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Alpha-MSH, the melanocortin-1 receptor and background adaptation in the Mozambique tilapia, *Oreochromis mossambicus*

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Abstract

The regulation of skin darkness in vertebrates is mediated by α-melanophore-stimulating-hormone (αMSH). For this action, αMSH binds to the melanocortin (MC)-1 receptor, a 7-transmembrane receptor located in melanophore cell membranes. The Mozambique tilapia, *Oreochromis mossambicus*, can change the hue of its body in response to a change in background, a process that may involve αMSH and the MC1R. Scale melanophores were isolated from tilapia that were acclimatised for 25 days to a black, control grey or white background and then tested for their sensitivity to des-, mono-, and di-acetylated αMSH. On all backgrounds, mono-acetylated αMSH was the dominant isoform present in pituitary homogenates. Mono-acetylated αMSH also had the highest potency to disperse melanosomes. Black background adapted fish showed the highest dispersing response to αMSH, independent of the isoform applied. We elucidated the nucleotide and amino acid sequence of the tilapia MC1R. We show that its expression in skin does not change when tilapia are acclimatised for 25 days to a black, grey or white background, while a clear change in hue is visible. This finding, combined with the absence of differential MC1R gene expression following background acclimation indicates that the increased sensitivity to αMSH is most likely a result of changes in the intracellular signalling system in melanophores of black background adapted fish, rather than up-regulation of the MC1R.

Keywords: αMSH isoforms; Tilapia; *Oreochromis mossambicus*; Melanocortion-1 receptor; Background adaptation; Melanophores

1. Introduction

α-Melanophore-stimulating-hormone (αMSH) is known for its role in the skin pigmentation of vertebrates (Bagnara and Hadley, 1973). Darkening of the skin and its derivatives (hair, fur, and feathers) in mammals and birds is slow and can take up to weeks to fully develop. However, in lower vertebrates such as amphibians and fish, skin melanophores quickly change appearance due to fast movements of dark pigment (melanin) granules, melanosomes, within the melanophore. This enables these animals to show a rapid change of the hue (observable colour of the skin), in response to changes of the background (Healey, 1999; Roubos, 1997). Both the slow darkening process in mammals and birds, and the rapid responses in lower vertebrates can be stimulated by αMSH.

Peptides are often modified post-translationally by glycosylation, amidation or acetylation. α-MSH is found in three different N-terminal acetylation isoforms: des-, mono-, and di-acetylated αMSH. In mammals, as in most other species where post-translational acetylation of αMSH occurs, the major form is

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diacetyl αMSH (Dores et al., 1993; Keller et al., 1994). In a number of fish species, however, including tilapia, the dominant form is mono-acetyl αMSH (Arends et al., 2000; Dores et al., 1993; Lamers et al., 1991). Acetylation modifies the bioactivity of the peptides (Keller et al., 1994).

The Mozambique tilapia, *Oreochromis mossambicus*, uses body pigmentation as a means to communicate with conspecifics. The social status of an individual is read from its darkness and pigmentation pattern of the skin. Next to that, tilapia is able to adjust the pigmentation of its skin to the background it is kept upon (Eys and vanPeters, 1981). A black skin pigmentation can be induced by in vivo administration of mono-acetylated αMSH. This indicates that αMSH has melanotropic potency in tilapia and may be involved in the process of background adaptation. Interestingly, during prolonged acid water stress, a shift occurs in the ratio between di- and mono-acetylated αMSH in plasma, in favour of the di-acetylated isoform. This led Lamers et al. (1992) to propose a corticotrope role for di-acetylated αMSH (Lamers et al., 1992). In a study by Rudman et al. (1983), it was shown that acetylation of the peptide increased the melanotropic potency in a frog skin bioassay (relative potency: di = mono > des-acetylated αMSH) and prevented the degradation of αMSH (di > mono > des-acetylated αMSH). In salmon, mono-acetylated αMSH was also more potent than des-acetylated αMSH to stimulate melanosome dispersion in a frog test (Kawauchi et al., 1984).

The bioactivity of a peptide is determined by the receptor(s) it binds to. In most vertebrates, the control of pigmentation of the skin by αMSH is regulated via the melanocortin (MC) 1 receptor that is localised in the membrane of melanocytes (Cone et al., 1996). This receptor was first designated the αMSH receptor, as binding of αMSH induced a darkening of the skin. In mammals, this MC1R has the highest affinity for αMSH of all five receptor subtypes (named MC1 to MC5 receptors; Schiöth et al., 1995). The MC2R is specific for ACTH and located mainly in the adrenal tissue. The MC3, MC4, and MC5 receptors can all be found in the brain, the MC3 (e.g., adrenal cortex, placenta) and MC5 (e.g., exocrine glands, muscle) receptors are also expressed in a multitude of peripheral organs (Cone et al., 1996). In fish, contradicting findings have been reported. Studies on Japanese pufferfish, *Takifugu rubripes*, and rainbow trout, *Oncorhynchus mykiss*, show that in both species most of the MC receptors have a higher affinity for ACTH than for αMSH (Haitina et al., 2004; Klovins et al., 2004).

In this article, we compare the scale melanophores of fish adapted to three different backgrounds (black, white, and grey background tanks) in their response to the three isoforms of αMSH. We present the cDNA and deduced amino acid sequence of the melanocortin-1- receptor of Mozambique tilapia and have quantitated expression of the MC1R in these background adapted fish.

2. Materials and methods

2.1. Animals

Male and female tilapia were obtained from laboratory stock ($n = 24$ per background). Fish weighed around 70 g and were kept in 50 L tanks containing tap water of pH 7.8. Water temperature was 24 °C and fish were kept at a day/night rhythm of 12 L:12 D. Fish were fed commercial tilapia food (Tilapia 3.0, Trouw, Putten, The Netherlands). The walls of each experimental tank were covered with self-adhesive black (black background; B) or white foil (white background; W). The control tanks (control full-glass, grey background; G) were fitted with light-permeable one-sided see-through foil. In this way, disturbance of the fish by external movements or other stimuli was kept at a minimum and was similar for all groups, and the fish kept on the full-glass, grey underground served as extra controls, comparable to the background situation experienced in the rearing tanks.

2.2. Sampling

For scale melanophore studies, three fish from every background were lightly anaesthetised in 0.1% (w/v) 2-phenoxyethanol (Sigma). Scales were taken from the left-hand side of the fish and placed in a physiological salt solution (169 mM NaCl; 5.4 mM KCl; 1.8 mM $CaCl_2$; 1.3 mM $MgCl_2$; 5 mM Tris; and 5.6 mM D-glucose) during transfer to the microscope. Fish were returned to the experimental tanks and not sampled again for a week to enable regrowth of scales.

For MC1R expression studies, six fish from every background were euthanized in 0.2% (w/v) 2-phenoxyethanol. Blood was drawn from the caudal vessels, with syringes containing Na_2EDTA as anticoagulant, and transferred to ice-cold Eppendorfs containing 1 TIU of aprotinin, a serine-protease inhibitor. The blood was spun at 4 °C for 10 min at 13,500 rpm, after which the supernatant plasma was stored in Eppendorf vials and quickly frozen. Fish were subsequently placed on ice, and a piece of head skin and several scales from the left-hand side of the fish were transferred to sterilised Eppendorfs and immediately frozen in dry ice. Samples were stored at −80 °C until RNA isolation.

2.3. αMSH determination

The αMSH concentration in the plasma and in the pituitary gland was determined as described by Arends et al. (1999). The antiserum used for the αMSH radio