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Ketamine treatment reverses behavioral and physiological alterations induced by chronic mild stress in rats

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ABSTRACT

Several studies have supported the idea that ionotropic glutamate N-methyl-D-aspartate receptor (NMDA) is an important player in the etiology of psychopathologies, such as anxiety disorders and major depression. Additionally, studies have shown that ketamine induces antidepressant effects in humans as well as in rodents subjected to animal models of depression. In this context, the present study was aimed to evaluate behavioral and physiological effects of acute and chronic administration of ketamine, a NMDA receptor antagonist, in rats exposed to chronic mild stress (CMS). After 40 days of CMS, rats were treated with ketamine (15 mg/kg) and sweet food consumption, body and adrenal gland weight, corticosterone and adrenocorticotropic (ACTH) hormone levels, and hippocampal BDNF protein levels were assessed. Our findings demonstrated that CMS evoked anhedonia, induced hypertrophy of adrenal gland, impaired gain of body weight and increased corticosterone and ACTH circulating levels in rats. Acute and chronic treatment with ketamine reversed the increase in adrenal gland weight, promoted regain of body weight, and normalized corticosterone and ACTH circulating levels. Repeated, but not acute, administration of ketamine reversed anhedonia-like behavior, although the treatment with ketamine *per se* increased sweet food consumption in non-stressed rats. Finally, acute and chronic ketamine treatment did not alter hippocampal BDNF protein levels in stressed rats. In conclusion, these findings support the idea of a putative role of NMDA receptors in mood-related symptoms, and rapid and robust effects of ketamine in reverting mainly physiological alterations induced by chronic mild stressful situations in rats.

1. Introduction

Major depression is a serious and recurrent disorder often manifested with psychological, behavioral and physiological symptoms. It affects 17–20% of the world resulting in premature death, major social deficits and economic consequences (Kessler et al., 1994). Among people with major depression, 75–85% have recurrent episodes (Keller et al., 1986; Mueller et al., 1999) and 10–30% recover incompletely, displaying persistent and residual depressive symptoms (Mann, 2005).

The pharmacotherapy of depression is widely prescribed by physicians, although less than half of treated patients attain complete remission after therapy with a single antidepressant. Others exhibit partial, refractory or intolerant responses to the pharmacological treatment, emphasizing the need to discover novel antidepressants (Pacher et al., 2001). The challenges for the design of new antidepressant agents are threefold: rapid onset of antidepressant response, broader efficacy, and fewer adverse effects. While progress has been made to reduce side-effects, currently available antidepressants do not show convincing evidence for a shorter delay of onset of therapeutic actions neither for improved efficacy on the treatment of major depression (Nutt, 2002).

Several studies have supported the idea that ionotropic glutamate N-methyl-D-aspartate receptor (NMDA) is an important player in the etiology of psychopathologies, such as anxiety disorders and major depression (Javitt, 2004; Krystal et al., 1999). In fact, preclinical studies have demonstrated that NMDA antagonists, such as MK-801, AP7, CPP, neramexane and others, display anxiolytic- and antidepressant-like effects in rats injected into specific brain areas, and subjected to distinct animal models of anxiety and depression (Kos et al., 2006; Molchanov and Guimarães, 2002; Menard and Treit, 2000; Matheus and Guimarães, 1997; Skolnick et al., 1996; Maj et al., 1992).

Ketamine is a NMDA receptor antagonist which displays high affinity to the phencyclidine binding site, within the ionotropic channel. The quite unique ability of ketamine to block the NMDA receptor is based on:

Abbreviations: ACTH, adrenocorticotropic hormone; BDNF, brain-derived neurotrophic factor; CMS, chronic mild stress; HPA, hypothalamic–pituitary–adrenal; NMDA, N-methyl-D-aspartate.

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1) higher affinity for the NMDA receptor; 2) much slower open-channel blocking/unblocking kinetics; 3) a different type of channel closure (i.e., "trapping block" as opposed to "partial trapping" properties); 4) a non selective blockade of voltage-sensitive Ca^{2+} channels, and opioid, monoaminergic and muscarinic receptors, than other NMDA receptor antagonist (Bolshakov et al., 2003; for a review: Hirota and Lambert, 1996). Some of these properties may explain why ketamine was found to have significant antidepressant actions. In fact, administration of ketamine has been shown to induce antidepressant effects in humans as well as in rodents subjected to animal models of depression (for a review see: Maeng and Zarate, 2007). In a pilot study developed by Berman et al. (2000), seven human patients with treatment resistant depression showed significant improvement in depressive symptoms within 72 h of ketamine treatment. Other clinical studies replicated these findings and confirmed a rapid onset of action for ketamine on the treatment of major depression (Zarate et al., 2006; Liebrenz et al., 2007a,b). Preclinical studies showed that acute and chronic administration of ketamine in rodents also induces robust and sustained antidepressant-like effects, as assessed in the learned helplessness and forced swimming tests (Chaturvedi et al., 2001; Yilmaz et al., 2002; Garcia et al., 2008a,b; Maeng et al., 2008).

