Neuroscience 300 (2015) 539–553

AN ENDOCANNABINOID SYSTEM IS PRESENT IN THE MOUSE OLFACTORY EPITHELIUM BUT DOES NOT MODULATE OLFACTION

C. R. HUTCH, [a,b†] **C. J. HILLARD,** [d] **C. JIA** [c‡] **AND C. C. HEGG** [a,b,c*]

[a] *Neuroscience Program, Michigan State University, 293 Farm Lane, East Lansing, MI 48824, USA*

[b] *Environmental and Integrative Toxicological Sciences, Michigan State University, 1129 Farm Lane, East Lansing, MI 48824, USA*

[c] *Department of Pharmacology and Toxicology, Michigan State University, 1355 Bogue Street, East Lansing, MI 48824, USA*

[d] *Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA*

Abstract—Endocannabinoids modulate a diverse array of functions including progenitor cell proliferation in the central nervous system, and odorant detection and food intake in the mammalian central olfactory system and larval *Xenopus laevis* peripheral olfactory system. However, the presence and role of endocannabinoids in the peripheral olfactory epithelium have not been examined in mammals. We found the presence of cannabinoid type 1 (CB1) and cannabinoid type 2 (CB2) receptor protein and mRNA in the olfactory epithelium. Using either immunohistochemistry or calcium imaging we localized CB1 receptors on neurons, glia-like sustentacular cells, microvillous cells and progenitor-like basal cells. To examine the role of endocannabinoids, CB1- and CB2-receptor-deficient ($CB1^{-/-}$/$CB2^{-/-}$) mice were used. The endocannabinoid 2-arachidonylglycerol (2-AG) was present at high levels in both C57BL/6 wildtype and $CB1^{-/-}$/$CB2^{-/-}$ mice. 2-AG synthetic and degradative enzymes are expressed in wildtype mice. A small but significant decrease in basal cell and olfactory sensory neuron numbers was observed in $CB1^{-/-}$/$CB2^{-/-}$ mice compared to wildtype mice. The decrease in olfactory sensory neurons did not translate to impairment in olfactory-mediated behaviors assessed by the buried food test and habituation/dishabituation test. Collectively, these data indicate the presence of an endocannabinoid system in the mouse olfactory epithelium. However, unlike in tadpoles, endocannabinoids do not modulate olfaction. Further investigation on the role of endocannabinoids in progenitor cell function in the olfactory epithelium is warranted.

Key words: cannabinoids, 2-AG, olfaction, progenitor cells.

INTRODUCTION

A cannabinoid system in the central olfactory system, i.e., main olfactory bulb, was first identified decades ago (Herkenham et al., 1991; Egertova and Elphick, 2000; Cesa et al., 2001; Egertova et al., 2003). In the mammalian olfactory bulb the two primary endocannabinoid ligands, *N*-arachidonoylethanolamide (AEA or anandamide) and 2-arachidonylglycerol (2-AG), are present and endocannabinoid synthesis enzymes are localized to neurons in the glomerular layer (Piomelli, 2003; Okamoto et al., 2007; Wang et al., 2012; Soria-Gomez et al., 2014). CB receptors are expressed in the granule cell layer of the olfactory bulb and in the glomerular layer where afferents from olfactory sensory neurons make their primary synapses (Wang et al., 2012). Only recently were cannabinoids identified in the peripheral olfactory system, i.e., the olfactory epithelium, of *Xenopus laevis* tadpoles (Migliarini et al., 2006; Czesnik et al., 2007). Tadpole cannabinoid type 1 (CB1) receptors are expressed on dendrites of neurons and 2-AG is synthesized in both neurons and glial-like sustentacular cells (Czesnik et al., 2007; Breunig et al., 2010a). To date, an endocannabinoid system has not been described in the mammalian olfactory epithelium.

