Pharmacological Research 122 (2017) 20–34

Contents lists available at ScienceDirect

Pharmacological Research

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

Role of the area postrema in the hypophagic effects of oleoylethanolamide

Adele Romano[a], Cristina Anna Gallelli[a], Justyna Barbara Koczwara[a], Fiona E. Braegger[b], Annabella Vitalone[a], Mario Falchi[c], Maria Vittoria Micioni Di Bonaventura[d], Carlo Cifani[d], Tommaso Cassano[e], Thomas A. Lutz[b,f], Silvana Gaetani[a,*]

[a] Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy
[b] Institute of Veterinary Physiology, Vetsuisse Faculty University of Zurich (UZH), 8057, Zurich, Switzerland
[c] Centro nazionale ricerca su HIV/AIDS, Istituto Superiore di Sanità Viale Regina Elena, 299, 00161, Rome, Italy
[d] School of Pharmacy, Pharmacology Unit, University of Camerino,Piazza Camillo Benso Conte di Cavour, 19f, 62032, Camerino, Italy
[e] Department of Clinical and Experimental Medicine, University of Foggia, Via Luigi Pinto, c/o Ospedali Riuniti, 71122, Foggia, Italy
[f] Center of Integrative Human Physiology, University of Zurich (UZH), 8057, Zurich, Switzerland

ARTICLE INFO

Article history:
Received 28 November 2016
Received in revised form 10 April 2017
Accepted 16 May 2017
Available online 20 May 2017

Chemical compound studied in this article:
Oleoylethanolamide: (PubChem CID: 5283454)

Keywords:
Feeding behavior
Area postrema
Nucleus of the solitary tract
Brainstem
Hypothalamus
Satiety

ABSTRACT

The satiety-promoting action of oleoylethanolamide (OEA) has been associated to the indirect activation of selected brain areas, such as the nucleus of the solitary tract (NST) in the brainstem and the tuberomammillary (TMN) and paraventricular (PVN) nuclei in the hypothalamus, where noradrenergic, histaminergic and oxytocinergic neurons play a necessary role. Visceral ascending fibers were hypothesized to mediate such effects. However, our previous findings demonstrated that the hypophagic action of peripherally administered OEA does not require intact vagal afferents and is associated to a strong activation of the area postrema (AP). Therefore, we hypothesized that OEA may exert its central effects through the direct activation of this circumventricular organ. To test this hypothesis, we subjected rats to the surgical ablation of the AP (APX rats) and evaluated the effects of OEA (10 mg kg^{-1} i.p.) on food intake, Fos expression, hypothalamic oxytocin (OXY) immunoreactivity and on the expression of dopamine beta hydroxylase (DBH) in the brainstem and hypothalamus. We found that the AP lesion completely prevented OEA's behavioral and neurochemical effects in the brainstem and the hypothalamus. Moreover OEA increased DBH expression in AP and NST neurons of SHAM rats while the effect in the NST was absent in APX rats, thus suggesting the possible involvement of noradrenergic AP neurons. These results support the hypothesis of a necessary role of the AP in mediating OEA's central effects that sustain its pro-satiety action.

1. Introduction

The central nervous system (CNS) and the gastrointestinal tract are constantly in reciprocal communication, through the so-called "gut-brain axis", a bidirectional system that involves neuronal, hormonal and immunological signals [1]. Many of these signals play a role in the control of energy homeostasis, such as leptin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), which have been the best characterized [2,3]. Among these "gut-derived signals" a great deal of attention has also been dedicated to oleoylethanolamide (OEA), an endogenous lipid generated in the intestine upon the ingestion of fat [4,5]. OEA is structurally

