Ethanolamine Produced from Oleoylethanolamide Degradation Contributes to Acetylcholine/Dopamine Balance Modulating Eating Behavior

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[1] **List of abbreviations:** acetylcholine (ACh); dopamine (DA); Oleoylethanolamide (OEA); N-acetylethanolamines (NAEs); ethanolamine (EA); nucleus accumbens (NAc); gamma aminobutyric acid (GABA); proliferator-activated receptor-α (PPAR-α)

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Oleoylethanolamide (OEA) is a well-recognized anorectic compound (1). Recent investigations pointed out that, when administrated at a dose of 10mg/kg, it affects food reward by influencing the acetylcholine (ACh)/dopamine (DA) balance in the cholinergic system (1). Recent published data support the effect of OEA on both the homeostatic and non-homeostatic regulation of food intake through the activation of proliferator-activated receptor-α (PPAR-α) in the small intestine, which mediates satiety signals to the brain *via* the afferent vagal fibers (2).

Mammals have the enzymes to synthetize and degrade N-acetylethanolamines (NAEs), including OEA. NAEs are biosynthesized "on-demand" from their precursors N-acylated ethanolamine phospholipids, through three different enzymatic pathways (3). The absolute concentration of NAEs in tissues is very low, in the order of a few nmol/g of tissue, the highest concentration being present in the brain and intestine (3).

NAEs, including OEA, can also be provided by certain foods, oatmeal, nuts, and cocoa powder being the major food sources, but the amount per gram of OEA found in these foods is very low (<2 μg/g) (4) compared to the amount used in clinical trials. OEA can also be synthetized upon ingestion of precursor-containing foods, such as olive oil. For example, the intake of a meal containing a high amount of oleic acid increased plasma concentration of OEA in rats and humans (5, 6) reducing the following energy intakes in humans (6, for a summary of human studies addressing the role of OEA on eating behavior see supplemental table1).

NAEs hydrolysis is catalyzed by the fatty acid amide hydrolase that has a strong activity in the intestine, liver, brain, and at the blood-brain barrier level (7), resulting in the production of fatty acids and ethanolamine (EA). After OEA is orally or intragastrically administrated in relatively abundant doses, *i.e.* 10mg/kg, it is likely that following its degradation, a peak in oleate and EA, occurs in several organs. The effect of oleic acid on the gut-brain-axis has been extensively investigated, whereas EA received scarce attention. One study (8) tested the effect of oral EA (1.88mg/kg, the equivalent amount released by 10mg/kg of OEA) and found no effect on food intake compared to a control group, in rats. However, in this study only food intake and no other

aspects of eating behavior, such as food preferences for high sugar/fat foods that may also be influenced by the activation of brain areas related to food reward, were investigated. On the contrary, there are scattered evidences from old and new recent researches, which underline the influence of EA on the cholinergic system.

Here, we briefly describe OEA and EA effects on the cholinergic system, suggesting that EA contributes to the overall balance between DA and ACh after OEA administration (9, 10).

The cholinergic system regulates food reward by interacting with the dopaminergic system

The cholinergic system modulates various brain functions through the release of the Ach (11). This occurs as a result of depolarization of the axonal termination that induces

a rise in the cytosolic Ca^{2+} concentration and the fusion between the axoplasmic membrane and the vesicles containing ACh.

Projection neurons constitute the main brain structure of the cholinergic system that originates mainly in subcortical regions, such as the basal forebrain, and extends into many brain regions, including cortical areas and the hippocampus. Interneurons with cholinergic activity were also identified in specific target areas of mesolimbic system, such as the nucleus accumbens (NAc) (11), which are involved in pleasure and motivation, contributing to the hedonic dimension of eating.

In the regulation of eating behavior, the cholinergic system constantly interacts with, and regulates the activity of one of the main neurotransmitters involved in reward, DA (12). DA is synthesized by dopaminergic neurons in the midbrain (*i.e.* ventral tegmental area and substantia nigra). The dopaminergic mesolimbic pathway is a neuronal circuit that is activated in order to translate motivation into action in a variety of behaviors, including eating (12).

