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NMDA Antagonist Ketamine Induces Significant Hyperactivity and Prepulse Inhibition Deficits in Gunn Rats: A Possible Hyperbilirubinemia-Induced Animal Model of Schizophrenia

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Abstract

Background: The Gunn rat, which is a mutant of the Wistar strain, has a genetic deficiency in glucuronyltransferase leading to unconjugated hyperbilirubinemia. The Gunn rat has potential as a schizophrenia animal model. Ketamine is widely accepted as a dissociative anesthetic, affecting glutamatergic activity *via* blockade of the N-Methyl-D-Aspartate (NMDA) receptor, which produces behavioral symptoms and cognitive deficits in healthy controls that resemble some symptoms of schizophrenia.

Methods: We compared the sensitivity of Gunn and Wistar rats to the NMDA antagonist ketamine in behavioral tests. The behaviors assayed in the study were open-field test locomotors activity and Prepulse Inhibition (PPI). The administration route was intraperitoneal injection for each.

Results: Gunn rats were affected by acute and chronic UCB neurotoxicity and is more vulnerable to the effects of NMDA antagonists than Wistar rats in regard to the open-field test locomotors activity.

Conclusion: It is possible to suppose the discrepancy between ketamine-induced behavioral changes in Gunn and Wistar rats in this study is attributed to differences in the sensitivity of the NMDA receptors in the rat strains.

Keywords: NMDA antagonist; Ketamine; Schizophrenia; Hyperbilirubinemia; Gunn rat

Introduction

Schizophrenia is considered as one of the neurodevelopmental disorders, which various genetic, epigenetic and environmental risk factors related to perinatal complications, can affect brain developmental processes. These factors could contribute to the emergence of the cognitive, positive and negative symptoms during adolescence and early adulthood [1].

From a neurodevelopmental viewpoint, it has been reported that hyperbilirubinemia has been associated with schizophrenia [2]. In the postnatal period, the serum elevated levels of Un Conjugated Bilirubin (UCB) which easily crosses the brood brain barrier, are occasionally found [3]. while in childhood, the prevalence of mental disorder associated with past histories of neonatal hyperbilirubinemia affecting neurodevelopment was significantly increased compared to a control group After childhood, patients with schizophrenia have a significantly higher frequency of hyperbilirubinemia relative to the general healthy population, moreover, relative to patients with other psychiatric disorders [4]. Moreover, there have been some neuroimaging findings, for instance, it was reported that Magnetic Resonance Spectroscopy (1H-MRS) of the hippocampus, basal ganglia, and vermin of cerebellum were reflected to show a decreased metabolism in the patient with both schizophrenia with hyperbilirubinemia which indicated, in part, as resulting from the bilirubin toxicity in the developing central nervous system.

Our previous studies have proposed that hyperbilirubinemia plays a role in the development of pathophysiology of schizophrenia from the standpoint of the heterogeneity [5]. We have reported that as the congenital hyperbilirubinemia rat the Gunn rat has potential as a schizophrenia animal model. The Gunn rat, which is a mutant of the Wister strain, has a genetic deficiency of the enzyme UDP glucuronosyltransferase leading to congenitally unconjugated hyperbilirubinemia. The total and unconjugated bilirubin level peaks at two weeks postnatal and falls to lower but still moderate elevated levels throughout their lives [6]. The neuropathological changes were detected in the hippocampus, basal ganglia, brainstem and cerebellum. It is also demonstrated that windows of developmental susceptibility to bilirubin toxicity exist for certain central nervous system structures. The developmental time of exposure to excess bilirubin is supposed as an important determinant of the specific pattern of neurological damage. We have detected that neuropathological features in the hippocampus of Gunn rats are identical to schizophrenia rodent models, for instance, the number of apoptosis cells was increased, while cellular activity and neurogenesis were depressed.

