



## NEUROPEPTIDES' ROLE IN FISH APPETITE REGULATION: A MINIREVIEW

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### ABSTRACT:

Neuropeptides comprise a class of evolutionarily well-conserved messenger molecules of enteric, sensory, autonomic, and central neurons. In recent decades, neuropeptides have been found to play a major role in food intake, energy homeostasis, and stress resilience. In regard to aquaculture, fish appetite management is a crucial area of study. Neurocircuitry that controls feeding may be unlocked by understanding how neuropeptides function. This brief review aims to summarize the functions of various neuropeptides in fish.

**Key words:** - Neuropeptides, Appetite regulation, Gut, Fishes, Food intake

### INTRODUCTION:

The most species-rich group of vertebrates is the fish, with around 33,700 species today ([www.fishbase.org](http://www.fishbase.org)). Half of all known vertebrate species are teleost, accounting for 95% of them (Nelson 2006). This diversity includes anatomic, ecological, behavioral, and genomic variations (Wootton 1991, Volff 2005). This makes fish an ideal species for studying the evolution of appetite-regulating mechanisms in vertebrates (Volkoff *et al.*, 2009).

Specific attributes which govern food intake in fish despite being equivalent, are distinctive. These variations can sometimes be attributed to the relatively few fish studies that are currently available. However, in most instances, the diversity of fish species, habitats, feeding preferences, and gastrointestinal (GIT) anatomy and physiology create very specific characteristics for fishes. Also, many other internal and external factors affect the feeding behaviour in fishes

which adds to the complexity (Hoskins and Volkoff, 2012).

### Endocrine and Neural control of Feeding responses and its regulation in Vertebrates

Feeding responses and its regulation is under multiple endocrine and neural controls in all vertebrates that involves both central and peripheral hormones and neuropeptides. In mammals', the mechanism and physiologies are rather well researched. But the scant information is available in lower vertebrates may not be representative of the complex group of fishes. Additionally, the adaption to a variety of feeding patterns may explain variances across fish species. Studies on interaction of neuropeptides modulating feeding behaviour have been available on different species of fishes.

### Interaction between brain and gut

In fish, the digestive tract shows remarkable diversity in the morphology and functional aspects. It takes an

intricate interplay between the brain and gut for the control of appetite, feed intake, and body weight to function in all vertebrates. Intestinal morphology of fish can be influenced by feeding habits, frequency of food intake as well as by body size and shape (Buddington *et. al.*, 1997). Literature survey reveals that certain peptides have dual distribution in brain and peripheral areas like gut (Dockary and Gregory 1980). The brain particularly the hypothalamus secretes key elements that either stimulate (orexigenic) or inhibit (anorexigenic) food intake.

In fish, most studies related to feeding and appetite focused on composition of diet and assimilation (Jobling, 2012) or effects of environmental factors (Bolliet *et. al.*, 2001). Only recently the work has been started to focus on neural regulation of appetite. The most potent stimulating component in fish is called neuropeptide Y (NPY). It has been discovered that NPY interacts with other significant orexigenic peptides, orexin A and B and galanin, in the regulation of food intake in a highly coordinated manner (Volkoff *et. al.*, 2004). The 29- amino acid peptide known as galanin (GAL) gets its name from the presence of an N- and C-terminal glycine residue. GAL is widespread throughout the hypothalamus and is expressed in both the stomach and brain and modulates various physiological processes including neuroendocrine secretion, feeding behavior, cognition, reproduction etc. Orexins are the recently discovered neuropeptides that are crucial for modulating physiological activities like food intake, sleep-wake cycles, and reproduction. (Wong *et. al.*, 2011). Orexin (Hypocretin) consists 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 precursors are also found in the gut and other peripheral tissues (Kirchgessner, 2002).

