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Review

Brain regulation of feeding behavior and food intake in fish☆

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Abstract

In mammals, the orexigenic and anorexigenic neuronal systems are morphologically and functionally connected, forming an interconnected network in the hypothalamus to govern food intake and body weight. However, there are relatively few studies on the brain control of feeding behavior in fish. Recent studies using mammalian neuropeptides or fish homologs of mammalian neuropeptides indicate that brain orexigenic signal molecules include neuropeptide Y, orexins, galanin and β-endorphin, whereas brain anorexigenic signal molecules include cholecystokinin, bombesin, corticotropin-releasing factor, cocaine- and amphetamine-regulated transcript, and serotonin. Tachykinins may also have an anorectic action in fish. The brain hypothalamic area is associated with regulation of food intake, while sites outside the hypothalamus are also involved in this function. There is correlation between short-term changes in serum growth hormone levels and feeding behavior, although possible mechanisms integrating these functions remain to be defined.

Keywords: Feeding; Neuropeptide; Growth hormone; Brain; Fish

1. Introduction

In mammals, stability of body weight and body composition over long periods of time requires that energy intake matches energy expenditure (Jéquier and Tappy, 1999). There are mechanisms that tend to maintain energy intake and energy expenditure in balance. In mature mammals, body weight regulation mainly concerns adipose tissue mass, as protein and carbohydrate stores vary relatively little. Chronic imbalance between food intake and energy expenditure results in changes in adipose tissue mass (Jéquier and Tappy, 1999). The 'lipostatic model' has been proposed to explain the well-regulated control of body weight and food intake, and predicts that secretions from fat cells are the key signal to the brain to regulate feeding and body-fat deposition (Inui, 1999). A large and rapidly growing literature supports this hypothesis (Woods et al., 1998; Seeley and Schwartz, 1999). In particular, recent cloning of the obese gene (*ob*) and identification of its encoded protein leptin produced from adipose tissue (Zhang et al., 1994) is a major breakthrough in the understanding of the neuroendocrine regulation of energy homeostasis. Leptin acts via hypo-

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thalamic receptors to inhibit feeding, decrease body weight, and increase locomotor activity and thermogenesis in rodents (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). Leptin and its receptor system provide an afferent negative feedback signaling system reflecting the amount of adipose energy stores to the brain hypothalamic centers (Schwartz and Seeley, 1997). Leptin targets in the hypothalamus include neuropeptides, such as neuropeptide Y (NPY), agouti-related peptides (AGRP), α-melanophore-stimulating hormone (α-MSH), corticotropin-releasing factor (CRF), galanin, melanocyte concentrating hormone (MCH), and orexins (Mercer et al., 1996; Cheung et al., 1997; Campfield et al., 1998; Håkansson et al., 1998, 1999), that modulate food intake and energy expenditure. Thus, a feedback regulatory loop with three distinct steps has been hypothesized, which include a sensor that monitors the level of energy, hypothalamic centers that receive and integrate through leptin receptors the intensity of the signal, and effector systems that influence energy intake and energy expenditure (Jéquier and Tappy, 1999).

There are relatively few studies on the brain control of feeding behavior in fish. From the 1960s to the early 1980s, a number of studies using electrical stimulation and lesioning of brain regions demonstrated hypothalamic involvement in the control of feeding behavior in fish (Peter, 1979; Demski, 1983). From the 1980s to the 1990s, a large number of brain neuropeptides and their receptors related to feeding regulation have been identified and characterized in mammals. Starting in the early 1990s, some of these mammalian neuropeptides or fish homologs of mammalian neuropeptides were examined to assess their regulatory effects on feeding behavior and food intake in fish. Goldfish have been used as a major model for these studies, and neuropeptides and neurotransmitters, such as NPY, orexins, galanin, CRF, β-endorphin, cholecystokinin (CCK), bombesin (BBS), cocaine- and amphetamine-regulated transcript (CART), tachykinins and serotonin, have been shown to be involved in the regulation of feeding in fish. These studies provide a framework for understanding mechanisms underlying the brain regulation of food intake in fish. The following review will briefly describe these studies in comparison with what has been achieved in mammalian models. We hope that this review will serve as a motivator for additional research on regulation of feeding in fish.

2. Brain regions involved in feeding regulation

In mammals, hypothalamic sites, such as the ventromedial nucleus (VMN), dorsomedial nucleus (DMN), paraventricular nucleus (PVN), and the lateral hypothalamus (LH), have been previously indicated as neural centers involved in the control of feeding behavior, largely based on the numerous studies employing either discrete lesions or surgical transection of neural pathways (Kalra et al., 1999). The dual center model for regulation of feeding hypothesized that the LH area served as a 'feeding center' and the VMN as a 'satiety center' (Anand and Brobeck, 1951). Over the past few years, identification of a number of orexigenic and anorexigenic signaling molecules and their receptors in the hypothalamus, and the neuronal sites of their production, release, and receptive fields has redefined the hypothalamic pathways involved in the regulation of feeding and body weight. In addition, evidence of morphological relationships among these signaling-molecule producing neurons, and co-localization or co-production of more than one signaling molecule in these neurons has demonstrated an interconnected circuitry in the hypothalamus to regulate feeding behavior (for a review, see Elmquist et al., 1999).

In LH, two neuropeptides, MCH and the orexins, also called hypocretin, were newly recognized (Bittencourt et al., 1992; de Lecea et al., 1998; Sakurai et al., 1998). It is now well known that MCH and orexins are orexigenic signaling molecules, and their producing neurons represent the anatomic components for the lateral hypothalamic feeding center (Qu et al., 1996; Sakurai et al., 1998). The central nervous system targeted sites for MCH and orexins are similar, and include the paraventricular thalamic nucleus, central gray, raphe nuclei, locus coeruleus, suprachiasmatic nucleus, PVN, arcuate nucleus (ARC) and supraoptic nucleus (Date et al., 1999). On the other hand, recent evidence has shown that neuropeptides that decrease food intake and body weight are also expressed in the LH area, and these may play a counteregulatory role, opposing the systems that increase food intake. These neuropeptides include CART and CRF, both of which are expressed in LH and decrease

