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# Central administration of oleate or octanoate activates hypothalamic fatty acid sensing and inhibits food intake in rainbow trout

Marta Librán-Pérez, Cristina Otero-Rodiño, Marcos A. López-Patiño, Jesús M. Míguez, José L. Soengas *

*Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo, 36310 Vigo, Spain*

## HIGHLIGHTS

- ICV treatment with oleate or octanoate decreased food intake in rainbow trout.
- Treatment directly activates fatty acid sensing systems in trout hypothalamus.
- Treatment decreased expression of NPY in trout hypothalamus.
- Treatment increased expression of POMC and CART in trout hypothalamus.

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## ABSTRACT

If levels of fatty acids like oleate and octanoate are directly sensed through different fatty acid (FA) sensing systems in hypothalamus of rainbow trout, intracerebroventricular (ICV) administration of FA should elicit effects similar to those previously observed after intraperitoneal (IP) treatment. Accordingly, we observed after ICV treatment with oleate or octanoate decreased food intake accompanied in hypothalamus by reduced potential of lipogenesis and FA oxidation, and decreased potential of ATP-dependent inward rectifier potassium channel ($K^{+}_{ATP}$). Those changes support direct FA sensing through mechanisms related to FA metabolism and mitochondrial activity. The FA sensing through binding to FAT/CD36 and subsequent expression of transcription factors appears to be also direct but an interaction with peripheral hormones cannot be rejected. Moreover, decreased expression of NPY and increased expression of POMC were observed in parallel with the activation of FA sensing systems and decreased food intake. These results allow us to suggest the involvement of at least these peptides in controlling the decreased food intake noted after oleate and octanoate treatment in rainbow trout.



## 1. Introduction

There is evidence that specialized neurons within mammalian hypothalamus detect changes in plasma levels of long-chain fatty acid (LCFA), but not short-chain (SCFA) or medium-chain (MCFA) FA through four different mechanisms [1–4]. These include: i) FA metabolism through inhibition of carnitine palmitoyltransferase 1 (CPT-1) to import FA-CoA into the mitochondria for oxidation; ii) binding to FA translocase (FAT/CD36) and further modulation of transcription factors like peroxisome proliferator-activated receptor type α (PPARα), and sterol regulatory element-binding protein type 1c (SREBP1c); iii) activation of protein kinase C (PKC) isoforms; and, iv) mitochondrial production of reactive oxygen species (ROS) by electron leakage resulting in an inhibition of ATP-dependent inward rectifier potassium channel ($K_{ATP}$) activity. Changes in these systems have been associated, through not completely understood mechanisms, with the inhibition of the orexigenic factors agouti-related protein (AgRP) and neuropeptide Y (NPY), and the enhancement of the anorexigenic factors pro-opio melanocortin (POMC) and cocaine and amphetamine-related transcript (CART) [1,2]. Altogether, these changes could be involved in the decreased food intake observed after rising circulating LCFA levels in mammals.

Most teleost fish are relatively intolerant to glucose thus relying more on amino acids and lipids to fuel metabolism [5–7]. A reduced food intake has been observed in fish fed with lipid-enriched diets or containing high fat stores [8–14] raising the question whether lipid sensing mechanisms regulating food intake may be present in fish [15]. Accordingly, in a previous study in rainbow trout *Oncorhynchus mykiss* [16] we observed that intraperitoneal (IP) acute administration of oleate (LCFA) or octanoate (MCFA) elicited an inhibition in food intake, and induced in hypothalamus a response compatible with FA sensing in which FA metabolism, binding to FAT/CD36, and mitochondrial activity were apparently involved. Changes in these hypothalamic

* Corresponding author at: Laboratorio de Fisioloxía Animal, Facultade de Bioloxía, Edificio de Ciencias Experimentais, Universidade de Vigo, E-36310 Vigo, Spain. Tel.: +34 986 812 564; fax: +34 986 812 556.
*E-mail address:* jsoengas@uvigo.es (J.L. Soengas).

