

Studying the behavioral changes in schizophrenic rats induced by chronic exposure to ketamine

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Abstract

Schizophrenia is a chronic and highly complex psychiatric disorder characterised by cognitive dysfunctions, negative and positive symptoms. The major challenge in schizophrenia research is lack of suitable animal models that mimic the core behavioural aspects and symptoms of this devastating psychiatric disorder. In this study, we used classical and atypical antipsychotic drugs to examine the predictive validity of ketamine-enhanced immobility in forced swim test (FST) as a possible animal model for the negative symptoms of schizophrenia. Repeated administration of a subanaesthetic dose of ketamine (0.1 mg / kg b.wt.) for 60 days enhanced the duration of immobility in FST 24 h after the final injection. Suggesting that the effects of ketamine on the duration of immobility in this study was neither due to motor dysfunction nor peripheral neuromuscular blockade. Our results suggest that repeated treatment with subanaesthetic doses of ketamine enhance the duration of immobility in FST, which might be a useful animal model for the negative symptoms (particularly the depressive features) of schizophrenia.

Key words: Schizophrenia, ketamine, forced swim test, rats.

Introduction

Schizophrenia (SCZ), one of the major psychoses, affects more than 1% of the whole human population (**Bhugra, 2006; Nagai et al., 2011**). SCZ patients are characterized by delusions, hallucinations, disorganized speech and behavior etc. (**APA, 2013**). These symptoms also include social dysfunction, which additionally affects family members and burden the society. Despite the importance, there is no cure for SCZ. Many partially effective therapies exist, but none can guarantee a cure to a specific case of the disease. The mechanism of all recently available antipsychotic drugs was discovered 60 years ago (**Carlsson and Lindqvist, 1963; and van Rossum, 1966**), their main therapeutic effect is the blockade of dopaminergic receptors (**Demeter et al., 2016**).

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Likewise, though increased immobility in a forced swimming test following chronic exposure of N-methyl-D-aspartate (NMDA) antagonists has been considered analogous to the induction of “emotional blunting” in schizophrenia, the specificity and pertinence of this read out remains debatable (Neill et al., 2014). Though the induction of immobility by neuroleptics/D2 receptor blockade might be claimed as related to “secondary negative symptoms”, it is hard to differentiate a putative impact of drugs on motor behavior, depressed mood and other factors from any putative induction of “emotional blunting”. Nonetheless, the enhanced immobility associated with chronic exposure to 1-phenylcyclohexylpiperidine (PCP) was reversed by D-Cycloserine (and sub-chronic clozapine, but not haloperidol) suggesting that further study is warranted (Murai et al., 2007; Turgeon et al., 2007).

Ketamine (Ket) is a dissociative anaesthetic with pharmacological effects similar to PCP, affecting glutamatergic activity via blockade of the NMDA receptor (Ellison, 1995). Ket was found to reinstate unique individual psychoses in remitted schizophrenic patients (Lahti et al., 1995 a, b). Therefore, this substance is also used to assess positive and negative symptoms of schizophrenia in humans such as deficits in auditory and visual context dependent processing, changes in [¹¹C]raclopride-specific binding, robust increases in 2-deoxyglucose (2-DG) uptake in limbic cortical regions, hippocampal formation, nucleus accumbens, and basolateral amygdala, increased cerebral blood flow in anterior cingulate cortex and reduced cerebral blood flow in the hippocampus and primary visual cortex, and visual perceptual alterations (Umbricht et al., 2000; Duncan et al., 2001) and animals (Miyamoto et al., 2001). It was demonstrated that repeated injection of sub anaesthetic doses of the substance induces subtle changes in cellular distribution of calcium sensor proteins, which in part resemble those recently described in postmortem brains of human schizophrenics (Bernstein et al., 2003).

The aim of the present work was to evaluate the effect of long exposure of ketamine on the behavior of rats. This goal could be achieved through assessment of forced swimming test of schizophrenic rats.

Materials and Methods

Chemicals and drugs

Ketamine was obtained from Trittau, Germany. All other reagents, solvents and chemicals used for analysis met the quality criteria in accordance with international standards.