Abbreviations: AP, area postrema; BBB, blood brain barrier; CCK, cholecystokinin; CNS, central nervous system; CTA, conditioned taste aversion; DAB, 3,3 diaminobenzidine-tetrahydrochloride; DBH, dopamine beta hydroxylase; FAAH, fatty acid amide hydrolase; GLP-1, glucagon-like peptide-1; GPR119, "orphan" G-coupling receptor; HDC, histidine decarboxylase; i.c.v., intracerebroventricular; i.p., intraperitoneal; NST, nucleus of the solitary tract; OD, optical density; OEA, oleoylethanolamide; OXY, oxytocin; PBS, phosphate buffer saline; PPAR-alpha, peroxisome proliferator-activated-receptor-alpha; PVN, paraventricular nucleus; PYY, peptide YY; sCT, salmon calcitonin; SDA, subdiaphragmatic vagal deafferentation; SolC, nucleus of the solitary tract commisural part; SolDM, nucleus of the solitary tract dorsomedial part; SolM, nucleus of the solitary tract medial part; SolVL, nucleus of the solitary tract ventrolateral part; SON, supraoptic nucleus; TMN, tuberomammillary nucelus; TRPV1, transient receptor potential cation channel vanilloid-1; VEH, vehicle.

* Corresponding author.
E-mail address: silvana.gaetani@uniroma1.it (S. Gaetani).

http://dx.doi.org/10.1016/j.phrs.2017.05.017
1043-6618/

analogous to the endocannabinoid anandamide, although it does not show any affinity for the cannabinoid receptors, nor does it induce cannabinomimetic effects [6]. Most of the consideration for OEA's biological roles and mechanism of action derived from its potential interest as a novel pharmacological target for the treatment of obesity and eating disorders [5,7]. In fact, as a drug, OEA reduces food intake and body weight gain [6,8–18] in both lean and obese rodents. These effects are mediated by the activation of the peroxisome proliferator-activated-receptor-alpha (PPAR-alpha), for which OEA shows a high affinity (IC_{50} = 120.0 ± 10.7 nM) [19]. The anorexigenic effect of OEA is due to the induction of a satiety state [20,21] and appears behaviourally selective: not linked to anxiety-like symptoms, visceral malaise, alterations in plasma corticosterone, changes of body temperature, pain threshold, or of glucose, leptin and insulin plasma levels [6]. Moreover, the prosatiety effect evoked by systemic OEA administration is associated to the induction of c-fos transcription in key brain areas involved in the control of food intake, such as the nucleus of the solitary tract (NST) and the hypothalamic tuberomammillary (TMN), paraventricular (PVN) and supraoptic nuclei (SON) [5]. In these areas, different neuronal pathways seem to be involved and they include oxytocinergic, noradrenergic, and histaminergic neurons [5]. In fact, OEA's effects can be prevented by the intracerebroventricular (i.c.v.) pre-administration of a selective oxytocin (OXY) receptor antagonist [11], or by the lesion of hindbrain noradrenergic fibers projecting to the PVN [12]; it is also significantly attenuated in mice lacking the histamine-synthesizing enzyme histidine decarboxylase (HDC-KO) or in mice acutely depleted of histamine via i.c.v. infusion of the HDC blocker α-fluoromethylhistidine [22]. OEA's mechanism of action might also involve the activation of vagal fibers, as suggested by the observation that its effect after intraperitoneal (i.p.) administration is blunted in rats subjected to a complete subdiaphragmatic vagotomy or to a pre-treatment with a neurotoxic dose of capsaicin, which destroys vagal and non-vagal unmyelinated afferent nerve fibers [6,19,23]. These observations, together with the finding that OEA does not reduce food intake when administered by i.c.v. infusion in rats [6], suggests that its mechanism of action could be peripheral rather than central and that the activation of both brainstem and hypothalamic areas might be indirectly mediated by ascending fibers. However, we recently showed that OEA does not require intact intestinal vagal afferents to reduce food intake [16], as observed in rats that underwent a selective subdiaphragmatic vagal deafferentiation (SDA), a surgery that removes all abdominal vagal afferents, but sparing approximately half of the efferents [24,25]. These findings did not seem to support the hypothesis of a strictly peripheral mechanism and suggest that the blockade of OEA's effects by total subdiaphragmatic vagotomy may be due to the absence of efferent rather than afferent innervation.

