STUDIES ON THE "COBALT" VARIANT OF RAINBOW TROUT

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ABSTRACT

The "cobalt" variant of rainbow trout *Oncorhynchus mykis* lacks most of the pars intermedia of the pituitary in which melanocyte-stimulating hormone (MSH) cells are distributed. An excessive accumulation of fat in the abdominal cavity is also observed in variant. In this study, the role of MSH on lipid metabolism of the trout and the possible relation to fat accumulation in the cobalt variant were investigated. In the light muscle, dark muscle, liver, and mesenteric fat, the cobalt variant showed higher contents of triacylglycerol (TG) than the normal trout. When α-MSH with an unacetylated –terminus (N-Des-Ac-α-MSH) was administered to the normal trout, circulating levels of fatty acid and lipolytic activities in the liver increased. N-Des-Ac-α-MSH also stimulated TG lipolysis in the cultured liver slices from both the normal and the cobalt variant of the trout. Those findings indicate the importance of MSH as a lipotropic hormone in the trout, and the lack of MSH cells in the pituitary remnant of the cobalt variant appears to be related to the abnormal accumulation of fat. Studies of the cobalt variant of trout may provide valuable information for pituitary research, for example, in studies of the functions of hormones produced by the pars intermedia and the early development of the pituitary.

INTRODUCTION

A blue-colored variant of rainbow trout *Oncorhynchus mykiss* appears rarely at trout experimental stations and commercial trout farms in Japan. This variant is often referred to as the "cobalt trout" because of the cobalt-blue body color. Another characteristic of this variant is the excessive accumulation of fat in the abdominal cavity, suggesting an abnormality in lipid metabolism (Kaneko et al. 1993).

Anatomical and histological studies reveal that the cobalt variant of rainbow trout has an irregularly-shaped pituitary which is completely detached from the hypothalamus (Fig. 1), and situated in the region ventral to the usual location of the pituitary. A normal adenohypophysis is divided into three regions: rostral pars distalis, proximal pars distalis, and pars intermedia. In the pituitary of the cobalt trout, there are few of the somatolactin and melanocyte-stimulating hormone (MSH) cells usually distributed in the pars intermedia of a normal pituitary (Kaneko et al. 1993). In higher vertebrates, MSH show a weak, but significant, lipolytic activity (Ramachandran et al. 1976). This study investigates the role of MSH on lipid metabolism of the rainbow trout and its possible relation to the accumulation of fat in the cobalt variant.

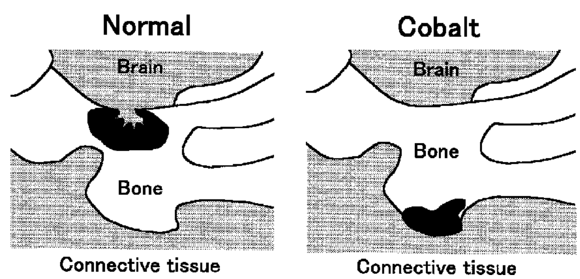

Figure 1. Location of the pituitary in the midsagittal plane of the normal and cobalt variant of the rainbow trout. Black area indicates the pituitary.

MATERIALS AND METHODS

Cobalt-variant were collected from Shiga Prefectural Samegai Trout Farm (Maibara, Shiga) and from Shizuoka Prefectural Fuji Trout Farm (Fujinomiya, Shizuoka). Normal rainbow trout were hatched and reared at the National Research Institute of Aquaculture, Nikko Branch. Both the cobalt variant and normal trout were reared separately in indoor rectangular tanks supplied with spring water (10 C). The light muscle, dark muscle, liver, and mesenteric fat were quickly removed, and lipids were extracted from these tissues using the method of Folch et al. (1957). Lipid classes were separated by thin-layer chromatography on CHROMATOROD S III (Iatron Laboratories, Tokyo, Japan), and triacylglycerol (TG) contents were quantified by a IATROSCAN MK-5 flame-ionization detector (Iatron Laboratories).

To investigate the effect of MSH on circulating fatty acid levels, normal trout were cannulated via the dorsal aorta, and a saline solution containing salmon α-MSH, α-MSH with an unacetylated –terminus (N-Des-Ac-α-MSH), β-MSH, or β-endorphin was injected via the cannula. Blood samples were then taken from the cannula into a syringe and centrifuged at 3000 x g for 5 min. Concentrations of plasma fatty acid were measured using a commercial kit; NEFA C-TestWako (Wako, Osaka, Japan).

