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Effect of MS-153 on the acquisition and expression of conditioned fear in rats

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Abstract

Pavlovian fear conditioning is one of the most extensively studied and reliable behavioral paradigms used to investigate the mechanisms involved in fear and anxiety. Increased glutamatergic neurotransmission may play an important role in mediating fear conditioning. The present study assessed whether (*R*)-(−)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153), a novel cerebroprotective agent that inhibits the release of glutamate and enhances glutamate uptake, affects the acquisition and expression of conditioned fear. The rats received administration of MS-153 (i.p.) at 3, 10, and 30 mg/kg, 30 min before footshock and 24 h after footshock. Freezing behavior was measured in the chamber where they had previously received footshock for the acquisition experiments. For the expression experiments, the rats received MS-153 (i.p.) at the same doses 23.5 h after footshock and 30 min before expression testing. MS-153 significantly attenuated the acquisition and expression of freezing behavior. In addition, MS-153 administration did not affect locomotor activity. The present results suggest that extracellular glutamate is involved in fear conditioning, and that MS-153 has an anxiolytic effect by decreasing endogenous glutamate neurotransmission.

Keywords: MS-153; Contextual fear conditioning; Glutamate uptake and release; NMDA receptor antagonist; Fear and anxiety

1. Introduction

Understanding the neuronal basis of fear and anxiety is very important for developing strategies to treat and cure anxiety disorders. Pavlovian fear conditioning is one of the most extensively studied and reliable behavioral paradigms used to investigate the mechanisms involved in fear and anxiety. In contextual fear conditioning, a type of Pavlovian conditioning, a neutral conditioned stimulus, such as an experiment chamber, is paired with an aversive unconditioned stimulus, usually footshocks. The conditioned stimulus then rapidly acquires aversive properties and elicits a series of motor behaviors, such as freezing and autonomic responses, when the animal is again placed in the chamber.

A great deal of evidence has shown that some brain areas, predominantly the amygdala, hippocampus, and prefrontal cortex, contain the neural circuits responsible for the acquisition and expression of Pavlovian fear conditioning (for review, see Fanselow, 2000). The cellular mechanism underlying this kind of emotional learning is suggested to be *N*-methyl-D-aspartate (NMDA) receptor-dependent long-term potentiation (Collingridge and Bliss, 1987; Rison and Stanton, 1995; Rogan and LeDoux, 1995; Rogan et al., 1997; Maren, 1999). This idea has been supported by numerous studies reporting that intraamygdala or hippocampal infusions of the competitive NMDA receptor antagonist, 2-amino-5-phosphonopentanoic acid (APV) (Miserendino et al., 1990; Campeau et al., 1992; Fanselow and Kim, 1994; Young et al., 1994; Maren et al., 1996), or the noncompetitive NMDA receptor antagonist, dizocilpine maleate (MK-801) (Zhang et al., 2001), effec-

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tively blocked the acquisition and the expression (Maren et al., 1996; Lee and Kim, 1998) of fear conditioning. In addition, infusion of the non-NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Kim et al., 1993), or 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (*F*)-quinoxaline (NBQX) (Walker and Davis, 1997) into the basolateral amygdala diminished the expression of fear conditioning.

The involvement of glutamatergic neurons in conditioning fear is also supported by the finding that, as measured with the glutamate, concentration is increased in the lateral amygdala in fear conditioning animals by measuring paired-pulse depression (PPD) or paired-pulse facilitation (PPF), which are measures of transmitter release (Zinebi et al., 2002; McKernan and Shinnick-Gallagher, 1998). Furthermore, fear conditioning induced a lasting increase in glutamate release in dentate gyrus synaptosomes in the hippocampus (Redini-Del Negro and Laroche, 1993). From the above evidence, it is conceivable that decreasing the overall extracellular concentration of glutamate in the central nervous system could affect fear conditioning.

MS-153 [(*R*)-(−)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153)] is a novel cerebroprotective agent that has been shown to inhibit glutamate release as well as enhance glutamate uptake (Umemura et al., 1996; Shimada et al., 1999). MS-153 reduces neuronal damage in the rat focal cerebral ischemia model, probably by specifically reducing the amount of extracellular glutamate due to enhanced uptake through glutamate transporters (Shimada et al., 1999).

It is therefore interesting to investigate whether MS-153 has the potential to affect fear conditioning in the light of its ability to diminish the amount of extracellular glutamate. So far, no studies of the effect of MS-153 on fear conditioning have been published. The present study aimed to investigate this hypothesis.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan), weighing 290–310 g at the time of testing, were housed in groups of four per cage and maintained on a 12-h light–dark cycle (light phase: 0630–1830 h), in a temperature-controlled environment (22±1 °C) with free access to food and water. Experiments began after a 1-week period of acclimatization. All experiments were performed between 0800 and 1300 h. In total, 114 rats were used in this study, of which 44 rats were used for acquisition experiments, 40 rats for expression experiments, and 30 rats for measurement of locomotor activity.