Whether OEA signal can directly or indirectly reach the brain remained to be fully elucidated. Although systemically or orally administered OEA quickly reaches the blood stream [20,21], indirect evidence suggests that it does not readily permeate the whole CNS, presumably owing to the high expression of its degrading enzyme, fatty acid amide hydrolase (FAAH), in the blood brain barrier (BBB) [26]. However, these findings do not necessarily exclude a possible action mediated by the circumventricular organs such as the area postrema (AP) in the brainstem. In fact, we recently reported that peripheral OEA induces a significant activation of AP neurons and in the subpostremal nucleus of the NST, as suggested by the increased transcription of c-fos in these areas [14]. The AP lacks a functional BBB by virtue of its lack of tight junctions and the presence of fenestrated capillaries, therefore circulating peptides and other peripheral signals can gain direct access to neurons of the AP. On the basis of these observations, we hypothesized that OEA may exert its central effects through the activation of the AP.

To test this hypothesis, we subjected rats to a surgical lesion of the AP (APX) and evaluated the effects of i.p. OEA administration (10 mg kg^{-1}) on food intake, on Fos expression, on OXY immunoreactivity at both PVN and neurohypophysial level and on the expression of dopamine beta hydroxylase (DBH) within the brainstem and PVN. Further, we aimed to assess the phenotype of neuronal populations activated by OEA in the brainstem of SHAM-lesioned and APX rats; to this aim, we assessed, also, whether OEA induced Fos expression co-localized with DBH as marker for noradrenergic neurons. Finally, as last step of our study, we investigated PPAR-alpha expression within the AP.

2. Materials and methods

2.1. Animals and housing

Forty-one male Wistar rats (Charles River, Sulzfeld, Germany) were used in this study. In particular, forty rats underwent AP lesion or SHAM surgery, while one drug-naïve rat was used to assess PPAR-alpha expression within the AP. All animals, weighing 200–250 g upon arrival, were individually housed in acrylic cages under a 12:12 dark-light cycle in a climate-controlled room (22 ± 2 °C and 60% relative humidity). All rats were fed with standard chow pellets (N 3430, Provimi Kliba, Gossau, Switzerland) *ad libitum*, unless otherwise stated. All experiments were performed upon the approval of the Veterinary Office of the Canton of Zurich and according to the European Community directives 2010/63/EU. The experimental timeline is depicted in Fig. 1A.

2.2. Area postrema lesion surgery

Rats were anesthetized using a mixture of ketamine (50 mg kg^{-1}; Narketan, Vetoquinol AG, Ittingen, Switzerland), xylazine (2.5 mg kg^{-1}, Xylazin; Streuli Pharma AG, Uznach, Switzerland) and acepromazine (0.75 mg kg^{-1}, Prequillan; Arovet AG, Dietikon, Switzerland) and placed on a stereotaxic apparatus to be subjected to either APX (N = 20) or SHAM (N = 20) surgery. To this aim, the head of each rat was flexed ventrally at an approximate 110° angle in a stereotaxic frame. Skin and three layers of neck musculature were cut and retracted for visualization of the foramen magnum. Under visual control with a surgical microscope, the cranial dura mater was penetrated and the cerebrospinal fluid was blotted. In the APX procedure, AP lesions were performed by aspirating the AP with a blunted cannula tip fixed to a flexible tube attached to a vacuum pump [27,28]. In SHAM-operated rats, the AP was exposed but not touched. Rats were given 2 weeks to recover from surgery, receiving post-operative care [29], and were habituated to i.p. injections of saline. One rat subjected to APX died during the recovery period.

As reported in previous studies [29,30], during the course of our experiment it became evident that APX rats were not hyperphagic and they did not gain more weight as compared to SHAM rats. Conversely, after surgery their weight gain was lower than SHAM operated rats and, on the test day, the body weight of APX rats was significantly ($P < 0.001$) lower that SHAM rats (SHAM rats: 363.8 g ± 5.65 g, APX rats; 265.1 g ± 8.74 g). Nevertheless, if normalized to body weight (g kg^{-1}), the daily consumption of food intake was similar between the two groups (data not shown).

Success and specificity of the AP lesion was verified both functionally and histologically [27,28,31,32]. In particular, the functional verification was based on the lack of the anorexigenic effects of peripheral salmon calcitonin (sCT, 5 μg kg^{-1}, i.p.), which requires an intact AP to inhibit food intake in rodents [29]. 24 h before the functional test, SHAM and APX rats were administered with saline