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Effect of ketamine on exploratory behaviour in BALB/C and C57BL/6 mice

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ABSTRACT

In this study, we evaluated the effect of ketamine on exploratory locomotion behaviours in the Balb/c and C57BL/6 strains of mice, which differ in their locomotion behaviours.

Intraperitoneal administration of ketamine at three different doses (1, 5 or 10 mg/kg, 0.1 ml/10 gr body weight) was performed on adult male Balb/c and C57BL/6 mice. The same volume of saline was applied to the control group. The open-field and elevated plus maze apparatus were used to evaluate exploratory locomotion.

In the open-field test, Balb/c mice less spend time in the centre of the field and was decreased locomotor activity compared to C57BL/6 mice ($p<0.01$). Ketamine treatment of Balb/c mice at 10 mg/kg dose caused an increase in locomotor activity and an increase in the amount of time spent in the centre in the open-field test, compared to the control group ($p<0.05$). In C57BL/6 mice, ketamine treatment (1 and 10 mg/kg) decreased locomotor activity ($p<0.05$). In C57BL/6 mice, the three different doses of ketamine application each caused a decrease in the frequency of centre crossing ($p<0.001$) and the spent time in the centre ($p<0.05$).

In the elevated plus maze, the number of open-arm entries, the percentage of open-arm time and total arm entries were decreased in Balb/c mice compared to C57BL/6 mice ($p<0.001$). Ketamine treatment of Balb/c mice at 10 mg/kg dose caused an increase in the open-arm activity ($p<0.001$). Ketamine application (10 mg/kg) decreased the open-arm activity in C57BL/6 mice ($p<0.05$).

A subanaesthetic dose of ketamine increased exploratory locomotion in Balb/c mice. In contrast, a subanaesthetic dose of ketamine decreased exploratory locomotion in C57BL/6 mice. In conclusion, hereditary factors may play an important role in ketamine-induced responses.

1. Introduction

The non-competitive receptor antagonist ketamine has been used in paediatric and adult patients as a short-term dissociative anaesthetic agent since the 1960s (Annetta et al., 2005). Ketamine application causes behavioural and neurochemical effects in humans and experimental animals (Dunn et al., 1989; Krystal et al., 1994; Lindefors et al., 1997; Silvestre et al., 1997; Babar et al., 2001; Zarate et al., 2006). In clinical and experimental studies, subanaesthetic doses of ketamine affected to locomotor behaviours (Imre et al., 2006; Mandryk et al., 2005). Previous studies suggest that ketamine can act through NMDA receptors as well as the serotonergic, dopimanergic and noradrenergic systems (Lannes et al., 1991; Lindefors et al., 1997; Duncan et al., 1998). It has been suggested that ketamine-induced locomotor behaviour may connected with all above systems.

NMDA receptor antagonists can affect local dopamine and serotonin receptors by indirectly regulating cortical GABA release in mice (Lindefors et al., 1997). Low doses of ketamine, phencyclidine and MK-801-like NMDA receptor antagonists have been shown to increase the level of extracellular dopamine in the prefrontal cortex (Hondo et al., 1994; Verma and Moghaddam, 1996) and in the extracellular 5-HIAA level of the frontal cortex (Löscher and Hönack, 1992). A decrease in the level of glutamic acid decarboxylase mRNA has been observed following MK-801 application, along with a decrease in GABA synthesis (Laprade and Soghomonian, 1995).

Rodents naturally experience fear and anxiety when exposed to a novel open field, which causes a behavioural response (Dolu and Özemsi, 2004; Bourin et al., 2007). The open field test has been used to evaluate locomotor activity and anxiety. Different strains of mice show different anxiety and locomotion responses in open-field tests (Carola et al., 2002; Tang et al., 2002). Balb/c mice have been found to exhibit decreased exploratory behaviours compared to C57BL/6 mice in an open-field test (Tang et al., 2002).

The elevated plus maze, has been extensively used in rodents as a test of fear, anxiety and exploratory behaviour (Lister, 1987; Benattia et al., 2011; Moreira et al., 2007). Previous research has shown that differences exist between exploratory and anxiety-like behaviours in the elevated plus maze of C57BL/6 and BALB/c. For example,

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some investigators have reported that Balb/c mice show higher levels of anxiety-like behaviours and lower of exploratory behaviours in the elevated plus maze than C57BL/6 mice (An et al., 2011). In addition to environmental factors, some studies have explored the genetic component of these effects and suggest that anxiety levels might be hereditary (Cle'ment et al., 2002).

In this study, we evaluated the effects of an NMDA receptor antagonist, using different subanaesthetic doses of ketamine (1 mg/kg, 5 mg/kg or 10 mg/kg), on exploratory locomotion in different strains of mice (Balb/c and C57BL/6).

2. Materials and methods

The experimental protocols were confirmed by the Local Ethics Committee of the Çukurova University Medical Sciences Experimental Search and Application Center. The procedures in the study were in accordance with the NIH Guide for Care and Use of Animals.

2.1. Animals

In this study, two different strains of mice (Balb/c and C57BL/6) were used and were bred in the Çukurova University Faculty of Medicine Physiology Laboratory. Adult (8–10 week-old) male mice (body weight 31.8 ± 0.4 for Balb/c mice, 29.2 ± 0.4 for C57BL/6 mice) were used for behavioural tests. Food and water were given without restriction. The room temperature was fixed at 21 ± 2 °C, and behavioural tests were performed at this temperature. Animals were housed in a room with a 12-hour light/dark cycle ($05^{:00}$–$17^{:00}$ light, $17^{:00}$–$05^{:00}$ dark). Behavioural tests were performed between $9^{:00}$–$12^{:00}$. Hand and room adaptations were applied to animals before behavioural tests were performed.