2.2. Drugs

MS-153 (a gift from the Institute of Biological Science, Mitsui Pharmaceuticals, Japan) was dissolved in 0.9% saline. MS-153 was administered intraperitoneally (i.p.) in a volume of 1 ml/kg.

2.3. Procedures

2.3.1. Conditioned fear stress-induced freezing

As described previously (Inoue et al., 1996), the rats were individually subjected to inescapable electric footshocks for a total of 2.5 min [five footshocks (2.5 mA scrambled shock, 30 s duration) delivered at intershock intervals of 35–85 s (mean 60 s)] in a shock chamber with a grid floor (19×22×20 cm; Medical Agent, Japan). Electric shocks were administered with a Model SGS-02D Shock Generator (Medical Agent). This provides a high-voltage, high-resistance circuit with resistance controlled by dial settings calibrated by the manufacturer in a short circuit current. At the setting of 2.5 mA, this generator actually gave a shock of 0.2-mA shock intensity to the rats (Inoue et al., 1996). Twenty-four hours after footshocks in the acquisition and expression experiments, the rats were placed individually in a shock chamber without shocks and were observed for 5 min. With these procedures, conditioned fear, as measured by freezing, develops to the contextual stimuli of the conditioned chamber (Fanselow, 1980). During the observation period, freezing behavior was recorded using a modification of a time-sampling procedure (Fanselow, 1980) previously described by Inoue et al. (1996). Freezing was defined as the absence of any observable movement of the skeleton and the vibrissae, except those related to respiration. All other behaviors were scored as activity. The total 5-min observation period was subdivided into 10-s intervals, in which an animal was scored as either freezing or active. The percentage score (percent freezing) represented the number of 10-s periods during which the animal froze for the entire 10 s. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee.

2.3.2. Effect of MS-153 administration on the acquisition of contextual conditioning fear

Forty-four rats were used in the acquisition experiments. Thirty minutes after saline or MS-153 (3, 10, and 30 mg/kg) administration, the rats received footshocks. Twenty-four hours after footshock, the rats were placed individually in the shock chamber without shocks and were observed for 5 min.

2.3.3. Effect of MS-153 administration on the expression of contextual conditioning fear

Forty rats were used in the expression experiments. Twenty-three and a half hours after footshock, the rats received a single intraperitoneal injection of saline in the

control group and MS-153 (3, 10, and 30 mg/kg) in the agent group. Thirty minutes after the injection, the rats were placed individually in the shock chamber without shocks and were observed for 5 min.

2.3.4. Motor activity

Thirty rats were used for measurement of locomotor activity. Motor activity was measured at two doses of MS-153 (10 and 30 mg/kg). The rats were housed individually for 3 days before testing, and their motor activity in the Plexiglass cages was automatically recorded by an infrared sensor that detected thermal radiation from the animals, as described by Ohmori et al. (1994). Saline or MS-153 (10 and 30 mg/kg) was administered at 30 min before measuring motor activity for 5 min. Horizontal movement was digitized and fed into a computer. Locomotion contributed predominantly to the count, but other body movements also contributed to the count when these movements contained substantial horizontal components. The rats were tested between 0800 and 0830 h. The number of rats per group for each experiment was 10.

2.4. Data analysis

All the data are presented as the mean±S.E.M. of the individual values for the rats from each group. Statistical analysis of the data was performed using a one-way analysis of variance (ANOVA) followed by Scheffe's test.

3. Results

3.1. Effect of MS-153 administration on the acquisition of contextual conditioning fear

MS-153 given 30 min before the footshock significantly attenuated conditioned freezing at 10 and 30 mg/kg compared with the effect of vehicle [one-way ANOVA, $F(3,40)=15.92$, $P<0.01$], when testing was performed 24 h after training for fear conditioning (Fig. 1A).

3.2. Effect of MS-153 administration on the expression of contextual conditioning fear

MS-153 significantly reduced conditioned freezing at 10 and 30 mg/kg compared with the effect of vehicle [one-way ANOVA, $F(3,36)=8.79$, $P<0.01$], when testing occurred 24 h after the footshock and 30 min after MS-153 administration (Fig. 1B).

