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

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

ZOOLOGICAL SCIENCE **17**: 1123–1127 (2000)

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

Takashi Yada[1]*, Teruo Azuma[1], Akiyoshi Takahashi[2], Yoshiro Suzuki[3], and Shigehisa Hirose[3]

[1]*Nikko Branch, National Research Institute of Aquaculture, Nikko, Tochigi 321-1661,*
[2]*School of Fisheries Science, Kitasato University, Sanriku, Iwate 022-0101,*
[3]*Department 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.

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

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.

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 administrated 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 fluoroimmunoassay (Yamada *et al.*, 1996).

***In vivo* effect on hepatic lipase activity**

Fish, weighing 100–150 g, were anesthetized with 0.02% 2-phenoxyethanol 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 $NaHCO_2$ (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 $MgSO_4$, 1 mM KH_2PO_4, 20 mM $NaHCO_3$, pH 7.5. The homogenate was centrifuged at 1,000×g for 10 min at 0°C. The infranatant 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 $MgSO_4$, 1 mM KH_2PO_4, 20 mM $NaHCO_3$, 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-^{14}C]-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

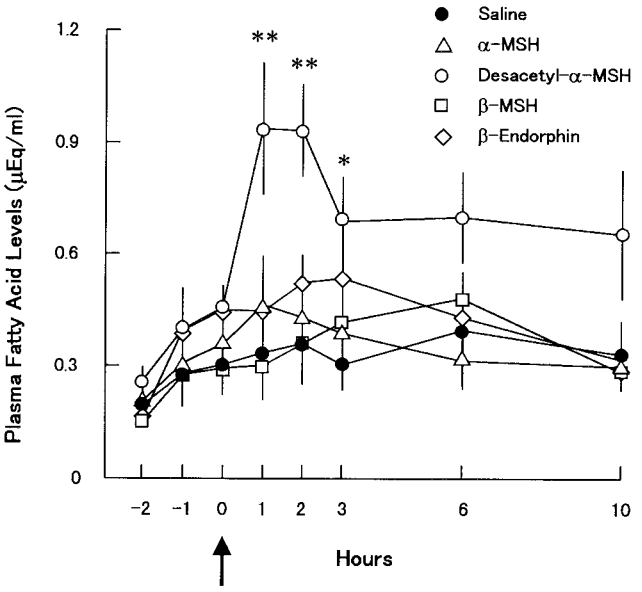

Fig. 1. Effect of POMC-derived peptides, α-MSH, desacetyl-α-MSH, β-MSH and β-endorphin on plasma FA levels in rainbow trout. Fish were injected with the peptides intra-arterially at a dose of 1 ng/g body weight at time 0 (arrow). Data are expressed as means ±SEM (n = 4). *,**Significantly different from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

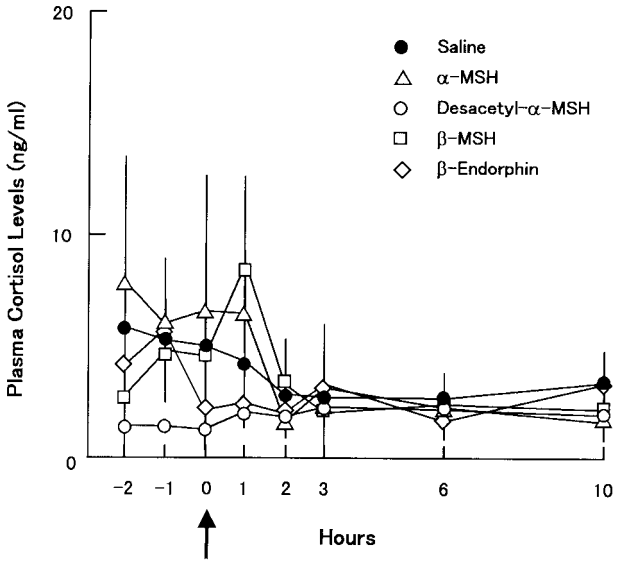

Fig. 2. Effect of POMC-derived peptides, α-MSH, desacetyl-α-MSH, β-MSH and β-endorphin on plasma cortisol levels in rainbow trout. Fish were injected with the peptides intra-arterially at a dose of 1 ng/g body weight at time 0 (arrow). Data are expressed as means ± SEM (n=4).