The role played by neurotrophic factors, particularly the brain-derived neurotrophic factor (BDNF), in neurogenesis has been considered important in the mechanism of action of antidepressant drugs (for a review see: Castrén et al., 2007). BDNF has important functions in the adult human brain as a regulator of neuronal survival, fast synaptic transmission, and activity-dependent synaptic plasticity (Lewin and Barde, 1996; Hashimoto et al., 2004; Blum and Konnert, 2005). However, in rodents, few studies have investigated the concentrations of hippocampal BDNF protein in antidepressant-treated animals, and available data indicate that classic antidepressant treatment did not affect the total amount of that hippocampal protein (Jacobsen and Mork, 2004; Garcia et al., 2008a,b). Very recently, we demonstrated that distinct from imipramine, a tricyclic antidepressant drug, the acute injection of ketamine increased BDNF protein levels in the rat hippocampus (Garcia et al., 2008a), thus suggesting that ketamine could be an innovative antidepressant able to induce distinct molecular effects on the rat hippocampus compared to classic antidepressants.

In this context, the chronic mild stress (CMS) model has been shown to evoke lower sucrose consumption (sweet food), postulated to reflect anhedonia (the loss of interest or pleasure) in animals, one of the two core symptoms required for diagnosis of a major depressive episode in humans (Katz et al., 1981; Willner et al., 1987; Willner, 1997). The exposure of rats to CMS also induces changes in hypothalamic–pituitary–adrenal axis, loss of body weight, and adrenal hypertrophy, which leads to corticosterone hypersecretion (Vollmayr and Henn, 2003). The CMS paradigm described by Willner et al. (1987) is a model of depression which consists of exposing animals sequentially to a variety of mild and unpredictable "stressors" (e.g. isolation, water and food deprivation, restraint, forced swimming, flashing light exposure) for a period of 4–6 weeks (Wilner, 2005). The CMS model requires intensive labor, space demanding and long stress procedure, although this inconveniences this protocol is regarded as being close to model the human situation, consisting more of daily hassles than traumatic events (Wilner, 2005).

In this context, the present study aimed to investigate behavioral and physiological effects of acute and repeated administration of ketamine in rats subjected to the CMS procedure. To reach this aim, rats were subjected to 40 days of chronic unpredictable stressful stimuli, and afterwards sweet food consumption was assessed through repeated sessions in saline- and ketamine-treated rats. Additionally, as physiological parameters, body and adrenal gland were weighed, and corticosterone and ACTH circulating levels were analyzed in the serum. The amount of hippocampal BDNF protein levels was also assessed in all experimental groups.

2. Materials and methods

2.1. Animals

Male Wistar rats (3–4 months, 220–310 g) were obtained from our breeding colony (UNESC). The animals were housed 5 to a cage with food and water available *ad libitum* (except for the stressed group during the period when the stressor applied required no food or no water) and were maintained on a 12-h light/dark cycle (lights on at 7:00 am). All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care.

2.2. Drugs and treatment

Ketamine (Fort Dodge Animal Health — Fort Dodge, IA, USA) at the dose of 15 mg/kg (as previously reported by Garcia et al. (2008a,b) to evoke antidepressant-like effects) was injected intraperitoneally, one day (acute treatment) or once a day across 7 days (chronic treatment) after CMS procedure. All treatments were administered in a volume of 1 ml/kg.

To develop this study we employed 90 animals ($n = 15$) separated in six groups, as described bellow: 1) non stressed — saline; 2) non stressed — acute ketamine; 3) non stressed — chronic ketamine; 4) CMS — saline; 5) CMS — acute ketamine; 6) CMS — chronic ketamine. The chronic treatment with ketamine was performed during the anhedonia test, once a day, across 7 days, 60 min prior to behavioral assessment. In the acute treatment, ketamine was administered in the first day of the anhedonia test 60 min before the behavioral test.

2.3. Experimental procedure

Chronic mild stress (CMS) protocol was adapted from the procedure described by Gamaro et al. (2003). Animals were divided in two groups: control (non stressed) and CMS (stressed) rats. During the 40 days of treatment, control rats were kept in their home cages according to conditions previously reported, being handled and weighed once a week (see above: Section 2.1). Forty days of chronic mild and unpredictable stress situations were used for assessing mood-related behaviors in ketamine-treated rats. Individual stressors and length of time applied each day are listed on Table 1. The following stressors were used: (i) 24 h of food deprivation; (ii) 24 h of water deprivation; (iii) 1–3 h of restraint as described later, (iv) 1.5–2 h of restraint at 4 °C; (v) forced swimming during 10 or 15 min as described later; (vi) flashing light for 120–210 min; (vii) isolation (2–3 days). Stressors stimuli were applied at different time everyday, in order to minimize the prediction.

Restraint was carried out by placing the animal in a 25×7 cm plastic tube and adjusting it with plaster tape on the outside, thus that does not allow the animal to move. There was a 1 cm hole at the end of the tube for breathing. Forced swimming was carried out by placing the animal in a glass tank measuring 50×47 cm with 30 cm of water at 23 ± 2 °C. Exposure to flashing light was made by placing the animal in a $60 \times 60 \times 25$ cm plywood made box divided in 16 cells of $15 \times 15 \times 25$ cm with a frontal glass wall. A 40 W lamp flashing at a frequency of 60 flashes/min was used.

2.4. Sweet food consumption (anhedonia test)

After 40 days of treatment, consumption of sweet food was used for measuring anhedonia. Animals were placed in a rectangular box ($40 \times 15 \times 20$ cm) with floor and side walls made of wood and divided into 9 equal rectangles by black lines placed in an illuminated room (150 lx in the center of the box). The open field arena was covered with a plastic adhesive, and it was cleaned with 10% alcohol between rats.