



Review

Aspects of the hormonal regulation of appetite in fish with emphasis on goldfish, Atlantic cod and winter flounder: Notes on actions and responses to nutritional, environmental and reproductive changes[☆]

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ARTICLE INFO

Article history:

Received 8 July 2008

Received in revised form 3 December 2008

Accepted 3 December 2008

Available online 7 December 2008

Keywords:

Fish

Feeding

Gene expression

Environments

Reproduction

ABSTRACT

In vertebrates including fish, food intake regulation involves intricate networks of hormones produced by both brain and peripheral tissues. Under optimum conditions, nutritional intake is adequate for basal metabolic needs, growth, development, reproduction, and deposition of energy stores. As fish represent a very diverse group, different fish species live in very different environments and are exposed to variations in a wide range of factors, including not only internal factors, such as nutritional/metabolic status and reproductive events but also environmental factors, such as temperature and photoperiod. These physiological responses often include changes in appetite that might occur through modulations of the gene expression and action of feeding-regulating hormones. Despite recent advances, our current understanding of the regulation of feeding in fish is still limited and based primarily on studies involving a few fish species. This review will give a brief overview of our current knowledge of the regulation of feeding by three central (NPY, OX and CART) and two peripheral (ghrelin and GRP) appetite-related factors in a freshwater species, the goldfish (*Carassius auratus*) and two marine species, cod (*Gadus morhua*) and winter flounder (*Pleuronectes americanus*).

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1. Introduction

In all vertebrates, feeding is regulated by key appetite-stimulating (orexigenic) or appetite-inhibiting (anorexigenic) endocrine factors, which act on feeding centers in the brain to mediate the regulation of short-term and long-term dietary intakes. These endocrine factors originate not only from the central nervous system (CNS), but also from peripheral organs such as the gastrointestinal (GI) tract, pancreas, liver and adipose tissue. Peripheral hormones convey

[☆] Contribution associated with the 6th International Symposium on Fish Endocrinology held in June 2008 in Calgary, Canada.

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information to central feeding centers either via the vagus nerve or by crossing the blood-brain barrier and acting directly through central receptors (Brightman and Broadwell, 1976). Central peptides include the orexigenic neuropeptide Y (NPY), orexins and galanin and the anorexigenic cocaine- and amphetamine-regulated transcript (CART) and corticotropin-releasing hormone (CRH). Peripheral signals include the satiety factors cholecystokinin (CCK), gastrin releasing peptide (GRP)/bombesin-like peptides, and amylin and only one known circulating appetite stimulant, ghrelin, which is produced by the stomach (Valassi et al., 2008). In recent years, a growing number of homologs of mammalian appetite-regulating peptides have been characterized in fish (Gorissen et al., 2006; Volkoff et al., 2005), suggesting that the regulation of food intake has been relatively well conserved along the vertebrate lineage. Similar to other vertebrates, some fish species are submitted to a number of environmental challenges, which might have led to a number of feeding adaptations, such as coping with long-term fasting. In addition, in fish, seasonal changes in feeding often coincide with spawning migration and reproduction, suggesting a link between nutrition and the reproductive axis. However, compared to mammals or other groups, fish represent a vast phylogenetic group, which shows a significant level of diversity with regards to morphology, ecology, behavior and genomes (Volkoff, 2004). This diversity suggests that the endocrine control of feeding in fish might also be diverse and involve species-specific molecules and mechanisms.

When examining the actions of a putative appetite-regulating hormone, quantifying food intake in fish treated by this hormone allows for an accurate assessment of its effects on feeding (Himick and Peter, 1994; Volkoff et al., 1999). Treatments can be administered either via peripheral (intraperitoneal, IP) or brain injections (intracerebroventricular, ICV) (Silverstein et al., 2001; Volkoff et al., 2003), orally (Gelineau and Boujard, 2001), and via intraperitoneally-implanted pellets (Johansson et al., 2005) or osmotic mini-pumps (Riley et al., 2005). When dealing with large or wild animals, treating the animals or quantifying food intake is not always feasible. The role of peptides in food intake can then be assessed indirectly by examining blood levels or protein/mRNA expression levels of these peptides in fish submitted to various conditions (e.g. fasting, environmental stresses).

