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Authors: Yada, Takashi, Azuma, Teruo, Takahashi, Akiyoshi, Suzuki, Yoshiro, and Hirose, Shigehisa

Source: Zoological Science, 17(8) : 1123-1127

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.17.1123>

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Effects of Desacetyl- α -MSH on Lipid Mobilization in the Rainbow Trout, *Oncorhynchus mykiss*

Takashi Yada^{1*}, Teruo Azuma¹, Akiyoshi Takahashi²,
Yoshiro Suzuki³, and Shigehisa Hirose³

¹Nikko Branch, National Research Institute of Aquaculture, Nikko, Tochigi 321-1661,

²School of Fisheries Science, Kitasato University, Sanriku, Iwate 022-0101,

³Department of Biological Science, Tokyo Institute of Technology,
Yokohama 226-8501, Japan

ABSTRACT—Effects of melanocyte-stimulating hormone (MSH) and β -endorphin on lipid mobilization were examined in the rainbow trout (*Oncorhynchus mykiss*). Plasma levels of fatty acid (FA) were measured after intra-arterial administration of α -MSH, desacetyl- α -MSH, β -MSH, or β -endorphin through a cannula in the dorsal aorta. Desacetyl- α -MSH at 1 ng/g body weight resulted in an increase in plasma FA levels 1–3 hr after the injection, whereas the other three peptides showed no significant effect at the same dose. There was no significant change in plasma levels of cortisol after administration of any of the peptides. Lipolytic enzyme activity in the liver was significantly increased in a dose-related manner 1 hr after single intra-peritoneal injection of desacetyl- α -MSH. The direct effect of desacetyl- α -MSH on lipolysis was examined in liver slices incubated *in vitro*. Lipase activity in the liver slice was stimulated in the medium containing desacetyl- α -MSH in a dose-related manner. The results indicate that desacetyl- α -MSH is a potent stimulator of lipid mobilization in the rainbow trout.

INTRODUCTION

α -Melanocyte-stimulating hormone (α -MSH) is produced in the pars intermedia of pituitary gland and is involved in the control of skin pigmentation and background adaptation in lower vertebrates. α -MSH is also known to possess immunomodulatory actions in teleost (Harris and Bird, 1998; Watanuki *et al.*, 1999). On the other hand, a variant of the rainbow trout (*Oncorhynchus mykiss*), termed “cobalt”, lacks most of the pars intermedia (Kaneko *et al.*, 1993). Sparseness of MSH cells in the pituitary seems to be related to a reduction in dermal melanophores and pale body color, which are the characteristics of this variant (Oguri, 1983; Kaneko *et al.*, 1993). The other characteristic of this variant is an abnormal fat accumulation in the liver and mesenteric adipose tissue (Oguri, 1976; Kaneko *et al.*, 1993). Because α -MSH is known to possess a lipolytic activity in mammalian adipocytes (Ramachandran *et al.*, 1976; Kawauchi *et al.*, 1984), the significant obesity in the “cobalt” variant implies an involvement of this hormone in lipid metabolism in the trout.

In chum salmon pituitary gland, α -MSH-related peptides as well as β -MSH and β -endorphin were identified as major products processed from a common precursor molecule,

proopiomelanocortin (POMC) (Kawauchi, 1983). Among salmon α -MSH-related peptides, α -MSH with the unacetylated N-terminus (desacetyl- α -MSH) is the most predominant form and exhibits the most potent lipolytic activity on rat adipocyte (Kawauchi *et al.*, 1984). In the present study, we examined the involvement of α -MSH, desacetyl- α -MSH, β -MSH and β -endorphin in lipid mobilization in the trout.

MATERIALS AND METHODS

Animals

Immature rainbow trout (*Oncorhynchus mykiss*) of both sexes were reared at the National Research Institute of Aquaculture at Nikko for successive generations in outdoor concrete ponds, supplied with a continuous flow of spring water at 10°C under natural photoperiods. They were fed commercial dry diet (Oriental, Chiba).