food intake when centrally administered (Koylu et al., 1997; Kristensen et al., 1998; Kelly and Watts, 1998). In ARC, a prominent hypothalamic site associated with hypothalamic integration of energy balance, neurons produce orexigenic peptides such as NPY, opioids, galanin (GAL), AGRP, dynorphin, and the pro-opiomelanocortin (POMC)-derived peptide, β-endophin (β-END), and anorexigenic peptides such as CART peptides and the POMC-derived peptide, α-MSH, and the amino aicds, γ-aminobutyric acid (GABA) and glutamate (Kalra et al., 1999). The terminal fields of these orexigenic and anorexigenic producing neurons in the ARC extend into various hypothalamic sites including the VMN, DMN, PVN, preoptic area (POA) and perifornical hypothalamus. In addition, the ARC appears to be the site for integrating circulating metabolic signals, such as the adrenal and gonadal steroids, leptin and insulin (Kalra et al., 1999). In VMN, there is no evidence so far for production of orexigenic or anorexigenic signals. However, it is suggested that VMN is a receptive field for a number of feeding regulatory signal molecules, and transfers the information flow to the DMN–PVN for the release of orexigenic signals (Kalra et al., 1999). DMH is one of the NPY-producing sites in the hypothalamus, and is suggested as a site of NPY and leptin interaction, and is thought to be involved in the attenuation or inhibition of feeding by leptin (Yokosuka et al., 1998). PVN is another NPY-producing site, and is thought to be one of the crucial sites for the release of orexigenic signals, and also one of the sites for interaction of neurohormones that inhibit feeding by diminishing NPY release (Kalra et al., 1991a; Dube et al., 1992).

Leptin controls energy balance through a negative-feedback loop that originates in adipose tissue, enters hypothalamus via the median eminence and acts on hypothalamic centers (Elmquist et al., 1999). Leptin receptor mRNA is highly expressed in the hypothalamic nuclei around the median eminence, such as ARC and parts of VMN, DMN and ventral premammillary nucleus, as well as in the LH area (Fei et al., 1997; Elmquist et al., 1998; Håkansson et al., 1998). Leptin receptors are expressed in NPY neurons co-producing NPY and AGRP (Broberger et al., 1998; Hahn et al., 1998). Leptin inhibits NPY-mediated neuronal activity in the hypothalamus, reduces levels of NPY mNRA in the ARC, and NPY levels in the ARC, PVN and DMN (Inui, 1999). In addition, leptin receptors are coexpressed with POMC neurons, which also produce CART peptides and POMC peptides (β-END and α-MSH) in the ARC and retrochiasmatic area (Cheung et al., 1997; Elias et al., 1998b). Recent anatomic studies have begun to illuminate the connection between leptin-regulated neurons in the ARC, and MCH and orexin neurons in the LH area. There is intense innervation of both MCH and orexin neurons in the LH by axons containing NPY, AGRP, and α-MSH, probably from the ARC (Broberger et al., 1998; Elias et al., 1998a). On the other hand, neural receptive sites for the anorexigenic signals overlap with sites containing receptors and terminal fields of orexigenic signals (Kalra et al., 1999). Therefore, the orexigenic and anorexigenic neuronal systems are morphologically and functionally connected, forming an interconnected network in the hypothalamus to govern food intake and body weight.

In fish, there are relatively few studies dealing with the neural control of feeding. Information on the neural substrates involved in the regulation of feeding are virtually all derived from studies using electrical stimulation and lesioning in the brain or transection of neural pathways, such as the olfactory tracts (for a review, see Peter, 1979; Demski, 1983). Electric stimulation studies, carried out in bluegill sunfish (Demski and Knigge, 1971), cichlids (Demski, 1973) and goldfish (Savage and Roberts, 1975; Roberts and Savage, 1978), demonstrated the involvement of the inferior lobes of the hypothalamus in the organization and control of feeding behavior. Feeding responses have also been evoked in nurse sharks by electrical stimulation of the inferior lobe of the hypothalamus (Demski, 1977). Feeding behavior in teleosts could also be evoked by stimulation of locations in the telencephalon or reduced by olfactory tract lesions (Grimm, 1960; Demski and Knigge, 1971; Stacey and Kyle, 1983). It was suggested that olfactory input and telencephalon output can serve to activate or arouse feeding behavior. The output from the telencephalon with regard to feeding behavior would presumably be via the medial forebrain bundle to the inferior lobe of the hypothalamus (Peter, 1979). In addition, electrical stimulation of the optic lobes in wrasse and sunfish has been shown to evoke feeding behaviors, indicating that an input from the optic tectum may also arouse feeding mechanisms in the hypothalamus (Peter, 1979).

In fish, several feeding-related neuropeptides, such as NPY (Blomqvist et al., 1992), MCH (Baker et al., 1995), galanin (Anglade et al., 1994; Wang and Conlon, 1994), POMC-derived peptides (Okuta et al., 1996; Arends et al., 1998), CRF (Okawara et al., 1988; Ando et al., 1999; Bernier et al., 1999) and CCK (Peyon et al., 1988), have been identified in the brain by isolation of the peptide or by cloning of their cDNA sequences. However, the studies on the potential regulatory effects of these neuropeptides on food intake are very limited. Generally, these studies indicate that the brain hypothalamic area is involved in the regulation of food intake by these neuropeptides. However, little work has been carried out on the precise mapping of these neuropeptides in the brain hypothalamic area in regard to their feeding effects. Interestingly, our recent studies on postprandial CCK and NPY gene expression have shown that sites outside of the hypothalamus may also play an important role in mediating regulation of feeding by these neuropeptides (Peyon et al., 1999; Narnaware et al., 2000).

3. Brain orexigenic signaling molecules

3.1. Neuropeptide Y

NPY is a 36-amino-acid peptide belonging to the pancreatic polypeptide family (Tatemoto, 1982), and is the most abundant peptide yet discovered in the mammalian brain, widely expressed in the central and peripheral nervous systems (White, 1993). In mammals, NPY is the most potent stimulator of appetite and food intake, and hypothalamic NPY-expressing cells represent an important and critical site of integration of peripheral hormonal signals with regulation of energy homeostasis (Kalra et al., 1999). The effects of NPY in stimulating food intake are mediated by NPY Y1 and Y5 receptor subtypes (Marsh et al., 1988; Wyss et al., 1998). In addition, NPY-producing cells in the brainstem and hypothalamus (ARC, DMN) innervate various hypothalamic sites including the ARC, VMN, DMN, PVN and surrounding regions, facilitating the interaction of NPY with other orexigenic and anorexigenic signals in the hypothalamus (Sahu et al., 1988a). Indeed, NPY-producing neurons coexpress GABA and AGRP in the ARC, and have synaptic contacts with GAL and POMC peptides (α-MSH and β-END) producing neurons in the ARC, with CART peptide perikarya in parvocellular PVN, and with orexin neurons in LH (Elmquist et al., 1999).