Endocannabinoids regulate neuronal activity and signaling in the glomeruli through canonical retrograde signaling and pre-synaptic inhibition (Wang et al., 2012). However, endocannabinoids also have a neuromodulatory role in the olfactory bulb and have been implicated in food intake (Di Marzo and Matias, 2005). A potential mechanism underlying sensory control of food intake is modulation of odorant sensitivity by endocannabinoids. In humans and other animals, sensitivity to and perceptual quality of food odorants are enhanced with hunger, and decrease with satiety (Berg et al., 1963; Crumpton et al., 1967; Aime et al., 2007). In addition, dysfunction

*Correspondence to: C. C. Hegg, 1355 Bogue Street, Room B439 Life Sciences Building, East Lansing, MI 48824, USA. Tel: +11-1-517-432-2339; fax: +11-1-517-353-8915.
E-mail addresses: hutchche@umich.edu (C. R. Hutch), chillard@mcw.edu (C. J. Hillard), JIAC01@mail.etsu.edu (C. Jia), hegg@msu.edu (C. C. Hegg).
† Present address: Department of Surgery, University of Michigan, 1500 E. Medical Center Drive, Ann Arbor, MI 48109, USA.
‡ Present address: Department of Biomedical Sciences, East Tennessee State University, Box 70582, Johnson City, TN 37614, USA.

Abbreviations: 2-AG, 2-arachidonylglycerol; 2-OG, 2-oxogluteric acid; AEA, *N*-arachidonoylethanolamide; CB1, cannabinoid type 1 receptor; $CB1^{-/-}$/$CB2^{-/-}$, CB1 and CB2 deficient; CB2, cannabinoid type 2 receptor; CK, cytokeratin; DAGLα, diacylglycerol lipase α; DAPI, 4′,6-diamidino-2-phenylindole; GFP, green fluorescent protein; IP3R3, inositol triphosphate receptor 3; MAGL, monoacylglycerol lipase; MASH1, mammalian achaete-schute homolog 1; OEA, oleoylethanolamide; OMP, olfactory marker protein; PEA, palmitoylethanolamide; WIN, WIN 55212-2.

http://dx.doi.org/10.1016/j.neuroscience.2015.05.056
0306-4522/

of the olfactory system occurs in eating-related obesity in humans (Richardson et al., 2004). Clearly, the sense of smell may be important in regulating food intake, and metabolic signals such as endocannabinoids may be able to modulate olfactory functions.

Soria-Gomez and colleagues (2014) demonstrated that CB1 receptor activity in the olfactory bulb influences neuronal activation, odorant detection, and food intake in fasted mice. A behavioral assay scoring exploration time of increasing concentrations of an odorant (almond or banana) was used to show cannabinoid treatment (via direct injection into the olfactory bulb or increased AEA production) decreased the odorant concentration at which mice spent significant times exploring compared to vehicle-treated mice (Soria-Gomez et al., 2014). Additionally, olfactory bulb-specific CB1-receptor-deficient mice displayed a physiological decrease in odorant detection threshold under vehicle conditions, suggesting that increased odorant detection in a fasted state is CB1 receptor-specific (Soria-Gomez et al., 2014). Mice with a selective CB1-receptor deficiency in the granule cell layer of the olfactory bulb ate less food 24 h after fasting than fasted control mice (Soria-Gomez et al., 2014). Collectively, these data suggest that endocannabinoid signaling via CB1 receptors helps link the physiological state of hunger to odorant detection thresholds and food intake.

The peripheral olfactory epithelium is the site where odorants are detected (odorant threshold) and the central olfactory structures, the olfactory bulb and olfactory cortex, are involved in the identification and the discrimination of odorants (Enwere et al., 2004; Kovacs, 2004). The cannabinoid system modulates odorant-evoked responses in the peripheral olfactory epithelium in larval *Xenopus* (Czesnik et al., 2007; Breunig et al., 2010a). Odorant threshold sensitivity is increased when 2-AG synthetic enzyme diacylglycerol lipase α (DAGLα) is inhibited pharmacologically, suggesting that endogenous olfactory sensory neuron odorant sensitivity is mediated by the endocannabinoid 2-AG (Breunig et al., 2010b). Exogenous cannabinoid stimulation after fasting decreases odorant threshold, thereby enhancing odorant detection (Breunig et al., 2010b), suggesting a role in food intake.

