Progress in Neuro-Psychopharmacology & Biological Psychiatry 59 (2015) 76–83

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

Cannabinoid CB1 receptors in the dorsal hippocampus and prelimbic medial prefrontal cortex modulate anxiety-like behavior in rats: Additional evidence

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ARTICLE INFO

Article history:
Received 11 September 2014
Received in revised form 7 January 2015
Accepted 8 January 2015
Available online 14 January 2015

Keywords:
Anandamide
Anxiety
CB1 receptors
Dorsal hippocampus
Endocannabinoids
Prefrontal cortex

ABSTRACT

Endocannabinoids (ECBs) such as anandamide (AEA) act by activating cannabinoid type 1 (CB1) or 2 (CB2) receptors. The anxiolytic effect of drugs that facilitate ECB effects is associated with increase in AEA levels in several encephalic areas, including the prefrontal cortex (PFC). Activation of CB1 receptors by CB1 agonists injected directly into these areas is usually anxiolytic. However, depending on the encephalic region being investigated and on the stressful experiences, opposite effects were observed, as reported in the ventral HIP. In addition, contradictory results have been reported after CB1 activation in the dorsal HIP (dHIP). Therefore, in the present paper we have attempted to verify if directly interfering with ECB metabolism/reuptake in the prelimbic (PL) portion of the medial PFC (MPFC) and dHIP would produce different effects in two conceptually distinct animal models: the elevated plus maze (EPM) and the Vogel conflict test (VCT). We observed that drugs which interfere with ECB reuptake/metabolism in both the PL and in the dentate gyrus of the dHIP induced anxiolytic-like effect, in both the EPM and in the VCT via CB1 receptors, suggesting that CB1 signaling in these brain regions modulates defensive responses to both innate and learned threatening stimuli. This data further strengthens previous results indicating modulation of hippocampal and MPFC activity via CB1 by ECBs, which could be therapeutically targeted to treat anxiety disorders.

1. Introduction

Endocannabinoids (ECBs) are lipid compounds derived from arachidonic acid that act by activating cannabinoid type 1 (CB1) or 2 (CB2) receptors (Piomelli, 2003). In the central nervous system, ECBs are produced on demand and released from the postsynaptic neuronal membrane (Wilson and Nicoll, 2002). CB1 receptors are the most abundant metabotropic receptors in the mammalian brain (Herkenham et al., 1990). They are predominantly located in presynaptic terminals where they inhibit release of several classical neurotransmitters such as glutamate and GABA (Egertova et al., 1998). One of the main ECBs is arachidonoylethanolamide (Anandamide, AEA), a neurotransmitter that can activate, in addition to CB1 receptors, potential vanilloid type 1 receptors (TRPV1) (Aguiar et al., 2014). AEA actions terminate after an internalization process followed by enzymatic hydrolysis by fatty acid amide hydrolase (FAAH) in the postsynaptic neuron (Cravatt et al., 1996).

Extensive expression of CB1 receptors in encephalic regions such as the hippocampus (HIP), the medial prefrontal cortex (MPFC), the cerebellum, the periaqueductal gray matter (PAG) and the basal nuclei (Tsou et al., 1998) is probably related to the effects of Δ9-tetrahydrocannabinol (THC), the main psychoactive compound of the *Cannabis sativa* plant, on memory, cognition and behavior. Several studies have shown CB1 receptors are implicated in anxiety (Haller et al., 2004; Rey et al., 2012), mood (Steiner et al., 2008) and extinction of aversion-related memories processes (Marsicano et al., 2002; Metna-Laurent et al., 2012). For example, the anxiolytic and anxiogenic-like effects of drugs that facilitate ECB signaling are associated to increases in AEA levels in the PFC and the HIP (Bortolato et al., 2006; Draycott et al., 2014; Kathuria et al., 2003; Laviolette and Grace, 2006; Tan et al., 2011), suggesting these structures are involved in cannabinoid effects.