Moreover, neuroinflammation due to microgliosis and astrogliosis has been detected [7]. We have performed behavioral analyses that Gunn rats were found to have increased locomotors activity, inappropriate social interaction, impaired cognitive function, and deficits of attention function, as assessed by Pre Pulse Inhibition (PPI), compared with Wistar rats [8]. In addition, antipsychotic drugs and consecutive electroconvulsive shocks were demonstrated to ameliorate the schizophrenia-like behaviors of Gunn rats. These features are referred to as the face validity of the schizophrenia animal model.

Non-competitive NMDA antagonists, such as phencyclidine, MK-801, or ketamine, at subanesthetic doses have frequently been used to establish behavioral models of schizophrenia Ketamine is widely accepted as a dissociative anesthetic, affecting glutamatergic activity *via* blockade of the NMDA receptor which produces behavioral symptoms and cognitive deficits that resemble some positive and negative symptoms of schizophrenia [9]. A growing body of evidence suggests that vulnerabilities to NMDA antagonists are characteristic of schizophrenia. Hence, administration of ketamine has provided a useful strategy to examine NMDA function and schizophrenia. The hyperactivity and decreased Pre Pulse Inhibition (PPI) evoking dose-dependently effects of NMDA antagonists have been characterized in rodents [10].

In the present study, we sought to examine how their locomotors activities and PPI respond to the administration of ketamine in Gunn rats compared to Wistar rats, which reflected the disruption of the NMDA receptors of the Gunn strain. The study could also be referred to as the validity of the schizophrenia animal model.

To prove the rationale for the validity of the schizophrenia animal model from the neurodevelopmental viewpoint, we examined whether ketamine would exacerbate the schizophrenia-like behavior of the Gunn rats [11]. In other words, we examined whether the ketamine-induced behavioral symptoms would become more enhanced than at the baseline which would be different from that on Wister rats, since Gunn rats are genetically characterized by some schizophrenia-like behaviors and ketamine can also induce schizophrenic symptoms as mentioned above. We studied whether the enhanced effect of ketamine was in a dose-dependent manner, and whether the behavioral changes would be due to the sole effect of ketamine or increased serum UCB levels induced by ketamine.

Materials and Methods

Animals

Male homozygous (j/j) Gunn rats (140 g-160 g) and male Wistar rats (200 g-220 g) were obtained from Japan SLC Inc they were 7 weeks old at the time of the experiments. The rats were housed in plastic cages two to a cage, under standard conditions with a room temperature of 23°C ± 2°C, humidity of 55% ± 5%, and a 12 h light, 12 h dark cycle Food was provided in the form of dry pellets, and water was given ad libitum [12]. One week before the beginning of the experiment, the rats underwent a one-time handling procedure that was aimed at reducing experimental stress. The handling procedure consisted of picking the rat up with a gloved hand and stroking it for 10 minutes. All procedures were performed with the approval of the Shimane University Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Japan.

Drugs

Ketamine hydrochloride was obtained from Daiichi Sankyo. Ketamine was dissolved in saline and administered at 0.1 ml per 10 g of body weight [13-15]. Gunn and Wistar rats were randomly selected and distributed into two ketamine groups (15 mg/kg and 10 mg/kg) and a control group (0 mg/kg). The control group was injected with 0.9% saline. The administration route was intraperitoneal injection.

Serum bilirubin determination

In order to examine the relation between the serum UCB levels and behavioral measures and the relation between the effect of the intraperitoneal injection of ketamine to UCB and total bilirubin of Gunn and Wister rats, serum UCB and total bilirubin concentrations were measured *via* enzymatic methods provided by the SRL corporation and respective correlations were computed in each of the two strains [16].

Behavior observations

The behaviors assayed in the study were locomotors activity and prepulse inhibition. The methods are described below in detail. Tests were performed between 9: 00 h and 15: 00 h under the light phase of cycle [17]. Animals were brought to the testing room in their home cages and immediately placed in the test chamber.