Hormones

α -MSH was purchased from Sigma. Chum salmon desacetyl- α -MSH which has free amino terminus, β -MSH I and β -endorphin I were synthesized with a peptide synthesizer (PSSM-8; Shimadzu, Kyoto), and purified by HPLC (Kawauchi and Muramoto, 1979; Kawauchi *et al.*, 1984; Takahashi *et al.*, 1995). Amino acid sequences of these peptides were confirmed by a gas phase amino acid sequencer (PSQ-1/C-R4A, Shimadzu, Kyoto). Amino acid sequences of chum salmon α -MSH and β -MSH I are identical to those of rainbow trout homologues (Salbert *et al.*, 1992). Chum salmon β -endorphin I is 93% identical with rainbow trout homologue. The difference of α -MSH and desacetyl- α -MSH is presence or absence of N-terminal acetyl group.

* Corresponding author: Tel. (808)236-7453;
FAX. (808)236-7443.
E-mail: yadat@nria.affrc.go.jp

Cannulation and blood sampling

Fish, weighing 0.8–1.0 kg, were cannulated via the dorsal aorta as described by Ishimatsu *et al.* (1988), and held individually in plastic boxes with a continuous flow of spring water at 10°C. The cannula (PE60, Clay Adams) was connected outside the box to a plastic syringe filled with heparinized saline (100 U/ml in 0.9% NaCl). Blood sampling began 1 day after surgery. The first 800 µl (mixture of blood and saline) were taken up into a syringe, and discarded to clear the cannula of saline. The actual blood sample (100 µl) was then taken from the cannula, centrifuged at 3,000×g for 5 min at 4°C and stored at -80°C until analysed. The cannula was immediately flushed with 0.6 ml saline. Blood was collected at various intervals before (-2, -1 and 0 hr) and after (1, 2, 3, 6 and 10 hr) hormone administration. Hormones were administered in 80–100 µl saline via the cannula, and the cannula was flushed with 0.6 ml saline. To prevent the possible effect of heparin on endothelium-bound lipoprotein lipase, saline without heparin was used for the solvent of hormones and for the flush of the cannula. Plasma fatty acid (FA) concentrations were measured using a commercial kit, NEFA C-TestWako (Wako, Osaka). Plasma levels of cortisol were measured by fluorimmunoassay (Yamada *et al.*, 1996).

In vivo effect on hepatic lipase activity

Fish, weighing 100–150 g, were anesthetized with 0.02% 2-phenoxxyethanol and injected intraperitoneally with desacetyl- α -MSH in 100–150 µl saline. They recovered from anesthesia in tanks with a continuous flow of water. Three hours after the injection, liver was removed, frozen quickly on dry ice, and stored at -80°C until determination of triacylglycerol lipase activity.

In vitro effect on hepatic lipase activity

Livers from intact fish, weighing 100–150 g, were placed on ice, and sliced at 1–2 mm thickness with a tissue slicer (ST-20; Narishige, Tokyo). The slices were incubated in a culture medium (RPMI 1640, Nissui, Tokyo) buffered with 18 mM NaHCO₂ (pH 7.8) at 20°C for 30 min. Then 3–5 slices (weighing about 1.5 g) were incubated at 20°C in 50 ml of the medium containing desacetyl- α -MSH in a plastic tube. A relatively large volume of the medium (50 ml) was necessary to maintain the medium pH at 7.8, since released FA from the slices is known to affect proton concentration in the culture medium (Sheridan, 1987). The tubes were subjected to continuous gyratory shaking at 100 rpm. After 3-hr incubation, liver slices were quick frozen on dry ice, and stored at -80°C until determination of lipase activity.

