



Starvation Induced NPY Variation In Gastrointestinal Tract Of Catfish *H. fossilis*

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Abstract:

Starvation condition is experienced in most species of fish during certain period due to various environmental conditions. The physiological condition of the fish is affected due to starvation. In fish appetite is regulated by appetite regulating factors which can be orexigenic or anorexigenic. NP-Y a 36 – amino acid peptide, is one of the important orexigenic factors. It known to be increased in regulation of feeding.

In present study, we examined the localization and distribution of NPY in the Gastrointestinal tract of fresh water catfish *H. fossilis* during 24 and 48 hr. starvation. Fishes were fed at 9.00 hr. daily for one week. Fish were then starved for 24 and 48 hr. and one group of fish was fed regularly and used as control. NP-Y localization was observed to increase with increased starvation period in the middle intestine and stomach showed complete absence of NPY immunoreactivity.

The results confirmed the role of NP-Y in regulating fasting and digestion of the teleost fresh water fish *H. fossilis*.

Keywords : Immunoreactivity, Starvation, Orexigenic, Localization, NP-Y (neuropeptide Y), Gastrointestinal tract (G.I. tract).

Introduction :

Neuropeptides in vertebrates and in invertebrates have been suggested to play key roles as signalling molecules in the regulation of physiology, behaviour and development, (Tensen *et al.*, 1998). These peptides also shows several effects on the digestive and feeding behaviour (Toni 2004 and Jensen 2001). In fish digestive tract more than 45 gastro-intestinal peptides have been restored (Kiliaan *et al.*, 1997; Girolamo *et al.*, 1999; Lucini *et al.*, 1999; Domeneghini *et al.*, 2000; Youson *et al.*, 2001; Burrin *et al.*, 2003). The key regulators to control appetite in fish includes neuropeptide Y (NP-Y), ghrelin (GRLN), cocaine and amphetamine regulated transcript (CART) and various others (Volkoff *et al.*, 2005).

They play important roles in the overall regulation of digestive processes such as nutrient absorption, gut motility and intestinal blood flow. In addition to potent stimulation of food intake, the involvement of NPY in energy balance regulation in fishes is a major research aspect in immunocytochemistry. Neuropeptide Y (NPY), a powerful stimulant of eating behavior in many species, including goldfish (Lopez-Patino *et al.*, 1999; Namaware *et al.*, 2000), is also a potential gene for the action of cortisol in the regulation of food intake.

Heteropneustes fossilis (Bloch, 1794), the stinging catfish of India is an important commercial fish due to high market value. It is highly appreciated due to low fat content and high amount of iron and calcium. *H. fossilis* also

reported for high value of genetic variability within and between the population.

The aim of the present investigations was to study the localization and distribution of endocrine cells that are NP-Y immunoreactive from the stomach and intestine of *H. fossilis*. Also this study emphasizes in providing new information on NPY regulation during starve condition by immunocytochemistry as a tool for analysis.

Materials and Methods

Animals and sample preparation :

H. fossilis fish of either sex (weigh 180-200 gram) were procured from local market. Approximately 45 fish were acclimatized in the aquaria at temperature 21°C. for one week. They were divided into three groups, a control group which were regularly fed for 900 hr. while the other two groups fish did not receive food i.e. they were starved for 24hrs and 48hrs respectively. Fish from each group were anesthetized by 2-phenoxyethanol (sigma cat = P1126 1l) and perfused transcardially with ice-cold PBS at pH 7.45 and further processed for immunocytochemistry.

Immunocytochemistry :

For immunocytochemistry, the tissue was passed through 10%, 20% and 30% sucrose solution. Streptavidin-biotin peroxidase method was employed to localize NPY in tissue sections at light microscopic level. All the incubations and washing procedure were performed in humid chamber. Briefly, the tissue sections were washed in PBS and treated with 1% Bouin Serum Albumin (BSA). Section were further incubated with

primary antibody against (NPY rabbit) (sigma cat # N- 9528) diluted in PBS (1:2000). After wash in PBS biotinylated secondary antibody IgG(GeNei,India,021031) was applied for 2 hour followed by streptavidin- peroxidase conjugate (GeNei India 091072) for 2 hour. The reaction was visualised through chromogen AEC (3-amino 9 ethyl carbazole). Section were rinsed twice in distilled water and mounted in glycerol gelatin.

Specificity of the Antibody :

Control procedure like (1) omission of the primary antibody from the reaction, (2). Replacing the anticera against NPY with bovine serum albumin, (3). Preabsorption of the antibody with NPY at 10^{-5} M for 24h before incubation resulted in total loss of immunoreactivity.

All the prepared slides were observed under the Olympus light microscope .

Result:

Effect of starvation on localization of Neuropeptide Y (NPY) in intestine of *H. fossilis* by immunocytochemical study

The experimental work for observation of effect of starvation was carried out by making three groups of the species *Heteropneustes fossilis* viz; 1) control with regular fed 2) 24 hours starvation 3) 48 hours starvation. The immunocytochemical study of stomach and intestine was carried out by using NPY as primary antibody. The NPY immunoreactivity was observed in the intestinal region where as stomach was completely devoid of immunoreactivity.