Open-field test

Locomotors activity in a novel environment was monitored in a computerized activity chamber measuring 42 cm × 42 cm × 42 cm. Activity was measured by infrared beam motion sensors at two levels, one at floor level that measured horizontal activity (ambulation) and one at 13 cm above the arena floor that measured vertical activity (rearing). Each rat was placed in the center of the open-field apparatus, and beam-break counts caused by horizontal and vertical activities were recorded for 10 min for acclimatization as described previously [18]. Following a 10 min baseline recording, animals received an intraperitoneal injection of ketamine or saline as control. Horizontal and vertical activities refer to the total number of beams broken. Data was collected every 1 min for a total of 70 min.

Startle response and prepulse inhibition

A startle response measurement system was used as described previously [19]. Rats were injected intraperitoneally with ketamine (10 mg/kg or 15 mg/kg) or saline as soon as they were placed in a Plexiglas cylinder. The test lasted for 50 min. They were exposed to background white noise at 65 dB for a 5

min acclimatization period. This was followed by four types of trials which consisted of a 20-ms burst of white noise at 120 dB, 20 trials burst of white noise at 70 dB followed by a 20-ms white noise at 120 dB 10 trials a 20-ms burst of white noise at 80 dB followed by a 20-ms white noise at 120 dB 10 trials; and background noise only 10 trials. The interval between prepulse and pulse was set at 100 ms. Trials were given in pseudo-random order with variable intervals between each trial. Startle responses were measured in sessions consisting of 50 trials. The startle response was recorded by a piezoelectric accelerometer mounted below the cylinder. In each trial, the maximum amplitude of the response in the detection window was measured. From pulse-only trials, we derived the maximum amplitude of the startle response (V_{max}) in each trial. The mean Vmax was used to calculate the degree of PPI (%PPI). %PPI was defined as the magnitude of inhibition due to the startle amplitude that was induced by the prepulse [20-25].

%PPI=(1-(startle magnitude after prepulse-pulse pair/startle magnitude after pulse-only)) × 100.

Statistical analysis

Statistical analysis of the data was carried out using SPSS (Version 22). The data are presented as the mean ± Standard Error of the Mean (SEM). To assess whether the dose of ketamine affected serum UCB levels and whether UCB levels affected open-field and PPI data, Pearson correlation coefficients were calculated separately for Gunn and Wistar rats [26]. The serum UCB, total bilirubin levels, Open-field and PPI data were analyzed by two-way ANOVA, with strain and ketamine dose as between-subjects factors. A post hoc Turkey-HSD test was used for the pairwise comparisons. Significance for the result was set at $p<0.05$.

Results

Serum bilirubin level

The mean serum UCB and total bilirubin levels after injections Strain was revealed to have an effect on the UCB levels. Ketamine dose and the interaction of strain and ketamine dose were not revealed to have an effect on the UCB. Strain was revealed to have an effect on the total bilirubin levels as well. Ketamine dose and the interaction of strain and ketamine dose were not revealed to have an effect, which indicated that the serum UCB and total bilirubin of Gunn rats were significantly higher than in Wistar rats, independent from ketamine treatment each data after experiments are respectively as shown in **Table 1**.

Table 1: Serum bilirubin level. Note: Data represent mean ± SEM. n=10 subjects in each genotype.

UCB (mg/dl)	Total bilirubin	(mg/dl)
WK0	0.55 ± 0.02	0.67 ± 0.03
WK10	0.55 ± 0.02	0.66 ± 0.02
WK15	0.55 ± 0.02	0.67 ± 0.03
GK0	4.36 ± 0.15	5.08 ± 0.15
GK10	4.57 ± 0.14	5.28 ± 0.14
GK15	4.42 ± 0.17	5.14 ± 0.17

No significant Pearson correlation coefficients between ketamine dose and serum UCB levels were detected in both strains which indicated that serum UCB levels were not affected by the administration of ketamine.

Furthermore, no significant Pearson correlation coefficients between serum UCB and behavioral valuables in Gunn rats were detected which indicated that there were no relations between behavior measures and serum UCB levels in the Gunn strain.

