Neuropeptides and the control of food intake in fish

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Abstract

The brain, particularly the hypothalamus, integrates input from factors that stimulate (orexigenic) and inhibit (anorexigenic) food intake. In fish, the identification of appetite regulators has been achieved by the use of both peptide injections followed by measurements of food intake, and by molecular cloning combined with gene expression studies. Neuropeptide Y (NPY) is the most potent orexigenic factor in fish. Other orexigenic peptides, orexin A and B and galanin, have been found to interact with NPY in the control of food intake in an interdependent and coordinated manner. On the other hand cholecystokinin (CCK), cocaine and amphetamine-regulated transcript (CART), and corticotropin-releasing factor (CRF) are potent anorexigenic factors in fish, the latter being involved in stress-related anorexia. CCK and CART have synergistic effects on food intake and modulate the actions of NPY and orexins. Although leptin has not yet been identified in fish, administration of mammalian leptin inhibits food intake in goldfish. Moreover, leptin induces CCK gene expression in the hypothalamus and its actions are mediated at least in part by CCK. Other orexigenic factors have been identified in teleost fish, including the agouti-related protein (AgRP) and ghrelin. Additional anorexigenic factors include bombesin (or gastrin-releasing peptide), \textalpha; melanocyte-stimulating hormone (\textalpha; MSH), tachykinins, and urotenin I. In goldfish, nutritional status can modify the expression of mRNAs encoding a number of these peptides, which provides further evidence for their roles as appetite regulators: (1) brain mRNA expression of CCK, CART, tachykinins, galanin, ghrelin, and NPY undergo peri-prandial variations; and (2) fasting increases the brain mRNA expression of NPY, AgRP, and ghrelin as well as serum ghrelin levels, and decreases the brain mRNA expression of tachykinins, CART, and CCK. This review will provide an overview of recent findings in this field.

Keywords: Fish; Peptides; Neuropeptide; Hormone; Food intake; Endocrinology

1. Control of appetite in fish

In all vertebrates, the regulation of appetite and body weight is a complex phenomenon involving elaborate interactions between the brain and peripheral signals. The brain, particularly the hypothalamus, produces key factors that either stimulate (orexigenic) or inhibit (anorexigenic) food intake. In fish, most studies examining feeding and appetite to date have dealt with diet composition and assimilation (Saether and Jobling, 1999) or with the effects of environmental factors such as photoperiod (Bolliet et al., 2001) and temperature...
(Talbot et al., 1999) on the level of food intake. Information on the neural regulation of appetite of fish has only recently begun to become available (De Pedro and Bjornsson, 2001; Jensen, 2001; Lin et al., 2000). Early studies using either electrical stimulation or lesioning of specific brain areas have shown that, in fish as in mammals, the hypothalamus is involved in the control of food intake (Demska and Northcutt, 1983; Peter and Crim, 1979). The hypothalamic inferior lobe in particular receives sensory information arising from the periphery and acts as an integration center that regulates feeding (Peter and Crim, 1979; Rink and Wullimann, 1998; Wullimann and Mueller, 2004). Indeed, electric stimulation of inferior lobes of the hypothalamus elicits a feeding response in several fish, including teleosts (Demska, 1973; Roberts and Savage, 1978) and elasmobranchs (Demska, 1977). Other regions of the fish brain also seem to be involved in the control of appetite of teleosts. For example, electrical stimulation of either the ventral telencephalon, the secondary gustatory nucleus or the optic tectum induces feeding behavior (Demska and Knigge, 1971), whereas feeding behavior is depressed by olfactory tract lesions (Demska and Knigge, 1971; Stacey and Kyle, 1983).

In fish, a number of peptides homologous to the mammalian appetite-regulating peptides have been isolated or their sequence deduced from cloned cDNA sequences. Such peptides include cholecystokinin, CCK (Volko et al., 2002; Wang and Conlon, 1994), somatostatin; UCN, urocortin; UI, urotensin I.

Information on the role of these neuropeptides in the control of food intake and their mechanism of action in fish is growing but is still very limited. This review examines our current knowledge on these appetite-regulating peptides in fish.

2. Appetite stimulators

2.1. Ghrelin

Ghrelin is a 28 amino acid peptide (Kojima et al., 1999) synthesized in the stomach (Sakata et al., 2002) and brain (Cowley, 2003) that is involved in the control of energy homeostasis and increases food intake in mammals (Horvath et al., 2003; Nakazato et al., 2001; Wren et al., 2001). Ghrelin receptors are localized in the hypothalamus, suggesting that the orexigenic actions of ghrelin are mediated by actions in the hypothalamus (Cowley, 2003; Inui et al., 2004).

In fish, ghrelin has been identified in several species including goldfish, Carassius auratus (Unniappan et al., 2002), tilapia, Oreochromis niloticus (Parhar et al., 2003), and Japanese eel, Anguilla japonica (Kaiya et al., 2003a,b). Ghrelin mRNA is highly expressed in fish stomach/gut and moderate levels are detected in the brain (Kaiya et al., 2003a,b; Unniappan et al., 2002). Ghrelin receptor cDNAs have been identified in the pufferfish, Spheroidees niphelus (Palyha et al., 2000) and the black seabream, Acanthopagrus schlegeli (Chan and Cheng, 2004) and show high levels of expression in pituitary and brain, in particular the hypothalamus (Chan and Cheng, 2004).