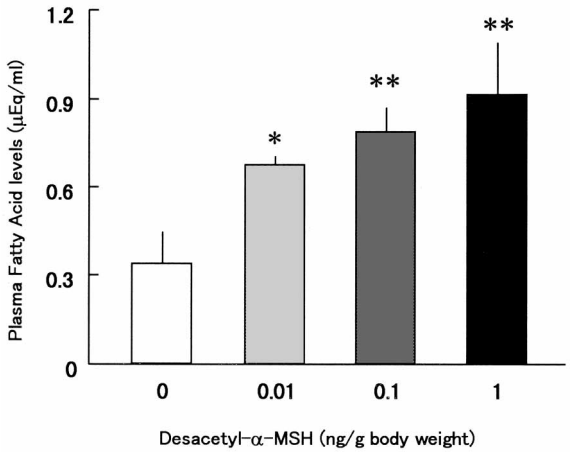

Fig. 3. Effect of desacetyl- α-MSH on plasma FA levels in rainbow trout 1 hr after intra-arterial injection. Data are expressed as means ± SEM (n=4). *,**Significantly different from saline-injected control (0 dose) at P <0.05, 0.01, respectively.

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, adrenocorticotropic 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

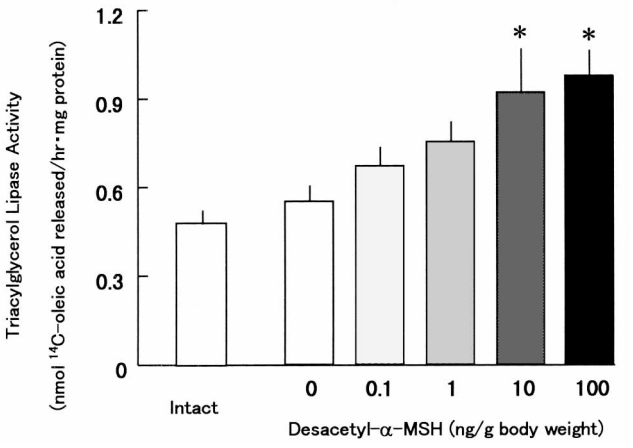

Fig. 4. Effect of desacetyl-α-MSH on triacylglycerol lipase activity in liver of rainbow trout 3 hr after intra-peritoneal injection. Data are expressed as means ±SEM (n=5). *Significantly different from saline-injected control (0 dose) at P <0.05.

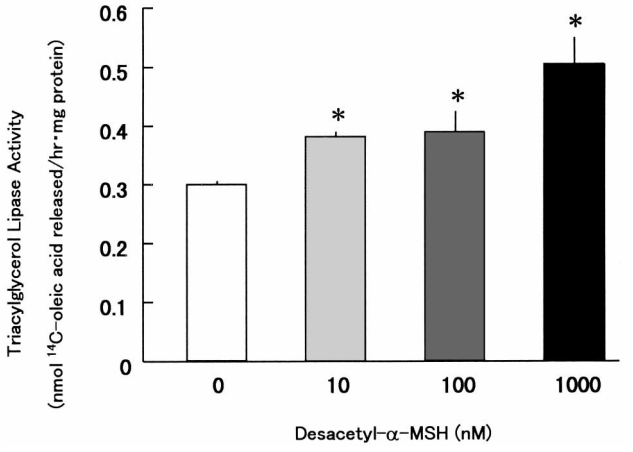

Fig. 5. *In vitro* effect of desacetyl-α-MSH on triacylglycerol lipase activity in liver of rainbow trout after incubation for 3 hr. Data are expressed as means ±SEM (n=4). *Significantly different from control (0 dose) at P <0.05.

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 desacethyl 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 μM (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.

