, 2021; Yuede et al., 2010). Accordingly, a dysregulation of the levels of apoptotic proteins has been observed in SZ patients. Post-mortem brain tissue from SZ patients has been reported to show a significantly higher Bax/Bcl-2 ratio and increased Bax, caspase-3, and caspase-9 levels compared to controls, indicating an increased proapoptotic brain state in those suffering from SZ (Dirican et al., 2023; Fredrik Jarskog et al., 2004; Szymona et al., 2019). Furthermore, reduced levels of the antiapoptotic protein Bcl-2 have been observed in the post-mortem brain tissue of SZ patients (Jarskog et al., 2000), while lower serum Bcl-2 levels have been reported to correlate with exacerbated positive and negative symptoms (Tsai et al., 2013). Additionally, a correlation between brain GLU metabolite levels and altered peripheral apoptosis markers has been seen in antipsychotic-naïve SZ patients (Batalla et al., 2015).

SZ treatment is based on the use of antipsychotic drugs that antagonise dopamine and serotonin receptors, improving the positive symptoms. However, current antipsychotics have a high percentage of non-responders (from a fifth to half of patients) and are not efficient for the treatment of negative and cognitive symptoms (Marder and Cannon, 2019; Miyamoto et al., 2005; Nucifora et al., 2019). Consequently, new therapies involving different targets have been proposed. Focusing on the glutamatergic hypothesis, drugs targeting the distinct metabotropic GLU receptor groups (group I, II, and III) have been developed. However, most of the efforts have focused on group II, which consists of the metabotropic GLU receptor types 2/3 (mGluR2/3) that can presynaptically reduce GLU release. There have been advances in developing highly selective agonists for these receptors, such as LY379268 (Imre, 2007) and LY2140023 (Kinon et al., 2011), as well as positive allosteric modulators (PAMs), such as LY487379 (Lundström et al., 2016) and JNJ-46356479 (JNJ) (Cid et al., 2016). JNJ treatment during adulthood has shown efficiency in ameliorating the cognitive and negative deficits present in a postnatal KET mouse model of SZ (Martínez-Pinteño et al., 2020). Additionally, early JNJ treatment during adolescence has been also shown to improve cognitive and negative symptoms in the same animal model (Martínez-Pinteño et al., 2023). At the brain molecular level, JNJ treatment partially normalized alterations in PV+ neurons and c-Fos expression observed in the prefrontal cortex (PFC) and hippocampus (HPC) of these animals postnatally exposed to KET (Martínez-Pinteño et al., 2020; Martínez-Pinteño et al., 2023). PFC and HPC are considered central to the brain circuitry accounting for memory and executive abilities in mammals due to their pivotal roles in behaviour and cognitive functions (Sigurdsson and Duvarci, 2016). PFC is responsible of the adaptative flexibility and memory processing, while HPC controls episodic memory (Song et al., 2022). Deficiencies in both tissues have been observed in SZ patients, such as reduced grey matter volume in the PFC or decreased myelinization and organization of white matter tracts in HPC (Karlsgodt et al., 2010). At the cellular level, JNJ attenuates the apoptosis, particularly caspase-3 activation, induced by dopamine and GLU in human neuroblastoma cell cultures (Gassó et al., 2023).

We hypothesised that targeting the GLU storm could be especially effective in ameliorating GLU-induced cytotoxicity in the brain and the highly apoptotic brain state that leads to patient deterioration, thus having a direct effect on SZ symptomatology. In this study, we aimed to evaluate whether JNJ treatment modified the protein levels of proapoptotic (caspase-3 and Bax) and antiapoptotic (Bcl-2) proteins in a mouse model of SZ postnatally exposed to KET. We also aimed to identify a correlation between these apoptotic protein levels and the behavioural deficits previously reported in these animals (Martínez-Pinteño et al., 2020).

2. Material and methods

2.1. Animals

C57BL/6 J mice (Charles River Laboratories, Wilmington, MA, USA) were used. The study sample consisted of 23 animals (11 males and 12 females). All experimental procedures involving animals were performed in accordance with European Union guidelines on the care and use of laboratory animals and were approved by the University of Barcelona Animal Care Committee (license number 525/16) and by the Department of the Environment of the Generalitat de Catalunya (order number 9208). Mice were housed in groups, with ad libitum access to food and water, and were maintained under a controlled temperature (22 °C) and an alternating 12-h light-dark cycle. Animal housing and in vivo experimental procedures were performed in the laboratory animal centre of the Faculty of Medicine at the University of Barcelona.

