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Research paper

The anorectic effect of central PYY_{1-36} treatment in rainbow trout (*Oncorhynchus mykiss*) is associated with changes in mRNAs encoding neuropeptides and parameters related to fatty acid sensing and metabolism

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ABSTRACT

We hypothesized that peptide YY (PYY) is involved in the metabolic regulation of food intake in fish. Therefore, we assessed in rainbow trout (*Oncorhynchus mykiss*) the effects of intracerebroventricular treatment with 10 ng/g PYY_{1-36} on food intake, expression of neuropeptides involved in food intake control, and the activity of fatty acid-sensing systems. The administration of PYY_{1-36} caused a significant reduction in food intake up to 24 h post-treatment. This anorectic action was associated with changes 2 h after treatment in mRNA abundance of neuropeptides involved in metabolic regulation of food intake in hypothalamus (decreased NPY and raised CART values) and hindbrain (increased POMCa1 values). We also observed that PYY_{1-36} treatment induced changes in mRNA abundance of parameters related to fatty acid sensing and metabolism in hypothalamus (decreased values of ACLY, PPARγ, and SREBP1c) and hindbrain (increased values of LPL, FAT/CD36, PPARα, PPARγ, and SREBP1c and decreased values of UCP2a). PYY_{1-36} treatment also increased mRNA abundance of mTOR. In general, it seems that mRNAs encoding some components of the machinery required for fatty acid sensing and metabolism are activated by PYY_{1-36}. The response observed was higher in the hindbrain than in the hypothalamus, supporting the greater importance of this brain area in mediating the modulatory effects of gastrointestinal hormones on feeding regulation.

1. Introduction

The detection of changes in nutrient levels in vertebrate brain is an essential process involved in the regulation of food intake and energy expenditure as demonstrated in mammals (see reviews Blouet and Schwartz, 2010; Morton et al., 2014) and fish (see reviews Delgado et al., 2017; Soengas et al., 2018). Accordingly, several mechanisms are present in brain areas, especially hypothalamus and hindbrain, detecting changes in the levels of nutrients like fatty acids as demonstrated in mammals (Bruce et al., 2017; Efeyan et al., 2015) and fish (Delgado et al., 2017; Soengas, 2014; Soengas et al., 2018). In previous studies in fish (Conde-Sieira and Soengas, 2017; Soengas, 2014) we demonstrated the capacity for detection of changes in the levels of specific long-chain fatty acids (LCFA) through fatty acid sensing mechanisms based on carnitine palmitoyl transferase-1 (CPT-1), fatty acid translocase (FAT/CD36), increased capacity of mitochondria to produce reactive oxygen species inhibiting ATP-dependent inward rectified potassium channel (K^+_{ATP}), and lipoprotein lipase (LPL) activity. These mechanisms are, in general, comparable to those described in mammals (Blouet and Schwartz, 2010; Morton et al., 2014; Magnan et al., 2015) with the exception of the ability of fish systems for detecting not only changes in the levels of LCFA, but also medium-chain fatty acid including octanoate and poly unsaturated fatty acid like α-linolenate. The activation of these systems in fish occurs in parallel with decreased activity of the energy sensor 5́-AMP-activated protein kinase (AMPK) and increased activity of mechanistic target of rapamycin (mTOR) (Velasco et al., 2017). The activation of these systems result in increased production of the anorexigenic peptides pro-opio melanocortin (POMC) and cocaine- and amphetamine-related transcript (CART), and decreased production of the orexigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP), ultimately leading to decreased food intake (Librán-Pérez et al., 2012, 2014; Velasco et al., 2016). The

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Table 1
Nucleotide sequences of the PCR primers used to evaluate mRNA abundance by RT-qPCR.

