Neuroscience Letters 500 (2011) 82–85

Contents lists available at ScienceDirect

Neuroscience Letters

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

Direct intra-accumbal infusion of a beta-adrenergic receptor antagonist abolishes WIN 55,212-2-induced aversion

Ana Franky Carvalho[a,b,c,*], Elisabeth J. Van Bockstaele[a]

[a] Department of Neurosciences, Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, PA, USA
[b] Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal
[c] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

ARTICLE INFO

Article history:
Received 14 December 2010
Received in revised form 2 June 2011
Accepted 6 June 2011

Keywords:
Cannabinoids
Lithium
Adrenergic receptors
Place conditioning
Aversion

ABSTRACT

The cannabinoid system is known to interact with a variety of neuromodulators in the central nervous system and impacts diverse behaviors. Previous studies have demonstrated that limbic norepinephrine is a critical determinant in the behavioral expression of cannabinoid-induced aversion. The present study was carried out to define the adrenergic receptor subtype involved in mediating cannabinoid-induced behavioral responses. An acute microinjection of the β1-adrenergic receptor blocker, betaxolol, directly into the nucleus accumbens (Acb), was able to prevent WIN 55,212-2-induced aversion, but not lithium-induced aversion, as measured in a place conditioning paradigm. These results suggest that noradrenergic transmission in the Acb is important for cannabinoid-induced aversion and that beta-adrenergic antagonists may be effective in counteracting negative side effects of cannabinoid-based agents.

Previous studies have shown an anatomical and functional interaction between the cannabinoid and noradrenergic systems in the brain. The cannabinoid receptor type 1 (CB1r) has been found in noradrenergic perikarya in the locus coeruleus (LC) [19] and the nucleus of the solitary tract (NTS) [3] as well as in its efferent projections including the prefrontal cortex (PFC) [12] and nucleus accumbens (Acb) [3]. Functionally, administration of the CB1r agonist WIN 55,212-2 increases norepinephrine (NE) release in the PFC and stimulates c-fos expression in the LC and is accompanied by an increase in anxiety-like behaviors [13,14]. Cannabinoids are known to differentially impact animal behaviors with low doses typically inducing reward-like responses and anxiolytic effects while higher doses are associated with aversive and anxiety-like behaviors [6,11]. In a previous study, we demonstrated that limbic NE is a critical determinant of cannabinoid-induced aversion but not cannabinoid-induced anxiety [4]. Although the study provided evidence for an important role of NE in the aversion induced by a cannabinoid agent, it did not establish the adrenergic receptor (AR) subtype involved and whether this effect was specific to cannabinoid-induced aversion or to other aversive stimuli. The present study was carried out using a combined pharmacological and behavioral approach to determine the role of the β1-AR in cannabinoid and lithium-induced aversion. It has been shown that acute and chronic injection of WIN 55,212-2 (3.0 mg/kg) leads to a decrease in β1-AR protein expression in the Acb [3], possibly reflecting a role of this receptor in WIN 55,212-2-induced aversion. The experimental design involved conditioning rats to the CB1r agonist, WIN 55,212-2 or lithium, in a place conditioning paradigm and antagonizing the β1-AR using an intra-accumbal microinjection of a β1-AR blocker, betaxolol, prior to testing the animals for aversion.

Thirty two male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 220–250 g were housed separately in a controlled environment (12-h light schedule (lights on 7:00), temperature at 20 °C, humidity at 55%). Food and water were provided *ad libitum*. The care and use of animals were approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University and were conducted in accordance with the NIH *Guide for the care and use of laboratory animals*. All efforts were made to minimize animal suffering and reduce the number of animals used.