Goldfish (*Carassius auratus*) are omnivorous temperate freshwater fish that usually live in a relatively stable environment. Atlantic cod (*Gadus morhua*) and winter flounder (*Pleuronectes americanus*) are both carnivorous cold-water species off the coast of Newfoundland (Canada). The latter two species are both submitted to decreases in food availability during the winter months but are able to withstand prolonged periods of fasting. Atlantic cod have been shown to survive up to 16 weeks of fasting (Beaulieu and Guderley, 1998). Studies have shown that Atlantic cod continue to feed during the winter months but have a reduced growth rate (Bjornsson et al., 2007). It has been suggested that during prolonged fasting, this species uses mainly lipids and glycogen stored from the liver (Hemre et al., 1993a) and slows down metabolism (Hemre et al., 1993b). In contrast, winter flounder stop feeding entirely and do not grow during the winter months. These fish enter a dormant-like state and undergo a period of natural fasting (Litvak, 1999; Stoner et al., 1999). It has also been suggested that lipid reserves and perhaps proteins are mobilized to provide energy during the fast (McLeese and Moon, 1989).

The differences in habitats, feeding habits and responses to fasting between goldfish, cod and flounder suggest that species-specific differences in the endocrine regulation of feeding might exist. The subsequent sections describe the responses of three central (NPY, OX and CART) and two peripheral (GRP and ghrelin) appetite-related factors to nutritional status and environmental changes in fish as well as their link to reproduction, with emphasis on goldfish, Atlantic cod and winter flounder.

2. Response of appetite-regulating hormones to feeding and fasting

2.1. Neuropeptide Y (NPY)

NPY is one of the most potent orexigenic agents known in mammals (Valassi et al., 2008). The amino acid composition of the mature NPY peptide is relatively well conserved among vertebrates, including fish (Cerdeira-Reverter et al., 2000; Sundstrom et al., 2005), suggesting similar physiological functions for NPY. Indeed, central injections of mammalian or fish NPY cause a dose-dependent increase in food intake not only in goldfish (de Pedro et al., 2000; Lopez-Patino et al., 1999; Narnaware et al., 2000), but also trout (Aldegunde and Mancebo, 2006) and catfish (Silverstein and Plisetskaya, 2000), suggesting that NPY regulates feeding in fish. In both goldfish (Narnaware and Peter, 2001) and Atlantic cod (Kehoe and Volkoff, 2007), forebrain NPY mRNA levels undergo peri-prandial variations, with highest levels around meal time, suggesting that NPY acts as a short-term hunger factor in these two species. In both goldfish (Narnaware and Peter, 2001) and winter flounder (McDonald, 2008), fasting (1–4 weeks) induces increases in hypothalamic NPY mRNA expression, suggesting that NPY might also act as a long-term regulator of feeding in fish. In Atlantic cod, however, NPY forebrain mRNA expression is not affected by short-term (1 week) fasting (Kehoe and Volkoff, 2007). Adult Atlantic cod experience prolonged natural periods of food deprivation during the winter months when food supply is limited (Blier et al., 2007). Previous studies have shown that in cod, 5–10 weeks of fasting only induces small decreases in total mass, length or condition factor (Belanger et al., 2002; Blier et al., 2007). When feeding is restored, however, cod undergo a “growth spurt”, known as compensatory growth, but this compensatory growth response only occurs following long periods of fasting, suggesting that short-term fasting does not induce “nutritional stress” in cod (Blier et al., 2007; Jobling et al., 1994). This resistance to long episodes of fasting might be due to modifications in metabolism and digestive structures resulting in the down-regulation of digestive performances during fasting. For example, pyloric caeca might regress during fasting whereas gut hypertrophy might occur during the re-feeding period (Blier et al., 2007). As NPY has been shown to affect both appetite and digestive functions in fish (Volkoff et al., 2005), it is possible that the maintenance of constant NPY mRNA levels during severe fasting might be part of this adaptation.