Control group (regular fed) :

The morphology of the intestine shows all four layers serosa, muscularis, submucosa and mucosa with villi like other teleost fishes.

NPY immunoreactivity in the proximal region of intestine was found scattered all around the mucosal gut fold. The NPY immunoreactive cells showed the cell body and the cytoplasmic

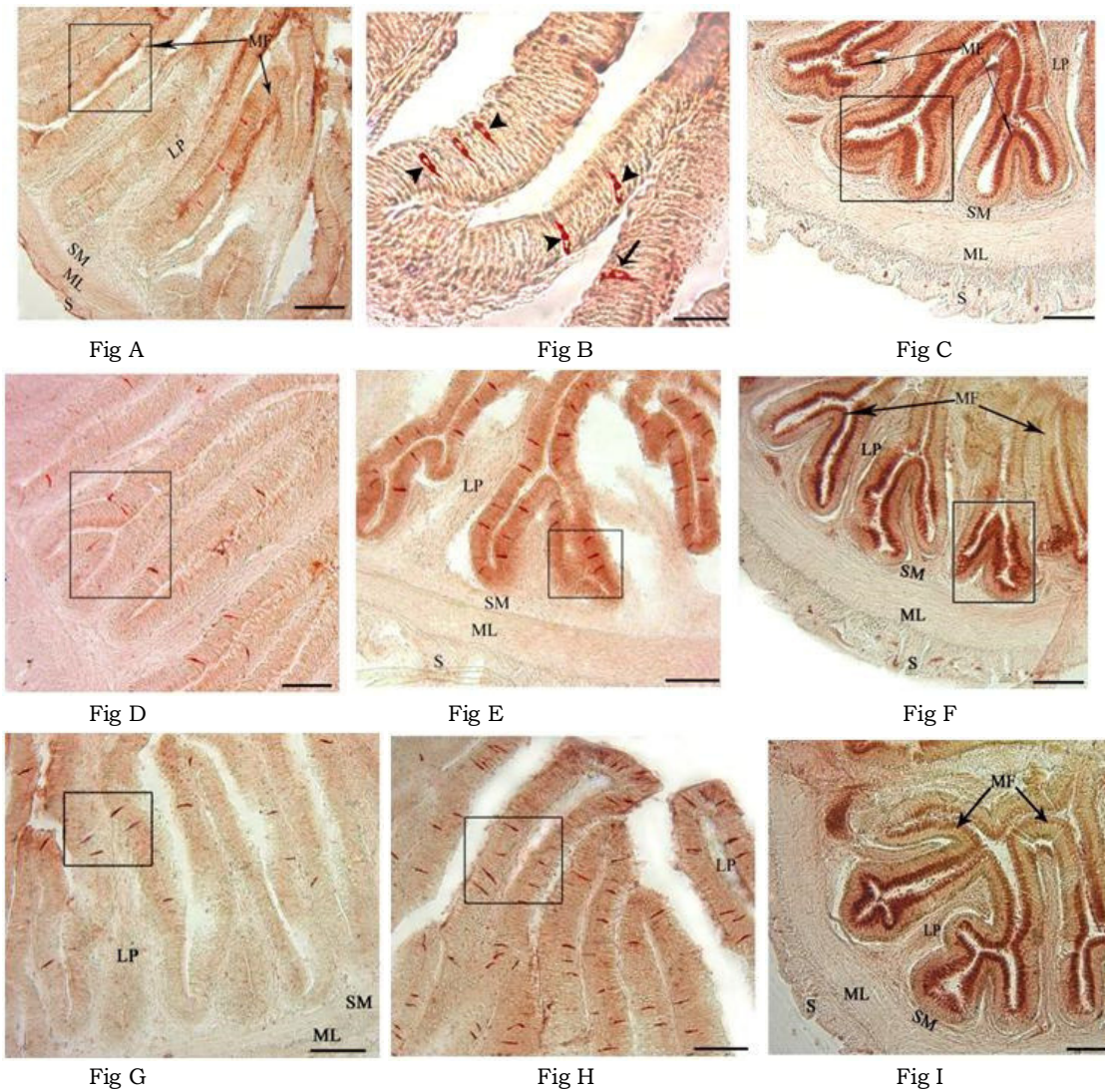
processes. The endocrine cells are open type tadpole and sac shape (Fig.A) were found in the proximal region. The middle intestinal region of control group showing intense immunoreactivity, the length of intestinal fold in the middle region were smaller than other region showing NPY immunoreactive open type slender shape and tadpole shape immunoreactive cells. (fig.B). In the distal intestinal region the NPY immunoreactivity was observed to have spread in the entire epithelial lining of the mucosa (Fig.C).

Thus, the control group shows moderate NPY immunoreactive endocrine cells in the proximal and distal intestine while intense immunoreactivity was confirmed in the middle intestinal region.