Pearson correlation coefficients between serum UCB and behavioral valuables in Wistar rats were also not detected which further indicated that there were no relations between behavior measures and serum UCB levels in Wistar rats.

Therefore, the behavioral changes caused by ketamine treatments were not caused *via* changes in UCB levels as shown in **Figures 1 and 2**.

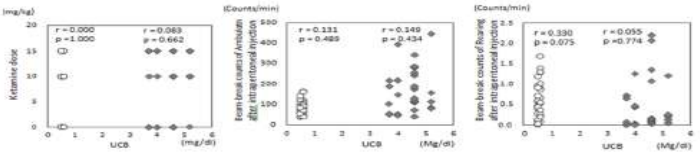

Figure 1: Pearson correlation coefficients.

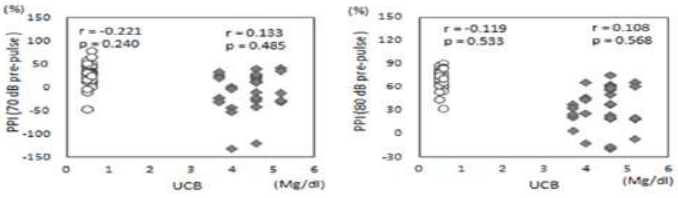

Figure 2: Pearson correlation coefficients of 70 dB and 80 dB.

No significant Pearson correlation coefficients were detected between UCB and intraperitoneal injection of ketamine, between UCB and behavioral measures, respectively, within Gunn rats, also within Wistar rats. There were 10 rats per group.

Open field test

The mean beam-break counts of ambulation and rearing before injection are shown in Figure 3. Strain was revealed to have an effect on the counts of ambulation. Ketamine dose and the interaction of strain and ketamine dose were not revealed to have an effect. Strain was revealed to have an effect on the counts of rearing. Ketamine dose and the interaction of strain and ketamine dose were not revealed to have an effect. Strain had an effect on beam-break counts of ambulation and rearing which indicated that before intraperitoneal injection of ketamine, Gunn rats showed significantly increased beam-break counts of ambulance and rearing compared to Wister rats shown in **Figure 3**.

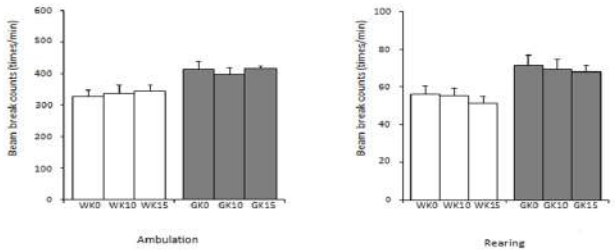

Figure 3: Open-field test before injection: The mean beam-break counts of ambulation and rearing for 10 min. Two-way ANOVA followed Turkey-HSD test revealed Gunn rats exhibited hyperactivity compared Wistar rats. Note: ☐ strain; ■ ketamine dose.

The mean beam-break counts of ambulation and rearing after injection are shown in Figure 4. Strain ketamine dose and the interaction of strain and ketamine dose were revealed to affect the counts of ambulation, which indicated that the mean beam-break counts of ambulation after injection is attributed to differences in the sensitivity of the NMDA receptors of the rat strains [27-30]. In other words, Gunn rats might have shown the tendency to have more sensitive NMDA receptors in the brain compared with Wistar rats. The ambulation of Gunn rats was affected significantly by administration of ketamine while Wistar rats were not affected further analysis revealed that a tendency toward dose dependency was exhibited by Gunn but not by Wistar rats. The numbers of beam-break ambulation for GK15 was significantly highest than all of each groups. The counts of GK10 was significantly higher than that of GK0. Indeed, intraperitoneal administration of ketamine to Gunn rats produced a significant increase in ambulation beam-break counts. A tendency toward dose dependency was exhibited by Gunn but not Wistar rats.