In human lean subjects, food cues stimulate DA release in the dorsal striatum (12) and during eating, DA response is positively correlated with the pleasantness of the food. These evidences indicate that, in lean subjects, DA may be considered as a biomarker of the hedonic value of foods. Conversely, in obese subjects, responses to food cues and food ingestion stimuli are blunted (12).

The dopaminergic and the cholinergic systems have a similar anatomical connectivity with

numerous functional neuronal endings, such as in the NAc. Here, cholinergic interneurons, together with dopaminergic projections of the ventral tegmental area, modulate the activity of gamma aminobutyric acid (GABA) projections neurons (12). In the NAc, ACh/DA fluctuations modulate eating behavior. For example, when rats are exposed to an adverse conditioned stimulus, there is a concomitant ACh increase and DA decrease. In addition, fasted mice in a free feeding state stop eating when the ACh/DA ratio begins to increase (12).

OEA influences cholinergic-mediated food-reward regulation

Some investigations also suggested that OEA influences the food-reward system by modulating DA release. High-fat-diet chronic consumption in mice produces a significant reduction in intestinal OEA synthesis, but not in other tissues, which is concomitant with a reduction of DA in the brain. In high-fat-diet-fed mice, infusion of OEA (10mg/kg) solution 25 min before intragastric lipid emulsion infusion restored DA release. No difference was found in DA increase in response to the calories content of the emulsion. In low-fat-diet-fed mice, OEA influenced the release of DA when stimulated by intragastric infusion of a low-calorie emulsion. In addition, in low-fat-diet-fed mice, the infusion of OEA decreased the intake of both low-calorie and high-calorie emulsions. Finally, the authors demonstrated that these effects were abolished in sub-diaphragmatic bilaterally vagotomised and in PPAR-α knockout mice (1). These evidences indicate that OEA interacts with the mesolimbic system, probably *via* activation of the PPAR-α on vagal afferent nerves. In contrast, another investigation provides evidences that OEA can regulate DA release through central mechanisms as well. In rats, when injected in the lateral hypothalamus, OEA increased extracellular DA levels in NAc (9). The molecular mechanisms of OEA–DA interaction have not been fully clarified yet.

Ethanolamine, a downstream metabolite of OEA, may serve as neuromodulator stimulating acetylcholine release in the brain

The quantitative degradation of 10mg of OEA may produce 1.88mg of EA and this is particularly interesting because it is a small molecule with a rather good ability to pass the blood-brain barrier and influence the activity of diverse brain areas (13). Several pioneering studies demonstrated that EA can be released from different brain regions following electrical or chemical depolarization (14). A more recent *in vitro* study demonstrated that, when synaptosomes and synaptoneurosomes were incubated with EA, this was stored in intracellular compartments, and released by classical exocytosis following depolarization (15). The authors pointed out that the concentration of EA released was low compared to a classical neurotransmitter, but they did not exclude the possibility that a discrete receptor exists post-synaptically for EA (15). Although EA cannot be considered as a pure neurotransmitter, all in all, these studies support the role of EA as a neuromodulator, a molecule that is not directly excitatory or inhibitory, but that can sustain both activities depending on the site and the time frame of its release. Therefore, it is convincing that the EA released from the degradation of NAEs in the intestine or at the blood-brain barrier level may play a role in the brain. In fact, it is known that EA can modulate neurotransmitters. It was shown that EA stimulates the release of aspartic and glutamic acids in the anterior hippocampus of rabbits (16), and it is also known to inhibit the enzyme GABA aminotransferase with the consequence of reducing GABA breakdown and increasing its concentration in the brain (17).