The presence of NPY in fish was first demonstrated in goldfish by immunological and chromatographic studies (Kah et al., 1989). In goldfish, NPY immunoreactive neurons are present in the ventromedial-posterior hypothalamus and hypothalamic inferior lobes (Pontet et al., 1989). Our previous studies have shown that NPY binding sites are also localized in the feeding regulatory center (Himick et al., 1995). The sequence of goldfish NPY cDNA has been determined and shows strong evolutionary conservation among vertebrate species (Blomqvist et al., 1992). The goldfish NPY has only five residues different from rat NPY (Blomqvist et al., 1992). In situ hybridization and Northern blot studies have shown that NPY mRNA is mainly expressed in forebrain regions of goldfish, particularly in the nucleus entopeduncularis of the ventral telencephalon, POA, olfactory bulbs, and various thalamic regions (Peng et al., 1994). In the midbrain of goldfish, NPY mRNA is present in the optic-tectum and locus coeruleus (Peng et al., 1994). A similar brain distribution of NPY mRNA has been described in coho salmon (Silverstein et al., 1998) by in situ hybridization using an antisense oligo probe corresponding to the goldfish NPY cDNA sequence. In addition, only the pre-optic area of the hypothalamus demonstrated an increase in NPY gene expression with fasting in this study, providing additional evidence for involvement of NPY in the regulation of food intake in fish (Silverstein et al., 1998).

The direct action of NPY on feeding behavior and food intake has been examined in our recent studies in goldfish (Narnaware et al., 2000). Goldfish trained to eat daily on a scheduled regimen (fixed time) for 10 days displayed a rapid and episodic elevation in NPY mRNA levels 1–3 h before the scheduled feeding time. The elevated mRNA levels associated with the daily feeding pattern suggest a physiological role for NPY in feeding in goldfish. Furthermore, brain intraventricular administration (i.c.v.) of goldfish NPY (gNPY) increased mean food intake in goldfish, in a dose-dependent manner. The maximum stimulation of food intake by gNPY was observed at 2 and 4 ng/g body weight (bw), while a higher

dosage (8 ng/g bw) of gNPY suppressed food intake. The i.c.v. injection of the NPY Y1 receptor antagonist, BIBP-3226, decreased basal food intake and also reduced food intake stimulated by gNPY in goldfish. These results indicate that NPY is a potent stimulator of food intake and feeding behavior in goldfish. The actions of the NPY Y1 receptor antagonist on feeding behavior also indicate that the central regulatory action of NPY on food intake is, at least in part, mediated by NPY Y1 receptors.

The significance of endogenous NPY synthesis in regulation of feeding has also been examined in goldfish. Food deprivation of goldfish for 24–72 h resulted in time- and brain region-dependent increase in NPY mRNA levels. In telencephalon-pre-optic and optic tectum-thalamus regions of goldfish brain, NPY mRNA levels were not increased by food deprivation until 72 h. In hypothalamus, NPY mRNA levels were significantly increased by food deprivation from 24–72 h, in a time-dependent manner. Food deprivation for 48 and 72 h also resulted in an increase in mean food intake, which lasted for at least 45–60 min after food deprivation. Finally, brain NPY gene expression was also modulated by the peripheral metabolic status of goldfish. Reduction in diet size by 50% of control levels (2% g bw) resulted in a significant increase in NPY mRNA levels in all brain regions examined (hypothalamus, telencephalon-POA, and optic tectum-thalamus). Taken together, these studies showed that NPY plays a physiological role in stimulating food intake and feeding behavior through its specific receptors in goldfish brain, consistent with the findings in the mammalian models.

In mammals, it has been shown that gonadal steroids modulate food intake and body weight gain, probably via their direct action on the NPY pathways in the ARC–PVN of the hypothalamus (Bonavera et al., 1994; Baskin et al., 1995). In goldfish, both estradiol and testosterone have been shown to induce a significant increase in NPY gene expression in the telencephalon-pre-optic area (Peng et al., 1994), implicating a possible regulation of feeding by gonadal steroids via NPY pathways.

3.2. Orexins/hypocretins

The orexin/hypocretin family of neuropeptides was recently discovered and characterized in rat and human by two separate laboratories (Sakurai et al., 1998; de Lecea et al., 1998). Orexin-A, a 33-amino-acid peptide, and orexin-B, a 28-amino-acid peptide, are produced from a single protein precursor by proteolytic processing. In rodents, orexin-containing neuronal perikarya are localized within and around the lateral hypothalamus, and in the dorsomedial hypothalamus and the perifornical nucleus (de Lecea et al., 1998; Sakurai et al., 1998; Date et al., 1999; Nambu et al., 1999). The i.c.v. injection of orexin-A and orexin-B stimulate feeding of the rat (Sakurai et al., 1998); however, the orexins are less effective than NPY in stimulating food intake. Orexin gene expression in rat hypothalamus was upregulated by fasting (Sakurai et al., 1998). These studies indicate that the orexins are the new mediators of the lateral hypothalamic feeding response. There is no published information on the presence and structure of orexins in nonmammalian vertebrates.

Recently, the effects of orexin peptides on feeding behavior in goldfish have been examined (Volkoff et al., 1999). The i.c.v. injection of human orexin-A or orexin-B in goldfish stimulated both appetite, as indicated by the number of feeding acts, and food consumption, as indicated by the total amount of food pellets consumed. Orexin-A was more potent than orexin-B, as orexin-A not only increased goldfish food intake at lower i.c.v. dosages than orexin-B, but also had a greater stimulatory effect than that of orexin-B at similar dosages. This suggests a role for orexin peptides in the regulation of feeding behavior and food intake in goldfish, similar to that described in the rat. This also suggests the presence of orexin-like peptides in the goldfish brain. In the rat, the effects of orexin-A are longer lasting than those of orexin-B, although both orexins increase food intake in a similar fashion (Sakurai et al., 1998). The longer lasting action of orexin-A might be due to the structure of orexin-A, which renders it more resistant to inactivation. The higher potency of orexin-A might also be due to the fact that orexin-A binds to both orexin receptors (OX1-R and OX2-R), while orexin-B binds to only the OX2-R receptors.