The mean beam-break rearing counts after injection of ketamine are shown in Figure 3. Ketamine dose was revealed to affect the counts. Strain and the interaction of strain and ketamine dose were not revealed to have an effect, which indicated that the beam-break counts were decreased due to administration of ketamine and were not different in the two strains. Further analysis revealed that the beam-break rearing counts with saline-treated groups were significantly higher than ketamine-treated groups [31]. When both rat strains were given ketamine, their rearing activity was significantly decreased shown in **Figure 4**.

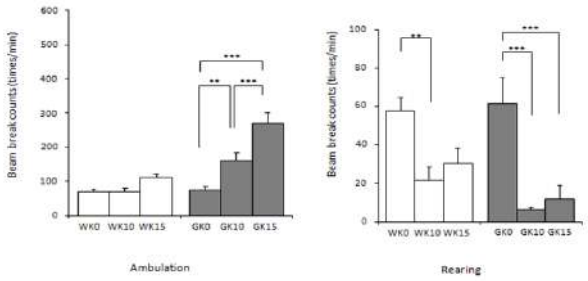

Figure 4: The mean beam-break counts of ambulation and rearing for 60 min. Two-way ANOVA followed Turkey-HSD test revealed Gunn rats exhibited ketamine-induced hyper locomotion, while Wistar rats did not. Gunn rats exhibited a tendency toward dose dependency. The beam-break rearing counts of saline-treated groups were significantly higher than ketamine-treated groups. When each bar represents mean ± S.E.M. both rat strains were given ketamine, their rearing activity was significantly decreased. Note: ☐ strain; ■ ketamine dose.

Prepulse inhibition test

The startle response magnitudes at 120 dB are shown in Figure 5. An interaction effect between strain and ketamine dose was not observed. Strain and ketamine dose were not revealed to have effects on the startle response amplitude, which also indicated that there were not significant differences between Gunn and Wistar rats for the startle response magnitudes at 120 dB are shown in **Figure 5**.

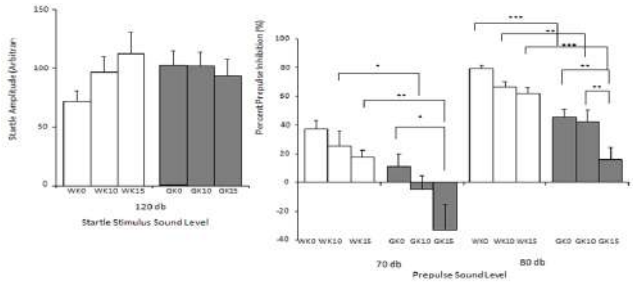

Figure 5: Startle response and prepulse inhibition. ☐ strain; ■ ketamine dose.

The percentage of prepulse inhibition: Gunn rats exhibited a tendency toward decreased PPI compared with Wistar rats and more interference by ketamine administration in proportion to the ketamine dose. The negative PPI value represents Pre Pulse Facilitation (PPF). The interference with the PPI of Gunn rats was more severe than that of Wistar rats in proportion to the ketamine dose.

(WK0: Wistar rats administered saline; GK0: Gunn rats administered saline; WK10: Wistar rats administered 10 mg/kg ketamine; GK10: Gunn rats administered 10 mg/kg ketamine; WK15: Wistar rats administered 15 mg/kg ketamine; GK15: Gunn rats administered 15 mg/kg ketamine).

The Percentage of Pre Pulse Inhibition (PPI) is shown for 70 dB and 80 dB. In the case of the 70 dB pre-pulse, there was no significant interaction effect between Strain and ketamine dose. Strain and ketamine dose were revealed to have effects, which indicated that PPI deficits were induced by ketamine in

proportion to the dose and that Gunn rats showed significant PPI deficits compared with Wistar rats [32]. It was also revealed that a tendency toward dose dependency was exhibited by Gunn but not by Wistar rats. %PPI in Gunn rats was significantly lower than Wistar rats at doses of both 10 mg/kg though there were no significant differences for administration of saline. Further analysis revealed there was a significant difference in %PPI of Gunn rats between administration of saline and the dose of 15 mg/kg. For doses of 10 mg/kg and 15 mg/kg, negative mean values for PPI were observed, suggestive of Pre-Pulse Facilitation (PPF). In the case of 10 mg/kg, this result cannot be considered to be facilitated due to the size of the error bars. In the case of 15 mg/kg, deficits of PPI resulted in PPF.