Triacylglycerol lipase activity

Triacylglycerol lipase activity was determined as described by Khoo and Steinberg (1981) and Sheridan *et al.* (1985). Liver sample was homogenized in two volume of 0.25 M sucrose, 130 mM NaCl, 5 mM MgSO₄, 1 mM KH₂PO₄, 20 mM NaHCO₃, pH 7.5. The homogenate was centrifuged at 1,000×g for 10 min at 0°C. The infranant fraction was centrifuged at 110,000×g for 1 hr at 0°C. The supernatant fraction was brought to pH 5.2 by the addition of 0.2 N acetic acid. After 20 min, the precipitate was collected by centrifugation at 3,000×g for 15 min at 0°C, and suspended in 130 mM NaCl, 5 mM KCl, 1.5 mM MgSO₄, 1 mM KH₂PO₄, 20 mM NaHCO₃, pH 7.5. The protein concentration of sample solution was measured by a Protein Assay Kit (Bio-Rad, Hercules, California) using bovine serum albumin as a standard. As a stock of the substrate, 12 µmol triolein (Sigma) and 0.17 MBq [carboxyl-¹⁴C]-triolein (NEN, Boston, Massachusetts) were dissolved in absolute ethanol, and made up to a final volume of 1 ml. To prepare the substrate mixture, 490 µl water, 200 µl 0.2 M phosphate buffer (pH 7.5), containing 10% bovine serum albumin, and 100 µl stock substrate were added to a plastic tube. A 50 µl aliquot was removed for the estimation of specific activity. Then a 100 µl sample solution was added to the substrate mixture and incubated at 20°C for 30 min. The incubation was stopped by adding 3 ml chloroform-methanol-benzene (1:2.4:2, v/v/v). The mixture was brought

to pH 11.5 with 0.1 N NaOH and mixed thoroughly. After centrifugation at 1,000×g for 10 min at 20°C, a 1.5 ml aliquot of the aqueous phase was assayed for radioactivity in a liquid scintillation counter (LS6500, Beckman, California).

Statistics

Significance of differences between two groups was analyzed by ANOVA followed by Duncan's multiple range test or Mann-Whitney U-test. Calculations were performed using a computer program, STATISTICA (Design Technologies Inc., Tokyo).

RESULTS

Changes in plasma FA levels after intra-arterial injection of α -MSH, desacetyl- α -MSH, β -MSH, or β -endorphin are shown in Fig. 1. Saline-injected fish showed no significant change in plasma FA levels. Single intra-arterial injection of desacetyl- α -MSH (1 ng/g body weight) caused significant increases in plasma FA levels 1 to 3 hr after the injection. On the other hand, injection of the other peptides had no significant effect. Although plasma cortisol levels fluctuated for the first 2 hr, no significant change was seen during the experiment in any of the groups (Fig. 2).

Figure 3 shows plasma FA levels 1 hr after intra-arterial injection of various doses of desacetyl- α -MSH. Plasma FA levels increased significantly at doses of 0.01 to 1 ng/g, and the effect was dose-dependent.

Effects of intra-peritoneal injection of desacetyl- α -MSH on hepatic triacylglycerol lipase activity were assessed by measuring breakdown of triacylglycerol *in vitro* by the lipase fraction partially purified from the liver. Saline injection did not affect the enzyme activity when compared to that of the intact

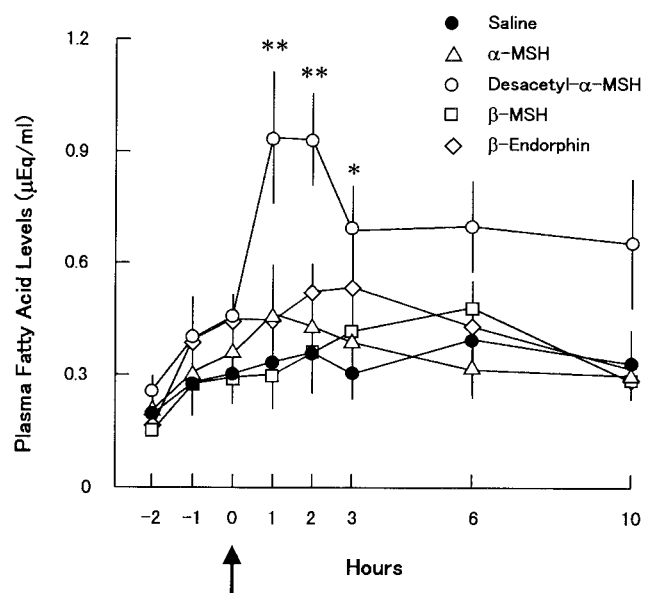


Fig. 1. Effect of POMC-derived peptides, α -MSH, desacetyl- α -MSH, β -MSH and β -endorphin on plasma FA levels in rainbow trout. Fish were injected with the peptides intra-arterially at a dose of 1 ng/g body weight at time 0 (arrow). Data are expressed as means $\pm$ SEM ($n = 4$). *, **Significantly different from saline-injected control at each time point at $P < 0.05$, 0.01, respectively.

