Behavioural Brain Research 336 (2018) 8–14

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Administration of riluzole to the basolateral amygdala facilitates fear extinction in rats

Azusa Sugiyama[a,b], Misa Yamada[a], Akiyoshi Saitoh[a], Jun-Ichiro Oka[b], Mitsuhiko Yamada[a,*]

[a] Department of Neuropsychopharmacology, National Institute of Mental Health, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashimachi, Kodaira, Tokyo 187-8553, Japan

[b] Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba 278-8510, Japan

ARTICLE INFO

Keywords:
Fear conditioning
Recognition memory
Anxiety
Riluzole
D-Cycloserine
Basolateral amygdala

ABSTRACT

A general understanding exists that inhibition of glutamatergic neurotransmission in the basolateral amygdala (BLA) impairs fear extinction in rodents. Surprisingly, we recently found that systemic administration of riluzole, which has been shown to inhibit the glutamatergic system, facilitates extinction learning in rats with a preconditioned contextual fear response. However, the mechanisms underlying this paradoxical effect of riluzole remain unclear. In this study, adult male Wistar rats were bilaterally cannulated in the BLA to examine the effects of intra-BLA administration of riluzole. We also compared the effects of riluzole with those of D-cycloserine, a partial agonist at the glycine-binding region of the *N*-methyl-D-aspartate (NMDA) receptor. In this study, intra-BLA administration of either riluzole or D-cycloserine facilitated extinction learning of contextual fear in conditioned rats. In addition, both riluzole and D-cycloserine enhanced the acquisition of recognition memory in the same model. However, intra-BLA injections of riluzole, but not D-cycloserine, had a potent anxiolytic-like effect when investigated using an elevated plus-maze test. Our findings suggest that riluzole-induced facilitation of extinction learning in rats with a preconditioned contextual fear reflects an indirect effect, resulting from the intra-BLA administration of the drug, and might not be directly related to inhibition of glutamatergic signaling. Further research is needed to clarify the mechanisms underlying the paradoxical effect of riluzole on fear extinction learning observed in this study.

1. Introduction

Contextual fear conditioning in rodents is a well-studied experimental model of the aversive expectations of danger that characterize anxiety in patients [1]. Contextual fear conditioning in experimental animals involves training to learn and remember an association between specific environmental cues (context) and aversive experiences. After conditioning, subsequent short-term re-exposure to the context elicits characteristic fear responses, including freezing. However, a longer re-exposure to the context without any aversive stimuli can induce extinction of the fear response [2]. In this situation, a new inhibitory association is formed, resulting in a reduced fear response. Fear extinction is critically dependent upon the basolateral region of the amygdala (BLA) [3,4]. Local inhibition of *N*-methyl-D-aspartate (NMDA) receptors, which are usually activated by glutamate or mitogen-activated protein kinase activity, impairs the acquisition of fear extinction [5–7]. Therefore, this learned inhibition of the conditioned fear response is thought to require activation of NMDA receptors within the BLA, and a general understanding exists that a drug that inhibits glutamatergic transmission impairs fear extinction in rodents.

Riluzole has been shown to inhibit glutamatergic signaling through a variety of mechanisms, both presynaptically or postsynaptically, including: inhibition of voltage-activated sodium channels [8]; inhibition of voltage-activated calcium channels [9]; inhibition of gamma-aminobutyric acid uptake [10]; potentiation of glutamate uptake [11]; and indirect inhibition of postsynaptic glutamate receptors [12]. Inactivation of glutamatergic signaling is known to decrease the occurrence of anxiety-like behaviors in rodents. We have previously demonstrated that systemic administration of riluzole has an anxiolytic-like effect in rat models, as indicated by behavioral tests for innate anxiety, including the rat elevated plus-maze test, the light-dark test and the open-field test [13]. In 2015, we reported that systemic administration of riluzole, which has been shown to inhibit glutamatergic signaling, facilitates extinction learning of contextual fear in rats [14]. However, the mechanisms underlying this paradoxical effect of riluzole remain unclear.

In this study, we investigated whether the effects of systemic administration of riluzole on extinction learning of contextual fear could be reproduced by administration of riluzole to the BLA using a rat

* Corresponding author.
E-mail address: mitsu@ncnp.go.jp (M. Yamada).

http://dx.doi.org/10.1016/j.bbr.2017.08.031
Received 9 June 2017; Received in revised form 15 August 2017; Accepted 17 August 2017
Available online 24 August 2017

