Yellowtail melanin-concentrating hormone 1: molecular cloning, tissue distribution, and response to fasting and feeding

Noriko HOSOMI, Toshiro MASUMOTO and Haruhisa FUKADA*

Abstract: Melanin-concentrating hormone 1 (MCH1) is an appetite-regulating hormone in vertebrates, but its functioning differs among species. Understanding the underlying mechanism of appetite regulation is important to improve production performance in aquaculture systems. The yellowtail Seriola quinqueradiata is one of the most cultured fish species in Japan, although little is known about its appetite-regulating hormones. In the present study, complementary DNA encoding for MCH1 was cloned in yellowtail. Cloned cDNA consisted of 599 bp, whereby the deduced mature MCH1 amino acid sequences showed high degree of identity with those from other teleosts. The phylogenetic analysis revealed that yellowtail preproMCH1 belongs to the MCH1 group. In terms of tissue distribution, the mch1 mRNAs were detected in all examined tissues (whole brain, telencephalon, optic tectum, hypothalamus, cerebellum, pituitary, retina, stomach, pyloric caeca, anterior intestine, liver, and kidney). The mch1 mRNA expression in the brain did not show significant differences after fasting when compared to the control group. However, mch1 mRNA expression in the hypothalamus decreased significantly after feeding, in fish previously fasted for 72 h. Further studies are needed to identify the role of MCH1 in feeding regulation in yellowtail.

Key words: Yellowtail; Melanin-concentrating hormone 1; Appetite; Brain

Melanin-concentrating hormone (MCH) is a peptide hormone originally isolated from the pituitary gland of chum salmon Oncorhynchus keta (Kawauchi et al. 1983). In fish, MCH comprises 15–17 amino acids in a cyclic structure linked by a disulfide bond (Kawauchi and Baker 2004; Kawauchi 2006). The main function of MCH is to concentrate melanin granules in the melanophores of the skin (Kawauchi et al. 1983). MCH has been intensively studied in teleost fishes, where it was seen to affect pigment aggregation and influence other pituitary hormones (Kawauchi 2006). MCH was also found to play an important role in feeding regulation of teleost, although the mechanism is still unclear. In the goldfish Carassius auratus, an intracerebroventricular (ICV) injection of MCH suppressed feed intake (Matsuda et al. 2006), and fasting decreased MCH immunoreactive neuronal cell bodies (Matsuda et al. 2007), suggesting that MCH works as an anorexigenic hormone in this species. However, an increase in brain mch mRNA expression after fasting was observed in zebrafish Danio rerio (Berman et al. 2009), barfin flounder Verasper moseri (Takahashi et al. 2004), winter flounder Pseudopleuronectes americanus (Tuziak and Volkoff 2012), Atlantic cod Gadus morhua (Tuziak and Volkoff 2013), and starry flounder Platichthys stellatus (Kang and Kim 2013), suggesting that in these species, MCH works as an orexigenic hormone.

cDNA cloning of MCH has been done in several species, such as the chinook salmon Oncorhynchus tshawytscha (Minth et al. 1989), coho salmon Oncorhynchus kisutch (Nahon et al. 1991), rainbow trout Oncorhynchus mykiss (Baker et al. 1995), tilapia Oreochromis
mossambicus (Gröneveld et al. 1995), and barfin flounder (Takahashi et al. 2004). Furthermore, a novel MCH (MCH2) was cloned in zebrafish (Berman et al. 2009), winter flounder (Tuziak and Volkoff 2012), starry flounder (Kang and Kim 2013), and barfin flounder (Mizusawa et al. 2014). MCH2 is also thought to be related with feeding regulation.