2.2. Drug treatment

A summary of the study design is shown in Fig. 1.

On postnatal days (PND) 7, 9, and 11, half of the animals were treated with saline, as vehicle and control group, while the other half were treated with KET (30 mg/kg). At adulthood (PND 80), the animals were subcutaneously treated daily with JNJ-46356479 (10 mg/kg) or vehicle (10% hydroxypropyl-β-cyclodextrin, HPβCD). All drugs were administered to the animals at 10 ml/kg. After 14 days (PND 94) of drug treatment, the behavioural assessment was initiated. Treatment was maintained during the behavioural assay (6 weeks). Animals were euthanised by cervical dislocation 1 h after the last drug administration and tissue from their PFC and HPC was obtained and maintained at −80 °C.

Animals were divided into four groups: the control group (Veh + Veh: $N = 6$, 3 males and 3 females), the JNJ group (Veh + JNJ: $N = 5$, 2 males and 3 females), the KET group (KET+Veh: N = 6, 3 males and 3 females), and the KET+JNJ group (KET+JNJ: N = 6, 3 males and 3 females).

2.3. Molecular tissue analyses

2.3.1. Tissue lysates

We used 10 μl/mg of 1× cell lysis buffer (Cell Signaling Technology, Danvers, MA, USA) containing 1 mM phenylmethylsulphonyl fluoride (PMSF) (Thermo Fisher Scientific, Rockford, IL, USA) for total protein isolation from the PFC and HPC tissues. Lysate protein concentrations were determined using a commercial Lowry assay kit (Bio-Rad, Hercules, CA, USA) and analysed using a Tecan Spark® 20 M microplate fluorescence reader (Tecan, Männedorf, Switzerland).

2.3.2. Western blot analysis

A total of 20 μg of total protein was separated on 4–12% NuPAGE Bis-Tris gels (Invitrogen, Carlsbad, CA, USA) using a commercial MOPS buffer system (Invitrogen, Carlsbad, CA, USA) during an electrophoretic run. The gels were transferred to polyvinylidene difluoride (PVDF) membranes (Invitrogen, Carlsbad, CA, USA) using the iBlot 2 Dry Blotting System (Invitrogen, Carlsbad, CA, USA). Non-specific binding sites were saturated with non-fat dry milk for 1 h at room temperature. The membranes were incubated with the primary antibodies overnight at 4 °C before being incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature. The membranes were visualised in the ChemiDoc imaging system (Bio-Rad, Hercules, CA, USA) using the Clarity Western enhanced chemiluminescence (ECL) detection kit (Bio-Rad, Hercules, CA, USA). Protein levels were quantified by measuring the intensities of the individual protein bands from the scanned film by densitometric analysis using ImageJ version 1.53 t

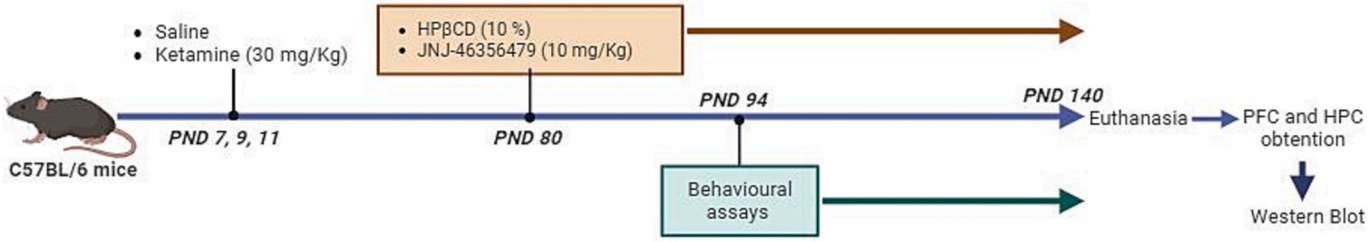

Fig. 1. *Study Design*: On PND 7, 9, and 11, C57BL/6 mice were treated with saline or ketamine. On PND 80, mice were subcutaneously treated daily with vehicle (10% hydroxypropyl-β-cyclodextrin, HPβCD) or JNJ-46356479. Behavioural assays were performed after 2 weeks of treatment and the treatment was maintained during this time period (6 weeks). Euthanasia was performed and tissue from the prefrontal cortex (PFC) and hippocampus (HPC) was obtained. Tissues were analysed by western blot. PND, postnatal day.

