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Effects of ketamine on different types of anxiety/fear and related memory in rats with lesions of the median raphe nucleus

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Abstract

The aim of the present study was to determine the involvement of the median raphe serotonergic system in the effects of ketamine on anxiety behaviours and related memory. The effects of ketamine pretreatment (3 and 10 mg/kg, i.p.) on three types of fear-motivated behaviours, unconditioned one-way escape, conditioned avoidance and freezing were tested. Experiments were performed with the inhibitory avoidance apparatus in rats with ibotenic acid lesions of the median raphe nucleus. It was found that 10 mg/kg ketamine had an anxiogenic-like effect on one-way escape type of fear and anxiolytic-like effect on conditioned freezing-related fear; these effects were unaffected by median raphe lesions. Both ketamine doses impaired freezing-related fear memory. Ketamine (10 mg/kg) also produced an anxiolytic-like effect on avoidance type of fear and impaired avoidance memory. The median raphe lesions attenuated the anxiolytic action of the drug on the avoidance type of fear and prevented ketamine-induced avoidance memory impairment. These results suggest that the anxiolytic-like effect of ketamine on avoidance-type fear is mediated through the median raphe serotonergic system. © 2001 Published by Elsevier Science B.V.

Keywords: Ketamine; Median raphe nucleus; 5-HT (5-hydroxytryptamine, serotonin); Anxiety; Fear; Memory

1. Introduction

Ketamine, a short-acting dissociative anaesthetic agent, produces psychotomimetic actions (Kamaya and Krishna, 1987; Krystal et al., 1994; Newcomer et al., 1999) and memory-disturbing effects in humans and animals (Ghoneim et al., 1985; Sharma and Kulkarni, 1991; Uchihashi et al., 1994; Lahti et al., 1995; Adler et al., 1998). Although ketamine has shown an anxiogenic-like profile (Silvestre et al., 1997), several clinical studies showed subanaesthetic doses of ketamine to have anxiolytic-like effects on anxiety/fear-related behaviours (Sappington et al., 1979; Gutstein et al., 1992; Roelofse et al., 1996; Diaz, 1997). For instance, Sappington et al. (1979) demonstrated that ketamine reduced the negative affect experienced during stressful situations in the human.

It is well known that ketamine is a non-competitive NMDA glutamate receptor antagonist (Anis et al., 1983; Thomson et al., 1985). Anti-anxiety effects of NMDA receptor antagonists have been demonstrated in animal models for anxiety (Koek and Colpaert, 1991; Xie and Commissaris, 1992; Plaznik et al., 1994; Wiley, 1997). Apart from NMDA-mediated actions of ketamine, there are several reports showing the influence of the drug on monoaminergic systems (Ylitalo et al., 1976; Irifune et al., 1997; Lindefors et al., 1997; DePetrillo et al., 2000). Although ketamine inhibits synaptic serotonin (5-HT) uptake (Smith et al., 1981; Martin et al., 1990), little is known concerning ketamine effects on anxiety-related behaviours depending on the 5-HT-ergic systems (Gandolfi et al., 1990; Suzuki et al., 1999).

It is widely recognised that the dorsal and median raphe nuclei of the brain stem consist of two major ascending 5-HT-ergic pathways of the forebrain (Azmita and Segal, 1978; Mokler et al., 1998) and are implicated in the regulation of different kinds of emotional states and behaviours (Srebro and Lorens, 1975; Gryer et al., 1976), including anxiety and related memory (Iversen, 1984; Handley and McBlane, 1993; Graeff et al., 1996; Melik et al., 2000; Treit et al., 2001). While some studies showed the possible anxiogenic feature of the dorsal raphe 5-HT-

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ergic system (Wise et al., 1972; Iversen, 1984; Gargiulo et al., 1996), clinical observations have shown that drugs which enhance 5-HT neurotransmission, such as antidepressants or 5-HT uptake inhibitors, attenuate many types of anxiety disorders (Nutt, 1991; Lopez-Rubalcava, 1996; Pine, 2001). It is hypothesised that the median raphe nucleus–hippocampal system attenuates anxiety (Graeff et al., 1996). It is reasonable to assume that the effects of ketamine on some types of anxiety/fear-related behaviours may be mediated by the median raphe 5-HT-ergic system. Therefore, the present study investigated the effects of subanaesthetic doses of ketamine (3 and 10 mg/kg) on different types of anxiety-related behaviours, such as unconditioned one-way escape fear, conditioned freezing and avoidance types of fear seen in the inhibitory avoidance apparatus in rats with neurotoxic lesions of the median raphe nucleus.