In vivo and *in vitro* experiments for lipolysis of TG essentially followed the protocol of Plisetskaya et al. (1989). For the *in vivo* experiment, fish were injected intraperitoneally with saline containing N-Des-Ac-α-MSH. Three h after injection, the liver was removed, frozen on dry ice and stored at -80 C. For the *in vitro* experiment, the liver slices were incubated in RPMI 1640 (pH 7.8) containing N-Des-Ac-α-MSH at 20 C. After a 3-h incubation period, the liver slices were frozen on dry ice and stored at -80 C. TG lipase activity was determined as breakdown of ^{14}C-triolein to ^{14}C-oleic acid *in vitro* (Khoo and Steinberg 1981; Sheridan et al. 1985).

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

RESULTS AND DISCUSSION

Triacylglycerol contents in the light muscle, dark muscle, liver, and mesenteric fat of the cobalt trout were significantly (*P* <0.01) higher than those of the normal trout (Fig. 2). They were also higher than the TG contents in tissues of the same species (steelhead trout) during parr-smolt transformation (Sheridan et al. 1983). These results suggest decreased lipolytic activities (hydrolysis of TG) in the cobalt variant of the trout.

Our previous study shows that the number of MSH cells in the pituitary of the cobalt trout is fewer than in the pituitary of the normal trout (Kaneko et al. 1993). MSH is derived from the precursor molecule, pro-opiomelanocortin (POMC), as isolated and identified from salmon pituitary tissue (Kawauchi and Muramoto 1979; Kawauchi et al. 1984). In rat and rabbit, MSH

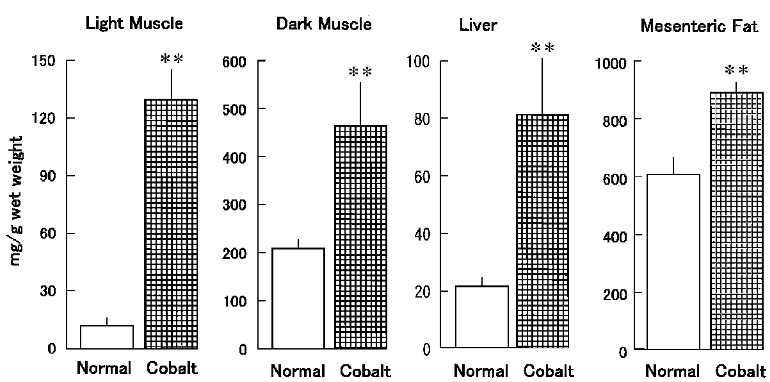

Figure 2. Triacylglycerol contents in the light muscle, dark muscle, liver and mesenteric fat of the normal and cobalt variant of the rainbow trout. Data are expressed as means ± SEM (n = 6). **Significantly different from the normal trout at *P* < 0.01.

cells show weak but significant lipolytic activity (Ramachandran et al. 1976). In salmon MSH cells, POMC is thought to be processed to α-MSH, N-Des-Ac-α-MSH, β-MSH and β-endorphin (Kawauchi et al. 1984). Changes in plasma levels of fatty acids after a single intra-arterial administration of these four peptides derived from POMC are shown in Fig.3. Administration of N-Des-Ac-α-MSH showed a significant ($P < 0.01$) increase in plasma levels of fatty acids, while the other 3 peptides showed no significant effect. The *in vivo* effect of various doses of N-Des-Ac-α-MSH on TG lipolysis in the liver are shown in Fig. 4. Intraperitoneal administration of N-Des-Ac-α-MSH stimulated TG lipase activity in the liver significantly ($P < 0.05$) at dosages of 10 and 100 ng/g BW. In isolated rabbit adipocytes, salmon N-Des-Ac-α-MSH shows the highest lipolytic activity among 7 peptides derived from salmon POMC; 3 types of α-MSH, 2 types of β-MSH, corticotrophin (ACTH), and N-terminal peptide of pro-opiocortin (Kawauchi et al. 1984). On the other hand, lipolytic activity of homologous POMC-derived peptides on salmonids is not known. This study revealed that salmon N-Des-Ac-α-MSH revealed a high potential to stimulate lipolysis in the rainbow trout.

