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Endocannabinoids, through opioids and prostaglandins, contribute to fever induced by key pyrogenic mediators

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ABSTRACT

This study aims to explore the contribution of endocannabinoids on the cascade of mediators involved in LPS-induced fever and to verify the participation of prostaglandins and endogenous opioids in fever induced by anandamide (AEA). Body temperature (Tc) of male Wistar rats was recorded over 6 h, using a thermistor probe. Cerebrospinal fluid concentration of PGE_2 and β-endorphin were measured by ELISA after the administration of AEA.

Intracerebroventricular administration of the CB1 receptor antagonist AM251 (5 μg, i.c.v.), reduced the fever induced by IL-1β (3 ng, i.c.v.), TNF-α (250 ng, i.c.v.), IL-6 (300 ng, i.c.v.), corticotrophin release factor (CRH; 2.5 μg, i.c.v.) and endothelin (ET)-1 (1 pmol, i.c.v.), but not the fever induced by PGE_2 (250 ng, i.c.v.) or $PGF_{2\alpha}$ (250 ng, i.c.v.). Systemic administration of indomethacin (2 mg kg^{-1}, i.p.) or celecoxib (5 mg kg^{-1}, p.o.) reduced the fever induced by AEA (1 μg, i.c.v.), while naloxone (1 mg kg^{-1}, s.c.) abolished it. The increases of PGE_2 and β-endorphin concentration in the CSF induced by AEA were abolished by the pretreatment of rats with AM251.

These results suggest that endocannabinoids are intrinsically involved in the pyretic activity of cytokines (IL-1β, TNF-α, IL-6), CRH and ET-1 but not the PGE_2 or $PGF_{2\alpha}$ induced fevers. However, anandamide via CB1 receptor activation induces fever that is dependent on the synthesis of prostaglandin and opioids.

1. Introduction

Fever is a preserved phylogenetic response to a wide variety of infectious and non-infectious triggers, which is characterized by an increase in body temperature induced by an elevation in the thermoregulatory set point. Although the importance of fever, as a defense mechanism is still a matter of debate, it is considered a cardinal symptom during sickness and it is one of first alert signal associated with infections, inflammatory diseases and poisoning (Roth and Blatteis, 2014; Malvar Ddo et al., 2014).

Activation of the cannabinoid system is involved in many physiological and pathological conditions (Mechoulam et al., 2014). Devane et al. (1992) identified arachidonoylethanolamide, which was named anandamide (AEA), as the first endogenous compound to bind to CB1 receptors (Devane et al., 1992). However, it is known that anandamide can activate CB1 and CB2 receptors (Howlett, 2002). Depolarization- and an agonist-induced increase in anandamide production was initially observed in primary culture of neurons and in rat brains (Di Marzo et al., 1994; Stella and Piomelli, 2001). Biological inactivation of anandamide occurs through a rapid uptake into cells followed by its intracellular hydrolysis, which is primarily mediated by fatty acid amide hydrolase (FAAH) (Cravatt et al., 2001). In 1995, a second endogenous ligand, 2-arachidonoylglycerol (2-AG) was identified. This endogenous ligand is more abundant than anandamide in the brain (Mechoulam et al., 1995). Similar to anandamide, inactivation of 2-AG occurs in two steps: a rapid uptake into cells followed by its intracellular hydrolysis by monoacylglycerol lipase (MAGL) (Beltramo and Piomelli, 2000). Kita et al. (2015) demonstrated that LPS-induced fever depends on monoacylglycerol lipase (Mgll) but not on cytosolic $PLA_{2\alpha}$. $Mgll^{-/-}$ mice showed a normal diurnal core body temperature variation, a normal cytokine production but a

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drastic reduction of the hypothalamic PGE_2 production after LPS administration.

The CB1 receptor was first identified by Matsuda et al. (1990) and it is expressed throughout the brain and is involved in the modulation of different neuronal pathways (Howlett, 2002; Matsuda et al., 1990; Tsou et al., 1998). CB2 receptors were cloned by Munro et al. (1993) and are expressed primarily on immune cells in the periphery, and during neuroinflammation on activated microglia in the CNS (Pacher and Mechoulam, 2011; Sanchez and Garcia-Merino, 2012) and mediates the immunoregulatory functions of cannabinoids such as inhibition of Th17 differentiation in experimental autoimmune encephalomyelitis and multiple sclerosis (Kong et al., 2014).

Studies from Van Sickle et al. (2005) showed the expression of CB2 receptor messenger RNA and protein localization on brainstem neurons of rats. Furthermore, Cabral and Griffin-Thomas (2009), Stella (2009) shown that CB2 receptors are involved in microglial function. In this line, Mecha et al. (2015) showed that CB2 receptors are expressed in an alternative phenotype of microglia and in acquired deactivated microglia. This induces an adjustment in the endocannabinoids machinery, which favors the selective synthesis of 2-AG and AEA, respectively, evidencing the significance of this system for the regulation of microglia activation in normal and pathological conditions.