Recent studies show that ghrelin is involved in the control of feeding in fish. Both central and peripheral injections of either goldfish or human ghrelin stimulate food intake in goldfish (Unniappan et al., 2002, 2004a,b). In goldfish, there is a postprandial decrease in preproghrelin mRNA expression in the hypothalamus and gut that is concomitant with a decrease in serum ghrelin levels (Unniappan et al., 2004a,b). The peri-prandial changes in ghrelin mRNA expression in the brain and gut and serum ghrelin levels provide further support to the orexigenic actions of ghrelin in goldfish. In goldfish, 7 days of starvation increases preproghrelin mRNA expression in the hypothalamus and gut (Unniappan et al., 2004a,b). In burbot, Lota lota, fasting reduces plasma ghrelin-immunoreactive peptide concentrations and this decrease is concomitant with a decrease in leptin-immunoreactive peptide concentrations (Nieninen et al., 2003). In addition, ghrelin-immunoreactive peptide concentrations are low in burbot during pre-spawning, a period that is characterized by high rates of liver glycoxylogenesis and lipid mobilization, but increased after

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1 Abbreviations used: 5-HT, serotonin-5-hydroxytryptamine; AgRP, agouti-related protein; ARC, arcuate nucleus; BBS, bombesin; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin; cDNA, complementary deoxyribonucleic acid; CNS, central nervous system; CRF, corticotropin-releasing factor; GAL, galanin; GALP, galanin-like peptide; GH, growth hormone; GHRH, growth hormone-releasing hormone; GI, gastrointestinal; GLP, glucagon-like peptide; GRP, gastrin-releasing peptide; HPA, hypothalamic–pituitary–adrenal; ICA, intracerebroventricular; IGF-I, insulin-like growth factor-I; LHA, lateral hypothalamic area; MCH, melanin concentrating hormone; mRNA, messenger ribonucleic acid; MSH, melanocyte-stimulating hormone; NKA, neuropeptide-A; NKb, neuropeptide-B; NMB, neuromedin B; NPY, neuropeptide-Y; PACAP, pituitary adenylate cyclase-activating peptide; POMC, pro-opiomelanocortin; PP, pancreatic polypeptide; PPT, preprotachykinin; PrRP, PRL-releasing peptide; PVN, paraventricular nucleus; PY, peptide-Y; PYY, peptide YY; SP, substance-P; SRIF, somatotropin-releasing inhibiting factor, somatostatin; UCN, urocortin; UI, urotensin I.
spawning when liver glycogenolysis is suppressed and the rate of gluconeogenesis increased (Mustonen et al., 2002). These results suggest that ghrelin has a major role in feeding, metabolism, and reproduction in fish.

2.2. Hypothalamic neuropeptides

2.2.1. MCH

Melanin-concentrating hormone (MCH) was initially isolated from the pituitary of chum salmon (Oncorhynchus keta) as a hormone mediating color change (Kawauchi et al., 1983). A mammalian MCH was subsequently identified and shown to play a major role in the control of feeding and energy homeostasis (Qu et al., 1996).

In fish, MCH is present in the lateral and caudal hypothalamic areas as well as in the pituitary (Baker and Bird, 2002). Two fish genes, MCH1 and MCH2 have been characterized to date and RNA encoding MCH receptors have been identified in zebrafish, Danio rerio and pufferfish, Fugu rubripes (Logan et al., 2003a,b; Pissios and Maratos-Flier, 2003). To date, the spatial expression of the fish MCH receptors has not been characterized. In fish, the main role of MCH is the regulation in color. MCH acts on melanophores to lighten the skin (Green et al., 1991), thus antagonizing the actions of α-melanocyte-stimulating hormone (α-MSH). Data suggest that, in contrast to mammals, MCH does not have a critical role in the regulation of feeding in fish. Transgenic medaka (Oryzias latipes) overexpressing the MCH gene displays changes in body color, but have normal growth or feeding behavior (Kinoshita et al., 2001).

In goldfish, central administration of MCH has no clear effects on food intake and fasting does not consistently modify hypothalamic MCH levels (Cerdá-Reverter and Peter, unpublished data).

2.2.2. Neuropeptide Y family of peptides

The neuropeptide Y (NPY) family of peptides consists of NPY, peptide YY (PYY), pancreatic polypeptide (PP), and peptide Y (PY). These peptides bind to a family of G-protein-coupled receptors that compose the Y family, which has five cloned members, namely Y1, Y2, Y4, Y5, and Y6 (Larhammar et al., 2001). In mammals, neuropeptide Y (NPY) is abundant in the CNS, particularly in hypothalamic nuclei such as the arcuate nucleus (ARC) and paraventricular nucleus (PVN) both key areas in the regulation of feeding (Halford et al., 2004). NPY is one of the most potent orexigenic agents known in mammals (Halford et al., 2004; Kalra et al., 1999). All fish produce two neuropeptide Y (NPY)-related peptides: NPY and peptide YY (PYY). Some teleost fish further produce pancreatic peptide Y (PY) (Cerdá-Reverter and Larhammar, 2000). NPY RNAs or peptide sequences have been determined for a large number of fish species (Cerdá-Reverter et al., 2000a,b,c; Doyon et al., 2003; Leonard et al., 2001). NPY neurons are widely distributed in the CNS of dipnoans (Trabucchi et al., 2000), elasmobranchs (Chiba, 2000), and teleosts (Cerdá-Reverter et al., 2000b; Doyon et al., 2003; Leonard et al., 2001; Peng et al., 1994; Silverstein et al., 1998), and NPY-immunoreactive fibers have also been identified in fish pituitary, pancreas and gastrointestinal tract, and nerve fibers surrounding blood vessels (Cerdá-Reverter and Larhammar, 2000; Chiba et al., 1996; Danger et al., 1991). To date, two Y receptor subtypes have been identified in fish. Y1-like receptors have been identified in several fish species (Larhammar et al., 2001), whereas Y2-like receptors have only been characterized in zebrafish and rainbow trout, Oncorhynchus mykiss (Fredriksson et al., 2004). Fish NPY receptors are expressed in brain but also in peripheral tissues such as eye and intestine (Fredriksson et al., 2004; Lundell et al., 1997).