(NIH, Bethesda, MD, USA). Protein expression levels were normalized by using the housekeeping β-actin as a loading control. The results are expressed as arbitrary units of densitometry.

2.3.3. Antibodies

The primary antibodies used were: rabbit anti-caspase-3 (1:1000, Cell Signaling Technology, #9662S, Danvers, Massachusetts, USA), rabbit anti-Bcl-2 (1:500, Cell Signaling Technology, #3498S, Danvers, Massachusetts, USA), mouse anti-Bax (1:1000, Cell Signaling Technology, #2772S, Danvers, Massachusetts, USA), and mouse anti-β-actin (1:5000, Sigma Aldrich, #A5441, St Louis, MO, USA). All primary antibodies were prepared in 5% bovine serum albumin (BSA) in Tris-buffered saline (TBS)-Tween (0.1%). The HRP-conjugated secondary antibodies used were a goat anti-mouse (1:10000) or a goat anti-rabbit (1:10000) antibody (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Animal behavioural assay

The mice had previously undergone a battery of behavioural tests including a: motor test battery, rotarod test, open field test, Y-maze, Three-Chamber Sociability and Social Novelty test, Five-Trial Social Memory test, and fear conditioning. Detailed information about the behavioural assay methodology and the behavioural deficits has been previously reported (Martínez-Pinteño et al., 2020). In summary, these animals presented impaired spontaneous alternation in the Y-maze test, suggesting deficits in spatial working memory, and a decrease in social motivation and memory, assessed in both the Three-Chamber and the Five Trial Social Memory tests. Following the JNJ treatment of adult mice these deficits observed in the animals exposed to KET were partially reversed in the three tests (Martínez-Pinteño et al., 2020). In this study, we particularly focused on the correlations between the results obtained in the behavioural tests where there were significant differences between the experimental groups (specifically the Y-maze test, the Three-Chamber Sociability and Social Novelty test, and the Five-Trial Social Memory test) and the altered brain apoptotic protein levels.

2.5. Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics software (version 27.0, IBM Corp, Chicago, IL, USA). The significance level was set at $p < 0.05$ for all tests. The data points that differed by 1.5 times the interquartile range (IQR) were classified as outliers and excluded from the analysis. Based on the Shapiro-Wilk test, the data in the groups followed a normal distribution. The possible confounding variables, including sex and the time of the experiment, were included as covariates in a linear regression model. No significant effect was detected for sex in any of the analysis, nor neither when its effect in the levels of the apoptotic proteins was analysed individually (Supplementary Fig. 1). A significant effect of the experimental time was detected for the Bax/Bcl-2 ratio in the HPC; therefore, differences between the groups were studied using two-way ANOVA. For the other analyses, differences between the groups were studied by one-way ANOVA. For pairwise comparisons, the Bonferroni post-hoc test was performed.

Correlations between the apoptotic protein levels in the PFC and HPC and the behavioural deficits were analysed using partial correlation, with the treatment group as a covariate.

3. Results

3.1. Effect of pharmacological JNJ treatment on apoptotic protein levels in the PFC and HPC

Fig. 2A and B show representative densitometry blots of caspase-3, Bax, Bcl-2, and β-actin in the PFC (A) and HPC (B) from the different study groups.

Caspase-3 protein levels showed significant differences between the treatment groups in the PFC ($F_{(3,19)} = 5.614$; $p = 0.007$). Specifically, caspase-3 levels were significantly increased in the KET+Veh group when compared with the Veh + JNJ group (140.67 ± 43.29 vs 42.54 ± 9.85, $p = 0.021$). No significant differences in caspase-3 levels were seen in the HPC (Fig. 2C).

Bcl-2 protein levels also showed differences between the treatment groups in the PFC ($F_{(3,19)} = 5.775$; $p = 0.007$), being significant after the Bonferroni post-hoc comparisons between the KET+Veh group and the KET+JNJ group (67.82 ± 13.08 vs 119 ± 23.23, $p = 0.018$). While the Bcl-2 levels were reduced when the mice had been exposed to KET, the protein levels were partially restored when these animals were treated with JNJ. Similar Bcl-2 protein levels were detected in the HPC (Fig. 2D).