Some investigations demonstrated that, similarly to OEA, EA could influence the cholinergic system. A first suggestion came from the neuronal cells' ability to use EA as a precursor of ACh. In chicken neuronal cultures, it was proved that EA is rapidly phosphorylated to phosphoethanolamine and converted to phosphatidylethanolamine that may also be methylated to phosphocholine (18). *In vivo*, intraventricular injections of ^{3}H-EA showed that the rat brain has the capacity to synthetize free choline *de novo* by stepwise methylation of EA, phosphoethanolamine and phosphatidylethanolamine (19). In the human cell line neuroblastoma LA-N-2, it was demonstrated that the same pathway exists and that the choline thus produced is a source of *de novo* synthesis of ACh (20). In contrast, Ansell and Spanner (21) described that after injection of labeled EA in the

ventricle side of the rat brain, labeled phosphatidylethanolamine was formed, but no labeled phosphatidylcholine was found. Similar results were obtained by Browning and Schulman (22) in slices of cerebral cortex of rats. They showed that labeled ACh was formed in the presence of labeled choline, whereas ACh was not labeled if choline was substituted with labeled EA, serine or methionine. Therefore it is accepted that, although the conversion of EA in ACh is possible, *in vivo* and in the presence of normal plasma choline levels, EA is not used for choline and ACh synthesis.

However, other investigations found that EA can increase ACh extracellular concentration in the brain by indirect mechanisms. In cultured explants of the medial septal nucleus from rat brains, EA enhances the ability to develop cholinergic neurons to utilize choline for the production of ACh (23). EA has been shown to increase extracellular levels of ACh in rat hippocampus slices during continuous infusion in a depolarizing KCl buffer. This increase was not mediated by a greater synthesis of ACh, but by a higher cellular release that would be stimulated by amino-alcohols such as EA (24). In a subsequent study, they suggested that the mechanism through which amino-alcohols facilitate ACh release in the hippocampus may involve the activation of calcium channels (25). Interestingly, this effect appears to be specific to cholinergic endings, since other neurotransmitters, such as norepinephrine and DA, were not affected. A similar result was obtained by Khairy et al. (10) who have shown that the application of EA on dorsal root ganglion (DRG) neurons cells, with a threshold of 10 nM, increases the amplitude and duration of the transport of KCl-stimulated Ca^{2+} release. Furthermore, EA stimulated voltage-activated K^{+} currents independently of the activation of Ca^{2+} channels. In another study, Liao et al. (17) in accordance Bostwick, also found that EA increased neurotransmitters release from brain synaptosomes, but they proposed different mechanisms of release. They demonstrated that synaptosomes incubated with EA, at a concentration from 0.31mM to 5mM, increased their capability to release neurotransmitters, *i.e.* [3H]-D-aspartic acid. They suggested that EA permeated into synaptic vesicles and increasing their filling capability. The enhanced capability of synaptic vesicles to load neurotransmitters explains how EA influence may regulate synaptic transmission. The

concentrations used in the study were within physiological levels of EA in the brain, *i.e.* between 197–870 nmol/g (17).

Therefore, even if the mechanisms are still not clear, there are strong evidences to support that EA influences neurotransmitters release in the brain. This is of great interest especially when the source of EA is OEA, because this NAE has itself an action on the cholinergic/dopaminergic system. It is also possible that there are differences in the overall effect on the brain between the administration of pure EA, as investigated by Nielsen et al (8) and EA released following OEA degradation, since this EA can keep acting on neurotransmitters previously stimulated by OEA.

In conclusion, these evidences support the hypothesis that OEA and its metabolite, EA, can interact in the regulation of the overall ACh/DA balance influencing brain areas involved in food-reward (**Figure 1**). Taking into account this perspective, further studies should investigate the kinetic of EA release following OEA administration and whether it can impact the cholinergic regulation of dopamine.

Statement of authors' contributions to manuscript: IM, GB and DV wrote the article, IM designed research and had primary responsibility for final content. All authors have read and approved the final manuscript.

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Figure Legends

Figure 1. Schematic representation of oleoylethanolamide (OEA) and ethanolamine (EA) effects on acetylcholine/dopamine (ACh/DA) balance. From intestine, OEA communicates *via* vagal afferent fiber with brain areas regulating DA release. OEA is then degraded by the enzyme fatty acid amide hydrolase in oleic acid and EA, which pass into the bloodstream and can in part penetrate the blood brain barrier. In the brain, EA acts as a neuromodulator being able to stimulate cholinergic neurons ACh release. ACh/DA fluctuations modulate eating behavior.