REFERENCES

Balm PHM, Hovens MLM, Wendelaar Bonga SE (1995) Endorphin and MSH in concert form the corticotropic principle released by tilapia (*Oreochromis mossambicus*; Teleostei) melanotropes. Peptides 16: 463–469

Bowley TJ, Rance TA, Baker BI (1983) Measurement of immunoreactive α-melanocyte-stimulating hormone in the blood of rainbow trout kept under various conditions. J Endocrinol 97: 267–275

Barton BA (1997) Stress in finfish: past, present and future — a historical perspective. In "Fish Stress and Health in Aquaculture" Ed by GK Iwama, AD Pickerling, JP Sumpter, CB Schreck, Cambridge University Press, Cambridge, pp 1–33

deRoos R, deRoos C (1992) Effects of mammalian ACTH on potential fuels and gluconeogenic substrates in the plasma of the spiny dogfish shark (*Squalus acanthias*). Gen Comp Endocrinol 87: 149–158

Harris J, Bird DJ (1998) Alpha-melanocyte stimulating hormone

(α-MSH) and melanin-concentrating hormone (MCH) stimulate phagocytosis by head kidney leucocytes of rainbow trout (*Oncorhynchus mykiss*) *in vitro*. Fish Shellfish Immunol 8: 631–638

Ishimatsu A, Iwama GK, Heisler N (1988) *In vivo* analysis of partitioning of cardiac output between systemic and central venous sinus circuits in rainbow trout: a new approach using chronic cannulation of the branchial vein. J Exp Biol 137: 75–88

Kaneko T, Kakizawa S, Yada T (1993) Pituitary of "cobalt" variant of the rainbow trout separated from the hypothalamus lacks most pars intermedial and neurohypophysial tissue. Gen Comp Endocrinol 92: 31–40

Kawauchi H (1983) Chemistry of proopiocortin-related peptides in the salmon pituitary. Arch Biochem Biophys 227: 343–350

Kawauchi H, Muramoto K (1979) Isolation and primary structure of melanotropins from salmon pituitary glands. Int J Peptide Protein Res 14: 373–374

Kawauchi H, Kawazoe I, Adachi Y, Buckley DI, Ramachandran J (1984) Chemical and biological characterization of salmon melanocyte-stimulating hormones. Gen Comp Endocrinol 53: 37–48

Khoo JC, Steinberg D (1981) Hormone-sensitive lipase from chicken adipose tissue including the separation and purification of monoglyceride lipase. In "Methods in Enzymology Vol 71" Ed by JM Lowenstein, Academic Press, New York, pp 627–636

Krieger DT, Liotta AS, Brownstein MJ, Zimmerman EA (1980) ACTH, β-lipotropin, and related peptides in brain, pituitary, and blood. Rec Prog Horm Res 36: 277–344

Loh, YP, Eskay RL, Brownstein M (1980) α MSH-like peptides in rat brain: identification and changes in level during development. Biochem Biophys Res Commun 94: 916–923

Li CH, Danho WO, Chung D, Rao AJ (1975) Isolation, characterization, and amino acid sequence of malanotropins from camel pituitary glands. Biochemistry 14: 947–952

Oguri M (1976) On the enlarged liver in "cobalt" variant of rainbow trout. Bull Japan Soc Sci Fish 42: 823–830

Oguri M (1983) Dermal melanophores and renal melanin-containing cells in the "cobalt" variant of rainbow trout. Bull Japan Soc Sci Fish 49: 1679–1681

Plisetskaya EM, Sheridan MA, Mommsen TP (1989a) Metabolic changes in coho and chinook salmon resulting from acute insufficiency in pancreatic hormones. J Exp Zool 249: 158–164

Plisetskaya EM, Ottolenghi C, Sheridan MA, Mommsen TP, Gorbman A (1989b) Metabolic effects of salmon glucagon and glucagon-like peptide in coho and chinook sakmon. Gen Comp Endocrinol 73: 205–216

Ramachandran J, Farmer SW, Liles S, Li CH (1976) Comparison of the steroidogenic and melanotropic activities of corticotropin, α-melanotropin and analogs with their lipolytic activities in rat and rabbit adipocytes. Biochim Biophys Acta 428: 347–354

Rodrigues KT, Sumpter JP (1984) Effects of background adaptation on the pituitary and plasma concentrations of some pro-opiomelanocortin-related peptides in the rainbow trout (*Salmo gairdneri*). J Endocrinol 101: 277–284

Rudman D, Brown SJ, Malkin MF (1963) Adipokinetic actions of adrenocorticotropin, thyroid-stimulating hormone, vasopressin, α- and β-melanocyte-stimulating hornones, fraction H, epinephrine and norepinephrine in the rabbit, guinea pig, hamster, rat, pig, and dog. Endocrinol 72: 527–543

