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Melanocyte-stimulating hormone facilitates hypermelanosis on the non-eyed side of the barfin flounder, a pleuronectiform fish

Takeshi Yamanome [a], Hiroaki Chiba [b], Akiyoshi Takahashi [b,*]

[a] *Iwate Fisheries Technology Center, Kamaishi, Iwate 026-0001, Japan*
[b] *School of Fisheries Sciences, Kitasato University, Ofunato, Iwate 022-0101, Japan*

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Abstract

A pleuronectiform fish, the barfin flounder, *Verasper moseri*, is promising for aquaculture and resource enhancement in Northern Japan due to its high commercial value. Hypermelanosis of its non-eyed side, which frequently occurs under culture conditions, diminishes its commercial value. Two peptide hormones, melanin-concentrating hormone (MCH) and melanocyte-stimulating hormone (MSH), having opposing actions, are associated with the color changes of fish. We have previously reported the positive effect of MCH in preventing hypermelanosis. Here, we examined the effects of MSH on the occurrence of hypermelanosis. A single injection of Des-Ac-α-MSH [0.01 nmol/g–10 nmol/g (0.016 μg–16 μg/g)] did not change the eyed-side body color, while a single injection of MCH [0.1 nmol/g (0.21 μg/g)] made the eyed-side color paler. No difference was observed in eyed-side lightness between fish injected with MCH (0.1 nmol/g) and those receiving MCH (0.1 nmol/g) and an increased amount of Des-Ac-α-MSH (0.01 nmol/g–10 nmol/g) simultaneously. These results indicate that MSH does not suppress the *in vivo* body color-paling effects of MCH in barfin flounders. On the other hand, implantation of a cholesterol pellet containing Des-Ac-α-MSH (280 μg, twice at 29-day interval) increased hypermelanosis of the non-eyed side of barfin flounders compared to control fish. Eyed-side bodies of MSH-treated fish were darker than control fish; thus, MSH is involved in morphological color change including ectopic melanin synthesis in non-eyed-side skin.

Keywords: Flounder; Hypermelanosis; Melanocyte-stimulating hormone

1. Introduction

Flatfish (Pleuronectiforms) are characterized by an asymmetrical and flat body shape, dark on the eyed side and white on the non-eyed side. Hypermelanosis on the non-eyed side (staining type; Norman, 1934) is a common problem in hatchery-reared flatfish, and decreases the market value of the whole fish. A number of factors are associated with this color anomaly (Bolker and Hill, 2000). Two hormones in the hypothalamo–pituitary system, melanocyte-stimulating hormone (MSH) and melanin-concentrating hormone (MCH), are associated with the color changes of fish, with opposing actions (Baker, 1993; Burton and Vokey, 2000). The long-term administration of α-MSH stimulates melanophore development in tilapia (van Eys and Peters, 1981), while MCH reduces the skin melanin content in rainbow trout (Baker et al., 1986). In flatfish, both exogenous and endogenous MCH inhibited hypermelanosis (Takahashi et al., 2004; Yamanome

* Corresponding author. Tel.: +81 192 44 1925; fax: +81 192 44 3934.
E-mail address: akiyoshi@kitasato-u.ac.jp (A. Takahashi).

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et al., 2005, 2007). In contrast to the known hypermelanosis-inhibiting effects of MCH, little is known about the effects of MSH on ectopic melanin synthesis.

Barfin flounder, *Verasper moseri*, is a promising flatfish species in aquaculture in Northern Japan. In this fish, two types of MSH, α-MSH and β-MSH, are generated from the precursor, proopiomelanocortin (POMC) (Takahashi et al., 2005). Barfin flounder was found to express three POMC mRNAs (A, B, and C types) in both the pars distalis and neurointermediate lobe of the pituitary gland (Amano et al., 2005; Takahashi et al., 2006). The final products in these areas differ because of tissue-specific posttranslational processing (Castro and Morrison, 1997; Smith and Funder, 1988; Takahashi and Kawauchi, 2006a,b). α-MSH with an acetylated N-terminus, Des-acetyl (Ac)-α-MSH with a free N-terminus, and β-MSH are preferentially produced in the neurointermediate lobe, and ACTH in the pars distalis (Takahashi and Kawauchi, 2006b); however, the pars distalis is another source of MSHs in the barfin flounder pituitary, as shown by the concomitant presence of ACTH, Des-Ac-α-MSH, and β-MSH (Takahashi et al., 2006).

