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Research report

Role of the amygdala GABA-A receptors in ACPA-induced deficits during conditioned fear learning

Mohammad Nasehi[a,**], Farnaz Roghani[b], Mohaddeseh Ebrahimi-Ghiri[c], Mohammad-Reza Zarrindast[d,e,f,g,h,*]

[a] Cognitive and Neuroscience Research Center (CNRC), Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran
[b] Department of Biology, Faculty of Basic Sciences, Islamic Azad University, Northern Branch, Tehran, Iran
[c] Department of Biology, Faculty of Sciences, University of Zanjan, Zanjan, Iran
[d] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[e] Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran
[f] Institute for Cognitive Science Studies (ICSS), Tehran, Iran
[g] School of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran
[h] Medical Genomics Research Center, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT

The basolateral amygdala (BLA) is a key structure for the emotional processing and storage of memories associated with emotional events, especially fear. On the other hand, endocannabinoids and CB1 receptors play a key role in learning and memory partly through long-term synaptic depression of GABAergic synapses in the BLA. The aim of this study was to explore the effects of GABA-A receptor agonist and antagonist in the fear-related memory acquisition deficits induced by ACPA (a selective CB1 cannabinoid receptor agonist). This study used context and tone fear conditioning paradigms to assess fear-related memory in male NMRI mice. Our results showed that the pre-training intraperitoneal administration of ACPA (0.5 mg/kg) or (0.1 and 0.5 mg/kg) decreased the percentage of freezing time in the contextual and tone fear conditioning, respectively. This indicated an impaired context- or tone-dependent fear memory acquisition. Moreover, the pre-training intra-BLA microinjection of GABA-A receptor agonist, muscimol, at 0.05 and 0.5 μg/mouse impaired context-dependent fear memory, while the same doses of GABA-A antagonist, bicuculline, impaired tone-dependent fear memory. However, a subthreshold dose of muscimol or bicuculline increased the effect of ACPA at 0.1 and 0.5 or 0.05 mg/kg on context- or tone-dependent fear memory, respectively. In addition, bicuculline at the lower dose increased the ACPA response on locomotor activity compared to its respective group. Such findings highlighted an interaction between BLA GABAergic and cannabinoidergic systems during the acquisition phase of conditioned fear memories.

1. Introduction

Fear conditioning is an animal model paradigm used to study emotional learning and aversive memory (Yaniv et al., 2004). It has also been reported that the cannabinoid CB1 receptor agonists are able to selectively impair the acquisition of hippocampus-dependent aversive memory in rats. Based on the above, cannabinoids may potentially be considered in modulating unpleasant memories (Pamplona and Takahashi, 2006).

The basolateral amygdala (BLA) is a brain structure involved in emotional control and fear conditioning (Davis et al., 1994). Endocannabinoid signaling in the BLA has been implicated in the extinction of aversive memories (Marsicano et al., 2002). Immunohistochemical studies have confirmed a dense expression of cannabinoid CB1 receptors not only on the GABAergic interneurons (Katona et al., 2001; Tsou et al., 1999) but also the glutamatergic pyramidal projection neurons in the BLA (Nyilas et al., 2008). The presynaptic CB1 receptors which are in fact $G_{i/o}$ protein-coupled, are negatively coupled with adenylyl cyclase (Howlett et al., 1986), positively coupled with mitogen-activated protein kinase (Bouaboula et al., 1995) and coupled with a variety of ion channels, including those of potassium and calcium (Howlett, 2002). As such, they are likely to modulate the release of GABA and glutamate from the BLA neurons.

The fast inhibitory synaptic transmission in the brain is largely

* Corresponding author at: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
** Corresponding author.
E-mail addresses: Nasehi@iricss.org (M. Nasehi), zarinmr@ams.ac.ir (M.-R. Zarrindast).

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mediated by GABA-A receptors (Barnard et al., 1998). These receptors are composed of five subunits drawn from a seven-subunit families (Nutt, 2006; Whiting et al., 1999). The most abundant composition consists of two α (1–6), two β (1–3) and one γ (1–3) subunits (Farrar et al., 1999; Sieghart et al., 1999). The inhibitory signaling in the amygdala plays an important role in modulating fear reactions at multiple levels (Samson et al., 2003) and is also a probable target for the anxiolytic effects of benzodiazepines (Nagy et al., 1979). It seems that information referring to contextual (conditioned stimulus; CS) and aversive/nociceptive (unconditioned stimulus; US) stimuli are converged at the level of the BLA, during fear conditioning (Fendt and Fanselow, 1999; Maren, 2001). This convergence of information is reinforced after each CS-US pairing through a process leading to an increased synaptic efficacy (Walker et al., 1997). The subsequent re-exposure to CS (context) which tend to provoke larger neuronal responses in the BLA (Quirk et al., 2003; Repa et al., 2001) is reported to be GABA-dependent (Rodriguez Manzanares et al., 2005). This information is relayed through GABAergic intercalated cells (Pare et al., 2004; Royer and Pare, 2002) to the central nucleus of the amygdala (CeA). In addition, studies have suggested that CB1 Receptors in the BLA are expressed on GABAergic interneurons expressing the anxiogenic peptide cholecystokinin (CCK), suggesting that CB1 receptor stimulation may reduce CCK peptide as well as GABA release (Chhatwal et al., 2009). In the same vein, behavioral studies have suggested an interaction between cannabinoidergic and GABAergic systems in memory processes including passive avoidance learning (Hasanein and Sharifi, 2015), spatial/object novelty detection memory (Yousefi et al., 2013), as well as the conditioned place preference learning (Nasehi et al., 2016b).