In the case of the 80 dB pre-pulse, there were no significant interaction effects between strain and ketamine dose. Strain and ketamine dose were revealed to have effects, which indicated that Gunn rats showed significant PPI deficits compared to Wistar rats and ketamine induced more PPI deficits in the 80 dB pre-pulse than in the 70 dB condition. It was also revealed that a tendency toward dose dependency was exhibited by Gunn but not by Wistar rats. %PPI was significantly lower for Gunn rats than Wistar rats in all doses of ketamine, which indicated that there were significant differences for administration of saline (F 10 mg/kg dose and the 15 mg/kg dose. Further analysis revealed that %PPI for GK15 was significantly lowest in each of the groups. Thus, intraperitoneal administration of ketamine to Gunn rats (15 mg/kg) produced a significant decreased PPI in the case of the 80 dB pre-pulse. Therefore, interference with the PPI of Gunn rats was more severe than that of Wistar rats in proportion to the ketamine dose [33-38]. The results indicated that Gunn rats showed significant PPI deficits in proportion to ketamine dose compared with Wistar rats in the both pre-pulse cases.

Discussion

In summary, ketamine exacerbated the schizophrenia like behavior of the Gunn rats. In other words, the ketamine-induced behavioral symptoms became more enhanced than at the baseline and that was different from that on Wistar rats [39]. The enhanced effect of ketamine was in a dose-dependent manner, and the behavioral changes were not due to increased serum UCB levels induced by ketamine but were due to the sole effect of ketamine.

Gunn rats showed horizontal hyperactivity compared with Wistar rats before injection for 10 min in the open-field test, while after administration of saline, there were no significant differences for the ambulation between Gunn and Wister strains in the “non-novel” environment open-field test for 60 min. The locomotors hyperactivity of Gunn rats was observed at the age of 7 w and 8 w [40-45]. It was also reported that the hyperactivity tendency was observed at 60 days and at 3-4 months old compared with Wister rats. No significant differences for the administration of saline was considered due to setting the 10 minute acclimatization period to the methodology as similarly and previously reported which was different from the previous studies of the “novel” environmental open-field test for 60 min. 10 min later, Gunn rats showed the tendency of non-remarkable hyperlocomotor activity compared with Wister rats. If the intraperitoneal injection of saline had been administrated in the “novel” environmental open-field test, the results might have been different. As mentioned above, there were no significant differences for the ambulation between Gunn and Wister strains in the case of the administration of saline, while the intraperitoneal administration of ketamine to Gunn rats produced a significant dose-dependent increase in ambulation beam-break counts. If the intraperitoneal injection of ketamine had been administrated in the “novel” environmental open-field test, the hyperlocomotor activity of Gunn rats might have been enhanced. Behavioral studies in focusing on locomotors hyperactivity as an measure of central neurotransmitter system activity has been to assess the effect of NMDA receptor antagonists It is possible to suppose hyperlocomotor activity after injection with ketamine is attributed to differences in the sensitivity of the NMDA receptors in the rat strains. Gunn rats might have shown the tendency to have more sensitive NMDA receptors in the brain compared with Wister rats [46].

Gunn rats in the case of the 80 dB pre-pulse were significantly lower than in Wistar rats for the administration of saline, while there were no significant differences in the case of the 70 dB pre-pulse. We previously reported that %PPI in innate Gunn rats in both cases of the 80 dB pre-pulse and 70 dB pre-pulse, were significantly lower than in Wistar rats. In the present study, the results might be related to the methodology, for instance, whether or not administration of intraperitoneal injection before measuring PPI and the difference of the statistical analysis. It is also reported that minor differences in the details of PPI test paradigm in the schizophrenia rodent models can have an impact on the results.

