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Dose–response characteristics of ketamine effect on locomotion, cognitive function and central neuronal activity

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Abstract

The present dose–response study sought to determine the effects of subanesthetic dosages (4–16 mg/kg) of ketamine on locomotion, sensorimotor gating (PPI), working memory, as well as c-fos expression in various limbic regions implicated in the pathogenesis of schizophrenia. In addition, we examined whether ketamine-induced locomotion was influenced by the dark/light cycle. We found that ketamine increased locomotor activity in a dose dependent manner, but found no influence of the dark–light cycle. Additionally, ketamine dose-dependently interrupted PPI, resulting in prepulse facilitation at doses of 8 and 12 mg/kg. The dose of 12 mg/kg also induced impairments in working memory assessed by the discrete-trial delayed-alternation task. C-fos expression indicated that the dose-dependent behavioral effects of ketamine might be related to changes in the activity of limbic regions, notably hippocampus and amygdala.

Keywords: Ketamine; Light/dark cycle; Hyperlocomotion; c-fos; PPI; Working memory; Schizophrenia; Limbic system

1. Introduction

A widely accepted and utilised psychopharmacological model of schizophrenia is based on the effect of non-competitive NMDA receptor antagonists, such as phencyclidine (PCP), dizocilpine (MK801) and ketamine [13,22].

A subanesthetic dose of ketamine produces several behavioural abnormalities in rodents such as disruption of sensorimotor gating (PPI) and working memory, which are analogous to the effects of ketamine in humans and mimic the symptoms of schizophrenic patients [14–16,18,31,33]. Such experimentally induced deficits provide a model with significant face, predictive and construct validity [14]. Ketamine and other NMDA antagonists also increase locomotor activity in rodents, but not in humans [14]. Thus, the relationship between rodent hyperlocomotion and schizophrenic symptoms lacks a proper degree of face validity [26]. However, because locomotor activity is associated with limbic-striatal function, ketamine-induced hyperlocomotion might be indirectly linked to schizophrenia since the latter is generally associated with limbic abnormalities [32].

The mechanism by which ketamine produces its adverse behavioural effects, at least partly, have been attributed to the blockade of NMDA receptors located on inhibitory GABAergic neurons limbic and subcortical brain regions [6,19,20]. This disinhibitory action leads to increase in the neuronal activity and excessive glutamate and dopamine release in the prefrontal cortex and limbic striatal regions [6–8,17,19]. In these studies, however, a higher dose range (10–35 mg/kg) was used, compared to the studies in which the behavioural effects of ketamine (2.5–12 mg/kg) were investigated [18,23,27,31]. Although Nishizawa et al. found a strong correlation between ketamine-induced locomotor responses and cell activity measured by c-fos expression, this study investigated only posterior cortical areas using considerably high doses of ketamine (20–50 mg/kg) [21]. Thus, the question arises which are the links between behavioural changes (hyperlocomotion and cognitive deficit) and brain activity induced by the lower doses of ketamine.

In Experiment 1, subanesthetic doses of ketamine (4–16 mg/kg) were used: (1) to characterise the nature and time course of its effect on the locomotion; (2) to define neuroanatom-

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ical regions where this treatment regime would affect neuronal activity assessed by c-fos expression; (3) and to investigate the correlation between behavioural changes and c-fos expression. Since rats are nocturnal animals, locomotor activity is more abundant in the dark phase of the dark/light cycle [34]. Nevertheless, behavioural studies investigating the locomotor activating effects of stimulants have been conducted during the light phase (i.e. the sleeping period) or do not mention this experimental conditions. Thus, we also compared the effects of ketamine on locomotion under different light conditions.

In a second study (Experiment 2) the dose–response characteristic of ketamine-evoked (4–12 mg/kg) cognitive deficits were assessed using two behavioral measures which are likely to have relevance to clinical symptomology: the prepulse inhibition of the startle reflex paradigm (PPI) and the discrete-trial delayed alternation task. PPI refers to the phenomenon that a weak stimulus (prepulse) reduces the behavioral response to a second, more intense, stimulus. PPI provides an operational measure of sensorimotor gating both in human and rodents [9]. The discrete-trial delayed alternation task is a working memory-related paradigm. Performance of this task depends on the functional integrity of the prefrontal cortex, and it is sensitive to the acute effects of psychostimulants [2].

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals

Twenty-five male Wistar rats (Harlan, The Netherlands) weighing 248 ± 8 g (5–6 weeks of age) at the start of the experiments were individually housed in plexiglas cages (45 cm × 28 cm × 20 cm) on a 12 h/12 h light/dark cycle (lights on at 7:00 h). After the second open field test, the light–dark cycle was reversed (lights on 19:00) and animals were allowed to acclimate to it for a period of 1 week. A piece of PVC tube (d = 8 cm, l = 17cm) was provided in the home cage as a shelter. Food (standard rat chow, Hopefarms) and water were available ad libitum. All animals were handled and weighed daily to minimise stress during the experiment. The Animal Ethics Committee of the University of Groningen approved the protocol (FDC: 2935).