Biological assay

Animals and treatment

All the experiment involving animals and tissue samples were conducted in accordance with the principles and guidelines for the care and use of laboratory animals of the National Institute of Health (NIH) (USA). This study was approved by the Ethical Committee for animal experimentation, National Research Centre, Egypt.

Twenty adult male albino rats of Wistar strain weighing 120 ± 10 gm at 90 days of age were enrolled in the present study. The animals were obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were housed throughout the experiment (8 rats/cage) in polypropylene cages under specific pathogen free (SPF) conditions with controlled illumination (12 h light/12 h dark cycle), a relative humidity (30-50%) and temperature (18-22 Co). Animals were fed with standard laboratory rat diet and water provided ad libitum. All animals received human care in compliance with the guidelines of the Ethical committee of Ain Shams University, Cairo, Egypt.

Rats were divided into two groups after 1 week of acclimation (con (n=10) which received a 0.9% saline solution and Ketaminized (n=10)) were received a daily orally administration of ketamine (0.1 mg / kg b.wt.) for 60 days to establish the animal model of schizophrenia according to (Fraga et al., 2011) as the SCZ group.

Behavioral assessment

Forced swim test (FST)

Schizophrenia-like behavior was assessed using the forced swim test. Each rat was placed individually in a transparent plastic cylinder (diameter: 40 cm, height: 60 cm) filled with water to a height of 40 cm. Rats were forced to swim for 5 min, the duration of immobility was recorded during the last 3 min. Stopping, struggling and floating on the surface of water indicated immobility. The test was video-recorded and analyzed later. Water was renewed each time and temperature was kept at 26 °C (Desikan et al., 2014).

Statistical analysis

The results of the present study were expressed as mean \pm S.E. of the mean. Statistical Package for the Social Sciences (SPSS) program, version 19.0 was used to compare the significance between every two groups. Difference was considered significant when $P < 0.05$. Percentage the difference representing the percent of variation with respect to the corresponding control group was calculated according to the following formula:

$$\% \text{ Difference} = [(Treated \text{ value} - \text{control value}) / \text{control value}] \times 100$$

Results

Behavioral assessment

Forced swimming test

In the FST, rats were forced to swim in a restricted space from which they could not escape, and as they were induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents that are therapeutically effective in human schizophrenia. The current results of the FST revealed that the orally administration of ketamine in adult male rats for 60 days leads to significant increase ($P < 0.05$) in the immobility time for each administered group compared with the immobility time for its corresponding group at zero time (Table 1). The SCZ group (after 60 days) recorded 182.32s vs 55.00s for its corresponding

group at zero time representing a percent of difference 220.30% increase in the immobility time corresponding group at zero time.

Table (1) Effect of ketamine on immobility duration in forced swim test in male rats. Data are represented as mean \pm S.E.M (n=10).

Parameter Groups	Immobility time (seconds)	
	Zero time	After 60 days of Ketamin administration
Con group	57.00 \pm 7.10	56.92 \pm 5.13
SCS group	55.00 \pm 5.26	182.32 \pm 7.15 ^a a (220.30%)

a: Significant change at P<0.05 in comparison with Con group.

Discussion

Schizophrenia is a chronic, life-long and debilitating disorder which is triggered in a complex, heterogeneous and multifactorial fashion by a diverse palette of genetic, epigenetic, developmental and environmental risk factors (Millan, 2013). The symptoms of schizophrenia are clustered into three categories: cognitive dysfunctions positive and negative symptoms (Crismon et al., 2008; Jones et al., 2011).

Increasing evidence supports the dysfunction of the glutamatergic system as a primary pathophysiological change in schizophrenia (Coyle et al., 2003). Pharmacological evidence for the role of glutamate in schizophrenia centers on findings that blockade of the NMDA receptor channel complex by non-competitive antagonists induce symptoms commonly seen in schizophrenia and produce psychotic symptoms in normal volunteers that are accompanied by progressive withdrawal and poverty of speech, akin to the negative symptoms of schizophrenia even at low doses. Non-competitive NMDA receptor antagonists, such as ketamine or PCP rekindle or exacerbate psychotic symptoms in both stabilised chronic and acute schizophrenic patients (Malhotra et al., 1997).