2.2. Orexins

Orexins (OXs or hypocretins) consist of two orexigenic peptides, orexin A and orexin B produced by cleavage of a single precursor, preproorexin (Valassi et al., 2008). In fish, mRNAs encoding for prepro-OX have been reported for pufferfish (Alvarez and Sutcliffe, 2002), zebrafish (Kaslin et al., 2004), stickleback, medaka (Faraco et al., 2006), goldfish (Hoskins et al., 2008; Miura et al., 2007) and cod (Xu and Volkoff, 2007). In fish as in mammals (Korczyński et al., 2006), orexins appear to have a role in the regulation of food intake. In goldfish, central injections of human OXs cause a significant increase in appetite (Miura et al., 2007; Volkoff et al., 1999) and the number of OX-like immunoreactive cells in the hypothalamus increases in fasted fish (Nakamachi et al., 2006). In cod, long-term food deprivation or food restriction (low rations) both induce an increase in brain preproOX mRNA levels, suggesting a role for OX as a long-term feeding regulator in fish (Xu and Volkoff, 2007). In cod, brain OX expression levels also display periprandial changes, with higher levels at meal time (Xu and Volkoff, 2007), but these changes can only be detected in fish fed a low ration, when OX levels are high. These data suggest that at least in cod, OX might be more important as a long-term regulator than as a short-term hunger signal. No data is available for the role of OX in flounder.

2.3. Ghrelin

Ghrelin is a peptide predominantly secreted by the stomach but also synthesized in the brain. To date, ghrelin is the only gastrointestinal peptide hormone with confirmed orexigenic properties (Valassi et al., 2008). Ghrelin has been isolated from several fish species (Kawakoshi et al., 2007; Manning et al., 2008; Olsson et al., 2008; Terova et al., 2008) but its role in the regulation of feeding and metabolism of fish is not clear. Studies have reported that both central and peripheral injections of either fish or human ghrelin stimulate food intake (goldfish, Matsuda et al., 2006; Miura et al., 2007; Unniappan and Peter, 2005; rainbow trout, Shepherd et al., 2007), while others show that peripheral ghrelin injections do not affect food intake (rainbow trout, Jonsson et al., 2007). In goldfish, gut preproghrelin mRNA expression displays periprandial variations (Unniappan et al., 2004a) and increases following fasting (Unniappan et al., 2004b), suggesting that ghrelin acts as a short term hunger factor. Upregulation of ghrelin gut mRNA expression during fasting has also been shown in sea bass (Terova et al., 2008), but fasting-induced decreases in plasma ghrelin levels occur in trout (Jonsson et al., 2007) and burbot (Nieminen et al., 2003). In Atlantic cod, it appears that fasting does not affect stomach ghrelin mRNA levels (Xu and Volkoff, in press), suggesting that ghrelin does not act as a long term hunger signal in this species. No data is available for winter flounder ghrelin.

2.4. Cocaine- and amphetamine-regulated transcript (CART)

CART is an anorexigenic neuropeptide originally isolated as an mRNA upregulated in rat brain following acute administration of psychomotor stimulants (Valassi et al., 2008). In fish, CART mRNA sequences have been published for goldfish (Volkoff and Peter, 2001) and cod (Kehoe and Volkoff, 2007). Central injections of human CART decrease food intake in goldfish (Volkoff and Peter, 2000) and periprandial changes in CART brain mRNA have been demonstrated in both goldfish and cod (Kehoe and Volkoff, 2007; Volkoff and Peter, 2001), suggesting that CART acts as a short-term regulator of feeding in fish. Short-term fasting (2–7 days) in both goldfish (Volkoff and Peter, 2001) and cod (Kehoe and Volkoff, 2007) induce decreases in CART mRNA brain levels, suggesting that CART has an important role in long-term regulation of feeding. Preliminary data in winter flounder suggests that hypothalamic CART mRNA expression is little affected by fasting (McDonald, 2008).

2.5. Gastrin-releasing peptide (GRP)

GRP is an anorexigenic peptide structurally related to bombesin (BBS), originally isolated in amphibian skin (Merali et al., 1999). BBS/GRP-like peptides have been detected in gastrointestinal tract and brain of fish (Bosi et al., 2005; Volkoff et al., 2000) but cDNA sequences are only available for goldfish and zebrafish. BBS/GRP-like peptides have been shown to regulate gastric functions such as acid secretion and gastric motility in fish (Thorndyke et al., 1990). In goldfish, BBS injections suppress food intake (Canosa and Peter, 2004; Canosa et al., 2005; Himick and Peter, 1994), suggesting that BBS/GRP-like peptides also act as satiety factors. However, in rainbow trout, plasma GRP levels are extremely low and appear not to be influenced by feeding (Jonsson et al., 2006). Similarly, in cod, there are no significant periprandial changes in GRP gut mRNA expression, suggesting that GRP is not crucial for the regulation of short-term feeding. However, GRP stomach mRNA expression is higher in cod fed high food ration compared to fish fed low rations (Xu and Volkoff, in press), suggesting that GRP might act as a long-term feeding regulator in cod. This hypothesis is confirmed by the fact that food deprivation for 1 or 4 weeks induces significant decreases in GRP stomach mRNA expression in starved fish compared to fed fish (Xu and Volkoff, in press).

