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Short communication

LY354740 attenuates the expression of long-term behavioral sensitization induced by a single session of foot shocks

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Abstract

Exposure of rats to a single session of foot shocks sensitizes behavioral responses to novel stimuli. There is evidence that metabotropic glutamate (mGlu) receptors play a role in sensitization processes. In the present study, we investigated the role of $mGlu_{2/3}$ receptors in the long-term (14 days) increase in defensive withdrawal behavior after a single session of foot shocks. Exposure to foot shocks increased defensive withdrawal behavior. The $mGlu_{2/3}$ receptor agonist LY354740 ((1S,2S,5R,6S)-(+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, 0.1 mg/kg, i.p.) normalized the increased latency and the decreased time in the light of the preshocked rats. We conclude that activation of $mGlu_{2/3}$ receptors attenuates the foot shock-induced expression of behavioral sensitization.

Keywords: Glutamate receptor; Stress-sensitization; (Rat)

1. Introduction

A stressful life event can induce long-lasting alterations in neuronal substrates which may play role in the development and maintenance of anxiety disorders such as post-traumatic stress disorder (Van der Kolk et al., 1985). Exposure of rats to a single session of uncontrollable foot shocks is a well-validated animal model to study the long-term effects of a stressful life event in humans (Yehuda and Antelman, 1993). Preshocked rats display increased anxiety-like behavior in the noise test which grows over time but remains stable from two weeks after the induction (Van Dijken et al., 1992). Increased anxiety-like behavior has also been found in the defensive withdrawal test and persists for at least 10 weeks after the foot shocks (Bruijnzeel et al., 2000). The progressive increase in anxiety-like behavior can be described as sensitization, an elementary form of nonassociative learning in which an animal learns to strengthen its defensive reflexes and to respond vigorously to a variety of previously neutral or indifferent stimuli after it has been exposed to a potentially threatening or noxious stimulus (Kandel and Schwartz, 1982).

A recent study demonstrated that the *N*-methyl-D-aspartate (NMDA) receptor antagonist (5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine hydrogen maleate (MK-801) injected intracerebroventricularly in rats before cat exposure prevents the resulting long-term increase in anxiety-like behavior in the elevated plus maze (Adamec et al., 1999), suggesting an important role for glutamatergic transmission in the induction of stress-sensitization. Moreover, the iontropic glutamate receptors NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) have been implicated in the induction and expression of drug-sensitization (Vanderschuren and Kalivas, 2000). However, the therapeutic value of drugs acting at the iontropic glutamate receptors is limited due to the severe side-effects (Danysz et al., 1996). The discovery of ligands for a new class of glutamate receptors, the G protein-coupled metabotropic glutamate (mGlu) receptors might help in the development of new therapeutics with less side-effects.

The mGlu receptors are divided in three groups based on sequence homology and second messenger coupling. The Group I mGlu receptors ($mGlu_1$ and $mGlu_5$) stimulate phosphoinositide hydrolysis and both the Group II ($mGlu_2$

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and $mGlu_3$) and Group III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) receptors are negatively linked to cAMP formation (Schoepp et al., 1999). Interestingly, the $mGlu_{2/3}$ receptor agonist LY354740 increases open arm exploration in the elevated plus maze and attenuates the fear potentiated startle response, suggesting an involvement of $mGlu_{2/3}$ receptors in anxiety-like behavior (Helton et al., 1998).

Nothing is known yet about the role of mGlu receptors in stress-sensitization, but there are indications that the mGlu receptors are involved in drug-sensitization. Cocaine affects the expression of $mGlu_5$ receptors in the brain (Ghasemzadeh et al., 1999) and rats exposed to amphetamine display a sensitized behavioral response to the Group I, II mGlu receptor antagonist (RS)-α-methyl-4-carboxyphenylglycine ((RS)-MCPG) (Kim and Vezina, 1998). The aim of the present study was to investigate the role of $mGlu_{2/3}$ receptors in the long-term expression of behavioral sensitization after a single session of foot shocks.

2. Materials and methods

2.1. Animals and housing

Male Wistar rats (Wistar: Unilever, Central Animal Facility, Utrecht University, The Netherlands) weighing 250–300 g were used. The rats were individually housed in macrolon cages measuring $40 \times 26 \times 20$ cm ($l \times w \times h$) with sawdust bedding at 21 °C under regulated lighting conditions (light on at 7:00 AM, off at 7:00 PM). Food (complete laboratory chow: Hope Farms, Woerden, The Netherlands) and water were freely available. Experimental procedures were approved by the Ethical Committee on Animal Experiments of the University Medical Center Utrecht.