Yellowtail *Seriola quinqueradiata* is one of the most important aquacultured species in Japan (Masumoto 2002). The cDNA of two yellowtail appetite-related hormones, cholecystokinin (CCK) and neuropeptide Y (NPY), has been previously cloned (Murashita et al. 2006; Hosomi et al. 2014). NPY was found to have an orexigenic function in response to fasting, whereas CCK did not show any significant changes (Hosomi et al. 2014). Increasing feed intake and consequently, improving growth is an important issue in aquaculture. Therefore, in the present study, the cDNA of MCH1, a hormone probably related to appetite-regulation in yellowtail, has been cloned, and its tissue distribution was examined. To investigate the relationship between *mch1* mRNA expression and appetite, the response to fasting and feeding has been analyzed.

**Materials and Methods**

*Animals*

Yellowtails used in this study were collected from Tosa Bay near Kochi, Japan and reared at an indoor aquaculture station (Konan, Kochi, Japan). Fish were fed a commercial extruded pellet (EP) diet (Been’s Nutra, Skretting, Fukuoka, Japan).

**Cloning of mch1 cDNA in yellowtail**

After 24h fasting, two yellowtails were killed by an anesthetic overdose of 2-phenoxyethanol (1 ml/l) (Nacalai Tesque Inc., Kyoto, Japan) dissolved in seawater. The pituitary glands were then collected from the fish brains, frozen in liquid nitrogen, and stored at ~80°C until further use. Total RNA was isolated with Sepasol-RNA I Super G (Nacalai Tesque Inc.) according to the manufacturer’s instructions. First-strand cDNA was obtained with 3 μg of RNA by using M-MuLV Reverse Transcriptase (Promega, Madison, WI, USA) according to the manufacturer’s protocol. Degenerate primer sets MCH1-F and MCH1-R (Table 1) were designed to clone partial brain *mch1* cDNA sequences of yellowtail based on the alignment of MCH sequences from other fish species. The polymerase chain reaction (PCR) parameters were 40 cycles at 94°C for 30 s, 55°C for 30 s, and 70°C for 1 min, with an additional initial 2 min of denaturation at 94°C and a 7 min final extension at 72°C. PCR products were sequenced by using a 2720 Thermal cycler (Applied Biosystems Inc., Carlsbad, CA, USA).

After PCR, the cDNA fragments were ligated into pGEM-T Easy vector (Promega) and transformed to *Escherichia coli* DH5α

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’-3’)</th>
<th>Primer direction</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCH1-F</td>
<td>CCRCTMCTTCTCGCCGCCACGCCT</td>
<td>Sense</td>
<td>Cloning</td>
</tr>
<tr>
<td>MCH1-R</td>
<td>CTASACATCCAGCAGTGGCC</td>
<td>Antisense</td>
<td>Cloning</td>
</tr>
<tr>
<td>GSP1</td>
<td>ATSSACATCCAGCAGTGGCC</td>
<td>Antisense</td>
<td>Cloning</td>
</tr>
<tr>
<td>GSP2</td>
<td>CKTCCCACATGCACCTCATG</td>
<td>Antisense</td>
<td>Cloning</td>
</tr>
<tr>
<td>Abridged anchor primer</td>
<td>GGCAGACCGTGCACACTATGCAGGGGGGGAAGGGG</td>
<td>Sense</td>
<td>Cloning</td>
</tr>
<tr>
<td>Abridged universal anchor primer</td>
<td>GGCACAGCGTCGACTAGTAC</td>
<td>Sense</td>
<td>Cloning</td>
</tr>
<tr>
<td>Not 1</td>
<td>AACTTGGGAGAGTTGCCCGGCCACGCA</td>
<td>Sense</td>
<td>Cloning</td>
</tr>
<tr>
<td>Not 2</td>
<td>CCRCTMCTCTGGCCGCCACGCGCT</td>
<td>Sense</td>
<td>Cloning</td>
</tr>
<tr>
<td>MCH1 sp F</td>
<td>GCTATGGCAGGAGCAGGAGGATT</td>
<td>Sense</td>
<td>rtq-RT-PCR</td>
</tr>
<tr>
<td>MCH1 sp R</td>
<td>GAAGCACAAGTACCTCTGTCCTCAA</td>
<td>Antisense</td>
<td>rtq-RT-PCR</td>
</tr>
<tr>
<td>MCH1 probe</td>
<td>CCCATGCCAAAGACGACGGATG</td>
<td>Sense</td>
<td>rtq-RT-PCR</td>
</tr>
<tr>
<td>18s rRNA sp F (Tom et al. 2004)</td>
<td>TACCCACATCCAAAGAGGGCA</td>
<td>Sense</td>
<td>rtq-RT-PCR</td>
</tr>
<tr>
<td>18s rRNA sp R (Tom et al. 2004)</td>
<td>TCGATCCGGAGATCCAA</td>
<td>Antisense</td>
<td>rtq-RT-PCR</td>
</tr>
</tbody>
</table>
Yellowtail MCH as appetite-regulating hormone

