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Prelimbic NMDA receptors stimulation mimics the attenuating effects of clozapine on the auditory electrophysiological rebound induced by ketamine withdrawal

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ABSTRACT

Ketamine (KET) is a non-competitive *N-Methyl-D-aspartate* (NMDA) receptors antagonist that intensifies sensory experiences, prompts hallucinations and delusions, exacerbates previously installed psychosis and disrupts physiological evoked potentials (AEPs). Pharmacologically, KET stimulates glutamate efflux in the medial prefrontal cortex, mainly in the prelimbic (PrL) sub-region. Efferences from this region exert a top-down regulatory control of bottom-up sensory processes either directly or indirectly. In the midbrain, the central nucleus of the inferior colliculus (CIC) plays a fundamental role in the processing of auditory ascending information related to sound localization, sensorimotor gating, and preattentive event-related potentials. Auditory hallucinations elicited during a psychotic outbreak are accompanied by CIC neural activation. Thus, it is possible that NMDA-mediated glutamate neurotransmission in the PrL indirectly modulates CIC neuronal firing. The aim of the present study was to assess the effects of KET on the latency and amplitude of AEPs elicited in the CIC of rats tested during KET effects and following withdrawal from the chronic administration. Changes on emotionally induced by KET treatment were evaluated with the use of the elevated zero maze (EZM). Unlike typical neuroleptics, the atypical antipsychotic clozapine (CLZ) potently blocks the disruption of the sensorimotor gating induced by NMDA antagonists. Therefore, the effects of KET withdrawal on AEPs were challenged with a systemic injection of CLZ. In addition, we further investigated the role of NMDA receptors of the PrL on the AEPs expression recorded in the CIC through intra-PrL infusions of NMDA itself. Our results showed that the processing of sensory information in the CIC is under indirect control of PrL. These data suggest that the long-term KET treatment disrupts the collicular auditory field potentials, possibly through influencing PrL glutamate activity on intrinsic 5-HT mechanisms in the dorsal raphe and CIC.

1. Introduction

Due to its reinforcing and rewarding effects, the non-medical use of ketamine (KET) has grown progressively worldwide in the past decades with the drug becoming increasingly popular, mainly among young people. KET is a non-competitive *N-Methyl-D-aspartate* (NMDA) receptors antagonist with strong dissociative and sedative anesthetic properties. KET administration elicits sensory and motor disturbances, memory deficits (Imre et al., 2006; Kos et al., 2006), hypermotility (Carlsson, 1993), stereotyped behavior, and ataxia (Tricklebank et al., 1989). In addition, KET prompts hallucinations and delusions, exacerbates previously installed psychosis (Krystal et al., 1994; Moghaddam and Krystal, 2012), and induces neurological and peripheral toxicity (Gable, 2004; Morgan et al., 2010). Besides changing behavioral and cognitive functions (Malhotra et al., 1997; Umbricht et al., 2000), KET can disrupt physiological auditory evoked potentials (AEPs) consistent with schizophrenia (Maxwell et al., 2006). Indeed, KET changes the reactivity of several brain regions to a variety of sensory inputs including the auditory ones, despite the drug does not promote a sensory blockade, an effect that has been called “dissociative” anesthesia (Domino et al., 1965). It has been supposed that KET disturbs coherent personality precisely by inducing sensory overload through breaking homeostasis of bottom-up sensorimotor gating processes (Mansbach and Geyer, 1989; Maxwell et al., 2006; Sandner et al., 2002).

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From the auditory system, the inferior colliculus (IC) is a midbrain region reputed to be one of the most important. Sound localization and the prepulse inhibition of the startle reflex (Braff and Geyer, 1990; Fendt et al., 2001; Li and Yue, 2002), are highly modulated in the IC (Li and Yue, 2002). Others preattentive event-related potentials such as mismatch negativity are also dependent on IC auditory mechanisms (Patel et al., 2012). Dysfunctions in this machinery are frequently observed following systemic KET, an indication that unconscious auditory attentional processes are modulated by glutamatergic neurotransmission at this midbrain level. Clinical studies have been shown that the performance of tasks related to prefrontal activity is strongly affected by KET (Honey et al., 2005, 2004); an effect comparable to those found in schizophrenic patients (Neill et al., 2010). Efferents from this region exert a top-down regulatory control of bottom-up sensory processes either directly or indirectly (Floresco et al., 2009; Vertes, 2004). Considering that, in the midbrain, auditory hallucinations elicited during a psychotic outbreak are accompanied by IC neural activation (Shergill et al., 2000), it is possible that glutamate-mediated neurotransmission in the prefrontal cortex may indirectly modulate IC neuronal firing.

So far, little is known about the impact of the repeated use of KET on the neural pharmacology and physiology of the auditory sensory system. Therefore, assuming that KET disrupts the midbrain auditory sensory gating and bearing in mind that the IC plays a fundamental role in the processing of auditory ascending sensory information, the aim of the present study was to assess the influence of KET on the AEPs elicited in the central nucleus of the IC (CIC) of rats tested during KET effects and following withdrawal from chronic administration. Possible changes in behavioral activity and emotionality induced by KET withdrawal were evaluated with the elevated zero maze (EZM). Unlike typical neuroleptics, the atypical antipsychotic clozapine (CLZ) potently blocks the disruption of the sensorimotor gating induced by NMDA antagonists (Bakshi et al., 1994). Thus, the effects of KET on AEPs were challenged with a systemic injection of CLZ. The gamma-band oscillations, a physiological indicator of a psychotic state, is highly increased by KET in the auditory cortex and prelimbic cortex (PrL) (Anver et al., 2011; McNally et al., 2011; Middleton et al., 2008; Oke et al., 2010). So, we further investigated the role of NMDA receptors of the PrL on the AEPs recorded in the CIC through intra-PrL infusions of NMDA itself. The CIC was chosen for AEPs investigation due to its exclusive role in the conduction of auditory ascending information (Aitkin et al., 2004). In the present report, the chronic method of daily KET injections was selected to model chronic KET abuse, as usually seen in humans.