2.2. Foot shock stressor

Between 5:00 PM and 6:30 PM, rats in the preshocked group were placed in a metal grid cage ($32 \times 32 \times 38$ cm, $l \times w \times h$) and underwent a single 15 min session of scrambled electric foot shocks (10×6 s, 0.5 mA, with randomized intervals of 20–210 s). Rats in the 'control' group spent 15 min in the grid cage without receiving shocks. Afterwards, rats were returned to their home cages, and left undisturbed apart from weekly changing of bedding material. The defensive withdrawal test was conducted 14 days after the shock box session.

2.3. Drugs and treatment

LY354740 ((1S,2S,5R,6S)-(+)-2-aminobicyclo[3.1.0]-hexane-2,6-dicarboxylic acid) was dissolved in sterile water neutralized to a pH of approximately 7.5 using 5 NaOH, and injected intraperitoneally (i.p.) in a volume of 1 ml/kg body weight (BW) 30 min before the defensive withdrawal test.

2.4. Statistics

Two-factor multivariate analysis of variance was used to test the effect of foot shocks and LY354740 followed by the Tukey's honestly significant difference test. Effects were considered significant when $P < 0.05$.

2.5. Defensive withdrawal test

Testing was conducted in a grey circular open-field (∅ 80 cm) in which a dark box measuring ($26 \times 20 \times 14$ cm, $l \times w \times h$) was placed, with a door that could be opened at one short end (∅ 8 cm). The box was placed with the longest side to the wall of the open field at a distance of approximately 5 cm. At the beginning of the test the rats were placed in the dark box via the upper side and the lid closed. The arena was illuminated by a fluorescent lamp shielded by a metal plate, the light intensity in the center of the open-field was 75 and 0 lux in the dark box. After calibration of the video tracking software (Ethovision, Noldus Information Technology, Wageningen, The Netherlands) which took 10 s, the box was opened on one side. After 10 min, the rats were returned to their home cage and the box and the open field were cleaned. During the test, we measured the following behaviors; latency to leave the dark box which is defined as placement of all four paws outside the dark box, number of entries in the open field, percentage of time spent outside the dark box and distance moved in the open field. The defensive withdrawal test was conducted between 9:00 AM and 1:00 PM.

3. Results

Fig. 1 shows the effects of previous exposure to foot shocks and acute treatment with LY354740 (0.1, 1 mg/kg BW, i.p.) on the behavior of preshocked and control rats in the defensive withdrawal test. Previous exposure to foot shocks induced a significant increase in the latency to emerge from the dark box ($F(1,43) = 27.26$, $P < 0.001$). Statistical analysis also revealed a significant preshock × LY354740 interaction ($F(2,43) = 3.418$, $P = 0.042$), LY354740 decreasing the latency of the preshocked rats without affecting the latency of the control rats. Foot shock exposure resulted in a significant decrease in the time spent outside the dark box ($F(1,43) = 20.36$, $P < 0.001$). A significant preshock × LY354740 interaction ($F(2,43) = 3.84$, $P = 0.029$) was also found for the time spent outside the box, acute treatment with LY354740 increasing the time spent outside the box of the preshocked rats without affecting this parameter in the controls. The foot