Activation of CB1 receptors by CB1 agonists injected directly into encephalic structures related to defensive responses such as the MPFC

Abbreviations: ECBS, Endocannabinoids; AEA, Anandamide; FAAH, fatty acid amide hydrolase; CB1, Cannabinoid type 1 receptor; CB2, Cannabinoid type 2 receptor; TRPV1, Transient potential vanilloid type 1 receptor; CBD, cannabidiol; PFC, Prefrontal cortex; MPFC, Medial prefrontal córtex; PL, Prelimbic; HIP, Hippocampus; dHIP, dorsal Hippocampus; PAG, Periaqueductal gray matter; THC, Δ9-tetrahydrocannabinol; EPM, Elevated plus maze; VCT, Vogel conflict test; AP, Anteroposterior; L, lateral; V, ventral

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http://dx.doi.org/10.1016/j.pnpbp.2015.01.005
0278-5846/

(Fogaca et al., 2012; Rubino et al., 2008) and the dorsolateral PAG (Moreira et al., 2007) is usually anxiolytic. There are, however, several contradictory results. In addition to producing an inverted U-shaped dose–response curve, these drugs can also be anxiogenic (Campos et al., 2010; Hakimizadeh et al., 2012; Moreira et al., 2012; Roohbakhsh et al., 2007), indicating anxiety modulation by CB1 receptors is probably more complex than initially thought. These opposite effects could depend, in addition to the encephalic region being investigated, also on the stress experience of the subjects before or at the time of behavioral tests (Campos et al., 2010; Lisboa et al., 2008, 2010). In line with this proposal, we observed that intra-ventral HIP injection of AEA reuptake inhibitor produces anxiogenic and anxiolytic effects in naïve rats tested in the elevated plus maze (EPM) and the Vogel conflict tests, respectively. The anxiogenic effect observed in the EPM turned into an anxiolytic effect when rats were previously stressed by forced restraint (2 h) 24 h before the EPM test (Campos et al., 2010). Similar model- and stress-dependent effects were found after intra-PL injection of cannabidiol (CBD) (Fogaca et al., 2014; Lemos et al., 2010), a phytocannabinoid that can, among other effects, inhibit the FAAH enzyme (for review, see Campos et al., 2012). The involvement of the ECB system in these effects, however, is not clear, since CBD can act by several other mechanisms, including facilitation of 5HT1A-mediated neurotransmission (Resstel et al., 2009). Therefore, in the present paper we will directly verify if interference with AEA metabolism/reuptake in the PL and the dorsal HIP would produce different effects in two conceptually distinct animal models of anxiety, the EPM and the VCT.

2. Material and methods

2.1. Animals

Male Wistar rats weighing 230–270 g were used. Animals were maintained at the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. Rats were housed in groups of 4 in plastic cages with free access to food and water and under a 12 h light/dark cycle (lights on at 06:30 h). Independent groups of animals were used in all experiments. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (protocol n° 143/2007). All efforts were made to minimize animal suffering, to reduce the number of animals used.

2.2. Stereotaxic surgery

Rats were anesthetized with tribromoethanol (Sigma-Aldrich; 250 mg/kg i.p.). After scalp anesthesia with lidocaine (2% epinephrine as vasoconstrictor; subcutaneous), the skull was surgically exposed and stainless steel guide cannula (26G) were bilaterally implanted into the PL or the dentate gyrus of the dHIP using a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). The Bregma was used as a reference point. Coordinates for cannula implantation into the PL (incisor: −3.3 mm; AP = −3.3 mm; L = 1.9 mm from the medial suture, V = −2.6 mm from the skull with a lateral inclination of 22°) or dHIP (incisor: −2.5 mm; AP = −4.0 mm from bregma; L = +2.8 mm from the medial suture, V: −2.1 mm from the skull) were based on the rat brain atlas of Paxinos and Watson (2006). Cannulae were fixed to the skull with dental cement and a metal wire was inserted into the cannula to prevent obstruction. After surgery, the animals received an intramuscular injection of a poly-antibiotic (Pentabiotico®, Fort Dodge, Brazil; 0.2 ml) and a subcutaneous injection of the nonsteroidal anti-inflammatory flunixine meglumine (Banamine®, Schering Plough, Brazil) for analgesia.