We previously found that the rate of hypermelanosis on the non-eyed side of the barfin flounder was increased by rearing it in a black tank (Amiya et al., 2005; Yamanome et al., 2005), showing higher expression levels of POMC-A, -B and -C genes in the pituitary than fish reared in a white tank (Takahashi et al., 2005). It is therefore conceivable that MSH is a key hormone inducing hypermelanosis in non-eyed-side skin. Here, we examined the acute and chronic effect of MSH on pigmentation by MSH injection and implantation of an MSH pellet, respectively, including the occurrence of hypermelanosis on the non-eyed side of barfin flounder skin.

2. Materials and methods

2.1. Synthesis of peptides

Expression levels of POMC-A and -B genes in the barfin flounder pituitary were higher than POMC-C gene, and the amino acid sequence of α-MSH encoded on POMC-A gene is identical to that on POMC-B gene (Takahashi et al., 2006). Thus, Des-Ac-α-MSH was synthesized based on the amino acid sequence deduced from cDNA nucleotide sequences of POMC-A and -B using an automated solid-phase peptide synthesizer (PSSM-8, Shimadzu, Kyoto, Japan) according to the method described previously (Takahashi et al., 2004). Barfin flounder MCH was synthesized as described previously (Takahashi et al., 2004) and intramolecule disulfide linkage was formed by the incubation of linear MCH (20 μg/ml) in 0.001% ammonia at 37 °C for 2 h. The synthesized peptide was purified by high-performance liquid chromatography using a TSK-gel ODS120T (Tosoh, Tokyo, Japan) column with a linear gradient of acetonitrile from 10 to 70% in 0.1% trifluoroacetic acid (TFA). The accuracy of the sequence was determined by a mass spectrometric method using the matrix solution of α-cyano-4-hydroxycinnamic acid saturated in 50% acetonitrile/0.05% TFA with AXIMA-CFR plus mass spectrometer (Shimadzu).

2.2. Fish

Barfin flounders were bred in Iwate Fisheries Technology Center, Iwate, Japan, and all experiments were conducted in accordance with guidelines for the care and use of animals of Kitasato University. They were reared in indoor running seawater tanks with a natural photoperiod and water temperature. They were fed commercial pellets (Higashimaru, Kagoshima, Japan) twice daily (0830 and 1600) until satiety.

2.3. Injection of Des-Ac-α-MSH and barfin flounder MCH

Barfin flounder, 5.6±0.03 cm total length and 2.1±0.04 g body weight on average (n=77), were transferred to black or white tanks (36 l) 1 week before experiment. They were injected intraperitoneally with synthesized Des-Ac-α-MSH [0.01 nmol/g–10 nmol/g (0.016 μg/g–16 μg/g)] under anesthesia with 0.05% 2-phenoxyethanol. These fish were reared in white tanks. Water temperature was 9.1 °C. Simultaneous injections of MCH [0.1 nmol/g (0.21 μg/g)] and different amounts of Des-Ac-α-MSH (0.01 nmol/g–10 nmol/g) were also administered. These fish were reared in black tanks. Water temperature was 8.5 °C. Control fish received 0.9% NaCl. Four hours after injections, photographs of the eyed-side were taken by digital camera (Camedia X-350, Olympus, Tokyo, Japan).

2.4. Administration of MSH pellets

A cholesterol pellet containing Des-Ac-α-MSH (MSH pellet) was prepared according to the method of Lee et al. (1986) with slight modifications. In brief, Des-Ac-α-MSH (13.4 mg) was dissolved in 3 ml of 70% ethanol and mixed completely with cholesterol powder (1 g) (Wako, Osaka, Japan). After drying overnight, the resulting pellet was pulverized into powder, and then