2. Materials and methods

2.1. Animals

Adult male Wistar rats, weighing 220–250 g, were used in this study. The animals were housed five per wire cage with free access to food and water. They were maintained under a 12-h light–dark (lights on at 0700 h) illumination cycle. The animals were handled and allowed to adapt to the experimental room. The ethics committee of the university approved the experiments.

2.2. Apparatus

All anxiety-related behaviours were assessed in the inhibitory avoidance apparatus, which consists of two compartments, separated by a sliding guillotine door and connected by a hole (7×7 cm, $w \times h$). The testing compartment ($17 \times 25 \times 17$ cm: $w \times l \times h$) had transparent walls and was illuminated with a 60-W light bulb placed 36 cm above the floor. The dark-shock compartment was an opaque box, $27 \times 27 \times 27$ cm. The shock compartment had a grid floor made of parallel stainless steel rods (3 mm in diameter) spaced 1-cm apart. A shock generator (Champden Inst., USA) was connected to the steel rods and provided scrambled footshock.

2.3. One-way escape task

The first 3 days of the experiment, the rats were placed in the shock compartment of the avoidance apparatus for 180 s/day with free access to the testing compartment for adaptation. On the trial day, the rats were confined for 3 s to the shock compartment and then a 1.0-mA, single-trial scrambled shock was applied to the grid floor for 2 s. Immediately after the shock, the slide door was raised, so that the rat could pass to the illuminated compartment. The time taken to get from the dark compartment to the illuminated compartment was measured as escape latency.

2.4. Avoidance and freezing responses

On the trial day, the animals from this group were placed in the illuminated compartment and then the slide door was raised. The time to enter the shock compartment was considered as entry latency, taken as measure of spontaneous preference for the dark compartment. After the rats had spontaneously entered the shock compartment, the slide door was lowered and after a 3-s delay, a 1.0-mA scrambled shock was applied to the grid floor for 2 s. After a 3-s delay, the rats were removed from the shock compartment and immediately placed in the illuminated compartment and observed for 180 s. Forty eight hours after the shock, the same rats were again placed in the illuminated compartment and observed for 300 s. Immediately ("drug" day) and 48 h after the shock (drug-free day), avoidance and freezing responses of the same rat were tested in the illuminated compartment with free access to the shock compartment without shock. The avoidance response was assessed from the step-through latency which was defined as the time it took an animal, the first time, to place four paws on the floor of the shock compartment. Freezing response was assessed from samples scored as freezing, which was defined as the lack of any observable movements of the body and vibrissae, except those related to respiration for 10 s. Freezing behaviour was recorded by using a time-sampling procedure (Fanselow, 1980). All other behaviours were scored as general activity. All experiments were carried out in the dark room by an observer who was ignorant of the treatment. The avoidance apparatus was cleaned with 5% alcohol solution and tap water after each subject.

2.5. Lesion surgery

The rats were randomly assigned to two groups, sham-lesion and median raphe-lesion. The animals were anaesthetised with pentobarbital sodium (40 mg/kg, intraperitoneally i.p). Neurotoxin, ibotenic acid (Sigma I2765), was dissolved in Locke's solution (without glucose) and injected stereotaxically through a 24-gauge cannula in a volume of 2 μl (containing 2 μg ibotenic acid) into the median raphe nucleus. The following coordinates were used: PA: 350–160 μm; L = 00.00; and Ventral 8000 μm from the surface of the skull, derived from the stereotaxic atlas of König and Klippel (1963). The infusion was made with a micropump (Cole Palmer, Model 210) over 10 min and the needle was withdrawn 5 min after completion of the infusion. Sham-lesion was carried out in the same way except 2 μl of Locke's solution was injected into the median raphe nucleus instead of ibotenic acid. The animals were allowed to recover for 20 days following these surgical procedures.