Hypothermia is a common effect of exogenous cannabinoid when injected in different experimental animal models (Rawls et al., 2002; Pertwee, 2005). Paradoxically, the CB2 receptor agonist AM1241 was not able to change the body temperature of rats and the CB2 receptor antagonist AM630 did not reduce the AEA-induced fever. However, the CB1 receptor antagonist AM251 dose-dependently inhibited the fever induced by AEA and reduced the fever induced by ACEA (Fraga et al., 2009).

Moreover, endocannabinoids seems to contribute to the fever induced by lipopolysaccharide (LPS), since the CB1 receptor antagonists SR141716 (Benamar et al., 2007) and AM251 (Fraga et al., 2009) reduced the fever induced by this stimulus. Importantly, CB1 receptor deficient mice did not develop fever in response to LPS which seems to be related to the low expression of Toll-like 4 receptors (Duncan et al., 2013).

Nakashima et al. (1995) suggested the involvement of an opioid receptor mechanism in the early but not in the late phase of the fever induced by IFN-α while our group showed the contribution of opioids through activation of μ-opioid receptors in the progress of fever induced by LPS and endogenous pyrogens TNF-α, IL-6, MIP-1α, CRH and ET-1 (Fraga et al., 2008). Furthermore, opioid- and cannabinoid-receptors are often co-expressed in the central nervous system cells (Pickel et al., 2004; Rodriguez et al., 2001) and both systems are characterized by various and sometimes synergistic interactions related with analgesic, psychotropic, and behavioral effects (Naef et al., 2003; Navarro et al., 2001).

In light of the above considerations, the present study was designed to address at what level(s) endocannabinoids are recruited to take part in the complex central signaling mechanisms triggered by LPS for fever development and if opioids are involved in their pyrogenic effect.

2. Experimental procedures

2.1. Animals

Experiments were performed on 246 male Wistar rats weighing 180–200 g, housed individually at 24 ± 1 °C under a 12:12 h light–dark cycle (lights on at 06:00 AM) with free access to food and tap water until the night before the experiment, when only water was made available. Each animal was used only once. The animals did not present any signals of stress such as freezing behavior, piloerection and tachycardia. Moreover, the handling of the animals during the experiments was easy and did not change their behavioral parameters (Kumar et al., 2013). Moreover, the care and use of the animals were in full compliance with the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA) and Guide for the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research (National Research Council, 1996), and this study was previously approved by the Animal Research Ethics Committee of the Faculty of Medicine of Ribeirão Preto, University of São Paulo (Protocol nr. 136/2007).

2.2. Drugs

N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251 – Tocris, UK) was diluted in propylene glycol 10%, Tween 80 1% and saline. TNF-α (R&D Systems, USA), IL-1β, IL-6 (NIBSC, UK), PGE_2, $PGF_{2\alpha}$, CRH, and ET-1 (Sigma Chem. & Co., USA), celecoxib, indomethacin and Naloxone (Merck Sharp & Dome, Germany). Except for indomethacin that was diluted in sterile TRIS–HCl, pH 8.2 (Reagen, Brazil) all others drugs were diluted in sterile saline.

2.3. Intracerebral cannula implantation and microinjections

Under anesthesia with sodium pentobarbital (40 mg kg^{-1}, i.p., Sigma Chem. Co., St, Louis, MO), a permanent 22-gauge stainless steel guide cannula (0.8 mm outer diameter, 10 mm long) was stereotaxically implanted into the right lateral ventricle at coordinates: 1.6 mm lateral to the midline, 1.5 mm posterior to the bregma and 2.5 mm under the brain surface and the incisor bar was lowered 2.5 mm below the horizontal zero (Paxinos et al., 1985). All procedures were conducted under aseptic conditions and the rats were treated with oxytetracycline hydrochloride (400 mg kg^{-1}, i.m., Pfizer, BR) and allowed to recover for 1 week prior to experimental use.

Pyrogenic stimuli were all injected between 10:00 and 11:00 am. Microinjections into the lateral ventricle (i.c.v.) were made aseptically, using a 30-gauge needle connected by polyethylene (PE10) tubing to a 25 μl Hamilton syringe, respectively. The needle protruded 2 mm beyond the cannula tip and a 2 μl (i.c.v.) volume was injected slowly (over 1 min) with Hamilton syringe. After injection, the needle remained in place for 30 s before it was withdrawn, to prevent backflow of the injection fluid through the cannula.

2.4. Temperature measurements

Body temperature (Tc) was measured by inserting a thermistor probe (Yellow Springs Instruments 402, USA) 4 cm into the rectum for 1 min, at 30 min intervals, for up to 6 h. For each measurement, the animal was picked up gently and held by the experimenter during temperature measurements, without removing the animal from its home cage. This procedure was performed at least three times on the day before the experiment to minimize temperature changes secondary to handling. On the day of the experiment, the basal Tc of each animal was estimated four times, at 30 min intervals, before any injection. Only animals displaying a mean basal Tc between 36.8 and 37.2 °C were selected for the study. The experiments were conducted during the light cycle in a temperature-controlled room of 28 ± 1 °C, the thermoneutral zone for rats (Gordon, 1990; Romanovsky et al., 2002).

2.5. Dosage of PGE_2 and β-endorphin

Cerebrospinal fluid (CSF) was collected according to the method described by Consiglio and Lucion (2000). Briefly, prior to the CSF