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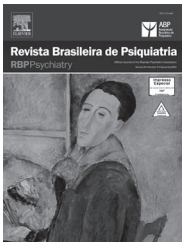

BRIEF COMMUNICATION

Effects of pregabalin on behavioral alterations induced by ketamine in rats

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DESCRIPTORS
Anticonvulsants;
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Abstract
Objective: The aim of this study is to investigate the effects of pregabalin on the behavior of rats under the influence of ketamine, an NMDA receptor antagonist that mimics the symptoms of schizophrenia. *Methods:* Rats were injected with saline or 25 mg/kg ketamine intraperitoneally. After that, behavior modifications were investigated by the evaluation of stereotypy and hyperlocomotion, after treating rats with pregabalin (at doses of 30 mg/kg or 100 mg/kg) or placebo (saline solution). *Results:* The administration of pregabalin reduced ketamine-induced hyperlocomotion. However, neither doses of pregabalin had a significant effect on ketamine-induced stereotypy. *Conclusion:* This is the first study to investigate the effects of pregabalin using an animal model of psychosis. Furthermore, our results indicate that behavioral changes induced by ketamine in rats can be reversed with the use of pregabalin, suggesting its potential to treat psychotic symptoms.

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DESCRITORES:
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Outras áreas das neurociências e outros aspectos de psicofarmacologia.

Efeitos da pregabalina sobre alterações comportamentais induzidas pela cetamina em ratos

Resumo
Objetivo: O presente estudo tem como objetivo investigar os efeitos da pregabalina sobre as alterações comportamentais em ratos induzidas pela cetamina, um antagonista do receptor glutamatérgico NMDA, utilizado em modelos animais de psicose. *Métodos:* Ratos receberam injeção com solução salina ou cetamina, na dose de 25 mg/kg, com posterior avaliação das alterações comportamentais induzidas, através da avaliação da estereotipia e hiperlocomoção, depois destes ratos terem sido tratados com pregabalina (30 mg/kg ou 100 mg/kg) ou placebo. *Resultados:* A administração de pregabalina reduziu a hiperlocomoção nos ratos sob o efeito da cetamina. No entanto, nenhuma das doses de pregabalina teve efeito significativo sobre a estereotipia induzida pela cetamina. *Conclusão:* Este é o primeiro estudo que investiga os efeitos da pregabalina em um modelo animal de psicose. Nossos resultados indicam que alterações comportamentais induzidas pela cetamina em ratos podem ser revertidas após uso da pregabalina, o que sugere um possível potencial desta no tratamento de sintomas psicóticos.

Introduction

The treatment of schizophrenia remains a great challenge, despite the advances in pharmacological and non-pharmacological approaches. The difficulty lies in the heterogeneity of the condition, expressed in the subjective and complex nature of its symptoms, and the lack of a comprehensive model to explain its pathophysiology.

The dopamine hypothesis for the pathophysiology of schizophrenia is insufficient to explain the disorder completely. The ineffectiveness of D2 receptor blockade in the treatment of negative symptoms, for example, highlights the need to search for other explanations and mechanisms that could be involved in schizophrenia. The glutamatergic hypothesis of schizophrenia postulates that the hypofunction of NMDA glutamatergic receptors in cortical-limbic areas could lead to alterations in interneuron transmission that would exacerbate dopaminergic function.[1] Cumulative data in the literature also suggest a role of GABA in schizophrenia.[2,3] One of the explanations suggests that a decrease in interneuron GABAergic activity induced by the lack of activation due to NMDA hypofunction could lead to an increased activity of excitatory glutamatergic transmission in cortical-limbic areas.[3] However, benzodiazepines, which increase GABAergic activity, are not particularly effective in treatment of schizophrenia.[4] Therefore, strategies that use more specific drugs to increase GABAergic action should be investigated. Pregabalin, a structural analogue of GABA, is an antiepileptic drug with broad-spectrum efficacy in the treatment of pain-related medical conditions and epilepsy, and it is also approved for the treatment of generalized anxiety disorder in the European Union.[5]

Two previous reports described the use of pregabalin in schizophrenic patients as an add-on treatment for schizophrenia-related anxiety.[5,6] The current study was aimed at testing the hypothesis that pregabalin itself can have antipsychotic properties. With that in mind, were investigated its effects on the behavior of rats under the influence of ketamine, an NMDA receptor antagonist that mimics the positive, negative, and cognitive symptoms of schizophrenia.[7]

Materials and Methods

Animals

Adult male Wistar rats (60 days old) were obtained from the Central Animal House of the Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care, with the approval of the Ethics Committee of the Universidade do Extremo Sul Catarinense.