No significant differences were seen in the Bax protein levels in any of the brain tissues (Fig. 2E). However, the Bax/Bcl-2 ratio showed significant differences between the treatment groups in the PFC ($F_{(3,18)} = 5.631$, $p = 0.007$) and also in the HPC ($F_{(3,18)} = 6.714$, $p = 0.009$). Both tissues showed higher ratios when the mice had been exposed to KET, with significant differences observed between the KET+Veh group and both the Veh + JNJ group in the PFC (170.79 ± 63 vs 64.97 ± 34.23, $p = 0.032$) and the Veh + Veh group in the HPC (199.09 ± 99.38 vs 100 ± 19.94, $p = 0.048$). The ratio was partially restored to control values when the animals exposed to KET were treated with JNJ (Fig. 2F).

3.2. Correlation between apoptotic protein levels and behavioural deficits

A negative correlation was found between the PFC caspase-3 protein levels and the sociability index assessed with the Three-Chamber Sociability and Social Novelty test ($R^2 = -0.702$, $p = 0.005$) (Fig. 3). The sociability index was calculated using the formula: $\frac{\text{Time spent in the chamber with the novel mouse}}{\text{Total time spent in both side chambers}} \times 100$. Mice with lower caspase-3 levels showed a higher preference for spending time sniffing an unfamiliar conspecific mouse than a familiar littermate.

The other correlations explored did not show significant differences.