Despite the existing data on: 1—the role of the BLA in fear conditioning, 2—the role of cannabinoid CB1 receptors in the acquisition of aversive memories, and 3—interaction between these two neurotransmitter systems in memory processes; the role of BLA GABA-A receptors in mediating conditioned fear memory impairments induced by the activation of CB1 receptor *via* ACPA has not yet been examined. This has prompted us to investigate the possible interaction between ACPA and GABA-A agents in the BLA and its subsequent effect on the context- and tone-dependent fear memory acquisition in a mice model.

2. Material and methods

2.1. Animals

Male NMRI mice (Institute for Cognitive Science Studies; Tehran, Iran) weighing 28–33 g were used as subjects. Animals were housed under standard laboratory conditions with a 12-h day/night cycle. Light was on at 7:00 and the room temperature was maintained at 22 ± 2 °C. Food and water were offered *ad libitum*. The experiments were performed during the light phase of the light/dark cycle. The entire experiment followed the international laws on animal experimentation and was approved by the ethics committee of Tehran University of Medical Sciences, Tehran, Iran. All procedures were carried out according to the guidelines for Animal Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 2010).

2.2. Stereotaxic surgery

Surgery was conducted as described previously (Nasehi et al., 2016a, 2016c, 2016d). Mice were anesthetized using ketamine (50 mg/kg, i.p.) and xylazine (5 mg/kg i.p.) from Alfasan Chemical Co, Woerden, Holland, and were fixed to a stereotaxic surgery apparatus. Two single guide cannulae (22 gauge) were then bilaterally implanted into the BLA (stereotaxic coordinates: AP = −1.58 mm from bregma, ML = ± 2.8 mm from midline, DV = 4.5 mm from bregma), according to the atlas of mouse brain (Paxinos and Franklin, 2001). The tip of indwelled microinjection cannulae (27 gauge) were inserted 1 mm below the tip of the guide cannulae. The cannulae were then fixed to the skull using dental cement. Following the above, animals were allowed to recover for at least 5 days before conditioning experiments.

2.3. Drugs and infusions

ACPA, Arachidonylcyclopropylamide (a potent and selective CB1 receptor agonist), and muscimol (a GABA-A receptor agonist) were purchase from Tocris Cookson, Bristol, UK. Injection solutions were prepared fresh just before each experiment except for ACPA, which was stored at −20 °C as a prepared solution dissolved in anhydrous ethanol at a concentration of 5 mg/ml, diluted to the required volume using sterile 0.9% saline before used in experiments. ACPA (at the volume of 10 ml/kg) was administered intraperitoneally at the doses of 0.05, 0.1 and 0.5 mg/kg. The above drugs were dissolved in 0.9% saline and were prepared for injections upon experiments. Bicuculline was purchased from Sigma St, Louis, Mo, USA and dissolved in one drop of glacial acetic acid with a Hamilton micro-syringe and made up to a volume of 5 ml with sterile 0.9% saline, then diluted to the required volume. The control animals received either saline or vehicle. Intra-BLA microinjections (muscimol, bicuculline or saline) at the volume of 0.3 μl/each side were done through a 27-gauge injection cannula. The injection cannula was 1 mm longer than the guide cannulae extending 1 mm beyond the tip of the guide cannulae into the center of the BLA. The injection cannula was attached with a polyethylene tube to a 1 μl Hamilton syringe. The injection cannula was left in the place for an additional 60 s to facilitate drug diffusion.

2.4. Fear conditioning experiments

The conditioning chamber had a cubic shape (25 cm × 25 cm × 25 cm) consisting of a house light (24 w), transparent plastic walls, and mouse shock grid- floor. The grid-floor was cleaned with 70% ethanol. The conditioning chambers were located in sound-proof isolation cubicles (55 cm × 53 cm × 67 cm), additionally insulated with acoustic foam. Tones for the conditioning procedure were generated by audio stimulus generators and applied by speakers mounted to the ceiling of the isolation cubicle over the respective context chambers. A small video camera was mounted on the ceiling of isolation cubicles to enable behavioral observation and digital recording. Upon training, mice were placed into the conditioning chamber. After 2 min, a 30 s tone (4 kHz, 35 dB) was presented terminating with a 2 s scrambled electric foot-shock of 1 mA. Mice were returned to their home cages 30 s after the shock was delivered. Twenty four hours after the training, mice were placed in the grid context for 300 s, without providing any aversive stimulus mice to investigate the context-dependent retention test. Tone-dependent retention test was done 1 h after context-dependent retention test. To conduct the tone-dependent retention test, mice were placed in a novel chamber different from the conditioning context in texture, bedding and shape. Mice were placed in this chamber for 2 min after which exposed to a 180 s period of tone presentation. The freezing behavior was assessed as a measure of fear. Freezing behavior, defined as the absence of all movements except for respiration, was continuously scored off-line by a trained observer blinded to the treatments. Raw data were used to calculate the percentage of freezing time [% Freezing; (sum of the freezing times/total time) × 100] and latency to first freezing (time elapsed till the onset of the first freezing behavior) for context- or tone-dependent retention test.

2.5. Measurement of locomotor activity

The locomotion apparatus (Borj Sanat Co., Tehran, Iran) was made