The study used 90 animals divided into six groups (N = 15 per group): 1) placebo (Sal+Sal), 2) pregabalin (30 mg/kg) + placebo (PG30+Sal), 3) pregabalin (100 mg/kg) + placebo (PG100+Sal), 4) placebo+ketamine (Sal+Ket), 5) pregabalin (30 mg/kg)+ketamine (PG30+Ket), and 6) pregabalin (100 mg/kg) + ketamine (PG100+Ket).

Pregabalin

Pregabalin was administered orally in doses of 30 mg/kg or 100 mg/kg at the time of the experiment.

Animal model of psychosis

The animals were injected with saline or 25 mg/kg of ketamine (CU Chemie Uetikon, Germany) intraperitoneally (i.p.), 20 minutes after administration of pregabalin or saline (SAL).

Locomotor activity

Locomotor activity was measured in an activity monitor (40x60 cm), surrounded by 50 cm high acrylic walls, containing 6 parallel bars, each bar containing 16 infrared sensors that detect a rat's exact position and movement, making possible a detailed analysis of animal's behavior. Information detected by the sensors over 60 minutes was transmitted to

a computer by the software Open Source version Interbase 6.01 (Activity Monitor – Insight Laboratory Equipment, Ribeirão Preto, SP).

Stereotypy

This parameter was analyzed simultaneously with locomotor activity. Stereotypy is considered by the software as an unstable movement; any time repetitive movements are recorded in sequential readings without the animals moving horizontally. The values are measured in millimeters (mm).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey multiple range test when F was significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS). A value of $p < 0.05$ was considered to be significant.

Results

The effects of ketamine on the behavior of rats

As expected and already described in the literature,[8,9] the use of ketamine alone in rats promoted alterations significantly different compared to the saline-only group (Sal+Sal), with ketamine use increasing both the distance traveled (17.9 m to 40.3 m, $p < 0.05$) and stereotypic movements (2,755 mm to 3,688 mm, $p < 0.05$) presented by the animals (Figure 1A & B).

The effects of pregabalin on ketamine-induced hyperlocomotion

First, the effects of pregabalin on basal locomotion were evaluated in groups that received saline solution prior to drug administration. The mean values for the distances observed (Figure 1A), showed an unexpected hyperlocomotion after pregabalin use at the higher dose of 100 mg/kg. In this group (PG100+Sal), the mean distance travelled of 38.1 m was close to the value of the ketamine-only group (Sal+Ket), and was significantly different from the control group ($p = 0.033$).

The administration of pregabalin before ketamine reduced hyperlocomotion indices at both doses (Figure 1A): the group that received pregabalin 30 mg/kg had a mean value of 22.2 m and the group that received pregabalin 100 mg/kg had a mean value of 20.2 m ($p = 0.035$).

The effects of pregabalin on ketamine-induced stereotypy

Neither doses of pregabalin had a significant effect on ketamine-induced stereotypy (Figure 1B).

Discussion

Both doses of pregabalin inhibited the effects of ketamine on the locomotion of rats, although not reducing general motor activity when administered alone (in fact, the high dose of pregabalin increased locomotor activity on its own), which suggests that this reduction was not secondary to motor impairment.

Animal models of psychosis that use NMDA receptor antagonists to induce behavioral changes like hyperlocomotion and stereotypy have often been used to assess the pharmacological effects of different candidate substances for the treatment of symptoms in schizophrenia,[8,9] with some evidence of parallels between those pharmacologically induced behavioral alterations in rats by decreases in NMDA function and behavioral alterations present in schizophrenia. For example, it has been proposed that increased locomotor activity in rats may be analogous to psychomotor agitation in schizophrenia.[10]

Pregabalin is a novel GABAergic substance that does not interact with either GABA-A or GABA-B receptors directly and is not an inhibitor of GABA uptake or degradation.[11] Its actions derive from complex effects on neurotransmission through its binding to the α2δ type 1 and 2 subunits of voltage-gated calcium channels, as well as by its actions in the glutamate-GABA cycle.[12] Our data indicate that pregabalin somehow enhances NMDA receptor function, since ketamine-induced behavioral alterations are explained by its NMDA receptor blockade. However, this enhancement of NMDA receptor mediated action after pregabalin use is not through direct action at the NMDA receptor, since this drug does not interact directly with these receptors.[11] At the same time, some consequences of NMDA receptor activation, such as modulation of glutamate release in the cerebral cortex, avoiding excess glutamate release,[7] are linked to the activation of NMDA receptors on GABAergic interneurons that modulate excitatory pathways and could possibly be influenced after pregabalin use due to the GABAergic action of this drug. In addition, this GABAergic action might be linked to the glutamate system metabolically since pregabalin has been shown to interact with glutamic acid decarboxylase (GAD) function, enhancing its activity and leading to an increase in GABA synthesis from glutamate.[13]

The finding of increased locomotor activity after pregabalin injection at the higher dose (100 mg/kg) in saline-treated rats, similar to what was observed with ketamine, although interesting, has already been observed in an animal model.[14] One possible explanation for this increase in basal locomotion by pregabalin, which has already been described with diazepam in mice, is that the anxiolytic effect of pregabalin - which was previously described in the literature[5] - could increase the observed basal locomotion at higher doses.[15] However, paradoxically this high dose reverses the hyperlocomotion observed with ketamine, suggesting that something other than an anxiolytic effect of pregabalin is involved in this particular drug-drug interaction; at the present, a clear explanation is not available.