Several studies have demonstrated that NPY is involved in the regulation of food intake in teleosts. Central injections of mammalian or fish NPY cause a dose-dependent increase in food intake in goldfish, C. auratus (De Pedro et al., 2000; Lopez-Patino et al., 1999; Narnaware et al., 2000), salmon, Oncorhynchus kisutch and catfish, Ictalurus punctatus (Silverstein and Pilserskay, 2000). In goldfish central injections with either a Y1 or a Y5 receptor agonist at low doses induce an increase in food intake, whereas Y2 agonist treatment has no effects (Narnaware and Peter, 2001b). In addition, co-administration of Y1 and Y5 agonists causes enhanced food intake that is additive of the individual doses alone and blocking one receptor does not influence the responsiveness of the other (Narnaware and Peter, 2001b). These data suggest that NPY acts centrally through Y1 and Y5 receptors, which act independently to stimulate food intake in goldfish. Further demonstration that NPY is a brain signal involved in feeding in fish comes from the fact that food deprivation induces an increase in the hypothalamic expression of NPY mRNA in goldfish (Narnaware and Peter, 2001a) salmon, O. kisutch and catfish, I. punctatus (Silverstein et al., 1999) and that re-feeding 72-h starved goldfish reverses the effects of food deprivation on brain NPY mRNA (Narnaware et al., 2000). Also, NPY mRNA levels undergo peri-prandial variations in goldfish, with an increase in NPY mRNA levels in the telencephalon–preoptic area and hypothalamus shortly before feeding and a decrease in brain NPY mRNA levels after feeding (Narnaware and Peter, 2001a). NPY gene expression in goldfish brain seems to be influenced by macronutrient intake. Goldfish fed either high carbohydrate or high fat diets exhibit changes in NPY brain expression whereas feeding diets with different protein contents do not influence NPY gene expression in goldfish brain (Narnaware and Peter, 2002). In fish as in mammals, NPY seems to closely interact with a number of appetite regulators, including...
CRF and cortisol, CART, leptin, orexins, and galanin. These interactions will be described in subsequent sections.

Although PY and/or PYY have been identified in several species (Cerdà-Reverter et al., 2000c; Soderberg et al., 2000), their physiological role in fish is unknown.

2.2.3. Galanin

Galanin has been identified in numerous vertebrate groups including mammals, birds, reptiles, and fish (Wynick et al., 2001). In mammals, galanin is widely expressed in the central nervous system and intestine and is involved in the regulation of feeding (Gundlach, 2002; Vrontakis, 2002). In mammals, galanin mediates its biological effects through its receptors, the GAL1R, GAL2R, and GAL3R and these receptors are widely distributed in central and peripheral tissues (Vrontakis, 2002).

Although the peptide galanin has been identified in several fish species (Anglaide et al., 1994; Wang and Conlon, 1994; Wang et al., 1999a,b), the nucleotide sequence of the galanin gene has only been published for goldfish (Unniappan et al., 2003). Galanin is widely distributed in fish brain and galanin-immunoreactive fibers are also found in the pituitary (Jadhao and Meyer, 2000; Jadhao and Pinelli, 2001; Rao et al., 1996; Rodriguez et al., 2003) as well as in peripheral tissues (Johnsson et al., 2001; Unniappan et al., 2002). In goldfish, preprogalanin mRNA expression is seen in the olfactory bulbs, telencephalon, hypothalamus, midbrain, and the posterior brain (Unniappan et al., 2002, 2004a,b).

Intracerebroventricular (ICV) administration of galanin stimulates food intake in goldfish (De Pedro et al., 1995; Volkoff and Peter, 2001b) and tench, Tinca tinca (Guijarro et al., 1999), whereas IP administration of galanin has been reported to have no effect (De Pedro et al., 1995; Guijarro et al., 1999). In goldfish, brain preprogalanin mRNA expression decreases postprandially but is not affected by 7 days of starvation, suggesting that galanin may be more important in the short-term regulation of food intake in fish than in the long-term adaptation to starvation (Unniappan et al., 2004a,b). ICV co-injections of low dosages of galanin with either orexin A or NPY result in a food intake higher than that observed in fish treated with galanin alone, suggesting that galanin acts synergistically with both orexin A and NPY (Volkoff and Peter, 2001b). Central injections of a specific galanin receptor antagonist (M40) decrease NPY-induced feeding but has no effect on orexin-induced feeding (Volkoff and Peter, 2001b), suggesting that the interactions between GAL and NPY systems are stronger than the interactions between GAL and orexin in fish.

2.2.4. Orexins

Orexins (hypocretins) consist of two peptides, orexin A (hypocretin-1) and orexin B (hypocretin-2) produced by cleavage of a single precursor, prepro-orexin. In mammals, orexins are produced mainly in the lateral hypothalamus but orexin neurons are also found in the gut and other peripheral tissues (Kirchghesner, 2002). In mammals, orexins act through two receptors, OX 1R, which has a high affinity to orexin A, and OX 2R, which binds both orexin A or orexin B with similar affinities and stimulates food intake (Ferguson and Samson, 2003; Ishii et al., 2004; Rodgers et al., 2002; Sakurai, 2002).

In fish, mRNAs encoding for prepro-orexin have been reported for pufferfish (Alvarez and Sutcliffe, 2002) and zebrafish (Kaslin et al., 2004). Fish prepro-orexins show a high degree of homology in their structure with other vertebrate prepro-orexins and appear to undergo a similar processing into orexin A and orexin B peptides (Kaslin et al., 2004). To date, orexin receptors have not been identified in fish. In zebrafish, both prepro-orexin mRNA and orexin protein are present in the hypothalamic nuclei believed to be involved in regulation of energy homeostasis. Orexin fibers also interact with both amine and cholinergic system, both involved in the regulation of sleep and vigilance in mammals (Kaslin et al., 2004). These anatomical data suggest that orexins may be involved in the regulation of states of wakefulness and energy homeostasis in fish (Kaslin et al., 2004). This putative role is consistent with physiological studies showing that central injections of orexins induce hyperphagia and increase locomotor activity in goldfish (Volkoff et al., 1999). In fish, as in mammals (Kirchghesner, 2002; Rodgers et al., 2002), orexins appear to interact with a number of appetite regulators, e.g., NPY, GAL, CART, and leptin. Orexin–GAL interactions are described in the previous section. Blocking of orexin receptors by treatment with high doses of orexin A results in a decrease in NPY-induced feeding, indicating that NPY is in part dependent on co-action with orexin A for the stimulation of food intake and feeding behavior in goldfish (Volkoff and Peter, 2001b). Conversely, blocking of NPY receptors reduces orexin A-induced feeding, suggesting that the effects of orexin A are mediated, in part, by the NPY pathway. In addition, co-injection with orexin A and NPY results in a food intake higher than that observed in fish treated with NPY alone and brain NPY mRNA expression increases following injection of orexin A (Volkoff and Peter, 2001b). In goldfish, CART (Volkoff and Peter, 2001a) and leptin (Volkoff et al., 2003) both inhibit orexin A-induced feeding. This data points to a functional interdependence between orexin and other peptidergic systems in the control of energy balance in goldfish.