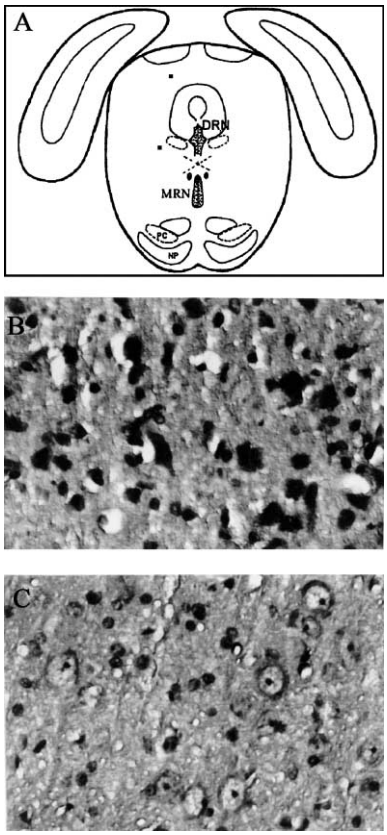

Fig. 1. Illustration of the position of the median raphe nucleus region of the brain stem according to König and Klippel rat brain atlas (A). Microphotograph showing undamaged brain tissue in the median raphe nucleus region of sham-lesioned animal (B) and severe polygonal cell body loss in the sample field taken at the ibotenic acid infusion site in a median raphe-lesioned animal (C). Haematoxylin–eosin ×800. Abbreviations: DRN, dorsal raphe nucleus; MRN, median raphe nucleus; NP, nucleus pontis, PC, pedinculus cerebri.

2.6. Drug

Ketamine was tested at the doses of 3 and 10 mg/kg i.p. Ketamine hydrochloride (Ketalar, Parke-Davis, 50 mg/kg) was used as the commercial preparation and further diluted with saline to the appropriate concentration, so that the desired dose could be given in a volume of 1 ml/kg/body weight. Saline (0.9% NaCl) in a volume of 1.0 ml/kg i.p was also given as control for conditioning and administration effects. Saline or ketamine was given 15 min before the behavioural procedure.

2.7. Histological analysis

Lesion placement was verified as described in a previous paper (Melik et al., 2000). Briefly, rats were perfused transcardially under deep pentobarbital anaesthesia with isotonic saline followed by a 10% formalin solution. Coronal sections were stained with haematoxylin–eosin. Histological analysis revealed that the ibotenic acid-infused rats showed polygonal cell body loss in the median raphe nucleus region, whereas they were mostly preserved in the sham control (Fig. 1).

2.8. Statistics

The data for latencies are expressed as group means ± standard error of the mean (S.E.M.). The samples scored as freezing for entire testing period were expressed as mean percentages ± S.E.M. The statistical significance of differences between groups was analysed by using two-way analysis of variance (ANOVA). When the overall ANOVA was significant, Post hoc testing was done with the Newman–Keuls test. The results were considered significant when $P < 0.05$.

3. Results

All 84 animals used in the experiments were considered for analysis ($n = 5$ per group for the one-way escape task; $n = 9$ per group for avoidance task). ANOVA on the data for one-way escape latency revealed a significant effect of ketamine ($F(2,24) = 9.6$, $P < 0.001$). As shown in Fig. 2, ketamine (10 mg/kg) reduced the time for intact and median raphe-lesioned animals to leave the shock compartment (all $P < 0.05$), which indicated an increase in unconditioned fear. ANOVA showed significance for neither the lesion effect ($F(1,24) = 0.008$, P n.s.) nor the lesion × drug interaction ($F(2,24) = 0.16$, P n.s.).

The data for freezing in the illuminated part of the inhibitory avoidance apparatus is shown in Fig. 3. ANOVA showed a significant effect of ketamine on freezing tested on a "drug" day ($F(2,48) = 21$, $P < 0.0001$) and drug-free day ($F(2,48) = 20$, $P < 0.0001$). Post hoc analyses revealed that in sham-lesioned animals, 10 mg/kg ketamine produced a significant decrease in freezing on "drug" day ($P < 0.001$); the 3 and 10 mg/kg doses of ketamine

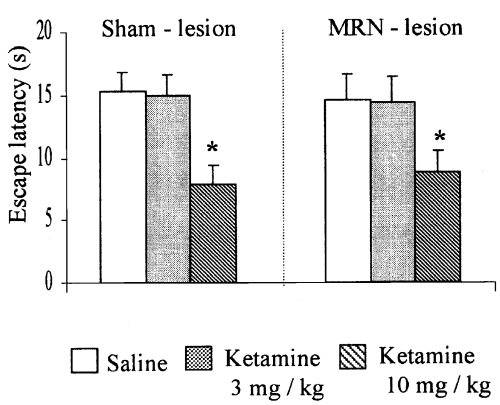

Fig. 2. The effects of ketamine (3 and 10 mg/kg i.p.) given 15 min before training on one-way escape latency in sham- and median raphe-lesioned rats. Bars represent means (±S.E.M.). $n = 5$ per group. The asterisks indicate significant differences from the corresponding saline control ($^{*}P < 0.05$).

reduced freezing on drug-free day ($P < 0.01$ and $P < 0.001$, respectively). ANOVA showed significance for neither the lesion effect on freezing immediately after the shock ($F(1,48) = 0.08$, P n.s.) nor the lesion × drug interaction ($F(2,48) = 1.31$, P n.s.). There was a significant lesion effect on freezing tested 48 h after the shock ($F(1,48) = 38$, $P < 0.0001$) and a significant lesion × drug interaction ($F(2,48) = 11.5$, $P < 0.0001$). These results indicated that median raphe lesions impair freezing-related fear memory without affecting acquisition. In the median raphe-lesioned animals, ketamine (10 mg/kg) produced a significant reduction of freezing on "drug" day ($P < 0.01$), but on drug-free day, there was no significant difference between the ketamine-treated groups and the saline control.