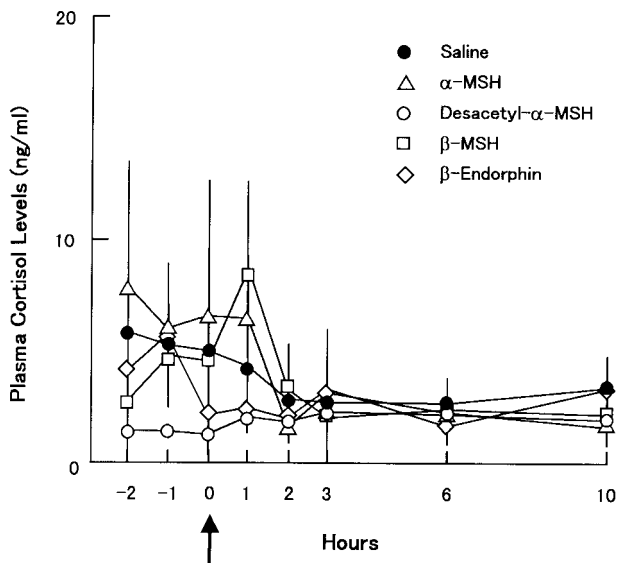


Fig. 2. Effect of POMC-derived peptides, α -MSH, desacetyl- α -MSH, β -MSH and β -endorphin on plasma cortisol levels in rainbow trout. Fish were injected with the peptides intra-arterially at a dose of 1 ng/g body weight at time 0 (arrow). Data are expressed as means $\pm$ SEM (n=4).

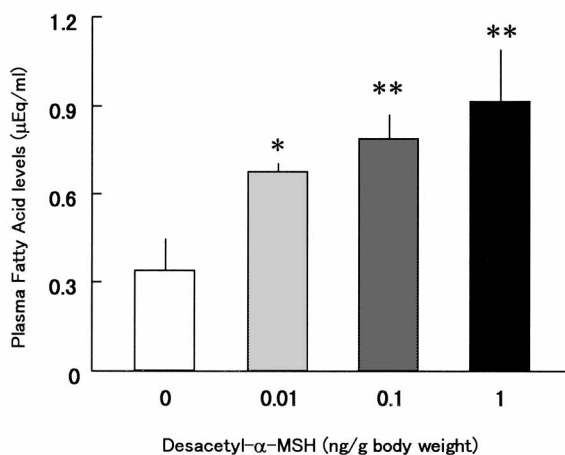


Fig. 3. Effect of desacetyl- α -MSH on plasma FA levels in rainbow trout 1 hr after intra-arterial injection. Data are expressed as means $\pm$ SEM (n=4). *, **Significantly different from saline-injected control (0 dose) at $P < 0.05$, 0.01, respectively.

fish. Significant stimulation by desacetyl- α -MSH was observed at 10 and 100 ng/g (Fig. 4). As shown in Fig. 5, an increase in triacylglycerol lipase activity was observed in the liver slices cultured in the medium containing the desacetyl- α -MSH at 10, 100 or 1000 nM, respectively.

DISCUSSION

In mammals and elasmobranchs, adrenocorticotrophic hormone (ACTH) has a significant lipotropic action (Rudman *et al.*, 1963; Ramachandran *et al.*, 1976; deRoos and deRoos, 1992). ACTH is one of the final products processed from POMC in the anterior pituitary (Krieger *et al.*, 1980). Both in

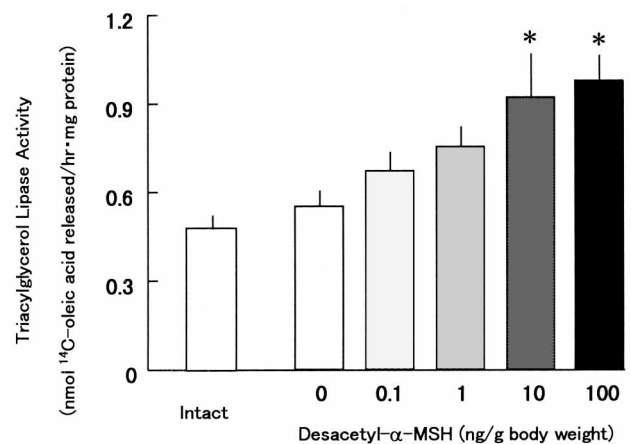


Fig. 4. Effect of desacetyl- α -MSH on triacylglycerol lipase activity in liver of rainbow trout 3 hr after intra-peritoneal injection. Data are expressed as means $\pm$ SEM (n=5). *Significantly different from saline-injected control (0 dose) at $P < 0.05$.