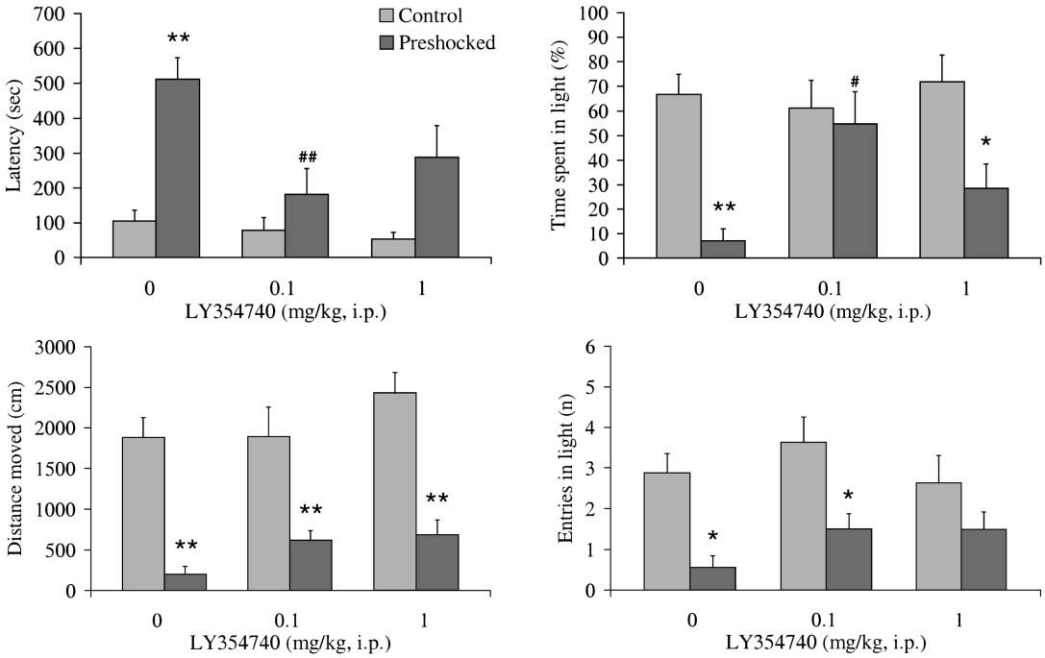

Fig. 1. Effects of LY354740 on defensive withdrawal behavior (in 10 min testing) in preshocked and control rats, 2 weeks after the foot shocks; $n = 8–9$ per group. Data are presented as mean ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ preshocked versus control with the same dose. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ preshocked LY354740 versus preshocked vehicle.

shock session decreased the distance moved outside the dark box ($F(1,43) = 72.24$, $P < 0.001$) and decreased the number of entries in the open field ($F(1,43) = 42.08$, $P < 0.001$), these parameters were not affected by treatment with LY354740. Post-hoc analysis showed that 0.1 mg/kg of LY354740 was most effective in decreasing the latency and to increase the time spent in the light of the preshocked rats compared to controls.

4. Discussion

The results of this study showed that there is a clear increase in anxiety-like behavior in the defensive withdrawal test two weeks after a single session of foot shocks. The $mGlu_{2/3}$ receptor agonist LY354740 normalized the latency and the time spent in the light of the preshocked rats.

Group II mGlu receptors are expressed throughout the brain, including brain areas involved in stress responses such as the frontal cortex, basolateral amygdala complex and the hypothalamic paraventricular nucleus (Ohishi et al., 1993a,b). The $mGlu_{2/3}$ receptors are primarily located perisynaptically on presynaptic terminals and do not affect neurotransmission under resting conditions, but when a large amount of glutamate is released, it diffuses and activates the perisynaptic $mGlu_{2/3}$ receptors resulting in a decrease in glutamate release (Cartmell and Schoepp, 2000).

In the present study, the $mGlu_{2/3}$ receptor agonist LY354740 normalized the latency and the time in the light of the preshocked rats at a dose which did not affect the behavior of the control rats. However, despite the fact that LY354740 increased the time spent in the light of the preshocked rats, it did not increase the distance moved in the open field in the same rats. It is unlikely that in our experiment LY354740 suppressed locomotor activity in the preshocked rats since LY354740 did not affect the behavior of the control rats. Moreover, it has been shown that LY354740, at a higher dose than used in the current study, does not affect locomotor activity during the first 10 min of an open field test (Moore et al., 1999). More research has to be done to assess whether the different parameters measured in the defensive withdrawal test also represent different forms of anxiety-like behavior.

In a recent study (Bruijnzeel et al., 1999), we found that a preshock experience sensitizes the electrified prod-induced expression of the marker for neuronal activation Fos. The expression of Fos was sensitized in brain areas involved in anxiety-like behavior such as the frontal cortex, basolateral amygdala complex and locus coeruleus. Interestingly, it has been shown that LY354740 can decrease the release of glutamate in the prefrontal cortex (Marek et al., 2000) and the locus coeruleus (Dube and Marshall, 1997), and that the $mGlu_{2/3}$ receptor agonist (2S,3S,4S)-2-(carboxycyclopropyl)glycine (L-CCG) suppresses synaptic transmission in the basolateral amygdala (Neugebauer et al., 1997). These studies demonstrate that $mGlu_{2/3}$ receptor agonists can diminish glutamate transmission in brain areas in which sensitized neuronal responses have been found. Therefore, we suggest that LY354740 blocks sensitized glutamate release in brain

areas considered to be part of the fear/anxiety-circuitry during the novel challenge, which may play a role in the increase in anxiety-like behavior as found in preshocked rats.

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