2. Materials and methods

2.1. Animals and housing conditions

In this study, we used 144 newborn male Wistar rats from the campus of Ribeirão Preto, University of São Paulo. The animals (weaned) were three weeks old, weighing 50 ± 10 g, at the beginning of the experiments. They were provided with food and water *ad libitum* and maintained in a colony in a temperature-controlled room (24 ± 1 °C) under a 12:12 h light–dark cycle (lights on at 07:00 a.m.).

2.2. Ethical and animal welfare issues

We declare that the present study received formal approval (protocol no. 16.5.738.59.4) from the Committee on Animal Research and Ethics (CEUA) of the University of São Paulo. Also, the experiments were conducted in agreement with the recommendations of the U. S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023) revised 1996. The number of animals used was the minimum required to allow for the reliability of the results. In keeping with accepted practice, every effort was made to minimize animal stress and suffering.

2.3. Drugs

The following drugs were used: KET hydrochloride (20, 40, and 80 mg/kg - Agener, Brasil), the atypical antipsychotic CLZ (10 mg/kg), and the glutamate receptors agonist NMDA (*N-Methyl-D-aspartate*, 7 nmol/0.2 µl – Sigma-Aldrich, USA). Physiological saline served as vehicle control for subcutaneous (sc.) KET. CLZ was obtained in a commercial form (Leponex, Novartis, Brazil), dissolved in a solution of 2% glacial acetic acid in isotonic physiological saline solution (0.9% sodium chloride), to obtain the required concentration of 10 mg/ml in a volume of 1 ml/kg body weight, and administered intraperitonially (ip.). The solution was cleaned with a syringe filter (Whatman GD/XP syringe filter nylon, pore size 20 µm, Maidstone, UK). The mix acetic acid/isotonic saline was used to control CLZ effects. NMDA was dissolved in phosphate-buffer saline (PBS), that was used to control NMDA effects. CLZ was administered ip. fifteen minutes before KET withdrawal trials. NMDA was locally administered (intra-PrL) five minutes before the test. The doses of drugs used were based on previous studies (Chatterjee et al., 2011; Nobre et al., 2004).

2.4. Implant surgery

For recording of the extracellular field potentials, one bipolar twisted electrode (12.0 mm length), made of stainless steel enameled wires (Plastic-One products, Roanoke, VA, USA), 150 µm in diameter, insulated except at the cross-section of the tip, was aimed at the CIC (−8.6 mm posterior, ± 1.5 mm lateral, −4.0 mm deep, relative to bregma). For the study with central infusions of NMDA, a stainless-steel cannula (22 G) was also implanted contralaterally in the PrL (3.00 mm posterior, ± 0.5 mm lateral, -2.0 mm deep, relative to bregma). Coordinates used were according to the atlas of Paxinos and Watson (2008). Animals were ip. anesthetized with a mix of 100 mg/kg KET/4.5 mg/kg xylazine (Agener, Brazil) and fixed in a digital stereotaxic frame. Lidocaine (20 mg/ml) was injected around the surgical field as a local complement to general anesthesia. Assemblies were fixed to the skull by means of acrylic resin and the three stainless steel screws. At the end of surgery, each animal received an intramuscular injection of a veterinary pentabiotic (120.000 UI, 0.2 ml) followed by an ip. injection of the anti-inflammatory and analgesic drug Banamine (flunixin meglumine, 2.5 mg/kg). The animals were allowed three days of post-surgery recovery.

2.5. Measuring collicular auditory-evoked potentials (AEPs)

KET administration and AEPs recording parameters were comparable to previous studies (Maxwell et al., 2006; Nobre, 2013). The very small electrical voltage AEPs were recorded from electrodes unilaterally implanted in the CIC. AEPs occurs in response to a repetitive stimulus along a specific brainstem auditory pathway and reflects neuronal activity in the auditory complex, mainly in the cochlear nucleus, superior olive, and IC (Long and Allen, 1984). Stimuli were pure tone clicks (50 ms duration; 3000 Hz square-wave pulses, 95 dB sound pressure level). AEPs were recorded after each of the 100 auditory stimuli as the voltage difference between the tips of the bipolar electrode (150 µm). This voltage difference was fed into an amplifier (TX001, Lynx, São Paulo, Brasil) through two noiseless shielded cables going through a hole in the roof of the Faraday cage and linked to a double-brush electric swivel (SL2C, Plastics-One, USA). The output of the amplifier was connected to an analog/digital converter (CAD 12/36) plugged into a microcomputer. The potential signals were sampled at a rate of 0.33 kHz and filtered (high-pass filter, 20 Hz, low-pass filter, 200 Hz). Filtering, amplification, and digitalization of the signals were performed by Sysdin system software (Lynx, São Paulo, Brazil). Sysdin software was set to sum up individual AEPs amplitudes. Average values were obtained at the end of the sessions. The data-acquisition sweep began 10 ms before the onset of the sound stimulus (latency to switch