competent cells (Takara, Kyoto, Japan). After colonies were incubated, plasmid vectors were extracted by using the alkaline lysis method. Extracted plasmids were cycle sequenced by using a BigDye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems Inc.) with the M13 primer set and purified by ethanol precipitation. The target inserts were sequenced automatically (ABI 3100 Genetic Analyzer, Applied Biosystems Inc.).

To obtain the 5´ and 3´ ends of the missing upstream and downstream regions of yellowtail MCH, we performed rapid amplification of cDNA ends (RACE). Primers for 5´ and 3´ RACE are shown in Table 1. A 5´ RACE system for Rapid Amplification of cDNA Ends (Invitrogen, Carlsbad, CA, USA) was used for 5´ RACE. First-strand cDNA was obtained with 3 μg of RNA by using gene specific primer (GSP) 1. The first PCR was performed by using GSP1 and the Abridged Anchor Primer (AAP) from the kit. Semi-nested PCR was carried out using GSP2 and the Abridged Universal Anchor Primer (AUAP) from the kit. For 3′-RACE, first-strand cDNA was obtained with Not I-dT primer. The first PCR was performed by using the Not I and GSP3 primers. All PCR products were subcloned into the pGEM-T Easy vector (Promega) and were sequenced with a BigDye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems Inc.).

Sequence and phylogenetic tree analysis

Nucleotide and deduced amino acid sequences were compared with the GenBank database by using a Basic Local Alignment Search Tool algorithm. After manual correction of the alignments, nucleotide sequences were aligned using ClustalW software (http://www.ddbj.nig.ac.jp/intro-j.html), and a phylogenetic tree was constructed by using neighbor-joining method. Values represent bootstrap scores of 1000 trials, indicating the credibility of each branch. Fig Tree v1.4.0 software (http://tree.bio.ed.ac.uk/software/figtree/) was used to graphically view the phylogenetic tree.

Real-time quantitative reverse transcription-PCR (qRT-PCR) for yellowtail mch1

Total RNA was extracted from tissues by using Sepasol-RNA I Super G (Nacalai Tesque Inc.). RNA integrity was verified based on an optical density (OD) at OD 260 nm/OD 280 nm absorption ratio of >1.7. For the measurement of mch1 mRNA expression, 200 ng/μl total RNA after DNase treatment was used to synthesize the first-strand cDNA. DNase treatment was performed in a reaction mixture (50 μl) containing 44 μl of total RNA (200 ng/μl), 5 μl of 10× DNase buffer, and 1 μl of deoxyribonuclease, incubated for 15 min at 37°C. Deoxyribonuclease (RT Grade) for heat Stop, NIPPOGEN GENE, Tokyo, Japan). Afterwards, 5 μl of stop solution was added, and the mixture was heated for 10 min at 70°C. RT was performed in a reaction mixture (10 μl) containing 2 μl of total RNA after DNase treatment, 0.5 μl of dNTPs (10 mM), 0.125 μl of random primers (Promega), 0.1875 μl of M-MuLV reverse transcriptase (Promega), and 5× buffer in a final volume of 2 μl. RT was performed for 10 min at 25°C, followed by 60 min at 42°C, and 5 min at 95°C. The qRT-PCR primers for yellowtail mch1 are shown in Table 1. Primers for mch1 were designed with the Primer Express Program (Applied Biosystems Inc.).