Rats were anesthetized with an intraperitoneal (i.p.) injection of a saline solution containing a cocktail of Ketamine HCl (100 mg/kg; Phoenix Pharmaceutical, Inc. St. Joseph, MO) and Xyla-Ject (2 mg/kg; Phoenix Pharmaceutical, Inc.) and subsequently placed in a stereotaxic surgical frame (Stoelting Corp., Wood Dale, IL). The anesthesia was maintained by administration of isoflurane (Webster Veterinary Supply, Inc., Sterling, MA) through a nose cone. Bilateral cannulae (22 gauge, 8 mm long, from PlasticOne) were implanted into the Acb (AP: 1.5 mm rostral to bregma, ML: +/−0.9 mm, DV: −6.4 mm), according to Rat Brain Atlas of Paxinos

* Corresponding author at: Life and Health Science Research Institute (ICVS), School of Health Sciences – University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. Tel.: +351 253 604929; fax: +351 253 604849.
E-mail address: anafranky@ecsaude.uminho.pt (A.F. Carvalho).

0304-3940/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2011.06.008

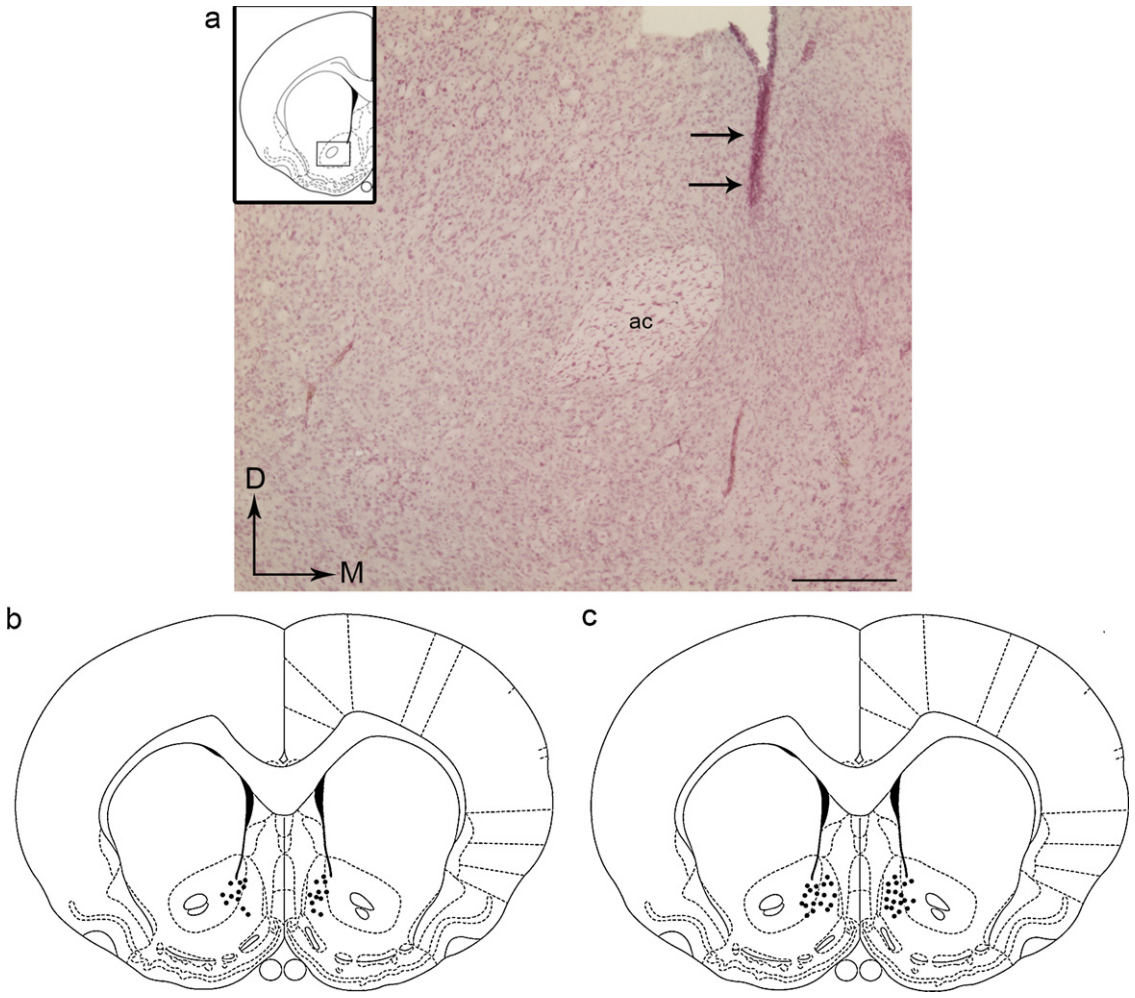

Fig. 1. Anatomical placement of cannulae in the Acb. (a) Photomicrograph of the Acb showing the placement of a cannula (arrows). (b) Schematic diagram of a coronal section through the rostral forebrain adapted from the rat brain atlas of Paxinos and Watson [16] showing sites of bilateral cannulae placements into the Acb of animals used for WIN 55,212-2-induced aversion ($n = 11$). (c) Schematic diagram showing sites of bilateral cannulae placements into the Acb of animals used for lithium-induced aversion ($n = 18$). Dots represent the tip of the cannulae. Scale bar, 25 μm.

and Watson [16] coordinates. Cannulae were affixed to the skull using acrylic cement and double stylets were placed in the cannulae to prevent blockage. Animals were given a week to recover from surgery before behavioral testing. For intracerebral microinjections, the obturators were removed and 28 gauge injector cannulae were lowered to the final site (1 mm past the guide). Infusions of 0.5 μl per side were made manually using a Hamilton syringe over a period of 30 s, as previously described [1].

