Pharmacology, Biochemistry and Behavior 101 (2012) 1–7

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Effects of the anandamide uptake blocker AM404 on food intake depend on feeding status and route of administration

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ARTICLE INFO

Article history:
Received 1 July 2011
Received in revised form 1 November 2011
Accepted 15 November 2011
Available online 22 November 2011

Keywords:
Oleoylethanolamide
N-acylethanolamines
Endocannabinoids
CB_1
PPARα
Fast
Satiety

ABSTRACT

Endocannabinoids (anandamide and 2-AG) are relevant modulators of appetite and energy expenditure through their action on cannabinoid CB_1 receptors. The actions of anandamide on feeding behavior are dependent both, on the anatomical location of CB_1 receptors (central nervous system versus peripheral tissues) and the feeding status. Anandamide uptake into cells, prior to its degradation by specific enzymatic systems, is a necessary step for the regulation of its extracellular levels. The present study explores the route and feeding stimulus dependency of the effects of the anandamide uptake blocker AM404. Peripherally, AM404 reduced feeding in partially satiated animals through a PPARα-independent mechanism, but not in food deprived ones. When AM404 was injected into the cerebral ventricles of food deprived rats, it resulted in hyperphagia that was antagonized by the cannabinoid receptor inverse agonist SR141716A. These results support the multimodal action of endocannabinoid signaling in feeding regulation, which depends on the anatomical site and the feeding status of the animal.

1. Introduction

The endocannabinoid system (ECS) has been detected throughout the central nervous system as well as in peripheral tissues involved in the control of energy balance (Matias et al., 2006). This system comprises cannabinoid receptors (at least two types CB_1 and CB_2), their endogenous ligands generically named endocannabinoids and the enzymatic machinery for their synthesis and inactivation. The most studied endocannabinoids are anandamide (*N*-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG) and both are polyunsaturated fatty acid derivatives (Devane et al., 1992; Mechoulam et al., 1995). In particular, AEA belongs to a family of bioactive lipid mediators, *N*-acylethanolamines (NAE, fatty acid ethanolamides), that includes another important molecule related to food intake and energy balance, *N*-oleoylethanolamine (OEA) (Rodriguez de Fonseca et al., 2001). NAEs are involved in a wide range of physiological activities, and even conflicting effects, through their variety in several targets (G protein-coupled receptors, nuclear receptors, channels…). In fact, despite the structural similarity of AEA and OEA, both NAEs exhibit an opposite role on feeding behavior. While OEA induces satiety via peroxisome proliferator-activated receptor alpha (PPARα), AEA displays an important appetite-inducing effect mainly via cannabinoid CB_1 receptors (Capasso and Izzo, 2008; Fu et al., 2003; Pavón et al., 2010). Together with the rest of NAEs, AEA shares common biosynthetic and degradative mechanisms that have been characterized in different cells, particularly in neurons (Matias et al., 2007; Rodriguez de Fonseca et al., 2005).

The inactivation mechanism of AEA includes two independent steps: cellular reuptake, by a putative AEA transporter that has not been isolated or cloned yet; and hydrolysis, mediated mainly by a fatty acid amide hydrolase (FAAH) (Basavarajappa, 2007; Piomelli, 2003). Focusing on AEA membrane transport, there are many drugs capable to inhibit such endocannabinoid transporter (e.g. AM404, VDM11, OMDM-1 or OMDM-2) (Beltramo et al., 1997; De Petrocellis et al., 2000; Lopez-Rodriguez et al., 2001; Ortar et al., 2003) in order to potentiate the activity of endogenous AEA, but little is known about the effects of this blockade on feeding behavior.

AM404 was originally identified as the first synthetic inhibitor for the AEA membrane transporter into neurons (Beltramo et al., 1997; Piomelli et al., 1999). However, AM404 also acts at multiple targets. It activates transient receptor potential vanilloid receptor 1 ($TRPV_1$), inhibits FAAH-mediated hydrolysis of AEA, inhibits cyclooxygenase

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doi:10.1016/j.pbb.2011.11.011

(COX)-1 and COX-2, and prostaglandin synthesis (Hogestatt et al., 2005). AM404 does not directly activate cannabinoid receptors, but it is able to potentiate and prolong several CB_1-mediated actions through an increase of endocannabinoid content (Giuffrida et al., 2001). It has been reported that AM404 promotes the extinction of fear memories in rats (Bitencourt et al., 2008), produces anxiolytic- and antidepressive-like effects in rats and mice (Adamczyk et al., 2008; Bortolato et al., 2006; Moreira et al., 2007; Patel and Hillard, 2006), reduces neuronal damage on cerebral ischemia in gerbils (Zani et al., 2007), displays antinociceptive properties in rodents (Hasanein, 2009; La Rana et al., 2008) and modulates addiction-related behaviors in rodents (Cippitelli et al., 2007; Del Arco et al., 2002).

However, the effects of AM404 on food intake have not been extensively explored yet. We found only two studies which have reported results on feeding with this AEA clearance inhibitor injected peripherally in rodents. Cippitelli et al. (2007) have described that an intraperitoneal administration of AM404 does not alter food intake in 24 h fasted rats and, similarly another study shows no effects on feeding following a subcutaneous administration in free-feeding rats (Chu et al., 2010). Recently, it has been demonstrated that central injections in nucleus accumbens of another endocannabinoid uptake inhibitor (OMDM-1) stimulate appetite in the same magnitude that AEA (Soria-Gomez et al., 2007). Thus, AM404 might represent a useful pharmacological tool to increase for extended periods of time endogenous levels of AEA, modifying feeding behavior via peripheral or central treatment without acting directly at CB_1.