3.3. Galanin

GAL is a 29-amino-acid peptide originally isolated from porcine small intestine (Tatemoto et al., 1983), shown to be widely distributed

throughout the gastrointestinal (GI) tract and central nervous system in mammals (Merchenthaler et al., 1993). GAL has been studied extensively for its appetite-stimulating action in the brain of mammalian species (Kalra et al., 1999). Microinjection of GAL in the PVN, LH, VMH and central nucleus of the amygdala stimulates feeding in rats, indicating the widely distributed receptive sites for GAL in stimulating feeding (Kyrkouli et al., 1990; Schick et al., 1993). A close anatomical and functional relationship exists between GAL and other orexigenic signal-producing cells in the rat brain. For example, GAL may partially mediate the NPY-induced feeding response, as the NPY-producing neurons have direct communication with GAL-producing neurons in the ARC and PVN of the rat brain (Horvath et al., 1996).

GAL was isolated and sequenced from rainbow trout, and has 79% homology to the porcine form of GAL (Anglade et al., 1994). GAL-like immunoreactive (IR) cell bodies and fibers were found in the GI tract and brain of several fish species, with most abundant accumulation of GAL-like IR perikarya and fibers in the hypothalamo-hypophysial region, particularly in the nucleus pre-opticus periventricularis, nucleus pre-opticus (NPO) and nucleus lateralis tuberis (NLT) (Prasada Rao et al., 1996a, and references cited therein). In addition, GAL-like peptide binding sites have been described in the brain of Atlantic salmon (Holmqvist and Carlberg, 1992), showing the highest density of binding sites in the posterior hypothalamus. These provide anatomic evidence for potential involvement of galanin in the regulation of food intake in fish. In goldfish, i.c.v. injection of GAL significantly stimulates food intake during the first 2 h after the injection, but has no effects during the rest of the food intake period (2–8 h) (De Pedro et al., 1995a). Intraperitoneal (i.p.) injection of GAL in goldfish was ineffective in stimulating food intake. These results suggest that GAL is involved in the central regulation of feeding in fish. In addition, the GAL-induced food intake can be blocked by i.c.v. administration of the GAL receptor antagonist galantide in combination with galanin, while the antagonist itself does not affect food intake in goldfish, suggesting that galanin regulates food intake through its specific receptor pathways in fish (De Pedro et al., 1995a). The stimulatory effects of galanin on food intake in goldfish can also be blocked by α2-adrenergic receptor antagonist but not by α1 adrenergic receptor antagonist (De Pedro et al., 1995a), indicating that the α2-adrenergic receptor pathway may be involved in the GAL regulation of food intake in fish; similar results have been reported in mammals.

3.4. Pro-opiomelanocortin gene derived peptides

POMC mRNA encodes a large precursor protein, which is processed into γ-melanophore-stimulating hormone (γ-MSH), adrenocorticotropin hormone (ACTH) and β-lipotropin hormone (β-LPH) (Nakanishi et al., 1979). Furthermore, ACTH and β-LPH contain small component peptides with biological activity; α-MSH and corticotropin-like intermediate-lobe peptide are derived from ACTH, whereas γ-LPH, β-MSH, β-END, and methionine-enkephalin are derived from β-LPH (Simth and Funder, 1988). Within the hypothalamus of mammals, POMC neurons are localized exclusively in the ARC, and innervate the VMN, PVN, DMN, and other areas of the hypothalamus (Khachaturian et al., 1985). β-END, along with dynorphin A and enkephalins, also produced by the hypothalamus, are members of the hypothalamic opioid peptides, which have been shown to stimulate food intake in mammals by activating the μ-, δ- and κ-opiate receptor subtypes, respectively (Kalra et al., 1999). Compared with NPY, feeding evoked by opioid peptides is generally short lived and relatively modest (Levine and Billington, 1989). Anatomic studies have shown morphological links between β-END, GAL, and NPY, and between β-END and GABA pathways in the hypothalamus, suggesting a role of β-END in the operation of the interconnected orexigenic network (Horvath et al., 1992, 1995, 1997). Indeed, NPY has been shown to stimulate β-END release in the hypothalamus (Kalra et al., 1995), and pre-treatment with the opiate receptor antagonist naloxone (NAL) attenuates feeding responses to NPY (Levine and Billington, 1989). On the other hand, NPY decreases POMC mRNA levels in the ARC, and NAL increases NPY mRNA levels in the ARC and NPY levels in the DMH (Garcia de Yebenes et al., 1995; Lambert et al., 1994).

In teleosts, POMC cDNA was cloned from chum salmon more than a decade ago (Kitahara et al., 1988). Since then, the POMC cDNA sequence has been determined from several fish

species, including sockeye salmon, common carp, goldfish and rainbow trout (Salbert et al., 1992; Okuta et al., 1996; Arends et al., 1998; Hui et al., 1999). Similar to its mammalian counterpart, fish POMC gene encodes a precursor potentially processed into α-MSH, β-END and ACTH. However, fish POMC lacks a coding region for γ-MSH. Endorphin peptides have been isolated from salmon (Kawauchi et al., 1980), and endorphin-like peptide immunoreactivity and binding sites have been found in fish brain (Vallarino, 1985; Rosenblum and Callard, 1988). In common carp and goldfish, POMC mRNAs are expressed in hypothalamus and some other regions of the brain (Arends et al., 1998; Hui et al., 1999). In goldfish, opioid peptides have been shown to be involved in regulation of several physiological and behavioral responses, including feeding behavior (De Pedro et al., 1995b). The i.c.v. injection of β-END induced an increase in food intake during the first 2 h postinjection, while no effects on feeding were observed in the next 6 h. In contrast, i.p. injection of the same dose of β-END did not affect food intake. The i.c.v. injection of NAL reduced food intake, and pre-treatment with NAL partially reversed the stimulatory effects of β-END on food consumption (De Pedro et al., 1995b). These results indicate that β-END stimulates food intake via opioid receptors in goldfish brain. The μ and κ3 receptors, but not δ or κ1 have been found in the goldfish brain by selective radioligand binding studies (Brooks et al., 1994). Recently, goldfish μ-opioid receptor cDNA was cloned and its mRNA detected in the hypothalamus, olfactory bulbs and cerebellum (Hui et al., 1999). A further study by de Pedro's group using different opioid agonists and antagonists showed that the stimulation of feeding by β-END is mediated by μ-opioidergic receptors in goldfish (De Pedro et al., 1996).

