Behavioural Brain Research 300 (2016) 114–122

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Modulation of cannabinoid signaling by amygdala α_2-adrenergic system in fear conditioning

Mohammad Nasehi [a,**], Majid Zamanparvar [b], Mohaddeseh Ebrahimi-Ghiri [c], Mohammad-Reza Zarrindast [a,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

HIGHLIGHTS

- Activation of CB1 receptors by ACPA impaired contextual and auditory fear memories.
- A same result was observed with intra-BLA microinjection of Clonidine or yohimbine.
- Subthreshold dose of clonidine did not alter deficit in both memories induced by ACPA.
- Subthreshold dose of yohimbine potentiated ACPA-induced contextual memory deficit.
- BLA α_2-adrenoceptors are only involved in ACPA-induced contextual memory deficit.

ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form 1 December 2015
Accepted 11 December 2015
Available online 14 December 2015

Keywords:
ACPA
Clonidine
Yohimbine
Fear memory conditioning
Mice

ABSTRACT

The noradrenergic system plays a critical role in the modulation of emotional state, primarily related to anxiety, arousal, and stress. Growing evidence suggests that the endocannabinoid system mediates stress responses and emotional homeostasis, in part, by targeting noradrenergic circuits. In addition, there is an interaction between the cannabinoid and noradrenergic system that has significant functional and behavioral implications. Considering the importance of these systems in forming memories for fearful events, we have investigated the involvement of basolateral amygdala (BLA) α_2-adrenoceptors on ACPA (as selective cannabinoid CB1 agonist)-induced inhibition of the acquisition of contextual and auditory conditioned fear. A contextual and auditory fear conditioning apparatus for assess fear memory in adult male NMRI mice was used. Pre-training, intraperitoneal administration of ACPA decreased the percentage freezing time in contextual (at doses of 0.05 and 0.1 mg/kg) and auditory (at dose of 0.1 mg/kg) in the fear conditioning task, indicating memory acquisition deficit. The same result was observed with intra-BLA microinjection of clonidine (0.001–0.5 μg/mouse, for both memories), as α_2-adrenoceptor agonist and yohimbine (at doses of 0.005 and 0.05 for contextual and at dose of 0.05 μg/mouse for auditory fear memory), as α_2-adrenoceptor antagonist. In addition, intra-BLA microinjection of clonidine (0.0005 μg/mouse) did not alter ACPA response in both conditions, while the same dose of yohimbine potentiated ACPA response at the lower dose on contextual fear memory. It is concluded that BLA α_2-adrenergic receptors may be involved in context- but not tone-dependent fear memory impairment induced by activation of CB1 receptors.

* Corresponding author at: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. P.O.Box 13145-784. Fax: +98 21 66402569.
** Corresponding author.
E-mail addresses: Nasehi@iricss.org (M. Nasehi), zarinmr@ams.ac.ir (M.-R. Zarrindast).

http://dx.doi.org/10.1016/j.bbr.2015.12.017
0166-4328/

1. Introduction

It has been suggested that stress effects on fear memory impairment or improvement require simultaneous noradrenergic and glucocorticoid activity in the basolateral amygdala (BLA) [1] and that the direction of the stress effects on fear memory depends on whether stress is experienced within or outside the context of the learning episode [2]. In addition, norepinephrine (NE) actions in modulating memory formation are dependent on the populations of adrenoceptors (ARs) in different brain locations and at different times in the sequence of memory processing [3,4]. The BLA receives a dense noradrenergic input primarily originating from the locus coeruleus (LC), which is heavily involved in stress and stress-related pathologies [5,6] and memory formation [7,8]. Studies have shown that stressful stimuli such as foot shock induce NE release in the rat amygdala [9]. Also, human functional magnetic resonance imaging (fMRI) studies show that elevated NE neurotransmission enhances BLA responses to fear signals [10]. Therefore, the LC is important for providing information about aversive stimuli to the BLA and generating appropriate responses to stressors, which suggests that the LC–NE circuit in the BLA could be a potential drug target for anxiety disorders [11]. NE can interact with three families of ARs: α_1, α_2 and $\beta_{(1-3)}$ receptors that exhibit different signal transduction. Like the β-adrenoceptor, the α_1-adrenoceptor subtype is located post-synaptically and activated by NE. In contrast, α_2-adrenoceptors are predominantly located pre-synaptically [12] and activation of these receptors inhibits the release of NE [13].