2.1.2. Locomotor behaviour

Open field tests (OF) were performed under both day-light (sleep period) and red-light (active period) condition in order to compare the effect of ketamine (Sigma, Germany) on locomotion in the inactive period with the active period of the animals. The open field consisted of a circular black arena with a diameter of 1 m. Rats were placed in the centre of the field at certain time points, i.e. at 0, 20, 40, 60 and 90 min post saline or ketamine (4, 8, 12, 16 mg/kg) injection (s.c.) and observed for a period of 5 min. This test was repeated 4 times, each performed 1 week apart: every rat served as its own control therefore each received a saline injection followed by a ketamine challenge under both normal- and reversed-light condition (Table 1) Five animals received only saline during this protocol in order to get control brain activity for c-fos comparison. Locomotor behaviour was recorded with a videotracking system (Etho Vision, 1.96, Noldus Information Techonology, Wageningen, The Netherlands). The distance moved within the arena was analysed.

2.1.3. Immunohistochemistry

The rats were euthanized 2 h after the last ketamine injection using sodium pentobarbital anesthesia (1 ml, 6%) and perfused with 50 ml heparinised saline and 300 ml of 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer (pH 7.4). The brains were removed and postfixed in the same fixative overnight at 4 °C. Following an overnight cryoprotection in a 30% sucrose solution, serial 40 μm coronal sections of the cerebrum were made with a cryostat microtome and collected in 0.02 M potassium phosphate saline buffer (KPBS). Fos immunostaining was performed as described previously [34]. Briefly, after rinsing with 0.3% H_2O_2 for 10 min the sections were thoroughly washed with KPBS and incubated with rabbit anti-Fos antibody (1:10000; Oncogene Research Products, San Diego, CA, USA) diluted in 0.02 M KPBS with 0.25% Triton X-100 and 2% normal goat serum for 72 h at 4 °C. After washing, the sections were subsequently incubated for 2 h with biotinylated goat-anti-rabbit IgG (1:1000 in 0.02 M KPBS) and avidin–biotin–peroxidase complex (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA, USA). After thorough washing, the peroxidase reaction was developed with DAB-nickel solution and 0.1% H_2O_2. Sections were washed for 15 min in buffer and mounted with gelatine solution and air-dried, dehydrated in graded alcohol and xylol solutions and then coverslipped with DePeX mounting medium (BDH).

In order to avoid differences in staining intensities, sections were stained according to the same procedure. The c-fos-labeled cells were quantified using computerized image analysis system by an observer who was blind to group assignment. The area of the region of interest (ROIs) was digitized using a Sony (SONY Corporation, Tokyo, Japan) chargecoupled device digital camera mounted on a LEICA Leitz DMRB microscope (Lecia Qwin version 2.3, Leica Microsystems Imaging Solutions, Wetzlar, Germany). The number of immunopositive nuclei was quantified in a single focus plane (at ×100) for each ROIs, which were outlined with digital pen and each digitized image was individually set at a threshold to subtract the background optical density. The average of immunoreactive cells was expressed as number of positive nuclei/0.1 mm^2.

2.2. Experiment 2

2.2.1. Animals

Twenty-four male Wistar rats (Harlan, The Netherlands) weighing 210 ± 5 g (4–5 weeks of age) at the start of the experiments were individually housed in plexiglas cage (45 cm × 28 cm × 20 cm) with a 12 h/12 h light/dark cycle (lights on at 7:00 h). A piece of PVC tube (d = 8 cm, l = 17cm) was provided in the home cage as a shelter. All animals were handled and weighed daily to minimise handling stress during the experiment. Food was available ad libitum 2 weeks following arrival, after which they were placed on restricted diet of 15 g per day per rat. The rats had ad libitum access to water throughout the duration of the experiment. The Animal Ethics Committee of the University of Groningen approved the protocol (FDC: D4116A).

2.2.2. Prepulse inhibition of the startle reflex (PPI)

After 1 week of acclimatization PPI tests were conducted using a TSE Startle Response Measuring System (Technical and Scientific Equipment GmbH, Bad Homburg, Germany). The rats were restrained in a small cage (27 cm × 10 cm × 12.5 cm) and placed on a transducer platform that registers movement. Acoustic stimuli were generated by means of high-quality high-linearity speakers situated on both sides of the cage. The whole setup

Table 1
Experimental protocol for open field-locomotor behaviour