Pharmacological Research 113 (2016) 100–107

Contents lists available at ScienceDirect

Pharmacological Research

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

Regular article

The hypophagic factor oleoylethanolamide differentially increases c-fos expression in appetite regulating centres in the brain of wild type and histamine deficient mice

Hayato Umehara[a,2], Roberta Fabbri[a,1], Gustavo Provensi[a], M. Beatrice Passani[b,*]

[a] NEUROFARBA Section of Pharmacology and Toxicology, Viale Pieraccini 6, Universita' di Firenze, 50139 Firenze (Italy)
[b] Dipartimento di Scienze della Salute, Viale Pieraccini 6, Universita' di Firenze, 50139 Firenze (Italy)

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form 27 July 2016
Accepted 16 August 2016
Available online 16 August 2016

Keywords:
Histidine decarboxylase
Tuberomamillary nucleus
Hypophagia
c-Fos
Feeding behavior

ABSTRACT

Histaminergic neurons in the hypothalamic tuberomamillary nucleus (TMN) establish connections with virtually all brain areas. Recent evidence suggests that feeding-related motivation is correlated with the activation of a subpopulation of histamine neurons in the ventral TMN that project to hypothalamic and subcortical areas controlling feeding behaviour. Oleoylethanolamide (OEA) is a hypophagic lipid-amide released by the small intestine in response to daily fat intake that indirectly activates hypothalamic oxytocin-neurons in the paraventricular (PVN) and supraoptic (SON) nuclei. We recently showed that OEA requires the integrity of neuronal histamine to fully display its hypophagic effect. Here we aimed to investigate if differences exist in OEA-induced c-Fos expression in several brain regions of fasted, histidine decarboxylase (HDC)-KO mice that do not synthesize histamine, and wild type (WT) littermates. All the brain regions examined receive histaminergic innervation and are involved in different aspects of feeding behaviour. We found that OEA increased c-Fos expression in the SON, arcuate nucleus (ARC) and the amygdala of WT mice, but not HDC-KO mice, whereas neither genotype nor treatment differences were observed in the lateral and dorsomedial hypothalamus. Furthermore, oxytocin-immunostaining was markedly increased in the neurohypophysis of WT and not in HDC-KO mice. Of note, OEA increased c-Fos expression in the nucleus of solitary tract of both genotypes. Our findings suggest that the TMN serves as a relay station to elaborate peripheral signals that control homeostatic and adaptive behavioural responses.

1. Introduction

The mechanisms for controlling eating behaviour involve the interplay between molecular signals secreted from the gut, the adipose tissue, neurohormones and central neurotransmitters. Food intake can be attenuated by multiple signals, including those associated with satiety, sickness and unpalatable tastants. The activity of histamine neurons that are clustered in the tuberomamillary nucleus (TMN) controls several homeostatic functions and behavioural responses such as sleep and aversive memory formation [28]. Brain histamine seems to have different roles in different aspects of feeding behaviour, [e.g. anticipatory vs consummatory phase [46]] and memory (e.g. consolidation vs extinction, [14]. Such a complex orchestration may be served by different histamine neuronal subpopulations that are recruited at different times during the unfolding of a specific behaviour [7]. Indeed, histamine neurons respond differently to endogenous [41] and exogenous [16] molecules, and they show heterogeneous expression of histaminergic receptors [7]. The activity of histamine neurons is also regulated, directly or indirectly, by molecules generated in the gastrointestinal tract and by neurohormones such as leptin, corticotropin-, thyrotropin-releasing hormones and nesfatin-1 (reviewed in Refs. [31,34,35]. These promote satiety at least in part through histaminergic neurotransmission [17,43]. Oleoylethanolamide (OEA) is a gut-derived satiety signal that inhibits eating and controls fat metabolism and energy expenditure, mainly through mechanisms involving activation of type-α peroxisome proliferator activated receptors (PPAR-α; [32,18]). The anorexic effect of OEA results from the engagement of PPAR-α in the gut and the recruitment of vagal

* Corresponding author.
E-mail address: beatrice.passani@unifi.it (M.B. Passani).
[1] Equal contributors.
[2] Present address: Division of Pharmacology, Niigata University, Graduate school of Medical and Dental Science. 1-757 Asahimachi-dori Chuo-ku, Niigata 951-8510, (Japan).

http://dx.doi.org/10.1016/j.phrs.2016.08.020
1043-6618/

afferents. The hypophagic effect is associated with the induction of c-Fos in brain regions that control food intake such as the nucleus of the solitary tract (NST), the PVN and SON, the area postrema [15,37]. OEA stimulates oxytocin neurosecretion from the PVN [37], and this hormone is involved in the regulation of homeostatic processes and eating behaviour [32]. Activation of the H_1 receptor in CNS induces hypophagia in rats [23,24], while the antagonism of this receptor increases meal size and duration [26,27]. Clinically, the importance of central histamine in the control of appetite is now accepted, as atypical antipsychotic drugs with high affinity for the histamine H_1 receptor determine weight gain and dysmetabolic disorders [20,22,10,34,35].

We recently demonstrated that the integrity of the central histamine system is necessary for OEA to suppress appetite and to activate oxytocic neurons in the PVN [33]. By using immunostaining for c-Fos we also demonstrated that a small population of histamine neurons responds to exogenous administration of OEA with increased activity [33]. Here, we further explored changes in neuronal activity induced by i.p. administration of OEA by assessing the pattern of c-Fos expression in brain regions that receive histaminergic innervation, in both mice deficient of the histamine-synthesizing enzyme histidine decarboxylase (HDC-KO), and their wild type (WT) littermates. We hypothesise that histamine mediates OEA hypophagic effect not only via oxytocin neurons activation, but also stimulating specific histaminergic pathways involved in the control of feeding and emotional behaviours.