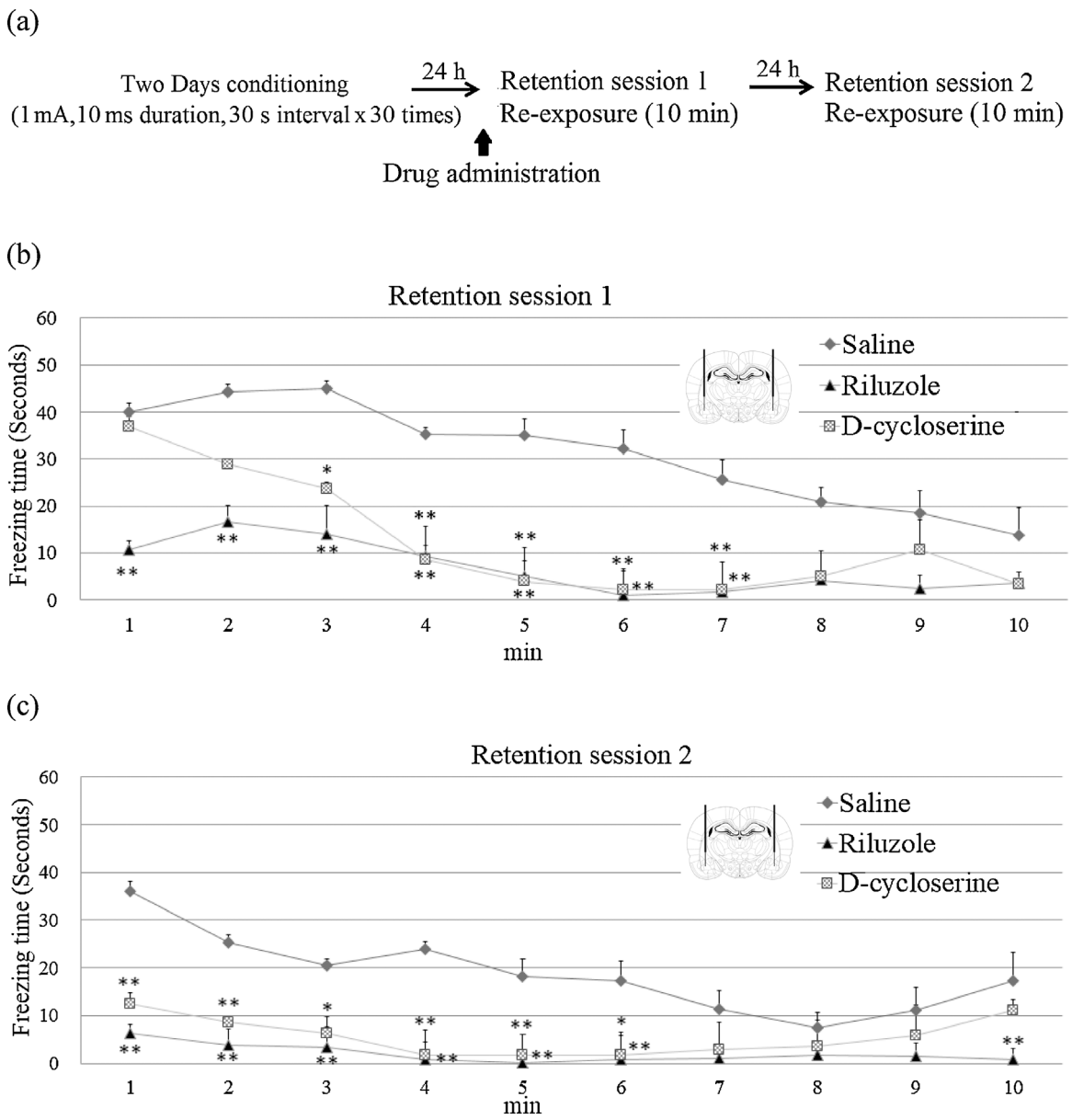

Fig. 1. Effect of drug treatments on freezing behavior during the extinction training session (retention session 1) and the test session (retention session 2). (*a*) General behavioral procedures: rats were re-exposed to the conditioned chamber without foot shock at 24 h after conditioning, and freezing behavior was observed for 10 mins (retention session 1). Riluzole, D-cycloserine and saline were administered into BLA bilaterally 5 mins before the session. Twenty-four hours after the retention session 1, rats were re-exposed again to the conditioning chamber without foot shock, and freezing behavior was observed for 10 mins (retention session 2). (*b*) Freezing time (seconds) of drug-treated rats during each 60-s period in retention session 1. (*c*) Freezing time (seconds) of rats during each 60-s period in retention session 2. Data are shown as mean freezing time ± SEM (saline, $n = 15$; riluzole, $n = 18$; D-cycloserine, $n = 14$) for each group. The statistical significance of data indicated by the rectangle was assessed using a one-way factorial ANOVA followed by Bonferroni's post-hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the saline-treated group.

model of anxiety. We examined the effects of intra-BLA injections of riluzole on anxiety-like behaviors and recognition memory in the conditioned rats. Furthermore, we compared the effects of riluzole with those of D-cycloserine, a partial agonist of the NMDA receptor that binds to the glycine-binding site. D-cycloserine does not have an anxiolytic effect when administered systemically, but has been shown to facilitate extinction of conditioned fear in animal models [3,14,15].

2. Materials and methods

2.1. Animals

Male Wistar rats (SLC, Shizuoka, Japan) were used for behavioral experiments. All rats were 12 weeks of age at the beginning of the behavioral experiments. All rats had free access to food and water in an animal housing facility maintained at 23 ± 1 °C with a 12-h light-dark cycle (lights were automatically switched on at 8:00 am). All rats were kept in this environment for at least 2 weeks prior to surgery. These studies were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of the Japanese National Center of Neurology and Psychiatry (Approval No. 2010001).

2.2. Drugs

We used riluzole hydrochloride and D-cycloserine (Sigma Chemical Co., St. Louis, MO, USA). The doses of riluzole (2 μM/0.2 μl/side) and D-cycloserine (0.1 μM/0.2 μl/side) were selected based on the findings of previous research [16]. Both drugs were dissolved in a saline solution.