Fig. 4 shows the mean latencies for inhibitory avoidance in each group. As illustrated in the figure, entry latencies were quite similar in all experimental groups. One-way ANOVA failed to show any significant between-group differences ($F(5,48) = 0.52$, P n.s.), indicating behavioural uniformity of the groups. ANOVA on step-through latencies on "drug" day and drug-free day showed significant ketamine effects ($F(2,48) = 28$, $P < 0.0001$; $F(2,48) = 12$, $P < 0.0001$, respectively). Post hoc analyses indicated that, in sham-lesioned rats, 10 mg/kg ketamine produced dramatic decreases in step-through latencies on "drug" day and drug-free day compared to the saline treatment (all $P < 0.001$). ANOVA showed no lesion effects on this parameter immediately or 48 h after the shock ($F(1,48) = 3.51$, P n.s.; $F(1,48) = 0.34$, P n.s., respectively) and significant lesion × drug interactions ($F(2,48) = 3.51$, $P < 0.05$; $F(2,48) = 8.9$, $P < 0.001$, respectively). As shown in Fig. 4, 10 mg/kg ketamine pretreatment significantly prolonged step-through latencies on "drug" day and drug-free day in median raphe-lesioned animals compared to the sham control ($P < 0.01$ and $P < 0.001$, respectively); indicating that median raphe lesions attenuate the ketamine-induced impairment in the acquisition of avoidance-type fear and prevented the impairing effects of the drug on avoidance memory storage.

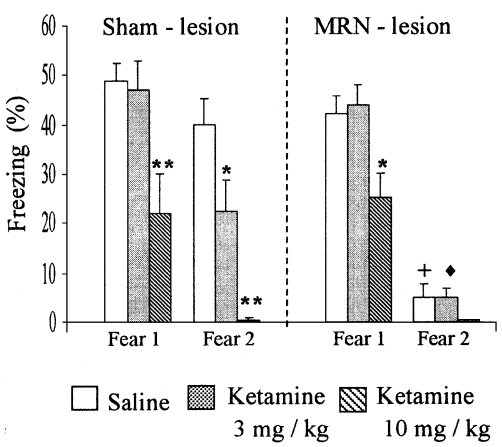

Fig. 3. The effects of ketamine (3 and 10 mg/kg i.p.) given 15 min before training on freezing responses observed in the illuminated part of the avoidance apparatus in sham- and median raphe-lesioned rats. Bars represent mean percentages (±S.E.M.). The significance of differences was determined by ANOVA; $n = 9$ per group. Fear 1 was measured immediately after the shock ("drug" day), Fear 2 was measured 48 h after the shock. $^{*}P < 0.01$, $^{**}P < 0.001$ as compared to the corresponding saline control; $+ P < 0.001$, ♦ $P < 0.01$ as compared to the corresponding sham control.

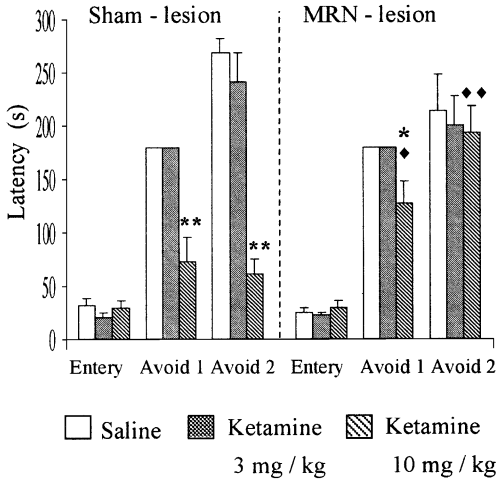

Fig. 4. The effects of ketamine (3 and 10 mg/kg i.p.) given 15 min before training on step-through latencies in sham- and median raphe-lesioned rats. Bars represent means (±S.E.M.). The significance of differences was determined by ANOVA; $n = 9$ per group; Avoid 1 was measured immediately after the shock ("drug" day) and Avoid 2 was measured 48 h after the shock. $^{*}P < 0.05$, $^{**}P < 0.001$ as compared to the corresponding saline control; ♦ $P < 0.01$, ♦♦ $P < 0.001$ as compared to the corresponding sham control.