Similar to mammals, fishes' brain centers ultimately control their appetite by receiving and analysing endocrine signals from both the brain and the periphery. These signals are made up of hormones that either promote or discourage feeding. Metabolic and peripheral neural inputs that provide signals for food intake and nutritional status also serve to control feeding centres. (Volkoff, 2006; Volkoff *et. al.*, 2009; Sobrino Crespo *et. al.*, 2014). The hypothalamic area was shown to be involved in feeding in earlier studies, which mainly used stimulation and lesion techniques in teleosts (Peter, 1979) and elasmobranchs (Demski, 2012), suggested that fish may have similar mechanisms to mammals. Although in mammals, the feeding centers appear to be restricted to the hypothalamus, work in this area suggests that they could possibly be more widespread in fish brains (Cerdá-Reverter *et al.*, 2009).

In the CNS, the hypothalamus is the key region involved in the regulation of appetite. It had previously been suggested that satiety was controlled by the ventromedial hypothalamic nucleus, and feeding was controlled by the lateral region. This early concept emerged into a much more elaborate and complex knowledge of the neural network responsible for the regulation of appetite which involving diverse pathways within specific nuclei of the hypothalamus, and various regulators (Perry and Wang, 2012).

Neuronal circuits which control feeding activities geared toward survival are very precisely controlled by a

central machinery. These neuronal circuits are mostly in excess which safe guards the perfect regulation and coordination. Due to the compensatory reactions of the neural circuits controlling food intake, which occasionally causes specific experimental challenges.

Several neuroendocrine systems have evolved in vertebrates to ensure a stable and balanced energy supply. Numerous studies have recently explored the role of neurotransmitters, neuropeptides, and hormone systems in modulating food intake in fish (Volkoff 2016, Ronnestad *et. al.*, 2017) and mammals (Rogers and Brunstrom, 2016).

The exact mechanisms behind the effects of metabolic state on feeding circuits are still not clearly understood, but evidence has emerged that both short- and long-term changes in metabolic state can lead to synaptic rearrangements and changes in the excitability of neurons in the hypothalamic circuit.

A perfect balance of food ingestion and expenditure accomplishes energy homeostasis over long periods. Feeding behavior shows a two fold arrangement a short-term (meal to meal) control of food intake mediated by central and peripheral signals, and a long-term (days to months) control of feedback fine-tuned by storage and food availability over long time periods. Both processes must run smoothly to maintain a balance between energy intake and expenditure. (Soengas, 2018).

There is a very comprehensive and lengthy list of hormones that control feeding in vertebrates. Here, emphasis will be on key hormones and recently studied appetite-

regulating elements that are known to influence and control fish feeding behaviour.

Husea *et. al.*, (2005) studied the distribution of orexin and melanin concentrating hormone (MCH) in the brain of the goldfish (*Carassius auratus*) focusing on regions related to feeding, sleep and arousal. These peptidergic systems were located in the hypothalamus and general visceral nuclei but not in locus coeruleus or raphe nuclei and support the view that these peptides originally played a role in regulation of energy balance and evolved secondarily to influence sleep-wake system in amniote vertebrates. Karila *et. al.*, . (1993) examined the existence of galanin-like immunoreactivity in nerves to the stomach of the Atlantic cod. According to research, a population of ganglion cells along the vagus nerve contain a peptide that resembles galanin. It's possible that some of these cells are a part of the autonomic parasympathetic pathways that innervate the gut and have direct excitatory effects on the smooth muscles of the gut wall and gut arteries.

An in-depth review about the role of various neuropeptides in the control of food intake in fish was carried by Volkoff *et.al.*, (2004). The complex interaction and role played by various neuropeptides like neuropeptide Y (NPY), galanin, orexin, ghrelin, cholecystokinin (CCK), bombesin, corticotrophin releasing factor (CRF), cocaine and amphetamine regulated transcript (CART) etc was reviewed in fish. The brain of an *Anguilla anguilla* treated to hyperosmolar and hypoosmolar circumstances was studied by Masini *et. al.*, in 2006. It was determined that in response to the two environmental situations,

different immunoreactive element distributions and intensities occur.