2.2.5. Agouti-related protein

In mammals, the agouti-signaling protein (ASP) regulates fur pigmentation and is a competitive antagonist of the melanocortin receptor subtypes 1 (MC1R) and 4 (MC4R) expressed primarily in the skin. Agouti-related
protein (AgRP) is the agouti protein homolog in the mammalian brain (Tritos and Maratos-Flier, 1999). AgRP is mainly expressed in the hypothalamic ARC and works mainly as an endogenous antagonist of MC3R and MC4R thus inhibiting the activity of melanocortins, i.e., α-MSH (MacNeil et al., 2002). AgRP is involved in the control of energy homeostasis and feeding in mammals (MacNeil et al., 2002; Wilson et al., 1999).

An mRNA encoding AgRP has recently been identified in goldfish (Cerdá-Reverter and Peter, 2003), zebrafish (Song et al., 2003), and pufferfish (Klovins et al., 2004). The goldfish AgRP gene encodes a 128 amino acid precursor and is expressed in a variety of tissues including brain and peripheral tissues. In the goldfish brain, AgRP is mainly expressed in the caudal portion of the hypothalamic lateral tuberal nucleus. Although the physiological role of AgRP in fish is not known, fasting up-regulates hypothalamic AgRP mRNA levels in both goldfish and zebrafish (Cerdá-Reverter and Peter, 2003; Song et al., 2003), suggesting a possible role of AgRP in the control of food intake in fish.

2.3. Somatotropic axis and GH

The somatotropic axis is one of the major players in the endocrine regulation of growth. It involves several molecules, including growth hormone (GH), insulin-like growth factor-I (IGF-I), and somatostatin (somatotropin-releasing inhibiting factor, SRIF). GH release by pituitary somatotrophs is stimulated by growth hormone-releasing hormone (GHRH), pituitary adenylate cyclase-activating peptide (PACAP), and GH secretagogues such as ghrelin, and inhibited by somatostatin (SRIF) and other factors including serotonin (5-HT). GH stimulates the liver and other tissues to produce insulin-like growth factor-I (IGF-I), a protein that mediates many of the growth promoting effects of GH (Anderson et al., 2004). All these peptides have been implicated in the control of feeding in mammals.

Most of the components of this axis appear to be present in fishes (Mommsen, 2001). To date, mRNAs encoding the GHRH/PACAP precursor have been isolated from several fish species (Adams et al., 2002; Jiang et al., 2003; Peng and Peter, 1997). GHRH receptors have been isolated from goldfish (Chan et al., 1998) and pufferfish (Cardoso et al., 2003) and shown to be expressed in the pituitary and throughout the brain. mRNAs encoding for SRIF-14 (preprosomatostatin-I or PSS-I gene) and fish SRIF-28 (iSRIF-28; PSS-II gene), which has not been described in mammals, as well as several SRIF-14 variants, including [Pro2]SRIF-14 (encoded by the PSS-III gene) have been identified in fish (Lin et al., 2000). Whereas in mammals five SRIF receptor subtypes (sst1–5) have been identified, 8 receptor subtypes (glsst1A, 1B, 2, 3A, 3B, 5A, 5B, 5C) have been cloned from goldfish brain (Lin and Peter, 2003; Lin et al., 2000). In addition, sst2 and sst3 homologues have been characterized in pufferfish (Bagheri-Fam et al., 2001) and black ghost knife-fish, Apterodonotus albifrons (Siehler et al., 1999; Zupanč et al., 1999). Both SRIF receptor mRNAs and three PSS mRNAs are widely expressed in goldfish brain, and in situ hybridization studies show that the three SRIF peptides are expressed in nuclei known to be involved in the control of pituitary function as well as food intake (Canosa et al., 2004). Rainbow trout and goldfish display postprandial increases in plasma SRIF-14 concentrations (Holloway et al., 1994) and peri-prandial variations in the forebrain expression of the SRIF precursor gene PSS-II (Canosa and Peter, unpublished results), respectively. Taken together these results suggest a wide range of physiological functions for SRIF peptides and a possible role in the regulation of feeding (Lin and Peter, 2003; Lin et al., 2000; Yunke et al., 2003). The effects of GHRH and PACAP on feeding have not been examined in fish.

GH and IGF-I have been isolated in several fish species (Mommsen, 2001; Wallis, 1996). GH stimulates hepatic IGF-I mRNA and circulating IGF-I in fish (Biga et al., 2004; Pierce et al., 2004; Silverstein et al., 2000). In fish, either growth hormone administration (Mclean et al., 1993; Silverstein et al., 2000) or GH transgenesis (Devlin et al., 1994) result in increased growth that might be attributed to increased feeding and improved food assimilation (Abrahams and Sutterlin, 1999; Silverstein et al., 2000). As GH treatment lowers dopaminergic activity in the hypothalamus of trout (Jonsson et al., 2003), it has been suggested that GH acts directly on the central nervous system (CNS) to modulate feeding behavior (Johnson and Bjorndahl, 1994). However, the role of GH on the control of feeding is still controversial. For example, ICV injections of GH in juvenile rainbow trout have no effect on appetite (Jonsson et al., 2003).