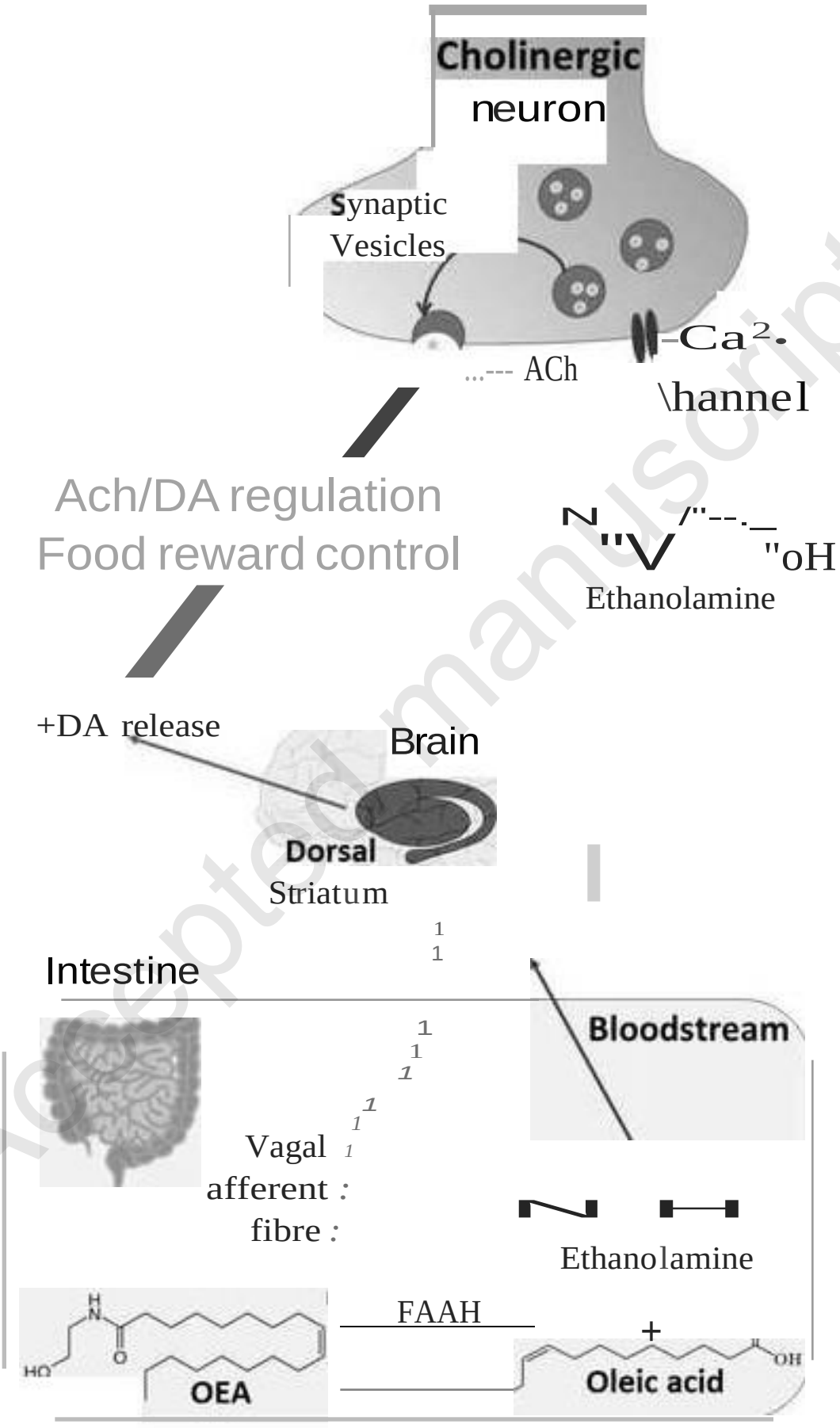

Figure 1