Neuropharmacology 101 (2016) 36–45

Contents lists available at ScienceDirect

Neuropharmacology

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

Acute food deprivation enhances fear extinction but inhibits long-term depression in the lateral amygdala via ghrelin signaling

Chiung-Chun Huang [a], Dylan Chou [b], Che-Ming Yeh [a], Kuei-Sen Hsu [a, b, *]

[a] Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan
[b] Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan

ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form
11 September 2015
Accepted 14 September 2015
Available online 15 September 2015

Keywords:
Fear extinction
Long-term depression
Food deprivation
Ghrelin
Lateral amygdala

ABSTRACT

Fear memory-encoding thalamic input synapses to the lateral amygdala (T-LA) exhibit dynamic efficacy changes that are tightly correlated with fear memory strength. Previous studies have shown that auditory fear conditioning involves strengthening of synaptic strength, and conversely, fear extinction training leads to T-LA synaptic weakening and occlusion of long-term depression (LTD) induction. These findings suggest that the mechanisms governing LTD at T-LA synapses may determine the behavioral outcomes of extinction training. Here, we explored this hypothesis by implementing food deprivation (FD) stress in mice to determine its effects on fear extinction and LTD induction at T-LA synapses. We found that FD increased plasma acylated ghrelin levels and enhanced fear extinction and its retention. Augmentation of fear extinction by FD was blocked by pretreatment with growth hormone secretagogue receptor type-1a antagonist D-Lys^3-GHRP-6, suggesting an involvement of ghrelin signaling. Confirming previous findings, two distinct forms of LTD coexist at thalamic inputs to LA pyramidal neurons that can be induced by low-frequency stimulation (LFS) or paired-pulse LFS (PP-LFS) paired with postsynaptic depolarization, respectively. Unexpectedly, we found that FD impaired the induction of PP-LFS- and group I metabotropic glutamate receptor agonist (*S*)-3,5-dihydroxyphenylglycine (DHPG)-induced LTD, but not LFS-induced LTD. Ghrelin mimicked the effects of FD to impair the induction of PP-LFS- and DHPG-induced LTD at T-LA synapses, which were blocked by co-application of D-Lys^3-GHRP-6. The sensitivity of synaptic transmission to 1-naphthyl acetyl spermine was not altered by either FD or ghrelin treatment. These results highlight distinct features of fear extinction and LTD at T-LA synapses.

1. Introduction

The lateral amygdala (LA) is known to be crucial for appetitive (Murray, 2007) and aversive emotional learning (Johansen et al., 2011). The LA receives converging synaptic inputs from the thalamus and the neocortex, and may serve as the sensory interface of the amygdala (Blair et al., 2001). The thalamic input synapses to the LA (T-LA) have been extensively studied as a site of associative learning-induced plasticity, and changes in synaptic efficacy at T-LA synapses are tightly correlated with fear memory strength. For example, auditory fear conditioning induces long-term potentiation (LTP)-like enhancement of synaptic transmission at T-LA synapses (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997). Extinction of consolidated fear memory reverses the conditioning-induced synaptic potentiation at T-LA synapses (Kim et al., 2007; Clem and Huganir, 2010). Based on these findings, it is speculated that long-term depression (LTD) and/or depotentiation may be a cellular mechanism underlying the extinction of fear memory (Park et al., 2014). Consistent with this possibility, augmentation of group I metabotropic glutamate receptor (mGluR)-dependent LTD at T-LA synapses was observed in fear conditioned mice (Clem and Huganir, 2013). Nonetheless, these findings apparently contradict the prevailing theory of fear extinction, which suggest that extinction does not erase the original fear memory but instead generates a new memory to inhibit the retrieval of the original memory (Herry et al., 2010). It has been reported recently that optical LTP protocol can reactivate auditory fear memory that was inactivated by LTD but does not reverse extinction, suggesting that extinction is not LTD (Nabavi et al., 2014). Considering these inconsistencies, further experiments are required to examine the association between T-LA LTD and fear extinction.

* Corresponding author. Department of Pharmacology, College of Medicine, National Cheng Kung University, No. 1, University Rd., Tainan 701, Taiwan.
E-mail address: richard@mail.ncku.edu.tw (K.-S. Hsu).

http://dx.doi.org/10.1016/j.neuropharm.2015.09.018
0028-3908/

The coexistence of two mechanistically distinct forms of LTD has been reported at T-LA synapses (Clem and Huganir, 2013; Park et al., 2014). The first form of LTD is induced by pairing low-frequency stimulation (LFS) at 1 Hz for 10 min with postsynaptic depolarization to −40 mV, and this form of LTD requires activation of N-methyl-D-aspartate receptors (NMDARs) and calcineurin. The second form of LTD at T-LA is induced by paired-pulse LFS (PP-LFS, 3 Hz for 3 min) paired with postsynaptic depolarization to −50 mV or pharmacological activation of group I metabotropic glutamate receptors (mGluRs) with the agonist (S)-3,5-dihydroxyphenylglycine (DHPG), and its induction relies on post-synaptic protein kinase C (PKC) activation and has been associated with calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (CP-AMPAR) removal. Intriguingly, mGluR- and CP-AMPAR-dependent LTD is selectively enhanced by auditory fear conditioning as a result of β-adrenergic receptor signaling (Clem and Huganir, 2013).