α-MSH, a nonopioid peptide encoded by the POMC gene, is distributed throughout the hypothalamus of rat (Jacobowitz and O'Donohue, 1978). Unlike β-END and other opiates, α-MSH inhibits food intake, probably through the type four melanocortin receptor (MC4-R), one of the five melanocortin receptor types cloned (Kalra et al., 1999). MC4-R mRNA is widely distributed in the brain, including PVN, VMH, DMN and nuclei that occupy the medial zone of the hypothalamus (Tatro, 1990; Mountjoy et al., 1994). MC4-R has now been shown to be pivotal in the central pathways for energy homeostasis in mammals (Flier and Maratos-Flier, 1998). Agouti protein, a 131-amino-acid protein normally expressed in the skin, is a natural antagonist of MC-R (Klebig et al., 1995; Ollmann et al., 1997; for a review, see Elmquist et al., 1999). Food intake and body weight are increased by administration of an agouti protein analog, and animals that ectopically overexpress the agouti protein are hyperphagic, obese and have yellow fur (Klebig et al., 1995; Fan et al., 1997). In addition, agouti-related protein, a homolog of agouti, is produced exclusively in the hypothalamus and acts as an antagonist at the MC4-R, as one member of an apparent yin–yang pair with α-MSH (Ollmann et al., 1997; Shutter et al., 1997). The output from the feeding inhibitory MC4 receptor may be determined by the ratio of agonist (α-MSH) and antagonist (AGRP) at MC4-R receptor neurons (Flier and Maratos-Flier, 1998). In fish, α-MSH has been intensively studied for its regulatory role in the control of skin color (Baker, 1993). However, the action of α-MSH on food intake and feeding behavior remains to be defined.

3.5. Melanin-concentrating hormone

MCH was first discovered in chum salmon pituitaries (Kawauchi et al., 1983). Salmon MCH regulates skin color by acting on melanosomes within pigmented skin cells (Baker, 1993). MCH and α-MSH are functional antagonists in the fish scale system; MCH lightens skin by inducing aggregation of melanosomes, while α-MSH induces skin darkening by dispersal of melanosomes (Baker, 1993). In mammals, MCH exists in the zona incerta and LH (Bittencourt et al., 1992), and i.c.v. administration of MCH stimulates feeding in rats (Qu et al., 1996). In addition, fasting increased expression of MCH mRNA in both control and genetically obese ob/ob mice, and MCH mRNA was elevated in ob/ob mice (Qu et al., 1996). Interestingly, MCH and α-MSH have been proposed, in models of auditory gating, grooming and feeding, to have opposing actions in rat brain, as they do in the fish scale system (Flier and Maratos-Flier, 1998). Notably, MCH has no affinity for melanocortin receptors, and MCH and α-MSH do not antagonize each other by acting on the same receptor, as in the case of AGRP and α-MSH. Recently, MCH has been recognized as a natural ligand for an orphan

G-protein-coupled receptor SLC-1 that is sequentially homologous to the somatostatin receptors (Chambers et al., 1999; Saito et al., 1999). SLC-1 mRNA and protein are expressed in the VMN and DMN of the hypothalamus of rat brain, consistent with a role for SLC-1 in mediating the effects of MCH on feeding (Chambers et al., 1999). Although MCH has been intensively studied for its control of skin melanophores in fish, its action on food intake and feeding behavior remains to be explored.

4. Brain anorexigenic signaling molecules

4.1. Cholecystokinin

CCK and gastrin constitute a family of peptides characterized by the common C-terminus of Trp-Met-Asp-Phe-NH_2 (Crawley and Corwin, 1994). In mammals, CCK exists in endocrine cells of the GI tract and within the central and peripheral nervous system (Vanderhaeghen et al., 1980). CCK acts as a satiation factor at the levels of the gut and centrally in specific brain regions in mammals (Silver and Morley, 1991). CCK/gastrin-like IR perikarya and fibers are widely distributed in the forebrain, midbrain and hindbrain of goldfish (Himick and Peter, 1994a). A highly concentrated and extensive CCK/gastrin-IR perikarya and fiber system is prominent in the posterior ventromedial and vertrolateral hypothalamus, and the hypothalamic inferior lobes of goldfish. A similar brain distribution of CCK-like IR has been described in the green molly and rainbow trout (Notenboom et al., 1981; Batten et al., 1990). Specific CCK/gastrin binding sites are found in these same brain regions in goldfish (Himick et al., 1996). Specific binding sites for [^{3}H]CCK have been described in the hypothalamic feeding area of the sea bass (Moons et al., 1992). Importantly, i.p. or i.c.v. injection of the sulfated form of CCK-8 acutely suppresses food intake in goldfish, supporting the action of CCK as a satiety factor in fish (Himick and Peter, 1994a).

Recently, we reported the complete nucleotide sequence of cDNA encoding CCK precursor from goldfish brain by molecular cloning and sequence analysis (Peyon et al., 1988). The deduced CCK precursor contains CCK8 near its C-terminus, with only one amino acid substitution (Met to Lys at position 5 of CCK8) relative to the mammalian CCK8 sequence. In situ hybridization studies showed that CCK mRNA is expressed in the posterior ventrolateral hypothalamus of goldfish (Peyon et al., 1988), consistent with immunocytochemistry findings. In addition, our recent studies showed a transient and acute postprandial increase in CCK mRNA levels in the goldfish brain, supporting that CCK synthesis and release occur following a meal in goldfish (Peyon et al., 1999). Notably, we have found that the hypothalamus as well as areas including the olfactory bulbs, telencephalon-pre-optic region and posterior brain, also have a postprandial increase in CCK gene expression. This implicates that sites outside the hypothalamus may be involved in the hypophagic actions of central CCK.

4.2. Bombesin

BBS is a tetradecapeptide that was first isolated from skin extracts of the fire bellied toad, *Bombina bombina* (Anastasi et al., 1971). In mammals, both BBS and gastrin-releasing peptide (GRP), a 27-amino-acid peptide sharing a similar decapeptide C-terminal sequence as BBS, are widely distributed in the GI tract and central nervous system (McCoy and Avery, 1990). BBS-related peptides have been shown to be potent in suppressing food intake when administered either i.p. or centrally within specific areas of the hypothalamus and/or the hindbrain in mammals (Gibbs et al., 1979; Flynn, 1991).