24 and 48 hour starvation group :

The NPY immunoreactive endocrine cells were located along the height of the intestinal region. The morphology of endocrine cells was similar to that of cells observed in control group. The distribution of the immunoreactive endocrine cells were found to decrease in the intestinal region of 24 hour starve group than those of the control group. Moderate amount of NPY immunoreactivity was confirmed in the proximal and distal intestinal region (Fig. D&F) while numerous in the middle intestinal region (fig.E) of the 24 hr starve group.

The NPY immunoreactive endocrine cells increased in the middle intestinal region of the 48 hr. starve group as compare to the control and 24 hour starve group. Huge amount of immunoreactivity was confirmed in the middle, moderate in distal while less in proximal intestinal fold (Fig. G,H,I). NPY immunoreactive tadpole, sac and slender shape endocrine cells observed in the apical part of the proximal intestinal fold (Fig G).



Photomicrographs of transverse section showing NPY immunoreactivity in *H. fossilis*

Fig A,B,C –proximal, middle and distal region for control group showing layers Serosa (S),Muscularis(ML),Submucosa(SM) & Mucosa (M) with Lamina propria (LP), Tadpole & Elongated shape immunoreactive endocrine cells.Fig D,E,F - –proximal, middle and distal region for 24 hr. starvation group showing tadpole,slender and sac shape endocrine cells. Fig. G,H,I – proximal middle and distal region for 48 hr. starvation group showing intense immunoreactivity in middle region.

A: Proximal intestine for control (regular fed), B: Middle intestine for control (regular fed)

C: Distal intestine for control (regular fed),D:Proximal intestine for 24 hr starvation group

E :Middle intestine for 24 hr starvation group ,F:Distalintestine for 24 hr starvation group

G:Proximal intestine for 48 hr starvation group, H:Middleintestine for 28 hr starvation group I:Distal intestine for 48 hr starvation group

Discussion :

Neuropeptide Y is the most potent orexigenic peptide involved in regulation of food intake of other animals and in fish. In the present study, there was a difference observed in the localization and distribution of NPY in the stomach and intestinal region of *H. fossilis*.

Studies of the gastrointestinal tract in *I. punctatus* (Min et al., 2009) and *pseudophoxinus antalyae* (Cinar et al., 2006) demonstrated that, rare NPY immunoreactive cells were present in the epithelial mucosa of the midgut whereas no cells were found in the stomach as well as posterior intestine. A similar observation was made in *Anguilla anguilla*, where NPY immunoreactive cells were rare and localized in the midgut and posterior intestine but reactivity was completely absent in the stomach (Domenghini et al., 2000). Similar observations made in *I. punctatus*, *P. antalyae*, and eel *Anguilla anguilla* and the studies made in *R. quelen* showed presence of numerous NPY immunoreactive cells in the initial portion of midgut and no immunoreactivity for NPY in the stomach region.

In *salmo trutta* (Tarakei 2012) NPY immunoreactive cells were observed in stomach with a very low frequency, whereas these immunoreactive cells were found many in pyloric caeca. In the present study pyloric caeca was found to be absent in *H. fossilis*.

To understand the mechanism of regulatory peptides in the gut of fishes, several studies have been performed (Holmgren, 1985; Holmgren et al., 1986). But no examination have been made in fishes to study whether the presence of neuropeptide containing endocrine cells get altered under different physiological conditions, such as starvation.

NPY is one of the most potent orexigenic agents, as in both goldfish (Narnaware and Peter, 2001) and winter flounder (McDonald, 2008), the fasting period induces increase in hypothalamic NPY mRNA expression, which suggesting that NPY acts as a long-term regulator of feeding in fish. Present study reveals that, NPY immunoreactive cells of middle intestinal region were increased with starvation period of 48 hr. Proximal intestinal region of control fish shows higher immunoreactivity than starved region, this result is coincide with that of *O. banariensis* which showed higher number of neuroendocrine cells immunoreactive to NPY in the proximal intestine, could indicate a role of this region as a primary

source of signals to stimulate food intake in the absence of food (Vagliano et al., 2011).

In fishes, *P. reticulata* and *L. idus melanotus* no changes occurs in the density of endocrine cells, nerve fibres or nerve cell bodies of the intestinal region after starvation (Patricia Burkhardt-Holm and Susanne Holmgren, 1989). Present results were contrast with that, there were change in the density of endocrine cells according to the starvation period.

Conclusion :

1. Immunocytochemical localization and distribution of NP-Y in stomach and intestine in control and starve condition revealed presence of this peptide only in intestine whereas its complete absence in stomach. From the above findings it appears that NP-Y does not have a specific role in the local digestive process.
2. Due to starvation alterations were observed in number of immunoreactive cells. It may be possible that exogenous starvation (forced starvation) had affected the distribution of peptide which showed an increase in the starvation period from 24 hr. to 48 hr. mainly in middle region of intestine. Our result confirmed the role of NPY peptide in feeding of the *H. fossilis*.

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