One strategy towards understanding the link between LTD and extinction is to demonstrate the mechanisms governing LTD may determine the behavioral outcomes of extinction. Although previous study has revealed that food deprivation (FD) stress can disrupt latent inhibition of auditory fear conditioning (De la Casa, 2013), so far there is no study demonstrating the possible effect of FD on extinction of conditioned fear responses. Moreover, to our knowledge, there are no data available regarding the effect of FD on the induction of LTD at T-LA synapses. Therefore, in the present study, we investigated whether the induction of LTD at T-LA synapses and the extinction of auditory fear conditioning are equivalently modulated by implementing FD in mice. It is surprising that, although FD enhanced fear extinction and its retention, it impaired the induction of PP-LFS- and DHPG-induced LTD at T-LA synapses.

2. Materials and methods

2.1. Animals

Adult (8–12 weeks old) male C57BL/6 mice were used in our experiments. Mice were housed in groups of four in a temperature (25 ± 1 °C) and humidity controlled room under a 12:12 h reversed light/dark cycle (lights on 06:00–18:00 h) with access to food and water *ad libitum* (AL) and were acclimated in the animal research facility for at least one week before the start of the experiments. Previous study has demonstrated that rats fasted for 24 h had significantly higher plasma corticosterone levels than did non-fasted controls, whereas a difference was not observed at 16 h or earlier time points (Nowland et al., 2011). To avoid the influence of corticosterone, mice were fasted either for 2 h or 16 h (overnight) with free access to water in our FD experiments. All handling, surgeries and behavioral procedures were performed during the light cycle between 10:00 and 15:00 h. To avoid interference effects between experiments, separate groups of mice were used for behavioral and electrophysiological experiments. All animal procedures described were executed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of National Cheng Kung University.

2.2. Auditory fear conditioning and extinction

The experimental design for behavioral training and testing is illustrated in Fig. 1A. Mice were transferred to a rectangular Plexiglas chamber (context A, 15.9 × 14.0 × 12.7 cm; ENV-307A, MED Associates) that was equipped with metal grid floor connected to an electrical current source and placed into a ventilated sound-attenuating isolation cubicle. Following a 3-min acclimation period, both AL and FD mice received six presentations of an auditory conditioning stimulus (CS; 80 dB sound at 2 kHz lasting 20 s) paired with an aversive footshock unconditioned stimulus (US; 0.8 mA lasting 2 s), separated by 30 s interval. Memory retrieval was tested 24 h after the last conditioning trial in a cylindrical Plexiglas chamber (context B). The CS-induced conditioned response (CR) was scored as the total time of the mouse spent in freezing during a 3-min test session. The extinction trials (CS-alone) were performed at 1 h after the retrieval test and consisted of 2 blocks of 20 trials (20 s CS per trial, with 30 s inter-CS interval) of each in context B, which were separated by 1 h interval. Twenty-four hours later, mice were returned to the extinction chamber (context B) and given 4 tone-alone presentations (20 s CS, 30 s interval) to measure spontaneous recovery of fear. One hour later, the same animals were placed back into the conditioning chamber (context A) and presented with 4 CS (20 s tone, 30 s interval) to assess renewal of extinguished fear. Average mean time spent in freezing across the 4 trials in the first of extinction block 1, the last of extinction block 2, spontaneous recovery and renewal of extinguished fear were calculated for comparisons. The behavior data were analyzed by differential subtraction of two consecutive images captured at 7.5 Hz to calculate the significant motion pixels (SMP). A freezing behavior was defined as the value of SMP <20 at any indicated time point.

2.3. Open field test

For the open-field test (OFT), mice were placed individually in the center of a test chamber to freely explore for 5 min under a low illumination (approximately 10 Lux). The test chamber consisted of a circular ground area (40 cm in diameter) with a 40 cm high wall set on a non-reflective white plastic base. The behavior of the animals was video recorded using a digital video camera and scoring was performed with the behavioral tracking system Ethovision (Noldus, The Netherlands). The activity was evaluated based on time spent in the central zone, and total distance traveled in the open field. The apparatus was thoroughly cleaned with 50% ethanol after each trial. The percentage of time spent in the center zone is defined as the percentage of time for the animals exploring the central 25% (20 cm in diameter) of the chamber.

2.4. Elevated plus maze

Elevated plus maze (EPM) experiments were performed as described previously (Pellow et al., 1985). The elevated plus maze was custom-made of black Plexiglas consisting of two open arms (25 × 5 × 0.5 cm) and two enclosed arms (25 × 5 × 16 cm) extending from a central square platform (5 × 5 cm) mounted on a wooden base raised 50 cm above the floor. Animals were placed on the center square platform facing an open arm and allowed to freely explore the maze for 5 min. The apparatus was illuminated with dimmed light (approximately 10 Lux). The behavior of the animals was video recorded using a digital video camera and scoring was performed with the behavioral tracking system Ethovision (Noldus). The activity was evaluated based on the number of entries into the closed arms and open arms and the percentage of time spent in the open versus closed arms. The apparatus was thoroughly cleaned with 50% ethanol after each trial.

2.5. Slice preparations and electrophysiology

Coronal brain slices containing the amygdala were prepared as described previously (Huang et al., 2014). In brief, mice were anesthetized with isoflurane and decapitated, and brains were rapidly removed and placed in ice-cold sucrose artificial