As shown in Fig. 5, *in vitro* administration of N-Des-Ac-α-MSH also stimulated TG lipase activity in the liver slices from the trout, suggesting the direct effect of this peptide on hepatocytes. Stimulation of lipolysis of TG by N-Des-Ac-α-MSH was observed in the normal and the cobalt variant of the trout. This result shows that the cobalt variant still has a lipolytic response to MSH, at least in the liver, and suggests that the lack of this hormone secreted from the pituitary is one reason for the significant obesity in the cobalt variant. Recent studies in higher vertebrates revealed several specific sequences which are necessary for pituitary organogenesis (Takuma et al. 1998; Treier et al. 1998; Lin et al. 1999). Further studies on the cobalt variant may provide valuable information of the pituitary

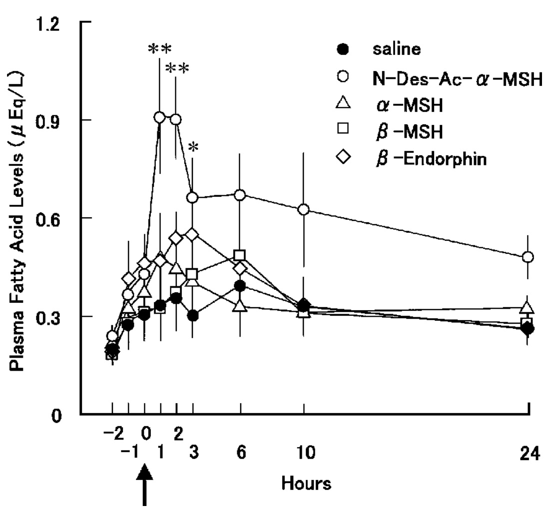

Figure 3. Effect of intra-arterial injection of POMC-derived peptides (1 ng/g body weight) on plasma fatty acid levels in the normal rainbow trout. Data are expressed as means ± SEM (n = 4). *,**Significantly different from control at $P < 0.05$, 0.01, respectively.

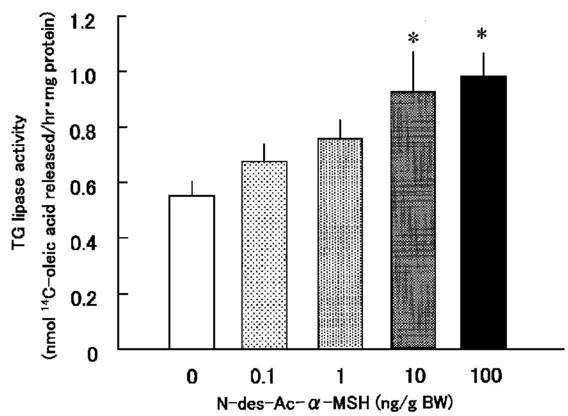

Figure 4. Effect of *in vivo* administration of N-des-acetyl-”-MSH on TG lipase activity in the liver of the normal rainbow trout 3 h after intraperitoneal injection. Data are expressed as means ± SEM (n = 5). *Significantly different from control at $P < 0.05$.

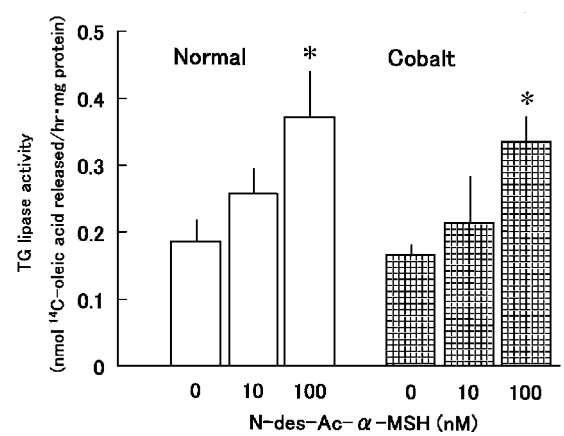

Figure 5. Effects of *in vitro* administration of N-des-Ac-”-MSH on TG lipase activity in the liver slices from the normal and cobalt variant of the rainbow trout. Data are expressed as means ± SEM (n = 4-5). *Significantly different from the control at $P < 0.05$.

ogranogenesis and the possible application of hypophyseal hormones to aquaculture.

ACKNOWLEDGMENTS

The authors thank Prof. S. Hirose and Dr. Y. Suzuki, Tokyo Institute of Technology, Prof. H. Kawauchi and Dr. A. Takahashi, Kitasato University, for their generous support during the course of experiments. Gratitude is also expressed to Prof. N. Okamoto, Tokyo University of Fisheries, Mr. T. Akutsu, Shizuoka Prefectural Fuji Trout Farm, and Mr. N. Sawada, Shiga Prefectural Samegai Trout Farm, for the provision of the precious fish and their valuable comments. This study was supported by a grant-in-aid from the Ministry of Agriculture, Forestry and Fisheries, Japan.

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