3. Appetite suppressors

3.1. CCK/gastrin

Gastrin and cholecystokinin (CCK) are structurally related peptides with a common C-terminal tetrapeptide sequence. CCK is found in both the brain and the gastrointestinal tract and has multiple biologically active forms, with CCK-8 being the most abundant form in the brain (Moran and Kinzig, 2004). Pro-CCK has three sulfated tyrosine residues and sulfation of the tyrosine residue in CCK is known to be important for its activity at CCK receptors (Beinfeld, 2003). CCK-related peptides bind to two receptor subtypes, the CCK-A receptor (or CCK-1) located primarily in the gastrointestinal tract and the CCK-B receptor (or CCK-2) located primarily in the brain. In mammals, CCK has many physiological
actions but functions primarily as a satiety signal (Cuppes, 2002).

CCK/gastrin-like immunoreactivity has been shown in the nervous system and gut of several fish species (Aldman et al., 1989; Barrenechea et al., 1994; Himick and Peter, 1994b; Jonsson et al., 1987; Kamisaka et al., 2001) and mRNAs encoding for CCK/gastrin have been determined for a number of fish including goldfish, rainbow trout, *O. mykiss* (Jensen et al., 2001), pufferfish and flounder, *Paralichthys olivaceus* (Kurokawa et al., 2003). Sequence analyses of fish CCK cDNAs reveal cleavage sites that suggest that the pro-CCK might be processed into octapeptides. The presence of octapeptides is indeed confirmed by chromatographic analysis. Fish appear to have different forms of CCK-8 peptides that differ by their amino acid at position 6, counting from the C-terminus (Asn, Leu or Thr). Although octapeptides are the major product of posttranslational processing, pro-CCK is also cleaved to a lesser extent into fragments of different lengths. For example, in trout, three forms consisting of 7, 8, and 21 residues have been isolated. All isolated peptides are fully sulfated (Jensen et al., 2001; Johnsen, 1998).

In all fish examined, CCK mRNAs are detected in the brain and intestine (Jensen et al., 2001; Johnsen, 1998; Kurokawa et al., 2003; Peyon et al., 1999). In goldfish, CCK mRNA is widely expressed in the brain, with the highest levels being found in the hypothalamus, and is also found in the pituitary, and in a number of peripheral tissues, including gill and gastrointestinal tract (Peyon et al., 1999).

It appears that a single primitive CCK/gastrin receptor exists in fish. CCK binding sites have been localized in the brain and gastrointestinal tract of several fish, including goldfish (Himick et al., 1996), seabass (Moons et al., 1992), and elasmobranchs (Oliver and Vigna, 1996). Within the brain, CCK/gastrin binding sites are found in the telencephalon and preoptic hypothalamus, as well as within hypothalamic nuclei associated with the brain feeding center (Himick et al., 1996).

In fish, CCK influences digestion and feeding processes. As in mammals, CCK is released in fish when food is present in the intestine (Aldman and Holmgren, 1995) and CCK-related peptides induce the contraction of the gallbladder in several teleosts (Aldman and Holmgren, 1995; Einarsso et al., 1997; Rajjo et al., 1988) and slow gastric emptying in salmonids (Olsson et al., 1999). CCK has been shown to affect appetite in fish. In goldfish, both central and peripheral injections of sulfated CCK-8 (CCK-8s) suppress food intake (Himick and Peter, 1994b; Volkoff et al., 2003) whereas treatment of trout with CCK antagonists induces an increase in food intake (Gélineau and Boujard, 2001). In addition, CCK mRNA levels increase 2 h after a meal in goldfish brain (Peyon et al., 1999). In goldfish, CCK appears to mediate in part the effects of leptin on food intake. Leptin potentiates the satiety actions of CCK, and CCK hypothy-
modulin, and is produced by the processing of pro-glucagon in the intestine and the CNS. In mammals and amphibians, GLP-1 has insulin-like physiological actions, controls gastric emptying and inhibits food intake (Drucker, 1998). The actions of GLP-1 are mediated via a specific G-protein-coupled receptor, the GLP-1 receptor (GLP-1R).

In fish, mRNAs encoding glucagon and GLP have been identified in several species including holocephalans (Conlon et al., 1987a), cyclostomes (Conlon et al., 1993; Irwin et al., 1999), and a number of teleosts (Andrews and Ronner, 1985; Irwin and Wong, 1995; Yuen et al., 1997; Zhou and Irwin, 2004). A GLP-1R receptor that binds fish GLP-1 and mammalian GLP-1 has been cloned in zebrafish (Mojsov, 2000) and goldfish (Yeung et al., 2002).

In contrast to mammalian GLP-1, fish GLP-1 is produced by both the pancreas and the intestine and appears to have glucagon-like activity rather than insulin-like activity (Plisetskaya and Mommsen, 1996). GLP-1 causes gastric emptying and has anorexigenic effects in fish. In channel catfish, I. punctatus, both central (ICV) and peripheral injection of GLP-1 induces anorexia but peripheral treatment has a weaker effect, suggesting that the major effects of GLP on food intake are centrally mediated (Silverstein et al., 2001a,b). However, the anorectic actions of GLP-1 in fish are not antagonized by central injection of the GLP-1 receptor antagonist exendin (9–39), or by immunoneutralization of GLP-1 by ICV injection of anti-salmon GLP-1 antisera (Silverstein et al., 2001a,b).