2.3. Drugs

The AEA transporter inhibitor 4-hydroxyphenylarachidonylamide (AM404; Tocris, Westwoods Business Park Ellisville, MO, USA) 50 pmol was dissolved in Tocrisolve TM 100 (a solvent that contains a 1:4 ratio of soya oil/water, emulsified with the block co-polymer Pluronic F68) as recommended by the manufacturer. Cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl Ester (URB597, inhibitor of FAAH enzyme; Calbiochem) 0.01 nmol was dissolved in DMSO 10% in saline (0.9% NaCl). The CB1 receptor antagonist N-(piperidin-1yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-Hpyrazole-3-carboxamide (AM251; Tocris, Westwoods Business Park Ellisville, MO, USA) 100 pmol was dissolved in DMSO 10% in saline (0.9% NaCl). The solutions were prepared immediately before use and were kept on ice and protected from the light during the experimental sessions. Tribromoethanol (Sigma, St. Louis, Missouri, USA) and Urethane (Sigma, St. Louis, Missouri, USA) were dissolved in distillated water. Morphine hydrochloride (5 mg/kg, Merck) was dissolved in saline (0.9% NaCl). AM404 and URB597 doses were chosen based on previous studies from our group showing these doses modulate anxiety-related behavior (Lisboa et al., 2008, 2010; Moreira et al., 2007) and from pilot studies showing these same doses modified anxiety-like behavior when injected into the dHIP or the MPFC. From these studies, we also choose a dose of AM251 that did not modify anxiety-like behaviors by itself.

3. Experimental procedures

In the test day, five to seven days after surgery, independent group of animals received two bilateral injections into the dHIP or PL. The first microinjection of vehicle (500 nl into the dHIP or 200 nl into the PL) or AM251 (100 pmol) was followed, 5 min later, by a second injection of vehicle, AM404 (50 pmol) or URB597 (0.01 or 0.1 nmol). Ten minutes later, the animals were submitted to the test session (Vogel or EPM).

In the experiments measuring tail withdrawal latency and water consumption, in which rats received only AM404 or URB, the animals were submitted to the tests 10 min after the drugs. Morphine hydrochloride 5 mg/kg (1 ml/kg) was injected systemically as a positive control in the tail flick test 30 min before evaluation.

3.1. Vogel conflict test

This test was performed in a Plexiglas box (42 × 50 × 25 cm) with a stainless grid floor. The metallic spout of a drinking bottle containing water projected into the box and the contact of the animal with the spout and the grid floor closed an electrical circuit controlled by a sensor (Anxio-Meter model 102, Columbus, USA). This sensor produced 7 pulses/s whenever the animal was in contact with both components. Each pulse was considered as a lick and after every 20 licks, a 0.5 mA/2 s shock was delivered in the metallic drinking spout. The sensor recorded the total number of licks and shocks delivered during the test period. The whole apparatus was located inside a sound-attenuated cage (Lisboa et al., 2008).

Animals were water deprived for 48 h before the test. After the first 24 h, the animals were allowed to drink freely for 3 min in the test box in order to find the bottle spout. The animals that did not find the spout were excluded from the experiment. After an additional 24 h period of water deprivation the drugs were injected into the dHIP or PL and 10 min later the animals were placed into the test box for the 3 min test session. The number of licks and shocks delivered were registered. Although the number of shocks delivered by the system was proportional to the number of licks performed by the rat (one shock at every 20 licks), sometimes at the end of the test the animal was still licking but had not yet received the next shock. Therefore, the number of licks is usually slightly higher than one would expect considering the number of shocks.

3.2. Water consumption evaluation

Apparatus was the same used in the test above; however, the electric shock delivering system was rendered inoperative.