Hrytsenko *et. al.*, (2007) studied the expression of insulin in the brain and pituitary cells of tilapia *Oreochromis niloticus* by using RT-PCR, qRT-PCR and Northern hybridization techniques. The findings showed that in adult tilapia, insulin expression was not only found in the endocrine pancreas cells but also in the neuronal cells of the brain and the pituitary gland, indicating that these tissues may represent the extra-pancreatic origin of insulin synthesis. Nakamachi *et. al.*, (2006) examined the effect of feeding status on the orexin like immunoreactivity and the expression of orexin mRNA in the goldfish brain. The results indicated that the orexin functions as an orexigenic factor in the goldfish brain. Major appetite regulating factors, central Orexigenic factors, Agouti-related protein (or peptide, AgRP) AgRP is a peptide released by hypothalamic NPY/AgRP neurons and is an endogenous antagonist of the melanocortin receptors MC3R and MC4R. In fish, AgRP has been identified in several species, including teleosts (Volkoff 2016).

A very intimate relation between energy and reproduction has been well known in mammals. Energy balance is maintained by a process that controls food consumption, energy expenditure, and energy storage. A number of hypothalamic neuropeptides including orexin, ghrelin, neuropeptide-Y(NPY), melanin concentrating hormone(MCH), pituitary adenylate cyclase activating polypeptide(PACAP), proopiomelanocortin(POMC)-derived peptides,

cholecystokinin(CCK), chicken gonadotropin-releasing hormone-II (cGnRH-II), 26RFamide (26RFa), galanin (GAL), and cocaine- and amphetamine-regulated transcript (CART) have been involved in the regulation of feeding behavior and energy balance. Additionally, hormones like leptin and ghrelin offer important hints on the availability of stored nutrients. (Shahjahan *et. al.*, 2014).

Ghrelin and obestatin are two gastrointestinal peptides obtained by post-translational processing of a common precursor, preproghrelin. The effect of obestatin on food intake is still not very clear. Simultaneous administration of ghrelin and obestatin-like found to decrease food intake, indicating that obestatin was able to provoke the effect of ghrelin. (Yuan X. *et. al.*, 2015). Besides its orexigenic effect, ghrelin was discovered to cause mice to exhibit anxiolytic and antidepressant behaviour (Lutter *et. al.*, 2008).

Nesfatin-1 is an 82 amino acid anorexigen encoded in a secreted precursor nucleobindin-2 (NUCB2). It was recently reported that NUCB1 encodes an insulinotropic nesfatin-1-like peptide (NLP) in mice. RT- qPCR showed NUCB1 expression in both central and peripheral tissues in fish. Western blot analysis and/or fluorescence immunohistochemistry exhibited NUCB1/NLP in the brain, pituitary, testis, ovary and gut of goldfish. NUCB1 mRNA expression in goldfish pituitary and gut showed a daily rhythmic pattern of expression. Altogether these results provide results indicating the anorectic action of NLP, and the regulation of tissue specific expression of goldfish NUCB1 (Sunderrajan *et. al.*, 2016).

The anterior intestine (J-loop) enteroendocrine cells of goldfish co-localized nesfatin-1- and ghrelin-like immunoreactivity. Additionally, the posterior nucleus lateralis tuberis of the goldfish hypothalamus, a part of the brain involved in the control of food intake, was found to co-localize ghrelin and nesfatin-1. These results point to a close connection between nesfatin-1 and ghrelin in goldfish. The findings reveal complex interactions between nesfatin-1 and ghrelin, CCK, and orexin as well as evidence that nesfatin-1 operates on other appetite-regulating peptides in a time- and feeding-status-dependent, tissue-specific manner in goldfish (Kerbel and Unniappan, 2012).

#### **Influence of factors like nutrients & stress on the release of appetite regulating neuropeptides**

It is observed that food and nutrients absorbed have enormous effect on the release of gastrointestinal food intake-regulating peptides. CCK has been found to be released early whereas GLP-1 and PYY are released later as nutrients go through the intestine, when nutrients reach the duodenum. This shows that anorexigenic peptides are released as a result of nutrients coming into direct touch with enteroendocrine cells. Digested lipids and proteins are the main sources of CCK stimulation, whereas intraduodenal glucose only slightly increased circulating levels of CCK in healthy participants. CCK release is induced by lipids and proteins, particularly fatty acids with more than 12 carbons (oleate) or proteins that have not fully or just partially been digested. GLP-1 is primarily induced by glucose, unlike the CCK.