Growing evidence suggests an interaction between the cannabinoid and noradrenergic system that has significant functional and behavioral implications. Importantly, cannabinoids can modulate noradrenergic transmission in both noradrenergic nuclei and target regions. This modulation seems to be circuit specific and may depend on the basal status of cannabinoid and NE levels. In addition, NE seems to be important for particular cannabinoid-induced behaviors [5]. Manipulation of the endocannabinoid system results in effects on mood and cognition that share similarities with the noradrenergic system. Briefly, increasing endocannabinoid tone has been shown to improve mood similar to increasing noradrenergic tone with antidepressants [14]. Taken together, the effects of manipulating the endocannabinoid system and modulating noradrenergic transmission suggest that the two systems may interact or share some common signaling pathways [5].

Augmented behavioral responses to aversive stimuli, including freezing, has been reported in a number of different learning and memory paradigms in animals subjected to prior stressful experiences [15,16]. The memory of learned fear can be assessed quantitatively using a Pavlovian fear-conditioning paradigm [17]. There is evidence suggests that both acute and chronic stress enhance fear conditioning, but the degree to which they might affect learning and memory processes requires more extensive analysis [18].

Given this background knowledge, the present study was designed to evaluate the role of α_2-adrenoceptors of the BLA in mediating arachidonylcyclopropylamide (ACPA; selective CB1 cannabinoid receptor agonist) fear learning. Therefore, we studied whether ACPA-induced fear learning deficit could be affected by intra-BLA microinjections of α_2-adrenoceptor agonist/antagonist.

2. Material and methods

2.1. Animals

All animal care and experimental studies were in compliance with the international laws on animal experimentation and approved by the committee of Ethics of Tehran University of Medical Sciences, Tehran, Iran. All procedures were carried out in accordance with the guidelines for animal care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 2010). Male NMRI mice were used in all experiments (Institute for Cognitive Science Studies; Tehran, Iran). At the beginning of the experiments, mice were weighed 28–33 g. The animals were maintained under controlled temperature ($22 \pm 2\,^{\circ}C$) and light (light–dark cycle from 8:00 to 20:00 h), with free access to food and water. The experiments were carried out between 9:00 am and 14:00 pm. The same conditions were maintained for all the behavioral tests.

2.2. Cannulae implantation

Prior to surgery mice were anesthetized with intraperitoneal injection of ketamine (50 mg/kg) plus xylazine (5 mg/kg) and fixed in a stereotaxic frame. A stainless steel guide cannula (22 gage) was implanted bilaterally in the amygdaloid complex (Basolateral amygdala nuclei). Stereotaxic coordinates for the guide cannulae placements were anterior–posterior (AP) = −1.5 mm from bregma, medial–lateral (ML) = ±2.8 mm, and dorsal–ventral (DV) = −4.5 mm [19]. Guide cannulae were anchored to the skull with screws and dental acrylic. At the end of the surgery each cannulae was temporarily sealed with a stainless steel wire to protect it from obstruction. Animals were given 5–7 days of post-operative recovery prior to the start of behavioral training. Cannulae placements were verified by dye (a 0.3 μl/side; 1% aquatic methylene blue solution) injections followed by coronal sectioning on the vibroslice. Animals in which the cannulae showed an incorrect placement were removed from the analyses.

2.3. Treatments

Drugs used were: cannabinoid CB1 receptor agonist, ACPA [Arachidonylcyclopropylamide] and α_2-adrenoceptor antagonist, yohimbine from Tocris, UK; and α_2-adrenoceptor agonist; clonidine hydrochloride from Sigma Chemical Co., St Louis, CA, USA. Clonidine and yohimbine were dissolved in sterile 0.9% saline, while ACPA was stored at $-20\,^{\circ}C$ as a prepared solution dissolved in anhydrous ethanol at a concentration of 5 mg/ml and then was diluted to the required volume using sterile 0.9% saline before related experiments. The highest concentration of ethanol and DMSO contained in a dose was used as the control vehicles 1 and 2, respectively. ACPA was administered intraperitoneally at 10 ml/kg, while other drugs were microinjected into the BLA at the volume of 0.3 μl/each side. The wait time for test sessions after drug injection was 5 min. In combination studies, each animal received intraperitoneal administration of ACPA, and after 5 min intra-BLA injection of α_2-adrenoceptor agents. Each mouse was tested once.

2.4. Intra-BLA microinjections

The animals were gently held in hand. A thin dental needle (27 gage) was introduced through the guide cannula until its lower end was 1 mm below its tip. The injection needle was connected to a 2.5 μl syringe Hamiltone by a polyethylene tube. A volume of 0.3 μl of the drug was injected over 60 s. The displacement of an air bubble inside the polyethylene tubing was used to monitor the microinjection.

2.5. Behavioral assessment

Fear conditioning was conducted using a protocol developed by Shoji et al. [20] for assessing conditioned fear in the mice. The fear conditioning apparatus consisted of a square chamber (55 cm × 53 cm × 67 cm) with acoustic walls and a light source, two