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Emotional arousal state influences the ability of amygdalar endocannabinoid signaling to modulate anxiety

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ABSTRACT

Systemic activation of cannabinoid receptors often induces biphasic effects on emotional memory and anxiety depending on the levels of emotional arousal associated to the experimental context. The basolateral nucleus of the amygdala (BLA) represents a crucial structure for the ability of endocannabinoid (eCB) signaling to modulate emotional behaviour, and receives dense projections from brainstem arousal system nuclei. We examined whether changes in emotional arousal state would influence the ability of acute eCB manipulations within the BLA to modulate anxiety. Rats were tested in an elevated plus maze (EPM) under low or high arousal conditions. The low emotional arousal group was extensively handled and habituated to the experimental room and tested under red light condition, the high emotional arousal group was not handled or habituated and tested under high light condition. We examined amygdalar eCB anandamide (AEA) and 2-arachidonoylglycerol (2-AG) levels immediately after the EPM and the effects of intra-BLA administration of the AEA hydrolysis inhibitor URB597 or the 2-AG hydrolysis inhibitor KML29 on anxiety behaviour. The modulation of anxiety-like behaviour by eCBs in the BLA was strictly dependent on the environmental-associated emotional arousal. Pharmacologically-induced elevations of AEA or 2-AG in the BLA decreased anxiety under conditions of low emotional arousal. Conversely, when the level of emotional arousal increased, local eCB manipulation was ineffective in the modulation of the emotional arousal-induced anxiety response. These findings suggest that, depending on the emotional arousal state, eCB system is differentially activated to regulate the anxiety response in the amygdala and help to understand the state-dependency of many interventions on anxiety.

1. Introduction

The endocannabinoid (eCB) system modulates a diverse array of behavioural and physiological processes (Katona and Freund, 2012; Mechoulam and Parker, 2013), through the activation of cannabinoid receptors type 1 (CB1) and type 2 (CB2), by the two major endogenous ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG). eCB signaling is primarily terminated by enzymatic degradation of AEA and 2-AG by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively (Kano et al., 2009; Piomelli, 2003).

Research in both rodents and humans has consistently shown that for many of the behavioural effects produced by activation of cannabinoid receptors, there are clear oppositional or biphasic effects, such that varying doses of a cannabinoid receptor agonist can have dramatically contrasting effects on the same behaviour (Micale et al., 2013; Morena and Campolongo, 2014; Zanettini et al.,

Abbreviations: AEA, Anandamide; 2-AG, 2-arachidonoylglycerol; BLA, Basolateral nucleus of the amygdala; CB1, Cannabinoid receptor type 1; CB2, Cannabinoid receptor type 2; CRH, Corticotropin releasing hormone; EPM, Elevated plus maze; eCB, Endocannabinoid; FAAH, Fatty acid amide hydrolase; HDIPS, Head dippings; HA, High Arousal; HC, Home cage; HPA, Hypothalamic–pituitary–adrenal axis; LA, Low Arousal; MAGL, Monoacylglycerol lipase; SAP, Stretch attend postures.

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2011). With the discovery that the primary neuronal cannabinoid receptor, CB1, existed on different neuronal populations, targeted cell-specific deletions of the CB1 receptor revealed that many of the dose-specific effects of cannabinoids related to the ability of these agonists to preferentially activate CB1 receptors on glutamatergic versus GABAergic neuronal populations at different concentrations (Bellocchio et al., 2010; Monory et al., 2007; Rey et al., 2012; Steindel et al., 2013). Independent of dose, however, there has also been a large body of work that has revealed that even a consistent dose of a CB1 receptor agonist can have oppositional effects on the same behaviour if there are alterations in environmental conditions. For example, the ability of a CB1 receptor agonist to influence cognitive performance, anxiety-like behaviour and activation of the hypothalamic–pituitary–adrenal (HPA) axis can be dramatically modified if the animal has been exposed to stress or is tested under highly aversive conditions (Campolongo et al., 2013; Fokos and Panagis, 2010; Hill and Gorzalka, 2004; Morena et al., 2015). Similar to the effects seen with exogenous cannabinoid ligands, manipulations of eCB signaling are also modulated by environmental variables. Specifically, the ability of both FAAH and MAGL inhibitors to modulate cognitive processes and anxiety-like behaviour is highly sensitive to environmental aversiveness (Aliczki et al., 2012; Bluett et al., 2014; Campolongo et al., 2012; Haller et al., 2009; Hill et al., 2013; Morena et al., 2014, 2015; Naidu et al., 2007; Sciolino et al., 2011; Kathuria et al., 2003). In addition to dose-dependent effects, these data suggest that the effects of both endogenous and exogenous cannabinoids on a given behavioural process, can also be significantly influenced by the emotional arousal state of an organism, often dictated by environmental conditions.