2. Material and methods

2.1. Animals

Housing, animal maintenance and all experiments were conducted in accordance with the Council Directive of the European Community (2010/63/EU) of the Italian Decreto Legislativo 26 (13/03/2014) and National Institutes of Health guidelines on animal care and approved by veterinarian supervision. HDC-KO mice and WT littermates (129/Sv background) were grown in the animal facility of NEUROFARBA-Section of Pharmacology and Toxicology, Università di Firenze. They were housed in a temperature-controlled room (22 ± 1 °C) with a 12:12-h light-dark cycle (light on 0700–1900 h), at a constant temperature and humidity with standard diet (4RF21; Mucedola s.r.l., Milan, Italy) and freely available water. HDC-KO and WT mice were used at 2–3 months of age (25–30 g). Mice were handled for one week before experiments. Mice genotype was confirmed by PCR according to Provensi et al. [33].

2.2. Immunostaining protocol

HDC-KO and WT littermates were maintained on standard chow diet and food-deprived for 12 h (between 20:00 and 8:00, water remained available) before i.p. administration of OEA (10 mg/kg) or vehicle. Mice were tested during light-on as in our previous study [33]. Two hours after OEA's injections mice were deeply anaesthetized with chloral hydrate (400 mg/kg, i.p.) and perfused transcardially with cold physiological saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. Brains were post-fixed in the same solution overnight (4 °C), and cryoprotected in 30% sucrose in PB. Forty μm thick sections were cut on a cryostat and collected in PB. Sections were preincubated in 0.75% H_2O_2 in PB for 30 min, in 0.2% BSA for 30 min and then incubated overnight in rabbit c-Fos primary antibody (1:5000; Sigma-Aldrich) at 4 °C. The immunoreactive product was detected with the avidin-biotin peroxidase system (Vectastain kit; Vector

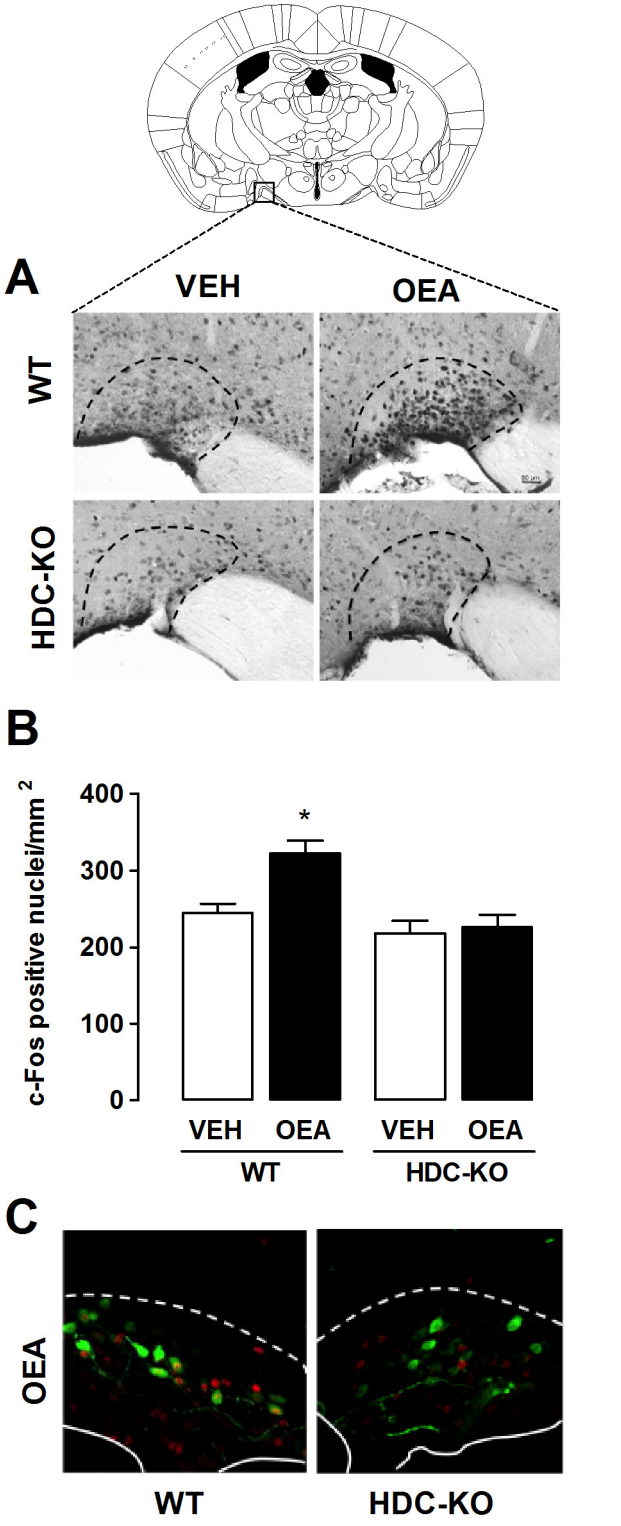

Fig. 1. OEA-induced c-Fos expression is blunted in the hypothalamic SON of HDC-KO mice. (A) Representative photomicrographs showing the effect of vehicle or 10 mg/kg OEA on c-Fos protein expression in the SON of WT and HDC-KO. (B) Quantitative analysis of data shown in (A). Data are expressed as means ± SEM of 4–5 mice per experimental group; *P < 0.05 vs respective controls by one-way ANOVA and Bonferroni MCT. (C) Immunohistochemical detection of oxytocin (green) and c-Fos (red) in the SON neurons of WT and HDC-KO mice treated with OEA.