3.3. Motor activity

MS-153 (10 and 30 mg/kg) administration given 30 min before failed to affect motor activity during the 5-min testing period compared with the effect of vehicle [control, 79.5±42.1 counts; 10 mg/kg, 65.2±36.4 counts; 30 mg/kg, 70.6±30.3 counts; one-way ANOVA, $F(2,27)=0.04$, NS].

4. Discussion

In the present study, we investigated the effects of acute MS-153 administration on the acquisition and expression of conditioned freezing. The results revealed that acute administration of MS-153, at 10 and 30 mg/kg, but not at 3 mg/kg, attenuated both the acquisition and expression of conditioned freezing. In addition, this study also showed that, at 10 and 30 mg/kg, MS-153 did not affect locomotion, which is consistent with the results of a previous study (Abekawa et al., 2002), so our results are unlikely to be due to nonspecific effects on motor activity.

The involvement of glutamate neurotransmission in behavior related to anxiety has been shown in a variety of animal models of the anxiolytic-like effects of drugs. Antagonists of NMDA and non-NMDA (AMPA) receptors reduced anxiety in the conflict test, social interaction test, elevated plus maze, and ultrasonic vocalization test (Dunn et al., 1989; Corbett and Dunn, 1991; Kehne et al., 1991; Benvenga et al., 1993; Wiley et al., 1995; Kotlinska and Liljequist, 1998). In addition, metabotropic glutamate receptor mGlu 2 agonists and mGlu 5 antagonists have

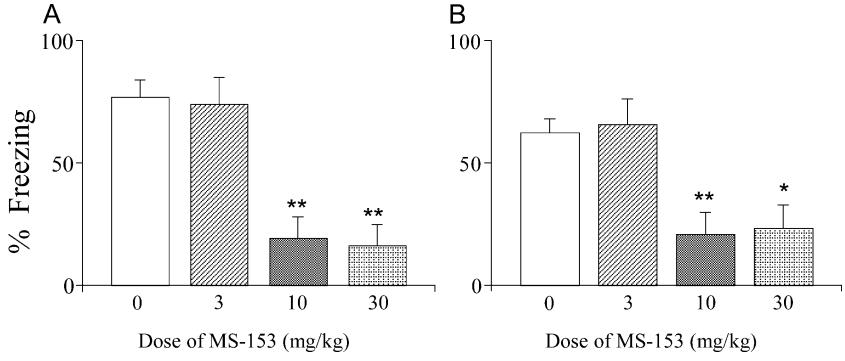

Fig. 1. Effect of acute MS-153 administration on the acquisition (A) and expression (B) of conditioned fear. (A) MS-153 was given (i.p.) 30 min before footshock. Twenty-four hours after footshock, freezing behavior was observed. (B) MS-153 was given (i.p.) 23.5 h after footshock and 30 min before the test in which freezing behavior was scored. Mean percentage±S.E.M. of freezing in a 5-min observation period is shown. Behavior was sampled at 10-s intervals. *$P<0.05$; **$P<0.01$ vs. vehicle controls. (A) n=8–16 rats; (B) n=8–16 rats.

also been reported to be anxiolytic in the conflict test and social interaction test (Klodzinska et al., 1999; Shekhar and Keim, 2000; Spooren et al., 2000).

With respect to fear conditioning, both NMDA and non-NMDA (AMPA) receptor antagonists abolished the acquisition and expression of conditioned fear by decreasing excitatory glutamatergic transmission (Miserendino et al., 1990; Campeau et al., 1992; Kim et al., 1993; Maren et al., 1996; Walker and Davis, 1997; Lee and Kim, 1998). In the present study, MS-153 also may have attenuated the acquisition and expression of conditioned fear by decreasing glutamate transmission due to the ability of this compound to both enhance glutamate uptake and inhibit glutamate release.

Another possible mechanism of MS-153 in this study, however, may be the blockade of acquisition of fear-related learning and impairment of fear-related memory because long-term potentiation, a candidate cellular mechanism for learning and memory of fear conditioning, is NMDA receptor-dependent (Collingridge and Bliss, 1987; Rison and Stanton, 1995; Rogan et al., 1997). In order to distinguish this possible property of MS-153 on learning and/or memory from its action on anxiety, other unconditioned anxiety paradigms should be used.

In conclusion, the present study suggests that MS-153 attenuated both the acquisition and expression of conditioned fear by ultimately decreasing the overall extracellular concentration of glutamate, and thus decreased glutamate neurotransmission could be the mechanism of anxiolytic action. This indicates that MS-153 may have applications not only as a neuroprotective drug but potentially also as an anxiolytic agent. In addition to blocking NMDA and/or non-NMDA receptors, prevention of an elevation of endogenous glutamate levels in the extracellular fluid represents another potential strategy to attenuate the acquisition and expression of fear and anxiety.

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