Cannabinoid effects on behavior vary depending on dose [6,11]. In the present study, we used a dose that has been shown (e.g. 3.0 mg/kg) by our group as well as by others to exert anxiogenic and aversive – like behaviors [4,15]. WIN 55,212-2 (Sigma–Aldrich, St. Louis, MO) was dissolved in 5% dimethyl sulfoxide (DMSO)(Fisher Scientific, Fair Lawn, NJ) in saline and injected i.p. (3.0 mg/kg) in a volume of 1 ml/kg body weight. Vehicle injections consisted of 5% DMSO in saline. Lithium chloride (LiCl; Sigma–Aldrich) was dissolved in saline and was given intraperitoneally (IP, 1 ml/kg body weight) in a dosage of 125 mg/kg. This dosage has been reported to be effective in producing a reliable place aversion [7,20]. Vehicle injections consisted of saline. Drugs were freshly prepared before each treatment trial. Betaxolol (Sigma–Aldrich) was dissolved in saline (1nmol/0.5 μl); betaxolol or saline were microinjected in a volume of 0.5 μl per side (as previously described [1]).

WIN 55,212-2-induced place aversion. The protocol used to induce WIN 55,212-2 aversion follows our previous report [4]. An unbiased place conditioning procedure was used, so that the side of the apparatus used to conditioned animals was counterbalanced in all the groups. The paradigm consisted of three phases: pre-test, conditioning and test. On pre-test day (day 1), animals were placed in the apparatus and allowed to freely explore both sides of the apparatus for 20 min. The time spent in each side was recorded by an investigator. During the conditioning phase (days 2–6), the rats were injected twice daily as follows: in the morning, animals were injected with vehicle and confined to one side of the apparatus for 45 min; in the afternoon, animals were injected with WIN 55,212-2 (3.0 mg/kg) and confined to the opposite side for 45 min. On the test day (day 7), animals received a microinjection of betaxolol in the Acb five minutes before being place in the apparatus and allowed to explored both sides for 20 min. Control animals received a microinjection of saline in the Acb. The time spent in each side was measured by an investigator. No WIN 55,212-2 or vehicle injection was given to the animals on the test day.

Lithium-induced place aversion. A different set of animals was conditioned to LiCl. LiCl aversion was achieved as for WIN 55,212-2 with the following modifications. Animals were allowed to explore the apparatus for 15 min in the pre-test and test sessions [7,20]. Conditioning phase lasted 4 consecutive days and animals were confined to one side of the apparatus for 30 min

At the conclusion of testing, animals were anesthetized with isoflurane (Isoflurane, USP, Webster Veterinary, Sterling, MA) and