3.4. Leptin

Leptin is a 16 kDa protein encoded by the obese (ob) gene that is produced and secreted mainly by adipocytes, but also by other tissues including brain and gastric epithelium (Harvey and Ashford, 2003). Peripheral administrations of leptin decrease food intake and induce weight loss in birds and mammals (Neyre et al., 2004). High levels of leptin receptors have been identified in a number of hypothalamic nuclei containing neurons expressing important orexigenic (such as NPY, GAL, AgRP, MCH, and orexins) and anorexigenic factors (such as POMC-derived peptides, CART, and CCK), suggesting that leptin affects food intake by inhibiting hypothalamic orexigenic pathways and stimulating anorexigenic pathways.

To date, leptin has only been isolated in mammals and birds (Doyon et al., 2001). However, recent evidence suggests that it is also expressed in lower vertebrates including fish. Leptin-like immunoreactive material has been detected in blood and tissues of amphibians (Muruzabal et al., 2002) and reptiles (Muruzabal et al., 2002; Paolucci et al., 2001) and peripheral injections of murine leptin decrease food intake and increase metabolic rates in lizards (Niewiarowski et al., 2000).

In fish, leptin-like immunoreactive material has been detected in several species (Johnson et al., 2000; Mustonen et al., 2002; Nieminen et al., 2003; Yaghoubian et al., 2001). Using Western blot analysis and anti-human leptin antibodies, Vegusdal et al. (2003) showed that a leptin-like protein of 15 kDa was present in salmon adipocytes. Leptin-treared green sunfish, Lepomis cyanellus display an increase in the enzymatic activity of several indicators of intracellular fat metabolism, suggesting that leptin induces an overall increase in fat metabolism (Londraville and Duvall, 2002). Studies in coho salmon, Oncorhynchus kisutch (Baker et al., 2000), catfish (Silverstein and Plisetskaya, 2000), and green sunfish (Londraville and Duvall, 2002) report no effect of leptin treatment on food intake or body weight. In goldfish, however, both peripheral and central injections of murine leptin decrease food intake (Volko et al., 2003). The different results among studies might be explained by the higher doses of leptin used in the goldfish studies. The fact that salmon with the greatest fat reserves after the parr-smolt transformation grow more slowly than fish that are ‘leaner’ at this time, is another clue suggesting that adiposity, and perhaps a leptin-like factor exerts a negative feedback on feeding in fish (Jobling et al., 2002). In goldfish, higher peripheral doses of leptin are required to cause a decrease in food intake than when administered centrally, suggesting that in fish, as in mammals, leptin acts primarily in the brain to control energy homeostasis (Volko et al., 2003). In goldfish, central injections of leptin accentuate the anorexigenic effects of CART and CCK and inhibit both NPY- and orexin A-induced food consumption, and these actions are associated with an increase in both CCK and CART mRNA expression and a decrease in NPY mRNA brain expression (Volko and Peter, 2001a; Volko et al., 2003). These data suggest that in fish, as in mammals, leptin interacts with hypothalamic pathways to inhibit food intake. As mentioned previously, data suggest that the actions of leptin in fish are mediated in part by CCK (Volko et al., 2003).

3.5. Hypothalamic neuropeptides

3.5.1. Melanocortin system:

POMC/melanocyte-stimulating hormone

Melanocortins are a group of pituitary hormones that include adrenocorticotropic (ACTH) and the α-, β-, and γ-melanocyte-stimulating hormones (MSH), all derived from the precursor molecule pro-opiomelanocortin (POMC) by tissue-specific posttranslational cleavage. In tetrapods, five melanocortin receptors (MC1R-MC5R) have been identified to date, all of which are G-protein-coupled receptors. In mammals, melanocortins are involved in the control of energy homeostasis (Ellacott and Cone, 2004). MSHs regulate appetite, as central administration of MC3R/MC4R receptor agonist and
antagonists inhibit and stimulate food intake, respectively (Schioth et al., 2003).

In fish, the POMC gene encodes for a number of MSHs. α-MSH and β-MSH have been detected in both bony fish (Alrubaian et al., 2003; Cerdá-Reverter et al., 2003a; Gonzalez-Nuñez et al., 2003) and cartilaginous fish (Dores et al., 2003; Takahashi et al., 2001). The γ-MSH sequence is absent from the POMC genes of euteleosts (Dores et al., 2003) and a fourth MSH sequence, δ-MSH is present in cartilaginous fish (Dores et al., 2003; Takahashi et al., 2001). Four receptor subtypes have been identified in pufferfish, *F. rubripes* (Logan et al., 2003a,b), six subtypes in zebrafish, *D. rerio* (Logan et al., 2003a,b; Ringholm et al., 2002) and two in goldfish, *C. auratus*, gMC4R and gMC5R (Cerdá-Reverter et al., 2003a,b,c). In goldfish, mRNA encoding these receptors is widely distributed in the brain and is present in a number of peripheral tissues such as spleen (Cerdá-Reverter et al., 2003b,c).

Several studies indicate that melanocortin receptors and MSHs are involved in the regulation of energy metabolism and food intake in fish. Desacetyl α-MSH, the major α-MSH observed in the salmon pituitary stimulates hepatic triacylglycerol lipase activity and increases the circulating level of fatty acids in rainbow trout (Yada et al., 2002). Also, trout with defective α-MSH synthesis have enlarged livers and accumulation of fat in the abdominal cavity (Yada et al., 2002), suggesting that the melanocortin system plays a role in the control of energy balance of fish by decreasing food intake, activating lipolytic activity, and enhancing energy expenditure. Contrasting with this view, goldfish MC4R and MC5R mRNAs show no or very low levels in the liver (Cerdá-Reverter et al., 2003a,b). Central administration of MTII, a gMC4R agonist, inhibits food intake in 24-h fasted goldfish in a dose-dependent manner, whereas treatment with the selective gMC4R antagonist, HS024, stimulates food intake in fed animals (Cerdá-Reverter et al., 2003b).

In goldfish, POMC is exclusively expressed within the lateral tuberal nucleus (homolog to mammalian ARC), but fasting does not significantly modify hypothalamic mRNA levels (Cerdá-Reverter et al., 2003a), thus suggesting that POMC neurons may exert a tonic inhibitory effect on feeding and energy storage via release of MSH at sites expressing melanocortin receptors.