In fish, BBS-like peptides have been detected within neurons and endocrine cells of the GI tract, cardiovascular system, and brain (Himick and Peter, 1994b, and references cited therein). BBS-like peptide has also been shown to be involved in the regulation of gut motility and visceral activity in several fish species (Holmgren and Jonsson, 1988; Bjenning et al., 1991). In goldfish, BBS/GRP-like IR are present in nuclear areas of the ventro-posterior hypothalamus and the hypothalamic inferior lobes, associated with the hypothalamic feeding center in fish (Himick and Peter, 1995a). High-affinity and specific BBS/GRP binding sites are also found in the same brain regions (Himick et al., 1995). Preliminary studies showed that BBS decreases feeding in carp following i.p. injection (Beach et al., 1988). In goldfish, BBS acts to acutely suppress food intake when administered either i.p. or i.c.v. (Himick and Peter, 1994b). Recently, we have isolated and sequenced

a cDNA-encoding BBS/gastrin-releasing peptide from goldfish brain (Volkoff et al., 2000). Studies are being conducted to examine the significance of gene expression and synthesis of BBS-related peptide in regulation of food intake in goldfish.

4.3. Corticotropin-releasing factor family of peptides

CRF is the primary hypothalamic hormone stimulating pituitary ACTH release, which in turn stimulates cortisol secretion from the adrenal glands (Antoni, 1986). In addition to the involvement of CRF in regulation of the pituitary–adrenal axis, CRF has been shown to be a potent anorectic substance in mammals. CRF inhibits food intake, increases energy expenditure, and produces sustained weight loss (Inui, 1999). The sites of anorectic action of CRF are within the PVN, possibly mediated by CRF R1 or CRF R2 receptor types (Monnikes et al., 1992; Heinrichs et al., 1993). Urocortin, a novel member of the CRF peptide family, shares 45% sequence homology with CRF and has been shown to be more potent than CRF in suppressing both fasting-induced and nocturnal feeding (Vaughan et al., 1995; Spina et al., 1996).

The isolation of CRF from several fish species and molecular cloning of CRF cDNA from white sucker (Okawara et al., 1988), and recently the cloning of CRF from sockeye salmon and goldfish (Ando et al., 1999; Bernier et al., 1999), illustrate that CRF sequences between mammals and fish are highly conserved, suggesting a conserved physiological role across evolution (Lovejoy and Balment, 1999). The NPO and NLT have been shown to be the principle sites of CRF peptide and mRNA in the brain of teleosts (Morley et al., 1991; Okawara et al., 1992; Ando et al., 1999). Indeed, CRF has been shown to act as a food intake regulator in goldfish (De Pedro et al., 1997, 1993, 1998a). The i.c.v. injection of CRF exerts an inhibitory effect on food intake at 2 h postinjection in goldfish, while i.p. injection of CRF has no effect, supporting that CRF acts centrally (De Pedro et al., 1993). The anorectic effect of CRF in goldfish can be reversed by i.c.v. injection of the antagonist α-helical CRF_{9-41} (De Pedro et al., 1997). This suggests that the anorectic action of CRF is mediated through specific receptors. The anorectic action of CRF appears to be independent of activation of the pituitary–adrenal system in fish, as increased plasma cortisol levels induced by i.p. administration of cortisol does not affect feeding (De Pedro et al., 1997). In addition, α1-adrenergic receptors and both D1 and D2 dopaminergic receptors have been shown to be involved in the anorectic effect of CRF in goldfish (De Pedro et al., 1998a).

Urotensin-I is a member of the CRF peptide family in fish, and urotensin I peptide has been isolated and sequenced from several fish species (Lovejoy and Balment, 1999). Complementary DNA encoding urotensin I has been determined from white sucker (Morley et al., 1991) and goldfish (Bernier et al., 1999). Goldfish urotensin-I has 62% sequence homology with rat urocortin, but only 45% homology with rat CRF. Phylogenetic analysis shows a close relationship between urocortin and urotensin-I (Lovejoy and Balment, 1999). Therefore, the fish urotensin-I could be the homolog of mammalian urocortin. The action of urotensin I in the regulation of feeding in fish remains undefined. However, fish urotensin I has been shown to be more potent than CRF in suppressing appetite and food intake in rats (Spina et al., 1996; Jones et al., 1998).

4.4. Cocaine- and amphetamine-regulated transcript

CART is a novel neuropeptide that has been shown to have a role in the control of feeding behavior in rats (Kuhar and Dall Vechia, 1999). CART was initially isolated using polymerase chain reaction differential display as mRNA produced primarily in the rat hypothalamus and transcriptionally regulated by acute administration of psychomotor stimulants such as cocaine and amphetamine (Douglass et al., 1995). CART is expressed in hypothalamic areas implicated in the control of feeding behavior (Douglass et al., 1995; Kristensen et al., 1998). Two CART transcripts of different length are generated by differential splicing, resulting in a mature peptide of either 102 (long form) or 89 (short form) amino acid residues (Douglass and Daoud, 1996). The mature peptide contains several potential cleavage sites and CART may be post-translationally processed into several biologically active fragments. In both rats and mice, i.c.v. injection of recombinant CART fragments has been shown to inhibit both normal and starvation-induced feeding (Lambert et al., 1998). The naturally occurring

$CART_{55-102}$ appears to be the most potent fragment, with a conserved secondary structure consisting of three disulfide bridges, but other CART fragments including $CART_{55-76}$ and $CART_{62-76}$ have also been shown to cause a dose-dependent feeding inhibition (Lambert et al., 1998). To further support the evidence that CART plays a major role in influencing appetite, it has been shown that administration of rabbit anti-$CART_{55-102}$ results in higher food intake in rats. Moreover, CART has been found to modulate the actions of two key regulators of food intake, leptin and neuropeptide Y (Kristensen et al., 1998).

Human $CART_{62-76}$ and $CART_{55-102}$ have been found to reduce food intake of both food-restricted goldfish and in fish subjected to NPY-induced feeding (Volkoff et al., 2000). As seen in rodents, $CART_{55-102}$ appears to be more potent than $CART_{62-76}$, as higher doses of the short fragment are necessary to elicit a significant response. This data suggests that CART peptides play a role in the regulation of feeding in goldfish.

4.5. Tachykinins

The tachykinins are a family of peptides sharing the common C-terminal sequence Phe-X-Gly-Leu-Met-NH_2 (Maggio, 1988). Mammalian nervous tissues contains the tachykinins, substance P (SP), neurokinin A (NKA), neuropeptide K (NPK) and neuropeptide γ, all derived from the preprotachykinin (PPT)-A gene (Carter and Krause, 1990). Alternative RNA splicing of the PPT-A gene primary transcript results in the generation of four mRNAs called α-, β-, γ- and δ-PPT mRNA (Carter and Krause, 1990; Khan and Collins, 1994). The SP sequence is encoded by all four PPT mRNAs; NKA is encoded only by β- and γ-PPT mRNAs, and NPK and Npγ are N-terminally extended derivatives of NKA. In mammals, the tachykinin peptides have been found in hypothalamic sites previously implicated in the control of reproduction and sexual and appetitive behaviors (Kalra et al., 1991b). It has been shown that NPK can acutely and consistently suppress feeding behavior, while the effect of i.p. administration is more effective than central treatment in decreasing food intake (Sahu et al., 1988b; Achapu et al., 1992). In addition, central and peripheral administration of SP acutely suppresses feeding behavior in rat (Dib, 1999). These suggest that tachykinins may function as endogenous anorexigenic peptides.