	Forward primer	Reverse primer	Annealing T (°C)	Amplicon size	Efficiency (%)	Data base	Accession Number
β-actin	GATGGGCCAGAAAGACAGCTA	TCGTCCCAGTTGGTGACGAT	59	105	91.2	GenBank	NM_ 001124235.1
ACLY	CTGAAGCCCAGACAAGGAAG	CAGATTGGAGGCCAAGATGT	60	149	94.5	GenBank	CA349411.1
AgRP	ACCAGCAGTCCTGTCTGGGTAA	AGTAGCAGATGGAGCCGAACA	60	87	90.8	GenBank	CR376289
AMPKα1	ATCTTCTTCACGCCCCAGTA	GGGAGCTCATCTTTGAACCA	60	131	95.2	GenBank	HQ40367
CART	ACCATGGAGAGCTCCAG	GCGCACTGCTCTCCAA	60	275	91.4	GenBank	NM_001124627
CPT1c	CGCTTCAAGAATGGGGTGAT	CAACCACCTGCTGTTTCTCA	59	187	90.3	GenBank	AJ619768
FAS	GAGACCTAGTGGAGGCTGTC	TCTTGTTGATGGTGAGCTGT	59	161	93.6	Sigenae	tcab0001c.e.06 5.1.s.om.8
FAT/CD36	CAAGTCAGCGACAAACCAGA	ACTTCTGAGCCTCCACAGGA	62	106	95.7	DFCI	AY606034.1
Kir6.x-like	TTGGCTCCTCTTCGCCATGT	AAAGCCGATGGTCACCTGGA	60	157	98.2	Sigenae	CA346261.1.s.om.8:1:773:1
LPL	TAATTGGCTGCAGAAAACAC	CGTCAGCAAACTCAAAGGT	59	164	94.3	GenBank	AJ224693
mTOR	ATGGTTCGATCACTGGTCATCA	TCCACTCTTGCCACAGAGAC	60	81	90.2	GenBank	EU179853
NPY	CTCGTCTGGACCTTTATATGC	GTTCATCATATCTGGACTGTG	58	247	89.2	GenBank	NM_001124266
POMCa1	CTCGCTGTCAAGACCTCAACTCT	GAGTTGGGTTGGAGATGGACCTC	60	95	95.6	Tigr	TC86162
PPARα	CTGGAGCTGGATGACAGTGA	GGCAAGTTTTTGCAGCAGAT	55	195	97.3	GenBank	AY494835
PPARγ	GACGGCGGGTCAGTACTTTA	ATGCTCTTGGCGAACTCTGT	60	171	98.1	DFCI	CA345564
SREBP1c	GACAAGGTGGTCCAGTTGCT	CACACGTTGGTCCGCATCAC	60	59	95.1	GenBank	CA048941.1
UCP2a	TCCGGCTACAGATCCAGG	CTCTCCACAGACCACGCA	57	423	93.2	GenBank	DQ295324

ACLY, ATP-citrate lyase; AgRP, agouti-related peptide; AMPKα1, AMP-activated protein kinase subunit α1; CART, cocaine- and amphetamine-related transcript; CPT1c, carnitine palmitoyl transferase type 1c; FAS, fatty acid synthase; FAT/CD36, fatty acid translocase; Kir6.x-like, inward rectifier K^+ channel pore type 6.x-like; LPL, Lipoprotein lipase; mTOR, mechanistic target of rapamycin; NPY, neuropeptide Y; POMCa1, pro-opio melanocortin a1; PPARα, peroxisome proliferator-activated receptor type α; PPARγ, peroxisome proliferator-activated receptor type γ; SREBP1c, sterol regulatory element-binding protein type 1c; UCP2a, mitochondrial uncoupling protein 2a.

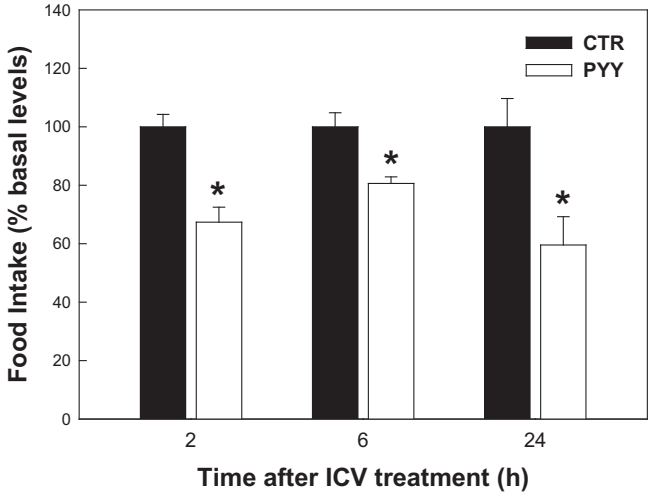

Fig. 1. Food intake of rainbow trout at 2, 6 and 24 h after intracerebroventricular administration of 1 μL.100 g^{-1} body mass of saline alone (control, CTR) or containing 10 ng/g of goldfish PYY_{1-36} (PYY). Food intake is displayed as mean + S.E.M. of the percentage of food ingested with respect to baseline levels (calculated as the average of food intake the 7 days previous to experiment). The results are shown as mean + S.E.M. of the results obtained in three different experiments in which 5 fish were used per group in each tank.