3.5.2. CART

Cocaine- and amphetamine-regulated transcript (CART) peptide was originally isolated using PCR differential display as the product of a transcript that was elevated in the rat brain following acute administration of psychomotor stimulants such as cocaine and amphetamine (Kuhar et al., 2002). CART is an anorectic neuropeptide in mammals (Kristensen et al., 1998; Kuhar et al., 2002; Vrang et al., 2000) and birds (Tachibana et al., 2003). In mammals, CART interacts with other appetite regulators, including CRF, orexins, and NPY, as well as leptin (Kuhar et al., 2002). No CART receptors have yet been identified in any species.

In fish, a complete gene sequence of CART has only been published for goldfish (Volkoff and Peter, 2001a), but mRNAs encoding pro-CART are also available for zebrafish and pufferfish in GenBank and the Fugu genome Project, respectively. Goldfish have two forms of CART peptide precursors, CART I, which encodes a 117-amino acid pro-CART, and CART II, which encodes a 120-amino acid pro-CART (Volkoff and Peter, 2001a). mRNAs for form I and II precursors have a widespread distribution and are present in the brain, pituitary, and a number of peripheral tissues including eye, gonad, and kidney (Volkoff and Peter, 2001a,b).

In goldfish, ICV injection of human CART fragments [CART (62–76) or CART (55–102)] decreases food consumption. An increase in CART mRNA levels is seen 2 h following a meal in the olfactory bulbs and hypothalamus for form I whereas no postprandial changes in form II mRNA levels are observed. Food deprivation induces a decrease in form I mRNA levels in several brain regions, including the hypothalamus, and in form II mRNA expression in the olfactory bulbs (Volkoff and Peter, 2000).

Administration of CART (55–102) inhibits both NPY- and orexin A-stimulated food intake in goldfish, suggesting an inhibitory action of CART on both NPY and orexin A systems (Volkoff and Peter, 2000). A synergistic interaction between leptin and CART has also been suggested (Volkoff and Peter, 2001a) (see leptin section above).

3.5.3. Tachykinins/substance P

The tachykinin family of peptides includes substance P (SP), neuropeptide γ, neurokinin A (NKA), and B (NKB), scyliorhinins and carassin, all derived from the preprotachykinin gene. In mammals, tachykinins are synthesized in the CNS and in the GI tract and function as neurotransmitters (Pennefather et al., 2004). In fish, tachykinins are present in the central and peripheral nervous system. Fibers containing NKA and/or SP are found in perivascular nerves innervating fish blood vessels and muscle (Funakoshi et al., 2000; Jensen and Holmgren, 1991; Johnsson et al., 2001). Several tachykinins have been isolated from fish intestine and brain including SP (Liu et al., 2002), scyliorhinins (Wang et al., 1999a,b), neuropeptide γ (Lee et al., 2003), and a NKA-like peptide (Wang et al., 1999a,b). A complete nucleotide sequence of γ-preprotachykinin (γ-PPT) mRNA encoding the neuropeptides SP, carassin, and NKA have been identified in goldfish brain and shown to be expressed in a wide range of brain areas as well as in peripheral tissues such as the intestine (Lin and Peter, 2003; Peyon et al., 2000). G-protein-coupled receptors
analogous to the mammalian tachykinin receptor also have been described in fish (Van Giersbergen et al., 1991; Jensen et al., 1993).

In fish, tachykinins have a variety of functions and they affect the vascular (Jensen and Holmgren, 1991) and immune system (Ndoye et al., 1992). It seems that tachykinins are somewhat involved in feeding and digestion processes in fish, although direct evidence for a role of tachykinin peptides in food intake in fish is lacking. SP-like immunoreactivity is present in the enteric nervous system in rainbow trout (Bjenning and Holmgren, 1988; Holmgren et al., 1982) and tachykinin/SP induce contractions of gut smooth muscle of several species of fish (Jensen and Holmgren, 1991; Johansson and Holmgren, 2003; Liu et al., 2002; Olsson and Holmgren, 2001). Also, in goldfish, postprandial changes in γ-PPT gene expression have been demonstrated in the brain, in particular the olfactory bulbs and hypothalamus (Peyon et al., 2000).

3.5.4. CRF-related peptides and cortisol

The corticotropin-releasing factor (CRF) system is comprised of a family of related peptides, two main receptor types, CRF-R1 and CRF-R2, and a CRF binding protein (Bale and Vale, 2004). The ligand family members include CRF, several urocortins (UCN), fish urotensin I (UI), and amphibian sauvagine (Lewis et al., 2001; Reyes et al., 2001). Fish appear to have four different CRF-related peptides, including CRF, UI, and two urocortins that are closely related to mammalian UCN3 (Lewis et al., 2001; Lovejoy and Balment, 1999). Whereas the mRNA sequences encoding CRF and UI have been reported for several fish species (e.g. Ando et al., 1999; Barsyte et al., 1999; Bernier et al., 1999; Doyon et al., 2003; Huisng et al., 2004; Van Enckevort et al., 2000), to date the two fish urocortins have only been identified from pufferfish (*Tetraodon nigroviridis* and *Takifugu rubripes*) genomes (Lewis et al., 2001). The mRNAs encoding orthologues of the mammalian and amphibian CRF-R1 and CRF-R2 have also been reported for several different fish (Arai et al., 2001; Cardoso et al., 2003; Huisng et al., 2004; Pohl et al., 2001) and a third CRF receptor type (CRF-R3) has been identified in the catfish, *Ameiurus nebulosus* (Arai et al., 2001). The CRF system in fish also includes a CRF binding protein (Huisng et al., 2004; Seasholtz et al., 2002). Overall, phylogenetic analyses of the CRF ligands, receptors, and binding protein reveal that all components of the CRF system are evolutionarily well conserved across vertebrates (Doyon et al., 2003; Huisng et al., 2004).