2.2. Ketamine treatment

Ketamine hydrochloride (Ketasol, Richter Pharma AG, 100 mg/ml) diluted in saline (% 0.9 NaCl) was applied to mice via intraperitoneum injection at doses of 1 mg/kg, 5 mg/kg and 10 mg/kg (at a volume/body weight ratio of 0.1 ml/10 gr) 15 min before behavioural testing. The same volume of saline was injected into the control group.

2.3. Apparatus

2.3.1. Open-field

The open-field apparatus used in the behavioural tests measured 60 × 60 × 24, which was made of black plexiglass, had an open top and a base that was divided into thirty-six squares and was enclosed by a 1 cm thick wall, forming a square box. The squares next to the wall were designated as “peripheral” and the others were designated as “centre.” Peripheral fields are safe and protected, whereas the centre field is nonprotective in the open-field apparatus. In open-field experiments, exploratory locomotor activity were measured using the following criteria: the distance travelled in the centre and peripheral fields, spending time in the centre versus the peripheral field, frequency of centre crossing, latency of entering the centre and frequency of defecation (Carola et al., 2002; Kinsey et al., 2007; Prut and Belzung, 2003). The frequency of rearing (vertical activity) was evaluated as well (Prut and Belzung, 2003). For the open-field test, mice were treated with ketamine or saline 15 min before the test and were placed anywhere in the apparatus. Behaviours were recorded for ten minutes using a video camera. The open-field apparatus was illuminated by 165 lx.

2.3.2. Elevated plus maze

The elevated plus maze consisted of two perpendicular open arms (30 cm × 5 cm) and two closed arms (30 cm × 5 cm × 15 cm) also in perpendicular position. The open and closed arms were connected by a central platform (5 cm × 5 cm). The maze was made of black plexiglass and was 40 cm above the floor. After treatment, the animal was placed at the centre of the plus-maze facing the enclosed arm, and observed for 5 min. In the elevated plus maze, exploratory locomotor activity were evaluated. The elevated plus maze was illuminated by 165 lx (the open arms). A video camera was used to monitor the animal's behaviour. The maze apparatus was cleaned after each trial. The following parameters were registered: number of entries in the open arm, total entries (open and closed arm) and time of permanence in the open arm. The percentage of time spent in the open arms was calculated. The animals were randomly ordered for testing. The person scoring the behaviour was blind to the treatment (Carola et al., 2002).

2.4. Statistics

Data values are expressed as mean ± S.E. For statistical analyses of strain versus drug interaction, two-way ANOVA was applied using the SPSS 11.5 computer programme. One-way ANOVA followed by the Tukey HSD test was used for comparisons between the groups. The Kruskal–Wallis test was applied to determine the normal distribution or variance of groups that were not homogenic. The Mann–Whitney-U test was applied for comparisons within the group. Significance levels were set at $p<0.05$.

3. Results

3.1. Open-field test

In the open-field test, the time spent in the centre and periphery of the apparatus, a two-way ANOVA-significant strain effect [$F(1,73)=5$ $p<0.05$)] and a strain × drug interaction were confirmed [$F(1,73)=34.02$ $p<0.001$)], whereas a drug effect was not confirmed [$F(1,73)=0.7$ $p>0.05$)]. The time spent in the centre of the apparatus was decreased in Balb/c mice compared to C57BL/6 mice, with a corresponding increase in the time spent in the periphery ($p<0.001$). In Balb/c mice, treatment with 5 mg/kg and 10 mg/kg doses of ketamine caused an increase in the time spent in the centre and a decrease in the time spent in the periphery compared to control animals ($p<0.05$). In C57BL/6 mice, application of 1 mg/kg, 5 mg/kg and 10 mg/kg doses ketamine caused a decrease in the time spent in the

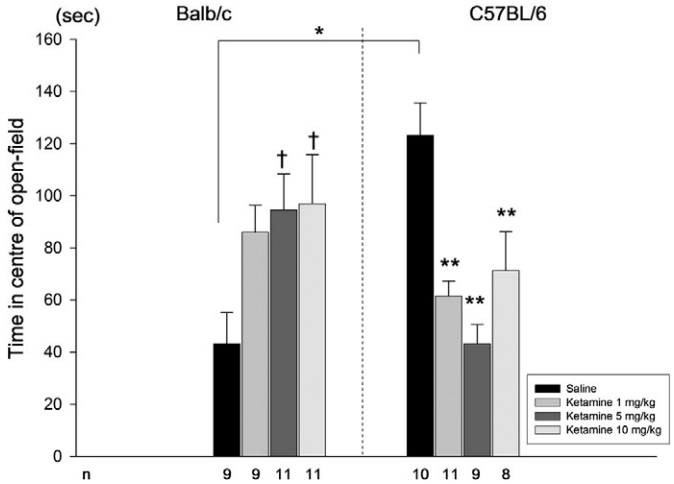

Fig. 1. Time in the centre of the open field (s). Data are expressed as mean ± SE. **$p<0.05$, *$p<0.001$ compared to saline-treated C57BL/6 mice and †$p<0.05$ compared to saline-treated Balb/c mice. Statistical analysis included a one-way ANOVA analysis followed by the Tukey HSD test.