Physiological arousal is elicited by a rapid autonomic sympathetic response (Pfaff, 2006). Neuroanatomically, the emotional arousal system consists of bilateral neuronal interconnections between the brainstem and forebrain. The activity of these ascending and descending neural projections influences the regulation of specific domains of anxiety states, such as vigilance, and enhanced levels of emotional arousal are associated to increased anxiety (McCall et al., 2015). The basolateral nucleus of the amygdala (BLA) is critical for the generation of appropriate behavioural responses to salient sensory stimuli and emotionally arousing events in the external world and plays a key role in the modulation of anxiety behaviour (Millan, 2003; Sah et al., 2003). Indeed, increased activity in the amygdala is associated with increased anxiety in humans and rodents (Etkin and Wager, 2007; Feinstein et al., 2013; Roozendaal et al., 2009). Optogenetic studies have similarly found that global activation of BLA somata produces a frank behavioural state of anxiety (Tye et al., 2011); however, localized optogenetic targeting of BLA terminals in discrete brain regions has demonstrated that activation of specific projection neurons can produce divergent effects on anxiety-like behaviour depending on the region of termination (see Janak and Tye, 2015 for detailed description). The amygdala receives dense innervations from arousal system nuclei in the brainstem (i.e. nucleus of solitary tract, locus coeruleus, A1/A2 cell groups) (Asan, 1998; Berridge and Waterhouse, 2003; Zhang et al., 2013) and is engaged when emotional stimuli elicit an arousal effect to modulate anxiety behaviour and processes of emotional memory formation (Atsak et al., 2015; McGaugh, 2015; McIntyre et al., 2002; Siuda et al., 2016).

With respect to eCB signaling and anxiety, it is interesting to note that the amygdala, particularly the BLA, appears to be an important hub for the regulation of stress and anxiety by eCB signaling (Bedse et al., 2014; Dono and Currie, 2012; Ganon-Elazar and Akirav, 2009; Gray et al., 2015b; Hill et al., 2009) reviewed in (Gunduz-Cinar et al., 2013a; Morena and Campolongo, 2014; Morena et al., 2016) and (Ramikie and Patel, 2012). However, to date, all studies examining the impact of changes in emotional arousal state on the ability of acute eCB manipulations to modulate anxiety have all been done in a systemic manner. Given the convergence of data that implicate the BLA as an important structure for the ability of eCB signaling to modulate emotional behaviour, and the target of afferent brainstem projections that modulate emotional arousal, the focus of this study was to explicitly determine if local manipulations of eCB signaling within the BLA were sensitive to changes in the animals emotional arousal state in the modulation of anxiety-like behaviour.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (350–380 g at the time of behavioural experiments; Charles River) were housed individually in a temperature-controlled (20 ± 1 °C) vivarium room and maintained under a 12 h/12 h light/dark cycle (8:00 a.m. to 8:00 p.m. lights on). Food and water were available ad libitum. All tests were performed during the light phase of the cycle between 10:00 a.m. and 2:00 p.m. All experimental procedures were in compliance with protocols approved by the University of Calgary Animal Care Committee and guidelines from the Canadian Council on Animal Care. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Surgery

Rats were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine (7 mg/kg). Subsequently, they were injected with 3 ml of saline (s.c.) to facilitate clearance of these drugs and prevent dehydration. The rats were then positioned in a stereotaxic frame (David Kopf Instruments), and two stainless-steel guide cannulae (23 gauge, 15-mm-long) were implanted bilaterally with the cannula tips 2 mm above the BLA (coordinates: anteroposterior, −2.8 mm and mediolateral, ±5.0 mm, from Bregma; dorsoventral, −6.5 mm, from skull surface) (Paxinos and Watson, 2005). The cannulae were affixed to the skull with two anchoring screws and dental cement. Stylets (15 mm-long 00 insect dissection pins) were inserted into each cannula to maintain patency. After surgery, the rats were retained in an incubator until recovered from anesthesia and were then returned to their home cages. Rats were allowed to recover from surgery for a week before testing.

2.3. Drug administration

The AEA hydrolysis inhibitor URB597 (10 ng/0.2 μl per side; Cayman Chemical) or the 2-AG hydrolysis inhibitor KML29 (200 ng/0.2 μl per side; Tocris Bioscience) or their vehicle was bilaterally infused into the BLA 30 min before the behavioural testing. To examine whether URB597 or KML29 effects were mediated via an activation of CB1 receptors, other groups of rats were administered a non-altering behavioural dose of the CB1 receptor antagonist AM251 (1 ng/0.2 μl per side; Cayman Chemical) or its vehicle together with URB597 or KML29 into the BLA 30 min before behavioural testing. Doses were selected on the basis of previous and pilot experiments performed in our laboratory, and others, indicating that these doses sufficiently elevate eCB levels and produce CB1 dependent behavioural effects (Gray et al., 2015b; Morena et al., 2014; Qi et al., 2015; Rubino et al., 2008). All drugs were dissolved in 5% polyethylene glycol, 5% Tween-80, and 90% saline. Bilateral infusions of drugs or an equivalent volume of