The CRF system is primarily recognized for its key role in the endocrine regulation of the stress response via the hypothalamic–pituitary–adrenal (HPA) axis (Bale and Vale, 2004; Lovejoy and Balment, 1999). However, the widespread central and peripheral tissue distribution of CRF-related peptides and receptors have revealed a much broader physiological importance for the CRF system (Dautzenberg and Hauger, 2002). One such extra-HPA function of the CRF system is in the regulation of food intake and energy balance (Bernier and Peter, 2001a; Heinrichs and Richard, 1999; Richard et al., 2002). In goldfish, ICV injections of either CRF or UI suppress food intake in a dose-related manner with UI being more potent than CRF (Bernier and Peter, 2001b; De Pedro et al., 1993). Pretreatment with the CRF receptor antagonist, α-helical CRF (9–41), reverses the reduction in food intake induced by either UI or CRF ICV injections (Bernier and Peter, 2001b; De Pedro et al., 1997). In addition, goldfish treated with either the glucocorticoid receptor antagonist, RU-486, or the cortisol synthesis inhibitor, metyrapone, show an increase in forebrain UI and CRF mRNA levels and a marked reduction in food intake that can be reversed by α-helical CRF (9–41), pretreatment (Bernier and Peter, 2001b). These results suggest that endogenous CRF-related peptides play a role in the control of fish appetite. Similarly, CRF may be involved in mediating the anorectic effects associated with subordination stress. Relative to dominant fish, socially subordinate trout are characterized by suppressed food intake and an increase in forebrain CRF mRNA levels (Doyon et al., 2003).

Beyond the identification of CRF and UI as potent anorexigenic peptides, relatively little is known about the role of the CRF system in the regulation of food intake in fish. Relative to mammals, where the different CRF-related peptides and receptors have been shown to have distinct functions in the regulation of food intake (Richard et al., 2002), the relative importance of the different CRF receptors and ligands in fish feeding behavior has not been investigated. Similarly, while the CRF binding protein has been implicated in the regulation of appetite and energy balance in mammals (Richard et al., 2002), its contribution to these physiological processes in fish is not known. In mammals, CRF-related peptides also affect the regulation of food intake through peripheral actions on gastrointestinal motility (Tache and Perdue, 2004). Whereas CRF-related peptides reduce gastric emptying through CRF-R2, they can also stimulate distal colonic transit through CRF-R1 (Maillot et al., 2000; Wang et al., 2001). In fish, the peripheral effects of CRF-related peptides on gut motility have yet to be assessed. Finally, although there is some evidence suggesting that CRF-related peptides in fish may mediate at least a portion of the anorectic effects of serotonin (De Pedro et al., 1998a,b), overall, our knowledge of how CRF-related peptides interact with the various orexigenic and anorexigenic systems is in its infancy (Bernier and Peter, 2001a; Bernier et al., 2004; Doyon et al., 2003).

Evidence also suggests that cortisol, the end product of hypothalamic–pituitary–interrenal activation in most fishes, may be involved in the regulation of food intake (Bernier and Peter, 2001a). In goldfish, moderate chronic increases in plasma cortisol stimulate food intake, decrease CRF and increase NPY brain expression,
whereas larger catabolic doses of cortisol decrease CRF mRNA levels but have no effect on food intake or NPY expression (Bernier et al., 2004). In contrast, intraperitoneal injections of cortisol have no acute effects on food intake in goldfish (De Pedro et al., 1997) but are associated with a reduction in food intake over several days in rainbow trout (Gregory and Wood, 1999). Overall, the mechanisms of glucocorticoid effects on food intake regulation in fish, as in mammals (Tempel and Leibowitz, 1994), appear to be complex and dose-dependent.

4. Conclusion

An increasing number of hormones are now recognized to play a role in the regulation of appetite and energy balance in fish. Fig. 1 summarizes our current

![Schematic diagram](image)

Fig. 1. Schematic diagram of the central and peripheral peptides known to regulate food intake in fish. Within the brain, central neuropeptide systems receive signals and integrate information from the periphery to adjust food intake. These central systems consist of both feeding stimulators (orexigenic factors) and feeding inhibitors (anorexigenic factors). Some of these neuropeptide systems interact with each other. These interactions are indicated by arrows. Peripheral hormonal signals from gut and possibly from adipose tissue reach the brain and influence the central neuropeptide signals. Peripheral signals are conveyed via sensory axons in the vagus nerve or reach the brain directly via the blood. Double arrowheads indicate an orexigenic action whereas square arrowheads indicate an anorexigenic action. AgRP, agouti-related protein; BBS/GRP, bombesin/gastrin-releasing peptide; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin; CRF, corticotropin-releasing factor; GH, growth hormone; GLP, glucagon-like peptide; MSH, melanocyte-stimulating hormone; NPY, neuropeptide Y; and POMC, proopiomelanocortin.
knowledge on the role of neuropeptides in the control of food intake in fish. NPY, orexins, GAL, ghrelin and possibly GH, AgRP, and MCH act as orexigenic signals whereas CCK, CART, CRF-like peptides, tachykinins, BBS/GRP, GLP-1 and possibly POMC, MSH, and a leptin-like factor act as anorexigenic factors. Omitted from this summary diagram are the potential role of the amines 5-HT, dopamine and noradrenaline, and steroids. The list of peptides regulating appetite in fish is bound to grow in the future. Peptides such as galanin-like peptide (GALP), prolactin-releasing peptide (PrRP), and γ-aminobutyric acid (GABA), all involved in the control of feeding in mammals, have been identified in fish and may also be found to be appetite regulators in fish. Neither the sites of synthesis and action of appetite-regulating peptides nor the mechanisms by which they elicit their effects on feeding are yet fully understood. Mechanisms appear to be complex and to involve a number of interactions. The use of transgenic/knockout fish could be of great interest in studies directed to the precise mechanisms by which these systems interact to regulate food intake and energy balance in fish.

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