Additionally, oral delivery of glucose, lipids, and proteins to healthy male and female individuals revealed that levels of the orexigenic peptide ghrelin dropped after consuming glucose and lipid meals, but not protein (Prinz and Stengel, 2017). The function of cerebral glucose sensing mechanisms and their presence in fish, notwithstanding the diminished significance of glucose metabolism, can be compared to that in mammals (Polakof *et al.*, 2012). However, important topics, such as the electrophysiological response of hypothalamic neurons to glucose, the role of carbohydrate-responsive element-binding protein (ChREBP) or the signalling pathway of sweet taste receptors are still unknown in fish. Saturated fats, Lipids are important elements found in fish and have an impact on a variety of processes (Polakof *et al.*, 2010). Similar to what has been observed in mammals, fish fed a lipid-rich diet with high plasma fatty acid levels or substantial lipid reserves typically experienced a decrease in food intake (Silverstein *et al.*, 1999).

The study of amino acid sensing in fish is much less extensive than that of other nutrients, and some significant issues remain unresolved. Teleost fish are highly dependent on amounts of dietary protein and amino acids due to their high dietary protein requirements. In contrast to mammals, fish choose amino acids as a carbon source for lipogenesis rather than glucose. In light of this, diets rich in protein and amino acids prevent people from eating, while unhealthy diets encourage them to eat (Soengas *et al.*, 2018).

Studies on feeding behavior indicate the

modulation in feeding pattern in fishes in response to stress. Fish typically reduce their food intake levels right away in response to acute stress, then resume normal levels once the stressful situation has passed (Leal *et. al.*, 2011, Guillot *et. al.*, 2016).

### CONCLUSION:

The majority of studies on the teleosts' appetite- controlling mechanisms are conducted on domesticated fish that have been bred in captivity for many generations (e.g., salmon, carp, and cod). In comparison to wild fish exposed to less favourable conditions, it is highly likely that these fish, who are accustomed to the most favourable habitat (e.g., no predators, constant light exposure and temperatures) and feeding (e.g., satiation, lack of struggle), may have changed their feeding behaviour and systems controlling appetite. Finding the similarities between wild and captive populations could reveal crucial details about how domestication affects feeding behaviour. Therefore, research on fish feeding patterns and fish sampling in their natural habitat would be tremendously beneficial (Ronnestad *et. al.*, , 2017). Teleosts and mammals share many of the same endocrine systems that regulate central control of food intake, showing a common thread of conservation throughout vertebrate evolution. Current studies on several fish hormones, such as insulin, endocannabinoids, and members of the glucagon family of peptides, are insufficient to make accurate comparisons. Only a few number of groups, such as the salmoniformes, perciformes, pleuronectiformes and cypriniformes, have undergone extensive research. Many fish species exhibit indeterminate growth, which means that

they keep expanding during the course of their whole lives. Growth of mammals and other model animals, such as zebrafish (*Danio rerio*), which reach a maximum length size as adults, is in contrast to this. Therefore, even though controlling one's hunger and food intake is frequently seen as a behavioural aspect of maintaining an energy balance, the basic notion of energy homeostasis needs to be applied with great caution in fish (Ronnestad *et. al.*, 2017). The effects on food intake of various neuroendocrine systems investigated in depth in fish, such as the NPY, melanocortin system, or CCK, were comparable to those in humans. In conclusion, we are unable to precisely identify evolutionary changes in all of their facets due to still limited knowledge about central control of food intake in fish compared to mammals. The material that is now available mostly refers to neuroendocrine signalling. As a result, it is impossible to identify any obvious evolutionary patterns in teleost fish with relation to the regulation of food intake. The majority of studies and all existing data on nutrient signalling point to homeostatic control of food intake. Research on response to various cues like stress, environmental factors, circadian rhythms, nutrients in fish is though in a preliminary state, it is comparable to mammals and imparts a definitive direction in fish research and has immense potential.

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