For data normalization, the 18S ribosomal RNA (rRNA) was used. The 18S primers were derived from a consensus of fish 18S sequences (Tom et al. 2004), and the primer pair used in the present study has been validated by qRT-PCR assay (Murashita et al. 2006). The expression of 18S showed stable values in our assay. The PCR reaction for the mch1 assays (20 μl) contained 10 μl of TaqMan Universal Master Mix II with UNG (Applied Biosystems Inc.), 1 μl of first-strand cDNA, 1.6 μM of each forward and reverse primers, and 5 μM of mch1 probe. The PCR for the 18S assay (20 μl) contained 10 μl of Power SYBR Green PCR Master Mix (Applied Biosystems Inc.), 1 μM of each forward and reverse primers, and 1 μl of the first-strand cDNA (1/100 dilution). Amplification and detection of samples were performed with the ABI7300 system with the
following thermal cycling conditions: 50°C for 2 min, 95°C for 15 s, and 60°C for 1 min (40 cycles). Three serial dilutions of sample cDNA were run to determine the efficiency (E) of PCR, which was calculated from the regression slope of the assay (E = 10^{-1/slope}). Steady-state yellowtail mch mRNA expression was calculated relative to the 18S rRNA gene by using the method developed by Pfaffl (2001).

Tissue distribution

Six yellowtails (mean weight = 527.3 g) were killed with an overdose of 2-phenoxyethanol (1 ml/l) after two days of fasting. The retina, pituitary, hypothalamus, telencephalon, optic tectum, cerebellum, whole brain, stomach, anterior intestine, liver, kidney, and pyloric caeca of each fish were collected and immediately frozen in liquid nitrogen. Tissues were stored at −80°C until subsequent analysis.

Response to fasting and feeding

Adult yellowtails were reared in an indoor aquaculture station (Konan, Kochi, Japan) in fiber-reinforced 1,100-l circular plastic tanks supplied with seawater maintained in a continuous flow-through system at ambient temperature (18.6°C–19.6°C) and photoperiod. Fish were distributed between two tanks (seven fish each) (Table 2). This experiment was duplicated and conducted from May 9 to May 23, 2013. The control group was fed a commercial EP diet (Beens’ Nutra, Skretting) to satiety once a day for two weeks, whereas the fasted group was deprived of feed. Fish were killed with an overdose of 2-phenoxyethanol (1 ml/l) before feeding, and then brain tissues were collected and stored at −80°C. Sampling was performed two weeks after the start of the experiment before feeding.

Juvenile yellowtails (mean weight = 168.6 g) were reared in an indoor aquaculture station (Konan, Kochi, Japan) in fiber-reinforced 1,100-l circular plastic tanks supplied with seawater maintained in a continuous flow-through system at ambient temperature (25.4°C–25.5°C) and photoperiod. Fish were distributed between two tanks (10 fish each), one of them was sampled before and the other after each feeding. This experiment was conducted from October 16 to October 17, 2014. Fish were fasted for 72 h and then fed a commercial EP diet (Beens’ Nutra, Skretting). Before feeding and 3 h after feeding, fish were killed with an overdose of 2-phenoxyethanol (1 ml/l). Hypothalamus were collected and stored at −80°C.

Statistical analysis

Tissue distribution analyses were performed by using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests. The two weeks fasting experiment and response to feeding results were analyzed by using Student’s t-test. Statistical analyses were performed with GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). Differences between groups were considered significant at \( P < 0.05 \).