Several forms of tachykinin have been isolated from the nervous and GI tissues of fish (Jensen and Conlon, 1992, and references cited therein). The presence of relatively high concentrations of tachykinin-like IR and tachykinin binding sites in fish hypothalamus have been demonstrated (Moons et al., 1992; Weld et al., 1994; Prasada Rao et al., 1996b). In goldfish, cDNA for γ-PPT has been characterized (Lin and Peter, 1997), which encodes SP, NKA and carassin, a 21-amino-acid N-terminal-extended form of NKA (Conlon et al., 1991). The γ-PPT mRNA is widely expressed throughout the brain of goldfish, with higher expression levels in olfactory bulbs and hypothalamus (Lin and Peter, 1997; Peyon et al., 2000). In our recent studies, the potential involvement of tachykinin peptides in regulation of food intake was studied by examination of postprandial γ-PPT gene expression in goldfish brain (Peyon et al., 2000). In olfactory bulbs, γ-PPT mRNA levels in fed fish were significantly increased at 120 min following a meal compared with levels at meal time and compared with unfed fish. The hypothalamic region of the fed fish exhibited a significant increase in γ-PPT mRNA levels at 30 and 120 min compared with levels at meal time and compared with unfed fish. These results indicated an acute postprandial increase in γ-PPT gene expression in olfactory bulbs and the hypothalamus of goldfish brain, suggesting an involvement of tachykinins in central regulation of food intake.

4.6. Serotonin

Serotonin (5-HT) has been implicated in the central control of feeding behavior and body weight in mammals. Stimulants of synthesis and release of hypothalamic 5-HT reduce food intake and weight gain, and increase energy expenditure (Leibowitz and Alexander, 1998). The regulatory action of 5-HT in feeding is mediated by 5-HT receptors located in various medial hypothalamic nuclei (Leibowitz and Alexander, 1998). In addition, the 5-HT(2A/2C) receptor pathway has been shown to antagonize NPY-induced feeding within the hypothalamic PVN (Currie and Coscina, 1997). Recently, a central anorectic action of 5-HT in teleosts has been demonstrated (De Pedro et al., 1998b). The i.c.v. injection of 5-HT significantly reduced food intake of goldfish at 2 h

postinjection, whereas i.p. injection of 5-HT did not affect food intake. In addition, pre-treatment with the CRF antagonist α-CRF_{9-41} partially blocked the inhibitory effects of 5-HT on food intake in goldfish, suggesting that CRF may, in part, mediate the feeding inhibition elicited by 5-HT in goldfish (De Pedro et al., 1998b). 5-HT has been previously shown to have an inhibitory effect on pituitary growth hormone release in goldfish (Somoza and Peter, 1991). The possible relationship between regulation of growth hormone (GH) and feeding by 5-HT remains to be defined.

5. Growth hormone and feeding

Growth in fish is regulated by the brain neuroendocrine-GH-insulin-like growth factor axis (Peter and Marchant, 1995). Growth of fish can be stimulated by manipulation of selected neuroendocrine regulators of GH; however, growth cannot occur without adequate food intake. Indeed, GH has been shown to play an important role in regulating food intake in fish (for a review, see Peter, 1995). Chronic administration of GH results in an increase in food intake and subsequent improved food conversion efficiency in several teleosts. Injection of GH into rainbow trout has also been shown to increase appetite (Johnson and Bjornsson, 1994). In goldfish, a short-term relationship between circulating serum GH levels and feeding has been demonstrated (Himick and Peter, 1995b). When fed a 2% wet bw ration, goldfish exhibit an acute elevation in serum GH at 30 min after feeding. This initial rise in serum GH is followed by a sharp decrease and then, over the next 3 h, a more gradual decrease to serum GH levels significantly lower than in unfed control fish (Himick and Peter, 1995b).

In fish, it has been well established that the control of pituitary GH secretion is mutifactorial, with a balance of stimulatory and inhibitory neurohormones acting directly on the pituitary somatotrophs on a seasonal basis (Peng and Peter, 1997). In addition, interactions among neurohormones could contribute to the regulation of GH secretion. Somatostatin (SRIF) is the major inhibitor, while a number of neuropeptides and amides stimulate GH release (Peng and Peter, 1997). Notably, several neuropeptides and amides, which have been shown to regulate food intake in fish and mammals, are also regulators of GH secretion (Himick and Peter, 1995b). In goldfish, treatment with CCK-8s or BBS concomitantly suppressed feeding and increased serum GH levels at 30 min following treatment (Himick and Peter, 1994a,b). CCK and BBS have been shown to stimulate GH secretion via direct actions on the pituitary (Himick et al., 1993; Himick and Peter, 1995a). Thus, CCK and/or BBS may integrate the regulation of satiation following a meal and the short-term postprandial increase in GH secretion.

NPY stimulates GH secretion in vitro and in vivo in goldfish (Peng et al., 1993). Our recent studies showed that the increase in NPY gene expression before a scheduled feeding was associated with increased serum GH levels (Narnaware et al., 2000). In addition, an increase in NPY gene expression following food deprivation was also associated with increased serum GH levels, which subsequently decreased after refeeding. These results suggest that NPY may be an important neuropeptide to integrate stimulation of feeding and GH secretion. On the other hand, GH may be involved in the pathways mediating NPY-induced feeding in fish. However, the mechanisms underlying the effects of GH on feeding remain unclear, and an increase in serum GH levels is not necessarily associated with an increase in food intake, as increased physiological levels of GH and decreased growth rates and hypophagia can occur together in goldfish (Peter et al., 1976).