neurons expressing neuropeptides are also responsive to the effect of hormones (Blouet and Schwartz, 2010). These include hormones providing information regarding metabolic stores or energy status such as leptin and insulin, and gastrointestinal tract (GIT) hormones providing information regarding absence/presence of food and its composition (Blouet and Schwartz, 2010). To date, the information available in fish concerning the endocrine modulation of central fatty acid sensing systems is scarce and restricted to insulin (Librán-Pérez et al., 2015) and ghrelin (Velasco et al., 2016). Information available regarding other GIT hormones and fatty acid sensing is scarce.

Peptide YY (PYY) is a 36-amino-acid straight chain peptide synthesized and released from enteroendocrine L-cells found in the GIT where it co-localizes with glucagon-like peptide 1 (GLP-1) (Batterham and Bloom, 2003). In mammals, two main endogenous forms have been characterized, the full-length peptide PYY_{1-36} and the truncated form PYY_{3-36} (Ballantyne, 2006; Grandt et al., 1994). They bind to a family of G-protein-coupled receptors of the Y family (Y1-Y6) (Bischoff and Michel, 1998; Larhammar et al., 2001). PYY participates in the regulation of gastrointestinal secretion, renal and vascular physiology; but also plays an important role in the regulation of appetite and weight control modulating food intake (Ballantyne, 2006). Accordingly, an anorectic effect has been reported after central or peripheral treatment with PYY_{3-36} (Batterham et al., 2002; Degen et al., 2005). In mammals, peripheral treatment with PYY_{1-36} resulted in decreased food intake (Chelikani et al., 2004), whereas central administration stimulates food intake (Hagan and Moss, 1995; Kanatani et al., 2000; Morley et al., 1985). PYY might be involved in the regulation of body weight, not only through modulation of food intake, but also by the control of energy expenditure and lipid metabolism (Guo et al., 2006). In this way, PYY over-expression resulted in increased fatty acid synthesis capacity due to a reduction in phosphorylated acetyl-CoA carboxylase and hepatic lipogenic capacity (Mells and Anania, 2013).

In fish, PYY has been identified in a large number of species and is widely expressed in both central and peripheral tissues (Cerdá-Reverter and Larhammar, 2000; Volkoff, 2015). The existence of Y-like receptors has been demonstrated in hypothalamus and hindbrain of several fish species, either for Y1 (Cerdá-Reverter and Larhammar, 2000; Cerdá-Reverter et al., 2000) or Y2 (Fredriksson et al., 2004; Larsson et al., 2006) types. Contrary to mammals, central administration of PYY_{1-36} decreases food intake in fish as demonstrated in goldfish (Gonzalez and Unniappan, 2016, 2010). Meanwhile, central or intraperitoneal administration of PYY_{3-36} had no effects on feeding in fish (Gonzalez and Unniappan, 2016, 2010) and channel catfish (Schroeter et al., 2015). This is due to the lack of endogenous PYY_{3-36}, as there is no extraction site for dipeptidyl peptidase 4 (DPP4), which is unable to cleave PYY_{1-36} to PYY_{3-36} in fishes (Unniappan et al., 2006). PYY seems to be also involved in the control of energy expenditure in fish, and PYY mRNA levels increased post-feeding in Atlantic salmon (Valen et al., 2011) and Atlantic halibut (Gomes et al., 2015). However, the impact of PYY on fatty acid sensing in fish has not been characterized. The only indirect evidence is the increase in PYY mRNA expression in the brain of Senegalese sole larvae in response to tube-feeding with specific fatty acids, an experimental approach that also decreased food intake (Bonacic et al., 2016). Therefore, in this study, we aimed to assess in rainbow trout (*Oncorhynchus mykiss*) if PYY_{1-36} central treatment results in decreased food intake as previously demonstrated in goldfish. We