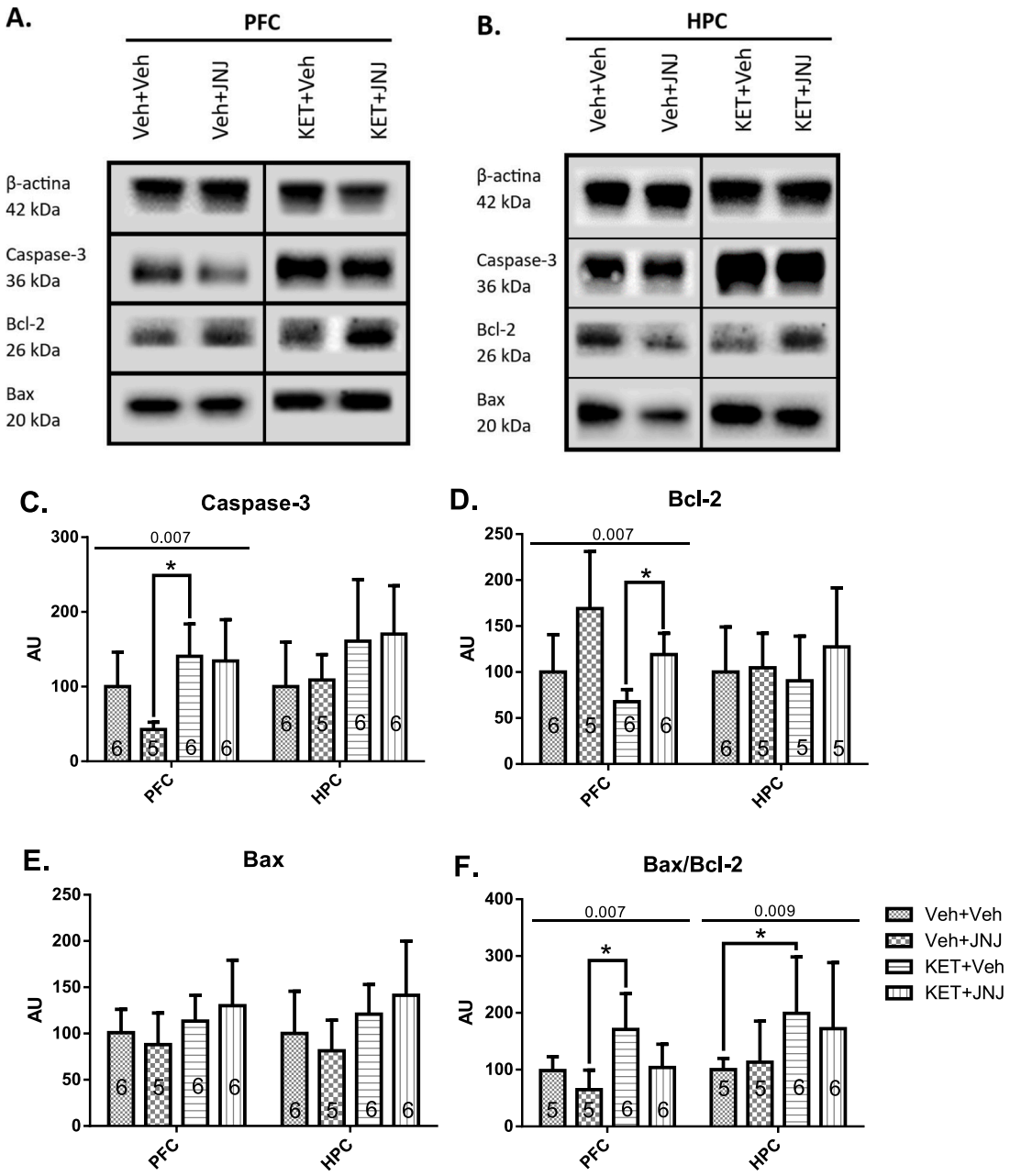

Fig. 2. Effects of JNJ treatment on the levels of apoptotic proteins in the prefrontal cortex (PFC) and hippocampus (HPC) of a postnatal ketamine mouse model: (A, B) Representative densitometry blots of caspase-3, Bax, and Bcl-2 from the different study groups in the PFC (A) and HPC (B). (C, D, E, F) Protein levels of caspase-3 (C), Bcl-2 (D), Bax (E), and the ratio between Bax and Bcl-2 (Bax/Bcl-2) (F) estimated from the quantification of the densitometric blots in the PFC and HPC tissues. * $p < 0.05$. AU, arbitrary units; Veh, vehicle; JNJ, JNJ-46356479; Ket, ketamine.

4. Discussion

The present study aimed to evaluate the capacity of JNJ-46356479, a PAM of mGluR2, to restore altered levels of brain apoptosis-related proteins in a postnatal KET mouse model (Jeevakumar et al., 2015) and to explore the relationship between apoptotic protein levels and SZ-related behavioural deficits in sociability and working memory (Martínez-Pinteño et al., 2020). The results showed that treatment with JNJ modified the levels of apoptotic proteins in the PFC and HPC of mice exposed to KET. Moreover, a correlation between increased levels of caspase-3 and a reduced preference for social novelty was observed in these animals.

Apoptosis may have a critical role in the manifestation of SZ. Neural apoptosis naturally occurs during neurodevelopment (Mazarakis et al., 1997; Sarić et al., 2022), but several studies have demonstrated a relationship between alterations in this process and SZ (Fatemi and Folsom, 2009). Our research group has previously observed increased apoptotic susceptibility in primary fibroblast cell cultures from antipsychotic-naïve patients with first-episode psychosis (Gassó et al., 2014), which show alterations in the expression of genes involved in the apoptotic pathways (Gassó et al., 2017). These alterations could be related to the accelerated dendritic pruning observed in the stages around the onset of SZ (Parellada and Gassó, 2021). Interestingly, the cortical volume loss present in SZ occurs without neuronal loss in the PFC and HPC (Bennett, 2011). Accordingly, local activation of apoptosis has not been observed in neuronal somas or axons (Williams et al., 2006; Williams and Truman, 2005). Increased dendritic pruning could be related to the presence of negative symptoms and cognitive deficits during the prodromal and

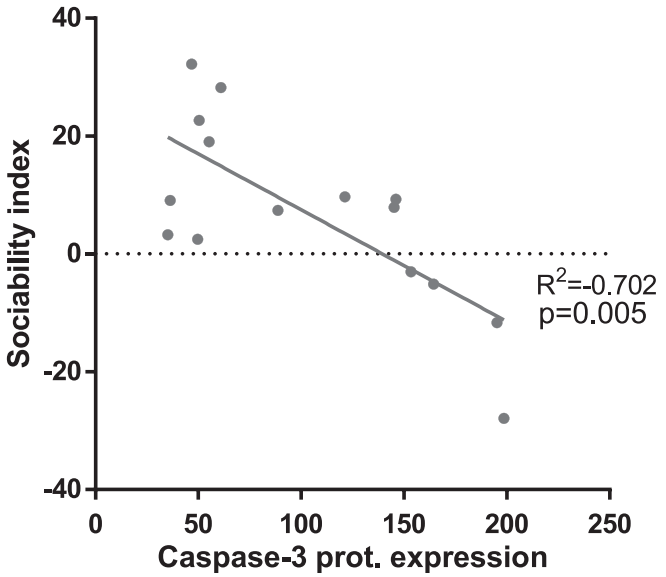

Fig. 3. Correlation between the protein levels of caspase-3 and the sociability index obtained in the Three-Chamber Sociability and Social Novelty test.