In mammals, growth hormone-releasing hormone (GHRH) and SRIF are major regulatory peptides of GH secretion. Both GHRH and SRIF are also involved in the central regulation of feeding, and integration of central and peripheral functions related to nutrition, metabolism and growth in mammals (Feifel and Vaccarino, 1994). In rats, i.c.v. injection of GHRH in picomoles increased feeding, while high dosages (nanomole) of GHRH suppressed feeding (Vaccarino et al., 1985; Vaccarino and Buckehnam, 1987). Picomole doses of GHRH are ineffective in stimulating GH release from the pituitary, and peripheral injections of GHRH have no effect or decrease food intake. Similarly, systemic administration of SRIF-14 decreased feeding in rats (Feifel and Vaccarino, 1990). Central administration of SRIF-14 in picomole doses increased food intake in rats, whereas significant suppression was produced with the nanomole dose tested (Feifel and Vaccarino, 1990).

GHRH has been isolated and characterized from common carp hypothalamus (Vaughan et al., 1992). GHRH cDNA has been cloned from several fish species (Peng and Peter, 1997). Interestingly, fish GHRHs are encoded by the same gene as the pituitary adenylate-cyclase activating polypeptide, while in mammals these two peptides are encoded by separate genes. Recently, a GHRH receptor has been cloned and characterized in goldfish (Chan et al., 1998). GHRH receptor mRNA is expressed in the pituitary and throughout the brain. Stimulation of GH release by synthetic human or carp GHRH has also been observed in several fish species (Peng and Peter, 1997). However, the effects of GHRH on feeding have not been examined in fish. On the other hand, SRIF peptides have been isolated and characterized in several fish species (Lin et al., 1998). The presence of multiple forms of SRIF has been demonstrated in fish by isolation of SRIF peptides or molecular cloning of cDNAs encoding for preprosomatostatin-I (PSS-I), probably processed into SRIF-14, and PSS-II, potentially processed into larger forms of SRIF (SRIF-25 or SRIF-28). In addition, several SRIF-14 variants have been identified in fish and other vertebrates (Lin et al., 1998). In goldfish brain, three PSS cDNAs have been determined, which encode PSS-I (SRIF-14), PSS-II (SRIF-28) and a third SRIF precursor PSS-III, potentially processed into [Pro^2]SRIF-14 (Lin et al., 1999a). SRIF-14 is a potent inhibitor of both basal and stimulated GH release in fish (Peng and Peter, 1997). [Pro^2]SRIF-14 also demonstrated an inhibitory effect on GH release in goldfish and rainbow trout, with similar potency to SRIF-14 (Nishii et al., 1995; Lin et al., 1999a). Recently, type one SRIF receptors have been cloned from goldfish brain (Lin et al., 1999b). SRIF receptor mRNAs and three PSS mRNAs are widely expressed in goldfish brain, providing an anatomic basis for a wide range of physiological functions of SRIF, similar to mammals. Effects of SRIF on feeding have not been investigated in fish. In rainbow trout, plasma SRIF-14 concentrations exhibited a significant diurnal rhythm, with a major increase associated with the postprandial period, implicating a possible involvement of SRIF-14 in feeding (Holloway et al., 1994).

In fish, gonadotropin-releasing hormone, thyrotropin-releasing hormone and dopamine are among other GH stimulators (Peng and Peter, 1997). The involvement of these factors in regulation of food intake in fish remains to be investigated.

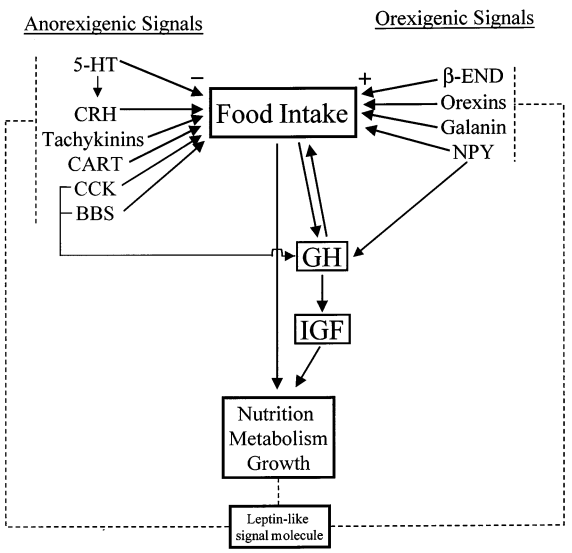

Fig. 1. A diagrammatic summary of the brain regulation of food intake and relationship between food intake and growth hormone secretion. Bombesin, BSS; cocaine- and amphetamine-regulated transcript, CART; cholecystokinin, CCK, corticotropin-releasing factor, CRF; growth hormone, GH; β-endorphin, β-END; insulin-like growth factor, IGF; neuropeptide Y, NPY; serotonin, 5-HT. +, stimulation; –, inhibition.

6. Concluding remarks

Fig. 1 summarizes our current understanding of brain regulation of food intake in fish, and the relationship between food intake and GH. The brain orexigenic signal molecules, NPY, orexins, galanin and β-END, stimulate food intake in fish, whereas brain anorexigenic signal molecules, CCK, BBS, CRF, CART peptide and serotonin, are satiation factors in fish. Tachykinins may also be involved in the anorectic action in fish. The brain hypothalamic area is associated with regulation of food intake, while sites outside the hypothalamus could also be involved in this function. There is correlation between short-term changes in serum GH levels and feeding behavior, although possible mechanisms integrating these functions remain to be defined. Some of the studies described in this review are only initial, and more work remains to be carried out with regard to the precise mapping of brain sites for synthesis

and release of neurohormones related to their feeding regulatory activities, and their innervation and the localization of their receptors in the brain. The possible morphological and functional interaction among orexigenic or anorexigenic signals, and between orexigenic and anorexigenic signaling systems, need to be elucidated. Immediate early gene (*c-fos*) expression, as a marker for neuronal activation by neuropeptide, allows the mapping of extended neuronal systems that respond to the administration of brain neurohormones. This technique would be applicable in mapping of neuronal systems involved in brain regulation of feeding in fish. Other approaches, such as antisense techniques, gene targeting and transgenic fish models, would be useful for studies on the physiological role of individual neuropeptides in feeding regulation or their interaction with other neuropeptides. Several aspects are open for future research. First, some fish neurohormones that have been currently characterized, such as MCH, α-MSH and glutamate and GABA, could be assessed for their regulatory effects on food intake in fish. Second, fish homologs of mammalian CART peptide, AGRP, and orexins and the receptors for these peptides and some known fish neuropeptides remain to be identified by molecular biology and/or protein chemistry approaches. Finally, identification of peripheral feedback signal molecule(s), such as leptin-like peptide, in fish would be useful for understanding the brain regulation of feeding.

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