4. Discussion

The findings of this study showed that, in intact animals, ketamine pretreatment enhanced the unconditioned escape type of fear, an effect considered as an anxiogenic-like action (Gargiulo et al., 1996) and attenuated conditioned freezing as well as the avoidance type of fear with memory storage impairment, effects considered as reductions in anxiety/fear and thus anxiolytic-like effects of a drug (Wiley, 1997). However, in the present study, we demonstrated for the first time that the median raphe lesions attenuated the ketamine-induced anxiolytic-like effect on the avoidance type of fear and prevented the impairing effect of the drug on avoidance memory storage.

Considering the effect of ketamine on one-way escape-type fear, which is triggered by native stressors and representative of innate, panic fear in the human (Gargiulo et al., 1996), it was found that 10 mg/kg ketamine enhanced anxiety in both intact and lesioned animals, an effect that was confirmed by a significant reduction in escape latency. This indicates that the median raphe system is not involved in the anxiogenic-like effect of ketamine. Earlier, Silvestre

et al. (1997) showed an anxiogenic-like action of ketamine on unconditioned fear in a non-conflict test, such as the elevated plus-maze. It is possible that the anxiogenic-like effect of ketamine is due to the potentiation of innate panic responses to native stressors rather than to the enhancement of pain sensitivity; otherwise, it would be difficult to explain the anti-anxiety effect of the drug on fear which is motivated by meta-stressors, as observed in our study. Based on results of the studies suggesting that unconditioned escape fear results in activation of 5-HT transmission within the dorsal raphe system (Kawahara et al., 1993; Maswood et al., 1998; Early et al., 1990; Maier et al., 1995), it is possible that ketamine exerts anxiogenic-like effects on the unconditioned one-way escape type of fear through the dorsal raphe system.

In the inhibitory avoidance test, ketamine reduced freezing in intact and median raphe-lesioned animals on "drug" day. The lack of a difference in freezing memory responses between ketamine and saline treatment in median raphe-lesioned animals might reflect a ceiling effect. Thus, results indicate that the anxiolytic-like effect of ketamine on the freezing type of fear is not dependent on the median raphe system. As noted above, ketamine decreased escape latency, a finding that precluded the analgesic effect of the drug as a cause of an anxiolytic-like action. Therefore, an anxiolytic-like effect of freezing-related fear can be explained by changes in the evaluation of meta-stressors. It is well recognised that NMDA receptors in the amygdala and hippocampus mediate expression and memory storage of the freezing type of fear in response to the meta-stress contextual cues (Fanselow and Kim, 1994; Maren and Fanselow, 1996; Plaznik et al., 1994; Xie and Commissaris, 1992). There is a possibility that the anxiolytic-like effects of ketamine on the freezing type of fear and related memory could be linked by the blockade of NMDA-receptors in these structures.

In the median raphe-lesioned animals, in contrast to its effect on freezing-related fear, ketamine failed to exert a marked anxiolytic action on the avoidance type of fear and did not affect avoidance memory storage. These results indicate that the median raphe nucleus is involved in the anxiolytic-like effects of ketamine on the avoidance type of fear. Histological findings in the present study showed that ibotenic acid infusion into the median raphe nucleus resulted in the loss of most of the large polygonal cell bodies which are shown to be 5-HT-ergic (Taber et al., 1960); and axons of these cells project mainly to the septo-hippocampal formation (Roberts et al., 1998). Drugs which increase 5-HT release have been reported to impair acquisition and memory storage of avoidance fear (Nutt, 1991; Lopez-Rubalcava, 1996). Electrolytic lesion of the median raphe in rat enhanced the ulcerogenic effect of restraint, whereas intrahippocampal microinjection 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) that leads to increase 5-HT release, reduced conditional anxiety in the elevated plus-maze as measured 24 h after restraint (Graeff et al., 1996). These findings provide the evidence that the ketamine-induced anxiolytic-like effect on avoidance-type fear is mediated through 5-HT-ergic transmission in the median raphe system.

It is considered that both avoidance and freezing conditioning are representative of an anticipatory/generalised type of fear in the human (Gargiulo et al., 1996). Hence, the present findings suggest that ketamine attenuates anticipatory fear through the median raphe 5-HT-ergic system.

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