Results

Cloning and sequencing of yellowtail mch1 cDNA

Yellowtail mch1 cDNA consisted of 599 bp (GenBank accession No. AB935990), encoding 135 amino acid residues. The positions of the start codon ATG and stop codon TGA were confirmed. The predicted MCH1 prepro-peptide contained a putative signal of 24 amino acids, an N-terminal peptide (N-proMCH) of 59 amino acids, a neuropeptide AL (NAL) of 32 amino acids, and a mature peptide of 17 amino acids (Fig. 1). The mature yellowtail MCH1 peptide is located at the C-terminus of the preproMCH1, preceded by two arginine residues, which are considered to be a cleavage site for proteolytic processing of mature hormones (Harris et al. 1998). The yellowtail preproMCH1 showed the highest degree of identity with preproMCH1 of Japanese flounder (76%), followed by Nile tilapia (72%), winter flounder (66%), Atlantic salmon (45%), rainbow trout (44%), goldfish (37%), and the lowest identity with teleost MCH2. The mature yellowtail MCH1 peptide had 100% identity with Japanese flounder MCH and a high identity with other teleost MCH1 (>94%) but a low identity to teleost MCH2.
Phylogenetic analysis showed that preproMCH comprises two clusters (MCH1 and MCH2) (Fig. 2). The cloned yellowtail MCH1 was grouped within the teleost MCH1 cluster.

**Tissue distribution**

$mch1$ mRNA expression levels were detected in all the examined tissues (whole brain, telencephalon, optic tectum, hypothalamus, cerebellum, pituitary, retina, stomach, pyloric caecae, anterior intestine, liver, and kidney; Fig. 3). The expression of $mch1$ mRNA in the hypothalamus was significantly higher than that in other brain regions and visceral organs. In the visceral organs, the expression of $mch1$ mRNA in the pyloric caeca was significantly higher than that in the stomach, anterior intestine, and liver.

**Response to fasting and feeding**

Initial and final mean body weight are shown in Table 2. After 2 weeks of fasting, the body weight of fasted fish were significant lower than that of control fish. No difference was observed in condition factor, but lower in fasted group. The $mch1$ mRNA expression in various tissues of
the fish fasted for two weeks showed higher values than those in fed fish. However, mch1 mRNA expression in the brain was used as 1 to normalize the data. Lowercase a and b indicate significant difference in all tissues; uppercase A and B indicate significant difference in brain tissues (b), and lowercase x and y indicate significant difference in other tissues (c). We used 18S ribosomal RNA (rRNA) to normalize data from real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR). Bars with different superscript letters differed significantly \((P < 0.05)\). Vertical lines represent standard error of the mean (SEM; \(n = 5\) and 6).

**Discussion**

In this study, the cDNA of MCH1, an appetite-related peptide, was cloned from the
Yellowtail MCH as appetite-regulating hormone

Mature MCH1 amino acid sequences of yellowtail indicated 100% identity with mature MCH from Japanese flounder and 93.8% identity with mature MCH1 and MCH from other teleost fish (Fig. 1). In the phylogenetic analysis of preproMCHs, teleost MCH was divided into teleost MCH1 (or considered MCH when MCH2 has not been identified) and teleost MCH2 (except chum salmon MCH2, which is an MCH1 isoform). Furthermore, yellowtail mature MCH1 amino acids displayed high identity to other vertebrate MCH (MCH1), while the N-terminal region was two amino acids shorter than that of mammalian MCH. In addition, C- and N-terminal regions of yellowtail mature MCH were four amino acids shorter than those of teleost MCH2. Therefore, the obtained cDNA seems to be yellowtail MCH1 belonging to the MCH1 group. Under white background color, the mch2 mRNA expression in the brain increased significantly (Mizusawa et al. 2014) and fish exhibit higher growth and feeding behavior (Sunuma et al. 2009), suggesting MCH2 might be involved in appetite regulation. The existence of MCH2 in yellowtail should be investigated in a future study.