3. Response of appetite-regulating hormones to environmental changes

Temperature and photoperiod are two of the most important environmental factors that might affect feeding in fish. However, under natural conditions, the distinct effects of the two parameters might be difficult to separate as fishes are subjected to seasonal cycles in which both factors vary. A relationship between temperature and food intake has been demonstrated in a number of fish species, where fish tend to increase their food consumption and growth rates with rising temperatures (Bendiksen et al., 2002; Sunuma et al., 2007). Little is known about the endocrine mechanisms regulating these temperature-induced changes in feeding. In Atlantic cod, fish kept at 2 °C have a lower food intake than that of fish kept at 11 °C or 15 °C (Kehoe and Volkoff, 2008). In this species, brain NPY mRNA expression is not affected by temperature, but brain CART mRNA expression levels are higher in fish held at 2 °C than in fish held at either 11 °C or 15 °C, suggesting that CART, but not NPY, may contribute to temperature-induced changes in appetite in cod (Kehoe and Volkoff, 2008). Feeding activity is affected by photoperiod and light regimens in some species including European sea bass (Sanchez-Vazquez et al., 1998), yellowtail (Kohbara et al., 2000), barfin flounder (Sunuma et al., 2007), but not in others, as in Senegal sole larvae (Canavate et al., 2006). The specific endocrine mechanisms behind these changes and the role of appetite-related hormones are unclear. In Atlantic cod, fish held at different photoperiods (24 h light, 24 h dark, 16 h light:8 h dark) do not display pronounced differences in either food intake or mRNA expression of either NPY, CART or OX in the brain or in ghrelin or GRP in the stomach, suggesting that in cod, photoperiod is not an important seasonal factor regulating feeding (Xu and Volkoff, unpublished). In winter flounder, it appears that the decrease in food intake seen during the winter month is accompanied by changes in hypothalamic NPY, but not CART, mRNA expression, suggesting that NPY is affected by seasonal parameters in this species (McDonald, 2008).

4. Links between appetite-regulating hormones and reproduction

Links between energy homeostasis and reproduction have been demonstrated in several vertebrates (Mircea et al., 2007) where animals usually reduce their food intake during the breeding season. Quantitative gender-specific differences have also been reported for several appetite regulators, including for tachykinins (Peyon et al., 2000), galanin (Rao et al., 1996), and ghrelin (Parhar et al., 2003). These data suggest that interactions might occur between appetite-related and reproductive hormones. An interaction has recently been proposed in goldfish, between orexin and gonadotropin-releasing hormone (form 2, GnRH2), a reproductive hormone that has been shown to stimulate spawning behavior in goldfish (Volkoff and Peter, 1999). In female goldfish, central injections of orexin at doses that stimulate feeding inhibit spawning behavior, and this inhibition is mediated by a decrease in the brain mRNA expression of cGnRH (Hoskins et al., 2008). Conversely, immature (Matsuda et al., 2008) or mature female (Hoskins et al., 2008) goldfish decrease their food intake when injected with cGnRH. In mature females, this decrease is mediated in part by a decrease in OX mRNA brain expression (Hoskins et al., 2008). It is likely that other appetite-related hormones influence reproduction in goldfish.

5. Conclusion

It appears that the regulation of food intake has been relatively well conserved in vertebrates as the same appetite-regulating peptide homologs are present in most groups and appear to have similar actions. However, increasing evidence suggests that in fish, species-specific differences might exist. In addition, within a same species, the actions and expression levels of these peptides can be affected by both

intrinsic factors and environmental factors. In order to characterize an appetite-regulating peptide in a specific fish, one must take into account not only the actions of the hormone and its interactions with other appetite-related hormones, but also the phylogeny of the fish, its physiological state (*i.e.* nutritional status and reproductive state) as well as the environment the fish evolves in (*i.e.* season, temperature...). Given the diversity of the fish group and the increasing number of appetite-related peptides discovered, it seems that much time and effort will be necessary to completely elucidate the mechanisms of endocrine regulation of feeding in fish.

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