Repeated administration of subanaesthetic doses of ketamine enhanced the duration of immobility of rats in the FST paradigm; the effect persisted for 21 days after withdrawal of the drug (Chatterjee et al., 2011). Depression is one of the major negative symptoms of schizophrenia (Barnes et al., 1989) and forced swim-induced immobility in rodent is an acceptable animal model of depression (Porsolt et al., 1978) that reflects a state of ‘despair’ in the rat and reduction in the immobility time serves as a specific and selective index of antidepressant activity (Page et al., 1999; Weiner et al., 2003).

In the FST, rats are forced to swim in a restricted space from which they cannot escape and are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents that are therapeutically effective in human depression (Willner, 1984). Moreover, El-Shamy et al. (2010) suggested that the increased immobility time in the FST may represent a condition similar to human depression. The results of the FST in the present study revealed significant increase in the immobility time of the group of rats administered ketamine for 60 days as compared to its corresponding group at zero time (non ketaminized rats). This finding is in agreement with El-Shamy et al. (2010)

Therefore, an increase in immobility time in the FST following repeated administration of a subanaesthetic dose of ketamine indicates an increase in the state of behavioral despair that connotes depression, which is an important characteristic feature of the negative symptoms of schizophrenia (Crismon et al., 2008).

The effect of ketamine on the immobility time was due to motor dysfunction, such as decrease locomotor activity or ataxia. Repeated administration of ketamine had an effect on motor coordination in the animals suggesting that the enhancement of the immobility time by ketamine might be elicited via central mechanisms, not by peripheral neuromuscular blockade (Chindo et al., 2003).

Moreover, studies appointed that acute and sub chronic ketamine administration induces negative, positive and cognitive symptoms of schizophrenia (Becker and Grecksch, 2004). This data showed that the administration of the acute doses of ketamine provoked an increase of the lipid peroxidation, verified in hippocampus and striatum of the control group. In fact, ketamine administration increases metabolism in hippocampus and provoke the energetic activation in this structure (Duncan et al., 1998). It has been suggested that blockade of NMDA receptors can modulate neuronal projections and alter the activation of hippocampus resulting in behavioral abnormalities (Csernansky et al., 2005).

Conclusion

Here, we reported on face validity (changed social behavior) in a potential animal model in schizophrenia research. To find out whether predictive validity is also given, social behavior in subchronically ketamine-treated animals.

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الملخص باللغة العربية

دراسة التغيرات السلوكية لمرض انفصام الشخصية في نموذج جرذان التجارب الناجم عن التعرض المزمن للكيتامين

المستخلص

انفصام الشخصية هو اضطراب نفسي مزمن ومعقد للغاية يتميز بخلل وظيفي إدراكي وأعراض سلبية وإيجابية. التحدي الرئيسي في أبحاث الفصام هو عدم وجود نماذج حيوانية مناسبة تحاكي الجوانب السلوكية الأساسية وأعراض هذا الاضطراب النفسي المدمر. في هذه الدراسة ، استخدمنا الأدوية المضادة للذهان الكلاسيكية وغير التقليدية لدراسة الصلاحية التنبؤية لعدم الحركة المعززة بالكيتامين في اختبار السباحة القسري (FST) كنموذج حيواني محتمل للأعراض السلبية لمرض انفصام الشخصية. تناولت الإدارة المتكررة لجرعة تحت جمالية من الكيتامين (٠.١ ملغم / كغم من وزن الجسم) لمدة ٦٠ يومًا مدة عدم الحركة في FST 24 ساعة بعد الحقن النهائي. مما يوحي بأن آثار الكيتامين على مدة الجمود في هذه الدراسة لم يكن بسبب الخلل الحركي ولا الحصار العصبي العضلي المحيطي. تشير النتائج التي توصلنا إليها إلى أن العلاج المتكرر مع جرعات الكيتامين دون التجميلية يعزز مدة الجمود في FST ، والتي قد تكون نموذجًا حيوانيًا مفيدًا للأعراض السلبية (خاصة الخصائص الاكتئابية) لمرض الفصام.