The tissue distribution of mch1 mRNA in yellowtail shows a widespread distribution, as observed in other fish species such as the winter flounder (Tuziak and Volkoff 2012), Atlantic cod (Tuziak and Volkoff 2013), and starry flounder (Kang and Kim 2013). The mch1 mRNA expression in the hypothalamus of yellowtail was significantly higher than that in other brain regions, similar to winter flounder (Tuziak and Volkoff 2012) and Atlantic cod (Tuziak and Volkoff 2013). In terms of immunohistochemistry, MCH-immunoreactive cells are found in the hypothalamic nuclei of barfin flounder (Amiya et al. 2008), medaka Oryzias latipes (Amiya et al. 2007), zebrafish (Berman et al. 2009), and goldfish (Matsuda et al. 2009), corroborating our mch1 mRNA expression results. In the visceral organs of yellowtail, mch1 mRNA expression was the highest in the pyloric caeca. mch1 mRNA expression levels in the gastrointestinal tract have been reported for several fish, including winter flounder (Tuziak and Volkoff 2012), Atlantic cod (Tuziak and Volkoff 2013), and starry flounder (Kang and Kim 2013). The relative high expression levels of mch1 mRNA in visceral organs was observed in the gall bladder, stomach, intestine, and testis of winter flounder (Tuziak and Volkoff 2012), liver, spleen, stomach, and ovary of Atlantic cod (Tuziak and Volkoff 2013), and gill and testis of starry flounder (Kang and Kim 2013). Expression levels in the gastrointestinal tract differ from that of yellowtail. Nevertheless, the mRNA expression of the other two appetite-regulating hormones (npy and cck) was also found to be high in the pyloric caeca of yellowtail (Hosomi et al. 2014). Since the pyloric caeca is an important organ regulating digestion in yellowtail, our data suggest that MCH1 might act as a brain/gut peptide and therefore, have a role in regulating appetite. The response of mch1 mRNA expression in the pyloric caeca to feeding condition should be confirmed in future experiments.

In the fasting experiment, retina and five brain regions were chosen to investigate the response of mch1 mRNA expression to fasting. Although an increasing trend of mch1 mRNA expression was observed in all tissues after the two weeks of fasting, this increase was not significant. In winter flounder, 10 days of fasting significantly increased mch1 mRNA expression in the optic tectum and the thalamus. The hypothalamic mch1 mRNA expression has also shown an increasing but not a significant trend to fasting (Tuziak and Volkoff 2012). In Atlantic cod, two weeks of fasting significantly increased mch mRNA expression in the hypothalamus (Tuziak and Volkoff 2013). Significant increases of brain mch mRNA expression were observed in starry flounder fasted for three days (Kang and Kim 2013) and in barfin flounder fasted for two weeks (Takahashi et al. 2004). Although fasting conditions were different, fasting increased mch mRNA expression in the brain, suggesting that MCH works as an orexigenic hormone in these species. In goldfish,
an intracerebroventricular (ICV) injection of MCH inhibits feed intake, while an ICV injection of anti-salmon MCH serum caused an immunoneutralization of MCH in the brain and increased feed intake (Matsuda et al. 2009). This suggests that, in goldfish, MCH works as an anorexigenic hormone. The role of MCH in appetite regulation differs among species. In yellowtail fasted for two weeks, although the hypothalamic npy mRNA expression significantly increased (Hosomi et al. 2014), mch1 mRNA expression did not change (this study).

An additional experiment (response to feeding) was performed to further investigate the function of MCH1 in regulating appetite in yellowtail. Since an increase in free amino acids, free fatty acids, and triglycerides in the plasma has been observed 2 h after feeding (Shimeno et al. 1993), tissue collection was performed 3 h after feeding the 72-h fasted yellowtails. In response to feeding, the hypothalamic mch1 mRNA expression decreased significantly 3 h after feeding. Such a rapid response of mch1 mRNA expression has not been described yet in teleosts, but a quick response of npy mRNA expression to feeding has been previously observed in some teleosts. Gene expression of brain npy mRNA decreased within 1-2 h after feeding in Brazilian flounder Paralichthys orbignyanus and channel catfish Ictalurus punctatus. The npy mRNA expression showed a constant response, suggesting an orexigenic role of NPY in response to both fasting and feeding. However yellowtail mch1 mRNA expression did not show any significant changes to fasting and only showed a significant decrease after feeding.