Rudman D, Hollins BM, Kutner MH, Moffitt SD, Lynn MJ (1983) Three types of α-melanocyte-stimulating hormone: bioactivities and half-lives. Am J Physiol 245: E47–E54

Salbert G, Chauveau I, Bonnec G, Valotaire Y, Jego P (1992) One of the two trout proopiomelanocortin messenger RNAs potentially encodes new peptides. Mol Endocrinol 6: 1605–1613

Sheridan MA (1986) Effects of thyroxin, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. Gen Comp Endocrinol 64: 220–238

Sheridan MA (1987) Effects of epinephrine and norepinephrine on lipid mobilization from coho salmon liver incubated *in vitro*. Endocrinol 120: 2234–2239

Sheridan MA (1994) Regulation of lipid metabolism in poikilothermic vertebrates. Comp Biochem Physiol 107B: 495–508

Sheridan MA, Kao Y-H (1998) Regulation of metamorphosis-associated changes in the lipid metabolism of selected vertebrates. Amer Zool 38: 350–368

Sheridan MA, Allen WV, Kerstetter TH (1983) Seasonal variations in the lipid composition of the steelhead trout, *Salmo gairdneri* Richardson, associated with the parr-smolt transformation. J Fish Biol 23: 125–134

Sheridan MA, Woo NYS, Bern HA (1985) Changes in the rates of glycogenesis, glycogenolysis, lipogenesis, and lipolysis in selected tissues of the coho salmon (*Oncorhynchus kisutch*) associated with parr-smolt transformation. J Exp Zool 236: 35–44

Sheridan MA, Plisetskaya EM, Bern HA, Gorbman A (1987) Effects of somatostatin-25 and urotensin II on lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus kisutch*. Gen Comp Endocrinol 66: 405–414

Sumpter JP (1997) The endocrinology of stress. In "Fish Stress and Health in Aquaculture" Ed by GK Iwama, AD Pickerling, JP Sumpter, CB Schreck, Cambridge University Press, Cambridge, pp 95–118

Sumpter JP, Pickering AD, Pottinger TG (1985) Stress-induced elevation of plasma α-MSH and endorphin in brown trout, *Salmo trutta* L. Gen Comp Endocrinol 59: 257–265

Takahashi A, Amemiya Y, Nozaki M, Sower SA, Joss J, Gorbman A, Kawauchi H (1995) Isolation and characterization of melanotropins from the lamprey pituitary glands. Int J Pept Protein Res 46: 197–204

Takashima F, Hibiya T. Phan-van N, Aida K (1972) Endocrinological studies on lipid metabolism in rainbow trout-II. Effects of sex steroids, thyroid powder and adrenocorticotropin on plasma lipid content. Bull Japan Soc Sci Fish 38: 43–49

Tilders FJH, Parker Jr. CR, Barnea A, Porter JC (1981) The major immunoreactive α-melanocyte-stimulating hormone (αMSH)-like substance found in human fetal pituitary tissue is not αMSH but may be desacetyl αMSH (adrenocorticotropin $_{1–13}$ NH_2). J Clin Endocrinol Metab 52: 319–323

van Eys GJJM, Peters PTW (1981) Evidence for a direct role of α-MSH in morphological background adaptation of the skin in *Sarotherodon mossambicus*. Cell Tissue Res 217: 361–372

van Raaij MTM, van den Thillart GEEJM, Hallemeesch M, Balm PHM, Steffens AB (1995) Effect of arterially infused catecholamines and insulin on plasma glucose and free fatty acids in carp. Am J Physiol 268: R1163–R1170

Watanuki N, Takahashi A, Yasuda A, Sakai M (1999) Kidney leucocytes of reinbow trout, *Oncorhynchus mykiss*, are activated by inteaperitoneal injection of β-endorphin. Vet Immunol Immunopathol 71: 89–97

Yamada H, Satoh R, Yamashita T, Iwata M, Kambegawa A (1996) Time-resolved fluoroimmunoassay (TR-FIA) of steroid hormones using antigen immoibilization method. Proc 3rd Int Sympo on Fish Endocrinology, Hokkaido University, Hakodate, p 169

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