It is well-known that ECS might regulate energy balance at several functional levels in brain (e.g. limbic/reward areas and hypothalamus) and periphery (gastrointestinal tract, pancreas, liver, adipose…) according to physiological and metabolic requirements under each feeding/nutritional status (Capasso and Izzo, 2008). However this signaling system is found to be dysregulated and overexpressed in eating disorders such as obesity (Engeli, 2008). For example, obese patients display increased levels of postprandial AEA in plasma compared to control patients (Gatta-Cherifi et al., 2011). These findings related to elevated levels of AEA were previously reported in binge-eating disorder, but also in anorexia and underweight patients (Monteleone et al., 2005). Therefore, a pharmacological modulation of endocannabinoid levels represents an interesting tool to further investigate the role of these lipid mediators in appetite and body weight regulation.

Taking in consideration then that endocannabinoid actions are dependent on anatomical location of CB_1 receptors and caloric status, the aim of this study was to test the relative efficacy of AEA transporter inhibitor AM404 on the regulation of feeding behavior. For that purpose, we evaluated the peripheral and central effects on food intake after acute administrations of AM404 under different nutritional conditions (both treatments under food deprivation and partial satiation).

2. Material and methods

2.1. Animals

Male Wistar rats (Charles Rivers Laboratories España, S.A., Barcelona, Spain) weighing 300–350 g at the beginning of the experiments were housed individually in a room with humidity and temperature control on a 12 h light/dark cycle (lights off 8:00 PM). Animals had ad libitum access to standard chow and water, except when restriction was required for experimental studies. In order to reduce stress associated with human contact, animals were habituated to handling at least 5 min once daily for 1 week prior to food intake experiments and/or intracranial surgeries by the experimenters.

Additional studies were performed on male mice weighing 25–30 g. Both wild-type (129S1/SvImJ, stock #002448) and PPARα-null (129S4/SvJae-Ppara^{tm1Gonz}/J, stock #003580) mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and maintained as an inbred colony of mice (Alonso et al., 2011; Suardiaz et al., 2007).

All animal procedures were performed in accordance with the European Communities Council Directive (86/609/EEC) and Spanish regulation (BOE 252/34367-91, 2005) for the care and use of laboratory animals.

2.2. Drugs

AM404 [*N*-(4-hydroxyphenyl)-arachidonoyl-ethanolamide; Tocris Bioscience, Bristol, UK], GW6471 [*N*-((2S)-2-(((1Z)-1-Methyl-3-oxo-3-(4-(trifluoromethyl)phenyl)prop-1-enyl)amino)-3-(4-(2-(5-methyl-2-phenyl 1,3-oxazol-4-yl)ethoxy)phenyl)propyl)propanamide; Tocris Bioscience, Bristol, UK] and SR141716A [rimonabant or *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide; Sanofi, Paris, France] were dissolved in different vehicle solutions according to the route of administration. Drugs were administered by intraperitoneal (i.p.) injection in a vehicle solution consisting of 5% Tween® 20 in sterile saline in a volume of 1 mL/kg. AM404 was also administered by intracerebroventricular (i.c.v.) injection in 70% dimethylsulfoxide (DMSO) diluted in saline, in a total volume of 5 μL after a previous surgery procedure.

2.3. Surgery procedure

For i.c.v. injections, stainless steel guide cannulas aimed at the lateral ventricle (LV) were implanted in the rats. Animals were anesthetized with an isoflurane/oxygen vapor mixture (1.5–2.0%) and mounted on a stereotaxic apparatus for implantation of a guide cannula (7 mm, 23 gauge) (David Kopf Instruments, Tujunga, CA). The stereotaxic coordinates were relative to Bregma point: AP, +0.6 mm; L ±2.0 mm; DV −3.2 mm (from the surface of the skull) (Paxinos and Watson, 1998). The cannula was placed 1 mm above the LV and secured to the skull by 2 stainless steel screws and cranioplastic cement. Dummy stylet wires were inserted into cannulas to prevent occlusion. After 7 days postsurgical recovery period, cannula patency was confirmed by gravity flow of isotonic saline through a stainless steel injector (8 mm, 30 gauge) inserted within the guide to 1 mm beyond its tip. This procedure allowed the animals to become familiar with the injection technique.

2.4. Drugs administration

AM404 was administered peripherally (i.p.) 15 min before starting the feeding studies at doses of 0.4, 2 and 10 mg/kg in a volume of 1 mL/kg for rats, and 0.3, 3 and 30 mg/kg in 10 mL/kg for mice.

For central (i.c.v.) administration of AM404 in rats, the stylet was removed from the guide cannula of each rat and an injector connected to 70 cm of calibrated polyethylene-10 tubing was lowered into the striatum. The tubing was then raised until flow began, and 5 μL of drug solution at doses of 0.4, 2 and 10 μg was infused over a 30–60 s period. The injector was left in the guide cannula for an additional 30 s and then removed, and the stylet was immediately replaced. Animals were tested in the corresponding food intake study 5 min after injections. The cannula placements were evaluated after each experiment by dye injection. Only rats with proper placements were included in the data analysis.

GW6471 and SR141716A were administered i.p. 30 min previously to food presentation in a volume of 1 mL/kg at a dose of 3 and 0.3–3 mg/kg respectively.

Pre-injection times were based upon previous studies with these feeding paradigms and cannabinoid drugs after their pharmacological characterization (Cani et al., 2004; Cippitelli et al., 2007; Chambers et al., 2004; Gomez et al., 2002; Pavon et al., 2006).