Our two experiments were conducted using different size of fish in different season. In winter flounder, the gene expression of hypothalamic cart and npy were lower in winter than those in summer (MacDonald and Volkoff 2009). Furthermore, the response of hypothalamic npy mRNA expression to fasting was different between summer and winter in winter flounder. A significant increase of hypothalamic npy mRNA expression by fasting was observed in only summer season. Those suggest that the response of appetite-related hormones to fasting might be changed by season including daylight and water temperature, which is one of possible reason for the lack of mch1 response to fasting in this study. In feeding experiment, smaller yellowtail fish were used compared to those used in the fasting experiment, furthermore the feeding experiment was conducted in higher water temperature. Deposition of fat in body is normally higher in large fish than in small fish and in low water temperature than in high water temperature in yellowtail (Shimeno et al. 1992). Fat is an important energy source for yellowtail, which also reflects nutrition status of fish. In fasting experiment, body weight decreased significantly in fasted fish, but no significant difference in condition factor between control (fed) and fasted groups. Fasted group might still keep enough energy stock in the body after 2 weeks of fasting. The response of mch1 mRNA expression to fasting could be observed by longer fasting and/or using smaller fish.

The function of MCH1 in appetite varied in different fishes as described in introduction. Direct evidence of MCH function was confirmed in only goldfish, in which an ICV injection of MCH suppressed feed intake (Matsuda et al. 2006). In in vitro study of goldfish, npy mRNA expression in forebrain, hindbrain and hypothalamus decreased significantly by the MCH treatment, suggesting anorexigenic function of MCH (Volkoff 2014). However, the function of MCH in goldfish remain unclear since MCH treatment in in vitro stimulated both orexigenic and anorexigenic hormones. Furthermore, the MCH worked differently in different brain regions. Similar in yellowtail, the function of MCH1 in appetite remains unclear since mch1 mRNA expression to fasting and feeding did not indicate consistent response. However yellowtail MCH1 is also probably involved in appetite regulation.

In the present study, the mch1 cDNA sequence was analyzed, and the mch1 mRNA expression was confirmed in the retina and brain regions of yellowtail. mch1 mRNA expression in the retina and brain regions did not vary after two weeks of fasting, whereas
the hypothalamic mch1 mRNA expression significantly decreased after feeding. In yellowtail, mch1 mRNA expression in the hypothalamus might be involved in feeding regulation, but its function remains unclear. Further studies are needed to identify the role of yellowtail MCH1 in appetite regulation.

Acknowledgements

We are grateful to Mr. Hiroshi Yabuki, Mr. Noriyuki Takahashi, and the staff of the Research Institute of Molecular Genetics, Kochi University, for their assistance during these experiments.

References


ブリのメラニン凝集ホルモン 1: cDNA クローニング,
組織分布および絶食と給餌への応答

細美野里子・益本俊郎・深田陽久

日本の養殖重要種ブリから食欲調整ホルモンの一種と考えられるメラニン凝集ホルモン (MCH1) の cDNA クローニングを行い、組織分布を明らかにした。さらに MCH1 は魚種によって食欲におかれる作用が異なることが知られているため、ブリで絶食と摂餌に対する応答を観察した。クローニングされたブリ MCH1 cDNA 配列から推定された mature MCH1 のアミノ酸配列は、硬骨魚類の MCH1 に対して高い相似性を示した。ブリ MCH1 遺伝子は、脳、網膜、胃、幽門垂、腸、肝臓、腎臓で発現していた。2 週間の絶食によって、網膜と脳における MCH1 遺伝子の発現量は変化しなかったが、72時間の絶食後の給餌 3 時間後に視床下部 MCH1 遺伝子発現量は有意に減少した。以上の事から、ブリ MCH1 は食欲の調整に関わっていると考えられるが、その作用を解明するためにはさらなる研究が必要とされた。