In the case of the administration of ketamine in both cases of 70 dB and 80 dB pre-pulses, the hypersensitivity of NMDA receptors of Gunn rats in the PPI were not revealed. This may have been associated with the significant difference between both strains. There might have been an association between the number of animals and the data dispersion. Due to the significant difference in PPI between both strains and the difference in response to the proportion of the dose-dependency of ketamine in PPI, the standard deviations have become wider and subsequently the statistical powers might have become weaker. Therefore, although the models may not be denied in the hypersensitivity to NMDA antagonists as shown in the open-field test, the models may not be adequate and could not reveal the hypersensitivity to NMDA antagonists in the PPI test. Gunn rats showed significant disruption in PPI resulting in PPF in proportion to ketamine dose compared with Wistar rats. Ketamine is known to induce a dose-dependent deficit PPI. In humans, it has been reported PPF was lower in schizophrenic patients than in healthy controls. Moreover, disruption in PPI and PPF has been repeatedly identified in patients with schizophrenia and in their unaffected blood relatives. However, there have been reports of normal levels of PPI and PPF in patients with schizophrenia utilizing a typical neutral or uninstructed methodology. It is important to understand the basis for these divergent findings, because these findings are

given the demonstrated utility of PPI and PPF as biomarkers and possible endophenotypes of schizophrenia. As mentioned above, minor differences in the details of PPI test paradigm in the schizophrenia rodent models can impact on the results, including technical details such as prepulse-pulse interval and stimulus modalities, and experimental protocol. The further study including other ketamine dose, in addition to using other NMDA antagonists, is crucial to clarify the NMDA sensitivity for PPI test and the mechanism of inducing PPF exhibited by Gunn rats in the current study.

It is known that UCB is responsible for the deleterious effects associated with unconjugated hyperbilirubinemia. UCB-induced neuronal injury appears to involve the NMDA receptors. The mechanism of UCB-induced neurotoxicity is related to the toxic accumulation of extracellular glutamate. The UCB-induced extracellular accumulation of glutamate is due to impaired glial functions of uptake and enhanced reactive secretion which indicates that UCB prolongs the presence of glutamate in the synaptic cleft. This overstimulation of NMDA receptors consequently leads to neurotoxicity Moreover, the activation of NMDA receptors was found in rat brain neurons exposed to UCB in culture. The mechanism by which ketamine induces behavioral changes in rats is attributed to the blockade of NMDA receptors located on Gamma-Amino butyric Acid (GABA)-ergic inhibitory interneurons. This disinhibitory action leads to an increase in glutamate and release of dopamine in the prefrontal cortex and limbic striatal regions.

Conclusion

The current study indicated that, Gunn rats were affected by acute and chronic UCB effect to neurodevelopment and is more vulnerable to the effects of NMDA antagonists than Wister rats, at least in regard to open-field test locomotors activity. It is possible to suppose the discrepancy between ketamine-induced behavioral changes in Gunn and Wister rats in this study is attributed to differences in the sensitivity of the NMDA receptors between the both rat strains. Moreover, our findings may provide a clue in the NMDA hypothesis of schizophrenia related to UCB neurotoxicity. In the present study, our findings are associated with the face validity, as schizophrenia animal models are supported by the disruptive effects of NMDA antagonists, as has already been described for other schizophrenia animal models. Further behavioral and neuropath logical analysis of Gunn rats is still needed.

Contributors

MH and TM designed the study. EL, IAA, MK, and SM collected data and supervised the study. MH, KT, and RA performed the experiments. MH, MI and TA analyzed the data. KM supervised about the data analysis. MN, RW, SH, and JH commented critically and revised the manuscript. TM was the study coordinator. MH and MI wrote the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest

The authors of this manuscript have no financial or personal relationships with other people or organizations to disclose that could inappropriately influence their work. These authors do not hold any potential conflicts of interest, including employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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