early stages of the disease (Moyer et al., 2015; Schmitt et al., 2007). One of the elements thought to trigger apoptotic pathways is the high GLU levels caused by NMDA receptor hypofunction in the inhibitory GABAergic interneurons, leading to a disinhibition of the glutamatergic neurons and the development of the GLU storm (Yuede et al., 2010). Our research group has also found (using spectroscopy (^{1}H-MRS)) a correlation between brain glutamate plus glutamine neurometabolites and altered levels of apoptosis markers in antipsychotic-naïve patients with first-episode psychosis (Batalla et al., 2015).

In the present study, a postnatal KET murine model showed an imbalance between pro- and anti-apoptotic protein levels in the brain, consisting of increased levels of caspase-3 in the PFC, reduced levels of Bcl-2 in the PFC, and an increased Bax/Bcl-2 ratio in the PFC and HPC, suggesting an increased proapoptotic brain state. Blocking the NMDA receptor with KET or similar antagonists, such as PCP or MK-801, has been involved in apoptotic-imbalance. The administration of PCP for 10 days in adult mice previously demonstrated to increase caspase-3 and Bax protein levels and reduce the Bcl-2 levels (Li et al., 2018), while KET administration in adult mice promoted cell death in the HPC by increasing caspase-3 activation (Majewski-Tiedeken et al., 2008). In the same way, the KET administration in SV-HUC-1 human cell cultures promoted accelerated apoptosis and enhanced oxidative stress (Shan et al., 2019). Additionally, early interventions with NMDA receptor antagonists have also demonstrated to promote apoptotic states. Mice and rat treatment with MK-801 or PCP on the PND 7 increased caspase-3 levels in the pups (Inta et al., 2016; Wang and Johnson, 2007). Interestingly, our results obtained in a mouse model exposed to KET during the PND 7, 9 and 11 also showed deficiencies in apoptosis induced during critical brain periods are maintained in the adult animal. Moreover, post-mortem tissue from SZ patients also shows increased levels of caspase-3 and Bax and reduced levels of Bcl-2 (Dirican et al., 2023; Fredrik Jarskog et al., 2004). These results are consistent with the neurodevelopmental hypothesis of SZ, in which the blocking of NMDA receptors promotes apoptotic environments.

The blocking of GLU release by PAMs of mGluR2, such as JNJ, could reduce the apoptotic brain state triggered by the GLU storm in the early stages of SZ. Accordingly, we observed in this study that Bcl-2 levels were partially reduced after JNJ treatment, with the modulation of the Bcl-2 protein levels restoring the Bax/Bcl-2 ratio to the levels of the control group without modifying the Bax protein levels. This suggested that JNJ activated the antiapoptotic Bcl-2 pathway. These results reinforce the putative neuroprotective effect of mGluR2/3 PAMs, specifically JNJ. Accordingly, our research group recently demonstrated that at the cellular level, JNJ attenuates the caspase-3 activation induced by dopamine and GLU in human neuroblastoma cell cultures (Gassó et al., 2023). These results could be associated with the capacity of JNJ to reduce environmental stress, since early treatment with JNJ has been previously shown to reduce brain nitrosative stress in the same mouse model used in this study (Treder et al., 2023). Additionally, JNJ alone did not promote an increased proapoptotic state. Other antipsychotics have been previously shown to decrease cell viability and promote apoptotic cell death, which has been linked to some adverse effects. Some examples of antipsychotics with proapoptotic activity are the first-generation antipsychotic haloperidol (Gassó et al., 2012; Jarskog et al., 2007) and the atypical antipsychotic quetiapine (Jarskog et al., 2007). Although a controversial topic in the literature, clozapine has been shown to promote caspase-3 activity in some studies (Gassó et al., 2023; Jarskog et al., 2007), while other studies have reported that the drug is involved in antiapoptotic activities (Lundberg et al., 2020; Morén et al., 2022). The antiapoptotic or non-proapoptotic capacity of some second-generation antipsychotics has previously been associated with their neuroprotective roles (Morén et al., 2022). Accordingly, our results reinforce the safety of mGluR2 PAMs previously demonstrated in neuroblastoma cell cultures, supporting the idea that JNJ could be associated with neuroprotective effects (Gassó et al., 2023). It is important to emphasise that it is the first time that effects of the treatment with a PAM of the mGluR2/3 modulator in apoptosis has been tested in vivo. However, previous research in mGluR2/3 modulation by agonists also demonstrates that the activation of these receptors is associated with reduced apoptosis (Bratek-Gerej et al., 2021; Bratek-Gerej et al., 2022). Additionally, the cell signaling axis Glu - mGluR2 - Extracellular-Signal-Regulated Kinase (ERK) has been seen involved in the apoptosis regulation (Liu et al., 2019). Consequently, we expect the regulation of mGluR2 through PAMs in other animal models promotes similar effects in apoptosis as those observed in these studies.

