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Research paper

Impaired central leptin signaling and sensitivity in rainbow trout with high muscle adiposity

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ABSTRACT

The hormone leptin has been identified in all vertebrate classes, but its physiological roles in non-mammalian vertebrates are not well defined. To elucidate leptin regulation in energy homeostasis in a teleost fish species, this study compares hypothalamic and pituitary leptin signaling systems in energetically divergent rainbow trout lines selected for low (lean line, LL) and high (fat line, FL) muscle adiposity under feeding and starvation conditions. In fed fish, hypothalamic gene expression and protein density of the full-functional leptin receptor ($LepR_L$), as well as a leptin binding protein (LepBP) expression, are lower in FL than LL fish. The FL fish have also lower activation of leptin-relevant signaling pathways involving protein kinase B (Akt) and extracellular signal-related kinase. These observations suggests impaired central leptin action in FL fish. During fasting, hypothalamic $LepR_L$ and LepBP expression, as well as active Akt levels are downregulated after one week, while pituitary $LepR_L$ expression is upregulated, in the LL fish only. After four weeks, hypothalamic $LepR_L$ protein levels return to normal levels in both fish lines and Akt is reactivated, although not to the same extent in FL as in LL fish, indicating that FL fish have low leptin sensitivity to nutritional changes. Neuropeptide Y and orexin expression is downregulated to similar levels in both fish lines after one-week fasting. The divergent leptin system profiles between the two fish lines demonstrate that phenotypic selection for high muscle adiposity affects leptin endocrinology, indicating regulatory roles for leptin in rainbow trout energy homeostasis.

1. Introduction

The peptide hormone leptin has now been identified in all vertebrate classes (Denver et al., 2011; Friedman-Einat et al., 2014; Huang et al., 2014; Londraville et al., 2014; Seroussi et al., 2016). Its amino acid sequence is not well conserved among or within classes (Londraville et al., 2014), with amino acid identity between teleost and human leptins being 13–25% (Gorissen and Flik, 2014). However, based on *in silico* modelling, the secondary and tertiary molecular structure of leptin is well evolutionarily conserved (Rønnestad et al., 2010; Denver et al., 2011). The tissue site of leptin production appears to have shifted through evolution. In teleosts, the liver is the primary site for leptin gene expression (Londraville et al., 2014; Douros et al., 2014) and thus assumed to be an important leptin-producing tissue. Adipose tissue may also contribute as rainbow trout (*Oncorhynchus mykiss*) adipocytes secrete leptin *in vitro* (Salmerón et al., 2015). In rainbow trout and Arctic charr (*Salvelinus alpinus*), plasma leptin levels do not correlate well with body adiposity, indicating that leptin does not function as indicator of adiposity in fish (Kling et al., 2012; Frøiland et al., 2012). In contrast, leptin is primarily produced and secreted by adipocytes in human and rodents, often resulting in positive correlation between plasma leptin levels and body adiposity. In these mammals, leptin is suggested to function as a lipostat signal to the brain (Liuzzi et al., 1999; Farooqi and O'Rahilly, 2014).

The physiological functions of leptin in appetite regulation, energy homeostasis and metabolism have been well studied in human and rodents. Leptin acts on the central nervous system, especially on the hypothalamic arcuate nucleus (ARC) and ventromedial hypothalamus, by suppressing appetite, increasing energy expenditure, and signaling states of negative energy balance and decreased energy stores (Allison and Myers, 2014; Park and Ahima, 2014). It stimulates the ARC neuronal population co-expressing proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART), and inhibits the neuronal population expressing agouti-related peptide (AgRP) and neuropeptide Y (NPY) (Xu et al., 2011). In addition, leptin inhibits food intake by reducing the expression of melanin-concentrating hormone (MCH) and orexins in the lateral hypothalamic area (Park and Ahima, 2014). Leptin has profound

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effects on glucose and lipid metabolism, stimulating glucose uptake and fatty acid oxidation through ARC and hypothalamic melanocortin system and hypothalamic-sympathetic nervous system axis (Bjørbaek and Kahn, 2004; Myers and Olson, 2012; Minokoshi et al., 2012).

Comparative data show the anorexigenic function of leptin being conserved from fish to mammals (Gorissen and Flik, 2014) at least partly through similar central mechanisms. Peripheral leptin treatment of rainbow trout transiently reduces *npy* expression, while stimulating *pomc-a* expression (Murashita et al., 2008). Intracerebroventricular leptin administration of rainbow trout acutely stimulates the expression of all *pomc* genes in the lateral tuberal hypothalamus, the homologous structure to the mammalian ARC (Gong et al., 2015). Long-term leptin administration of Atlantic salmon (*Salmo salar*) reduces growth by affecting food intake through the central POMC pathway (Murashita et al., 2011). However, the roles of leptin in long-term energy homeostasis in teleost fish are still not well defined.

Starvation or food restriction leads to elevated plasma leptin levels in salmonids (Kling et al., 2009; Frøiland et al., 2012; Trombley et al., 2014; Salmerón et al., 2015; Johansson and Björnsson, 2015), a change which is reversed during refeeding (Johansson and Björnsson, 2015). These leptin responses are contrary to those in mammalian model species, where nutritional restriction and overfeeding usually decreases and increases leptin levels, respectively (Wisse et al., 1999; Ahima, 2000). In rainbow trout, increased adiposity due to high energy intake reduces leptin secretion from adipocytes *in vitro* (Salmerón et al., 2015). High fat stores associated with low plasma leptin levels are also observed in Atlantic salmon, and food restriction leads to upregulation of leptin receptor (LepR) gene expression in the pituitary (Trombley et al., 2014). These observations suggest that leptin is involved in energy homeostasis in salmonids, but through different regulatory interactions between adiposity/energy status and plasma leptin levels than in mammals.