Pregabalin at both doses decreased the stereotypy behavior induced by ketamine to levels near the control group (Figure 1B), but the reduction was not quite statistically significant at either doses ($p = 0.05$). More comprehensive dose and time studies should be done before we can suggest that pregabalin may be useful for treating the stereotypy induced by ketamine.

In conclusion, our results indicate that the locomotor changes induced by ketamine in rats can be reversed with the use of pregabalin, suggesting potential of pregabalin as an add-on drug option for schizophrenia. Two previous clinical reports described the use of pregabalin in schizophrenic

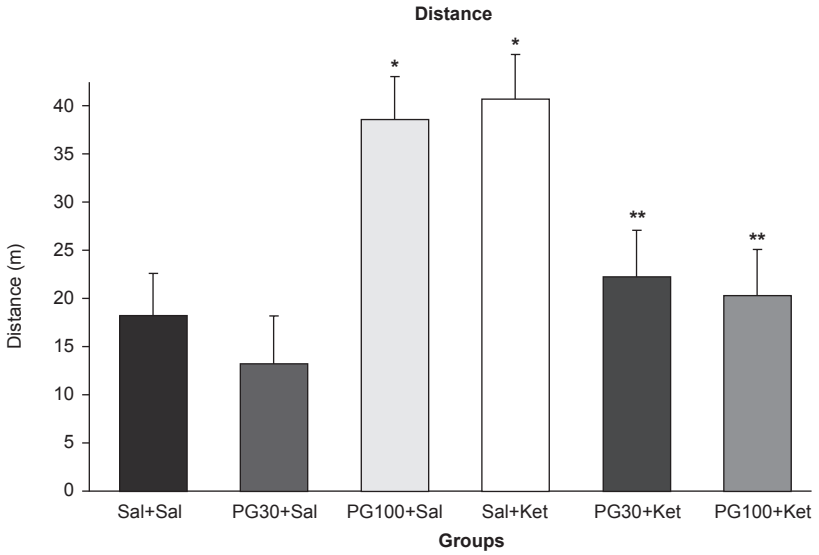

Figure 1A Effects of pregabalin (PG30+Ket, PG100+Ket) on hyperlocomotion induced by ketamine. *p < 0.05 compared to control (Sal+Sal). **p < 0.05 compared to ketamine group (Sal+Ket).

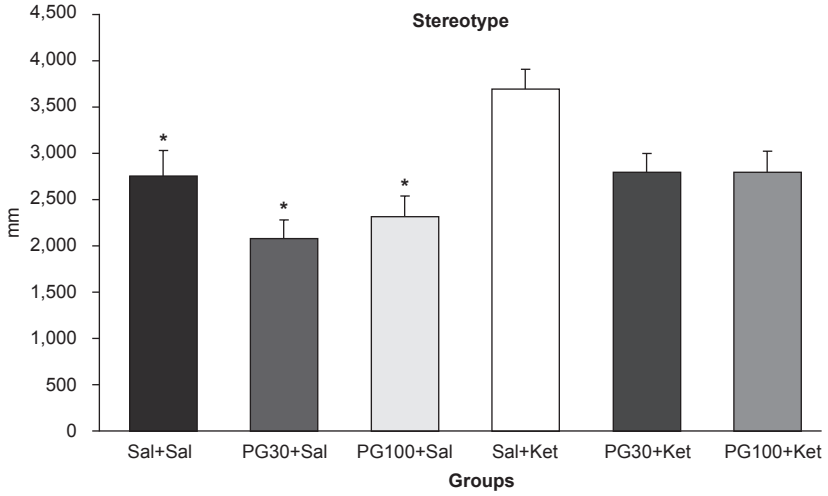

Figure 1B Effects of pregabalin (PG30+Ket, PG100+Ket) on stereotype induced by ketamine. *p < 0.05 compared to ketamine + saline group (Sal+Ket).

patients as an add-on treatment for schizophrenia-related anxiety,[5,6] but to our knowledge, this is the first study to investigate the direct effects of pregabalin using an animal model of psychosis.

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Disclosures

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Authors declare no conflict of interest.
** Modest*
*** Significant*
**** Significant. Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.*

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