The mouse olfactory epithelium is pseudostratified and contains multiple cell types. Olfactory sensory neurons have a cell soma located in the middle third of the epithelium and an unmyelinated axon that projects to the olfactory bulb. Non-neuronal sustentacular cells and microvillous cells have large cell bodies located in the upper third of the epithelium and thin cytoplasmic extensions that terminate in an endfoot process. Globose basal cells and horizontal basal cells are proliferative multipotent progenitor cells that lie near the basal lamina and give rise to both olfactory sensory neurons and non-neuronal cells (Holbrook et al., 1995; Huard et al., 1998; Chen et al., 2004). These progenitor basal cells proliferate, differentiate and mature to maintain homeostasis in the olfactory epithelium throughout life. The regulatory mechanisms of olfactory epithelium tissue homeostasis have not been clearly elucidated.

In the adult nervous system, the endocannabinoid system regulates progenitor stem cells in restricted neurogenic areas including the subventricular zone and dentate gyrus. Progenitor cell function is inhibited in CB receptor-deficient mice in these brain regions (Jin et al., 2004; Aguado et al., 2005). It is not known if endocannabinoids also regulate olfactory epithelium tissue homeostasis. CB receptor-mediated regulation of progenitor/stem cell number and fate could have functional and behavioral consequences for mice lacking these receptors. Here, we examined for the presence of an endocannabinoid system in the mouse olfactory epithelium. Using a CB1/cannabinoid type 2 receptor (CB2)-deficient mouse model, we examined the hypothesis that cannabinoid receptor signaling regulates tissue homeostasis and olfactory-mediated behaviors.

EXPERIMENTAL PROCEDURES

Animals

Adult male (6–8 weeks old) Swiss Webster (CFW) and C57BL/6 wildtype control mice were obtained from Charles River, Portage, MI. Cannabinoid receptor 1 and cannabinoid receptor 2-deficient ($CB1^{-/-}$/$CB2^{-/-}$) mice were kindly provided by Dr. Norbert Kaminski (Michigan State University, MI) who obtained them from Dr. Andreas Zimmer (University of Bonn, Germany) (Jarai et al., 1999). Some of the experiments used mice in which the first exon of the Itpr3 gene was replaced by the coding region of a fusion protein of tau and green fluorescent protein (GFP), designated inositol triphosphate receptor 3 (IP3R3)-tauGFP mice (Hegg et al., 2010; Jia et al., 2013). Both heterozygous $IP3R3^{+/-}tauGFP^{-/+}$ and homozygous $IP3R3^{-/-}tauGFP^{+/+}$ mice allow for the visualization of a subtype of microvillous cells. Mice were given food and water *ad libitum*. Animal rooms were kept at 21–24 °C and 40–60% relative humidity with a 12-h light/dark cycle. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as approved by the Michigan State University Institutional Animal Care and Use Committee.

Reverse transcriptase-polymerase chain reaction

All reagents used for reverse transcriptase-polymerase chain reaction were of molecular biology grade and were purchased from Promega (Madison, WI, USA), unless otherwise noted. Anesthetized (65 mg/kg ketamine with 5 mg/kg xylazine, i.p.) C57BL/6 and Swiss Webster (CFW) adult male animals were decapitated. The olfactory epithelia were immediately dissected and stored at −80 °C. Total RNA was isolated using Tri Reagent (Molecular Research Center, Cincinnati, OH, USA) and the polymerase chain reaction amplification was performed as previously described (Kaplan et al., 2005).

Western blot

Anesthetized adult male (6–8 weeks old; 65 mg/kg ketamine with 5 mg/kg xylazine, i.p.) C57BL/6 mice