The mammalian leptin signaling system is known to include one full-length, functional receptor (LepRb), four truncated receptors, and a soluble Lep binding protein (LepBP) (Lee et al., 1996). Leptin-LepRb interaction activates intracellular signals through several signaling pathways, including the Janus kinase 2 and the signal transducers and activators of transcription 3/5 (Jak2-Stat3/5), SH2-containing protein tyrosine phosphatase 2 – extracellular signal-related kinase (SHP2-Erk), phosphatidylinositol 3 kinase – protein kinase B (Pi3k-Akt), mammalian target of rapamycin-p70 S6 kinase (mTor-S6k) and 5′ adenosine monophosphate-activated protein kinase (Ampk) pathways (Park and Ahima, 2014). In rainbow trout, the leptin signaling system is so far known to include five LepR isoforms; the functional full-length $LepR_L$ which has 30% identity with the human LepRb, a truncated receptor ($LepR_T$) with shorter intracellular sequence and three soluble leptin binding proteins (LepBPs) (Gong et al., 2013; Gong and Björnsson, 2014). Both $LepR_L$ and $LepR_T$ genes are highly expressed in the rainbow trout brain, especially in the hypothalamus. Homologous ligand binding to the rainbow trout $LepR_L$ activates the Jak2-Stat3 and Pi3k-Akt pathways (Gong and Björnsson, 2014; Gong et al., 2015). The $LepR_T$ is suggested to function as a dominant negative regulator in $LepR_L$ signaling (Gong and Björnsson, 2014). The long LepBP, termed $LepR_{S1}$, with the complete extracellular domain, as well as the short LepBPs, termed $LepR_{S2}$ and $LepR_{S3}$, with partial extracellular domain, can specifically bind leptin in the circulation and modulate local leptin action (Gong et al., 2013).

To elucidate possible relationships between central leptin action and energy homeostasis in teleost fish, this study used two rainbow trout lines selected for low (lean line, LL) and high (fat line, FL) muscle adiposity (Quillet et al., 2005) over seven generations. The two fish lines have diverged in terms of glucose and lipid metabolism (Kolditz et al., 2008a; Skiba-Cassy et al., 2009; Kamalam et al., 2012). The FL fish have higher capability than the LL fish to utilize and store glucose and maintain glucose homeostasis (Kolditz et al., 2008a; Jin et al., 2014a). High lipogenic potential is suggested as a key mechanism responsible for high muscle adiposity in the FL fish (Jin et al., 2014b).

The two energetically divergent rainbow trout lines were studied under normal feeding conditions as well as after 1, 2 and 4 weeks of fasting, as previous studies have shown that 3–4 week fasting induces changes in plasma leptin and LepBP levels in this species (Kling et al., 2009; Gong et al., 2013; Johansson and Björnsson, 2015). The responses of central leptin signaling system to the nutritional changes were compared between the two lines. LepR gene expression was measured in the hypothalamus and pituitary gland. The $LepR_L$ protein density and leptin signal activation were examined in the hypothalamus. Hypothalamic NPY, orexin and POMC gene expression levels were also assessed to elucidate links between leptin and appetite-regulating neuropeptides.

2. Materials and methods

2.1. Fish and experimental design

The experiment was carried out at the Institut National de la Recherche Agronomique (INRA) experimental facilities in PEIMA, Drennec, Sizun, France, and its design has been described in detail in Johansson et al. (2016). Briefly, fifty rainbow trout, regardless of sex, were stocked into each of 16 circular, outdoor tanks, each with water volume of 1.8 m^3 and water flow of 3 $m^3\ h^{-1}$ and oxygen levels >6.0 mg L^{-1}. Eight tanks were stocked with LL fish (average body weight 262 g) and eight tanks with FL fish (average body weight, 238 g). The fish were acclimated for three weeks under ambient light and temperature conditions. The daily ration of feed was calculated once a week based on fish size and water temperature, and increased from about 1.16–1.25% BW day^{-1} over the duration of the study. The water temperature rose gradually from 10.6 to 13.5 °C during the three-week acclimation and four-week experimental period (mid-April to early June, 2013).

After acclimation, a four-week feeding/fasting experimental protocol was initiated. The experimental fish were grouped into four feeding regimes (duplicate tanks for each fish line for each regime): 1) fish fed throughout the four weeks; 2) fed for three weeks followed by one-week fasting; 3) fed for two weeks followed by two-week fasting; 4) fish fasted throughout the four weeks. The experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), under the official license of L. Laurent (29–036). The PEIMA facility is approved for animal experimentation through license C29-277-02.

2.2. Tissue sampling

Ten fish were sampled from each tank, thus 20 LL fish and 20 FL fish per group. Fish were netted from the four tanks in a sequence, alternating between LL and FL tanks and anesthetized with isoeugenol (ScanAqua) in a lethal dose (160 mg l^{-1}). Body weight (BW) and length (BL) were measured and blood was drawn from the caudal vessels. The fish were predominantly (90%) immature and their sex was not determined, while 10% could be identified as females in the initial stages of gonadal maturation (gonadosomatic index $\leqslant$0.3). The fish were decapitated, and the hypothalamus (without saccus vasculosus) and pituitary gland were sampled for gene expression analysis. The tuberal hypothalamic region, which was visible as a small ridge on the ventral surface of the diencephalon, was isolated from the hypothalamus for immunoblotting. All tissue