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pathways can be also related to the control of food intake, since changes in mRNA levels of specific neuropeptides such as NPY and POMC were also noted [16]. The main difference in the responses with those known in the mammalian model [2] is the effect of octanoate. This could be related to the fact that body lipids in teleosts contain considerable amounts of MCFA [17,18]. Accordingly, MCFA have been used as a dietary lipid source in fish [14], and in rainbow trout there is no preferential oxidation of MCFA compared to LCFA [14]. In a subsequent study [17] we observed that rainbow trout hypothalamus in vitro (in the absence of extrahypothalamic regulatory mechanisms) displayed responses in parameters related to FA-sensing similar to those previously observed after IP treatment suggesting that the increase of circulating LCFA or MCFA levels in rainbow trout is directly sensed in hypothalamus. However, we cannot reject that the effects of IP treatment with FA [16] could be attributed to an indirect effect mediated by changes elicited in levels of peripheral hormones. Therefore, in the present study, we evaluated in rainbow trout the effects of intracerebroventricular (ICV) treatment with oleate or octanoate on food intake, and in parameters related to putative FA sensing systems in hypothalamus. If FA are directly sensed in hypothalamus, central administration should elicit effects similar to those of intraperitoneal treatment, as we reported in rainbow trout [16], and as described in mammals [20,21].

## 2. Materials and methods

### 2.1. Fish

Rainbow trout (*O. mykiss* Walbaum) were obtained from a local fish farm (A Estrada, Spain). Fish were maintained for 1 month in 100 liter tanks under laboratory conditions and 12L:12D photoperiod in dechlorinated tap water at 15 °C. Fish weight was 101 ± 2 g. Fish were fed once daily (09.00 h) to satiety with commercial dry fish pellets (Dibaq-Diproteg SA, Spain; proximate food analysis was 48% crude protein, 14% carbohydrates, 25% crude fat, and 11.5% ash; 20.2 MJ/kg of feed). The experiments described comply with the Guidelines of the European Union Council (2010/63/UE), and of the Spanish Government (RD 55/2013) for the use of animals in research, and were approved by the Ethics Committee of the Universidade de Vigo.

### 2.2. Experimental design

Following 1 month acclimation period, fish were randomly assigned to 100 liter experimental tanks. Fish were fasted for 24 h before treatment to ensure basal hormone levels were achieved. On the day of experiment fish were lightly anesthetized with MS-222 (50 $mg \cdot l^{-1}$) buffered to pH 7.4 with sodium bicarbonate, and weighed. ICV administration was performed as previously described [22]. Briefly, fish were placed on a Plexiglas board with Velcro© straps adjusted to hold them in place. A 29 1/2 gauge needle attached through a polyethylene cannula to a 10 μl Hamilton syringe was aligned with the 6th preorbital bone at the rear of the eye socket, and from this point the syringe was moved through the space in the frontal bone into the third ventricle. The plunger of the syringe was slowly depressed to dispense 1 $\mu l \cdot 100\ g^{-1}$ body mass of Hanks' saline alone or containing 1 μmol oleate (O-1008, Sigma Chemical Co.) or octanoate (C-2875, Sigma Chemical Co.). To safely deliver FA they were solubilized in 45% hydroxypropyl-β-cyclodextrin (HBP) to a final concentration of 17 mM [21]. The HPB–FA solution was diluted in saline to the appropriate concentration used for each injection. HPB alone at a similar concentration as in the FA studies was used in controls (no effects of the vehicle alone were noted for any of the parameters assessed, data not shown).

In a first set of experiments food intake was registered for 3 days before treatment (to define basal line data) and then 6 and 24 h after ICV treatment with saline–HPB alone (control, n = 10 for each time point) or containing oleate (n = 10 for each time point) or octanoate (n = 10 for each time point). After feeding, the food uneaten remaining at the bottom (conical tanks) and feed waste were withdrawn, dried and weighed. The amount of food consumed by all fish in each tank was calculated as previously described as the difference from the feed offered [23–25]. The experiment was repeated three times, and therefore results are shown as the mean ± SEM of the data obtained in three different tanks per treatment.