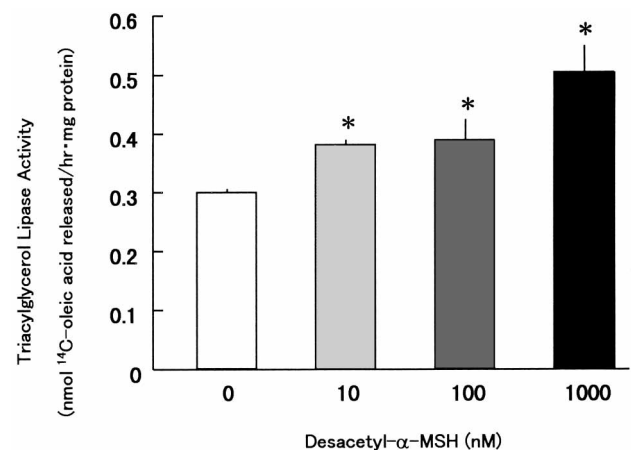


Fig. 5. *In vitro* effect of desacetyl- α -MSH on triacylglycerol lipase activity in liver of rainbow trout after incubation for 3 hr. Data are expressed as means $\pm$ SEM (n=4). *Significantly different from control (0 dose) at $P < 0.05$.

mammals and teleosts, the amino acid sequence of α -MSH is identical with residues 1–13 of ACTH. The lipotropic action of α -MSH has been examined in comparison with ACTH in several mammalian species (Rudman *et al.*, 1963; Ramachandran *et al.*, 1976). According to Takashima *et al.* (1972), however, intra-peritoneal administration of ACTH had no effect on lipid mobilization in the rainbow trout. β -Lipotropin is another final product from POMC in the anterior pituitary in mammals, and is known to affect lipid mobilization (Krieger *et al.*, 1980). However, β -lipotropin has not been detected in the salmon pituitary; β -lipotropin seems to be further processed to β -MSH and β -endorphin in the intermedial lobe of the pituitary (Kawauchi, 1983).

Acetylation of the N-terminus of α -MSH causes a decrease in lipolytic potency in rabbit adipocytes *in vitro* (Li *et al.*, 1975; Kawauchi *et al.*, 1984). On the other hand, acetylation of the N-terminus prolongs the half-life of α -MSH and increases its potency *in vivo* (Rudman *et al.*, 1983). In this study,

intra-arterial administration of desacetyl- α -MSH increased circulating levels of FA, whereas α -MSH had no effect. In the mammalian brain, desacetyl- α -MSH is the major α -MSH (Loh *et al.*, 1980). In the pituitary gland, however, desacetyl- α -MSH is one of the minor types except for human fetal pituitary (Tilders *et al.*, 1981). In the tilapia (*Oreochromis mossambicus*) pituitary, contents of acetyl α -MSH are higher than the desacetyl form (Balm *et al.*, 1995). By contrast, desacetyl- α -MSH is the major α -MSH observed in the salmon pituitary (Kawauchi *et al.*, 1984). Although the β -MSH content is also high in the salmon pituitary (Kawauchi *et al.*, 1984), administration of β -endorphin did not affect plasma FA levels significantly. Results obtained in the present study suggest that desacetyl- α -MSH has an important role in stimulating lipid mobilization among the peptides secreted from MSH cells in the trout.