Interestingly, our research group has previously demonstrated that JNJ can ameliorate the cognitive and negative symptoms present in the same postnatal KET mouse model (Martínez-Pinteño et al., 2020). As previously mentioned, apoptosis could be an essential element mediating the progressive neuropathological changes occurring around the onset and early phases of SZ. Consequently, apoptotic protein levels could have a direct effect on SZ symptomatology, especially on primary negative symptoms and cognitive impairment. Serum Bcl-2 levels in SZ patients have been observed to negatively correlate with the scores obtained in the Positive and Negative Syndrome Scale (PANSS), with the symptomatology worsening with decreasing Bcl-2 levels (Tsai et al., 2013). Similarly, Bax levels in the peripheral blood of SZ patients have been reported to positively correlate with the PANSS score (Szymona et al., 2019). Furthermore, previous studies have observed that patients with SZ present higher serum levels of apoptotic markers, while patients suffering from a deficit syndrome of SZ, characterised by enhanced negative symptoms, present even higher serum levels of apoptotic markers than the patients without the syndrome (Beyazyüz et al., 2016). In our study, increased brain caspase-3 protein levels correlated with a reduced preference for social novelty in mice. All these results support the idea that a greater apoptotic state could be associated with worse patient symptomatology. Although caspase-3 and caspase-9 expression has been previously seen to be increased in the blood of SZ patients, no significant correlation has been observed between the clinical parameters and protein expression (Dirican et al., 2023). However, in animal models, caspase-3 overexpression has been previously demonstrated to promote deficits in the working memory of mice, as assessed with the odour span task (Young et al., 2007).

We must mention the main limitations of the present experimental design that included a reduced sample size and the use of a semi-quantitative approach to study the apoptotic protein levels, which may have hindered the detection of small differences between the study

groups.

5. Conclusion

In conclusion, this study demonstrates that JNJ may affect brain apoptotic protein levels in vivo and suggests that there is a link between neuropathological deficits and SZ symptomatology. Current non-dopaminergic antipsychotics still show promising results in alleviating SZ symptoms. Several studies support mGluR2/3 PAMs as safe drugs with high tolerability that could be used to develop new pharmacological approaches. In addition, these drugs could be useful during critical neurodevelopmental periods, ameliorating or even preventing SZ progression. In this sense, the effects that these drugs could have against cell toxicity and apoptotic pathways might be particularly relevant. Our findings provide a new contribution to the understanding of the involvement of apoptotic-related processes in SZ pathophysiology.

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Author statement

All authors have seen and approved the final version of the manuscript being submitted. Authors declare that the article is an original work that hasn't received prior publication and isn't under consideration for publication elsewhere.

Ethical statement

Authors have read and agree with the journal's ethical standards. All authors declare they have non conflict of interest.

CRediT authorship contribution statement

David Olivares-Berjaga: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Albert Martínez-Pinteño:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Natalia Rodríguez:** Methodology, Writing – review & editing, Data curation. **Santiago Madero:** Methodology, Writing – review & editing. **Llucía Prohens:** Data curation, Methodology, Writing – review & editing. **Irene Martínez-Serrano:** Methodology, Writing – review & editing. **Sergi Mas:** Formal analysis, Writing – review & editing. **Constanza Morén:** Data curation, Methodology, Writing – review & editing. **Eduard Parellada:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Patricia Gassó:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declared no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2024.110955.

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