In a second set of experiments fish were ICV injected with saline–HPB alone (control, n = 15 at 2 h and n = 15 at 6 h) or containing oleate (n = 15 at 2 h and n = 15 at 6 h) or octanoate (n =15 at 2 h and n = 15 at 6 h) with the same concentrations described above. After 2 h or 6 h, fish were lightly anesthetized with MS-222 (50 $mg \cdot l^{-1}$) buffered to pH 7.4 with sodium bicarbonate. Blood was collected by caudal puncture with ammonium-heparinized syringes, and plasma samples were obtained after blood centrifugation, deproteinized immediately (using 0.6 M perchloric acid) and neutralized (using 1 M potassium bicarbonate) before freezing on liquid nitrogen and storage at −80 °C until further assay. Fish were sacrificed by decapitation and hypothalamus were taken, snap-frozen in liquid nitrogen, and stored at −80 °C. At each time, 10 fish per group were used to assess enzyme activities and metabolite levels whereas the remaining 5 fish were used for the assessment of mRNA levels by qRT-PCR.

In both experiments, we were interested in comparing the differential effects of oleate or octanoate, and therefore we used similar molar concentrations. Due to the different molecular weight of both FA this results in a difference in the utilizable energy of both treatments, which we cannot discard to be responsible, at least in part, of the differences observed.

### 2.3. Assessment of metabolite levels and enzyme activities

Levels of FA, total lipids, triglyceride, glucose, and lactate in plasma were determined enzymatically using commercial kits (Wako Chemicals, Neuss, Germany, for FA; Spinreact, Barcelona, Spain for total lipid, triglyceride, and lactate; Biomérieux, Grenoble, France, for glucose) adapted to a microplate format. The determination of total lipid is based on the sulfo-phospho-vanillin method [26].

Samples used to assess hypothalamic metabolite levels were homogenized immediately by ultrasonic disruption in 7.5 vol. of ice-cooled 0.6 M perchloric acid, and neutralized (using 1 M potassium bicarbonate). The homogenate was centrifuged (10,000 g), and the supernatant used to assay tissue metabolites. Tissue FA, total lipid, and triglyceride levels were determined enzymatically using commercial kits as described above for plasma samples. Samples for enzyme activities were homogenized by ultrasonic disruption with 9 vol. of ice-cold-buffer consisting of 50 mM Tris (pH 7.6), 5 mM EDTA, 2 mM 1,4-dithiothreitol, and a protease inhibitor cocktail (Sigma, St. Louis, Mo, USA). The homogenate was centrifuged (10,000 g) and the supernatant used immediately for enzyme assays. Enzyme activities were determined using a microplate reader INFINITE 200 Pro (Tecan, Männedorf, Switzerland) and microplates. Reaction rates of enzymes were determined by the increase or decrease in absorbance of NAD(P)H at 340 nm or, in the case of CPT-1 activity, of 5,5′-Dithiobis(2-nitrobenzoic acid)–CoA complex at 412 nm. The reactions were started by the addition of supernatant (15 μl) at a pre-established protein concentration, omitting the substrate in control wells (final volume 265–295 μl), and allowing the reactions to proceed at 20 °C for pre-established times (3–10 min). Enzyme activities are expressed in terms of mg protein. Protein was assayed in triplicate in homogenates using microplates according to the bicinchoninic acid method with bovine serum albumin (Sigma) as standard. Enzyme activities were assessed at maximum rates by preliminary tests to determine optimal substrate concentrations. ATP-citrate lyase (ACLY, *EC* 4.1.3.8), fatty acid synthase (FAS, *EC* 2.3.1.85), and CPT-1 (*EC* 2.3.1.21) activities were determined as described previously [16,19].