Lipids are stored in adipose tissue in higher vertebrates, while fish store lipids in several tissues including the liver (Sheridan, 1994). Triacylglycerol is the primary lipid storage molecule in vertebrates, and lipid mobilization is controlled by an intracellular triacylglycerol lipase (Sheridan and Kao, 1998). Intra-arterial injection of desacetyl- α -MSH caused a significant increase in plasma FA levels at 0.01 ng/g, although the other peptides derived from POMC did not show any significant effect even at 1 ng/g. Administration of desacetyl- α -MSH stimulated hepatic triacylglycerol lipase both *in vivo* and *in vitro*. Effective doses of desacetyl α -MSH on the cultured liver slices *in vitro* were comparable to those of catecholamines effective in the liver slices of coho salmon (*Oncorhynchus kisutch*); the minimum effective dose of norepinephrine was 1 nM and the maximum stimulation occurred at 10 μ M (Sheridan, 1987). Increased plasma FA levels after intra-arterial injection of this type of α -MSH seem to be a result of the stimulated breakdown of lipids and release from the storage tissues.

Several endocrine systems such as catecholamines, insulin, glucagon, somatostatin, thyroid hormones, growth hormone and prolactin are known to regulate lipid metabolism in fish (Sheridan, 1986, 1987; Sheridan *et al.*, 1987; Plisetskaya *et al.*, 1989a, b; van Raaij *et al.*, 1995). Estrogen is also thought to stimulate lipid deposition related with vitellinogenesis (see Sheridan, 1994). Cortisol is the major corticosteroid in teleosts, and also is known to stimulate lipase activity (Sheridan, 1986). Plasma levels of cortisol are increased in response to stress (see Barton, 1997; Sumpter, 1997). In brown trout (*Salmo trutta*) and tilapia, plasma levels of α -MSH are also increased by stress (Sumpter *et al.*, 1985; Balm *et al.*, 1995). Plasma cortisol levels in the cannulated trout in this study were essentially similar to the basal levels observed in the previous study (Sumpter *et al.*, 1985), suggesting that intra-arterial injection of the hormones and blood sampling was done under minimum stress. Furthermore, lipolysis in the liver slice was stimulated by desacetyl- α -MSH *in vitro*. Thus, it is unlikely that the effect of desacetyl- α -MSH on lipolysis is mediated by cortisol.

In this study, single intra-peritoneal injection of desacetyl- α -MSH did not affect skin color of the trout (data not shown).

This observation is in agreement with the finding that tilapia treated with acetyl α -MSH did not show visible change in skin color (van Eys and Peters, 1981). In contrast to effects in amphibians and elasmobranchs, MSH does not regulate rapid background adaptation of teleosts (Bowley *et al.*, 1983; Rodrigues and Sumpter, 1984). In teleosts, MSH is involved in background adaptation by morphological processes including an increase in the number of dermal melanophores and melanin content in the melanophores (van Eys and Peters, 1981). At any rate, the duration of MSH administration in this study seems to be too short to affect skin color and background adaptation.

This study shows the stimulatory action of α -MSH on lipid mobilization in the trout. In the "cobalt" variant of the rainbow trout, sparseness of MSH cells in the pituitary seems to be related to an abnormal fat accumulation in the liver and the mesenteric adipose tissue (Oguri, 1976; Kaneko *et al.*, 1993). The findings in the present study seem to support the hypothesis that lack of endogenous MSH causes abnormal lipid mobilization in the "cobalt" variant. On the other hand, qualitative changes in lipid composition occur during the period of parr-smolt transformation in salmonids (Sheridan *et al.*, 1983, 1985). Although several hormones are implicated in the changes in lipid metabolism during parr-smolt transformation in salmonids, involvement of POMC-derived peptides including α -MSH is still unclear (Sheridan and Kao, 1998). Further studies using "cobalt" are now in progress in order to clarify the role of endogenous MSH in physiological changes in lipid metabolism in the trout.

ACKNOWLEDGMENTS

We are grateful to Professor Tetsuya Hirano and Ms Claire Ball, University of Hawaii, for valuable discussions and critical review of the manuscript. We are indebted to Professor Hiroshi Kawauchi, Kitasato University, and Professor Nobuko Naito, Showa University, for their encouragement during the course of this study. We are also indebted to Mr. Saburo Oda and Mr. Kohji Muto, National Research Institute of Aquaculture at Nikko, for their help in maintaining the fish in good condition. This study was supported by a grant-in-aid from the Ministry of Agriculture, Forestry and Fisheries, Japan.

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(Received April 5, 2000 / Accepted May 12, 2000)