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A preliminary investigation of the role of melanin-concentrating hormone (MCH) and its receptors in appetite regulation of winter flounder (*Pseudopleuronectes americanus*)

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ABSTRACT

In order to better understand the role of melanin-concentrating hormone (MCH) in the regulation of appetite in fish, the mRNAs of two forms of MCH, prepro-MCH and MCH2, and two forms of MCH receptors, MCH-R1 and MCH-R2, were isolated from winter flounder (*Pseudopleuronectes americanus*). In addition, the mRNA expressions of these peptides and their receptors were determined under fed and fasted conditions. Both MCHs are expressed in forebrain and midbrain, as well as peripheral tissues including gut and gonads. Both MCH-Rs are ubiquitously expressed in the brain and periphery. Fasting induced an increase in the expression levels of MCH and MCH-R1 mRNAs in optic tectum/thalamus and hypothalamus but had no effect on either MCH2 or MCH-R2 mRNA expressions. Our results suggest that MCH and MCH-R1, but not MCH2 and MCH-R2 might have a role in the regulation of appetite in flounder.

1. Introduction

The endocrine mechanisms of appetite regulation in fish consist in a complex system. Numerous intrinsic (i.e. energy and reproductive status) and extrinsic (i.e. photoperiod and environmental color) factors influence how food intake is synchronized with the energy requirements of an individual (Volkoff et al., 2010). The regulation of feeding in vertebrates has been shown to be under the control of peptides that are produced either within the brain or in the periphery. Central feeding-stimulating (orexigenic) hormones include orexins (OX) (Volkoff et al., 1999; Nakamachi et al., 2006; Yokobori et al., 2011) and neuropeptide Y (NPY) (Lopez-Patino et al., 1999), whereas α-melanocyte-stimulating hormone (α-MSH) (Shimakura et al., 2008a,b) cocaine-amphetamine regulated transcript (CART) (Volkoff and Peter, 2001) and gonadotropin-releasing hormone (GnRH) (Hoskins et al., 2008; Matsuda et al., 2008) are central appetite-inhibiting (anorexigenic) peptides. Ghrelin is an example of an orexigenic hormone found in the periphery (stomach) (Terova et al., 2008; Miura et al., 2009), while leptin, synthesized in liver (Johnson et al., 2000) and adipose tissue (Ronnestad et al., 2010) represents an anorexigenic peptide.

Melanin-concentrating hormone (MCH) is a 17-amino acid peptide that was first identified in fishes as a hormone that regulates color change (Kawauchi et al., 1983). Although in most teleosts only one form of MCH has been identified, two or more variants of MCH have been isolated in Japanese flounder (*Paralichthys olivaceus*), zebrafish (*Danio rerio*) (Berman et al., 2009), green-spotted and Japanese pufferfish (*Tetraodon nigroviridis* and *Takifugu rubripes*, respectively) and salmonids (Ono et al., 1988; Baker et al., 1995). In zebrafish, one of the forms, MCH2, is most similar to mammalian MCH (Berman et al., 2009), while the other, MCH, appears to have closer resemblance to other teleost MCH amino acid sequences.

Early hypothalamic lesion studies in mammals showed that MCH is released by the ventromedial hypothalamus (VMH), a brain area implicated in the control of feeding (Deray et al., 1994; Griffond et al., 1995). Recent studies in mammals and fish have demonstrated that, in addition to its role in regulating skin color, MCH can indeed act as a neuromodulator of food intake directly or via other appetite-related peptides (Santollo and Eckel, 2008; Shimakura et al., 2008a,b). In rodents, MCH treatments increase food intake (Rossi et al., 1997; Gomori et al., 2003), MCH-deficient animals display decreases in feeding and body weight (Shimada et al., 1998) and fasted animals display greater brain MCH mRNA expression than fed animals (Presse et al., 1996; Qu et al., 1996), suggesting that MCH acts as an orexigenic factor in mammals.

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In fish, the exact role of MCH in the regulation of feeding remains unclear as few studies have been conducted. In barfin flounder (*Verasper moseri*), MCH mRNA levels and numbers of brain immunoreactive (ir) cell bodies are higher when fish are fasted, suggesting an orexigenic effect of MCH in this species (Takahashi et al., 2004). In zebrafish, MCH2 mRNA abundance increases with fasting suggesting that MCH2 enhances food intake (Berman et al., 2009). On the other hand, goldfish (*Carassius auratus*) injected with MCH display decreased food intake via the melanocortin-4 receptor (MC4-R) signaling pathway, suggesting an anorexigenic effect of MCH (Matsuda et al., 2006; Shimakura et al., 2008a,b). α-MSH, the MC4-R ligand, is a potent anorexigenic factor that antagonizes the effects of MCH in goldfish. Double-staining immunofluorescence analyses in goldfish demonstrate the connections between MCH-ir nerves endings and α-MSH cell bodies throughout the diencephalon, but more specifically in the hypothalamus (Shimakura et al., 2008a,b). The mediation of the actions of MCH by MC4-R has also been observed in rats (Cerdá-Reverter et al., 2003).

MCH regulation has been linked to background color, as MCH brain mRNA expression and plasma levels increase in several fish species when animals are placed in white tanks as compared to dark tanks (Green et al., 1991; Groneveld et al., 1995; Amiya et al., 2008). Correlations between background color, somatic growth and MCH mRNA expression have been determined in barfin flounder (Takahashi et al., 2004), where both greater growth rates and high MCH are seen in white-adapted animals and could be indicative of an increase in food intake.

In humans (*Homo sapiens*) and lower vertebrates, there are at least two G-protein-coupled MCH receptors (MCH-R), whereas only one receptor, MCH-R1, has been isolated in rodents. Post-mortem analyses of cachectic (with loss of weight and muscle mass) humans demonstrate 1.6 times higher MCH-R1 mRNA expression in the arcuate nucleus, a known feeding center in the hypothalamus, than in normal humans, which suggests a role for this receptor in energy homeostasis (Unmehopa et al., 2005). Furthermore, MCH-R1-deficient mice tend to be hypophagic and resistant to obesity with high-fat diets (Chen et al., 2002) and do not increase their food intake when injected intracerebroventricularly (ICV) with MCH (Marsh et al., 2002). In addition, MCH-R1 antagonists (Takekawa et al., 2002; Shearman et al., 2003) and agonists (Shearman et al., 2003) have been shown to mimic and inhibit the orexigenic stimulation of MCH on feeding, respectively.

Few studies have looked at the functional importance of MCH-R2 in animals. In mammals, MCH-R2 has only been characterized in dogs (*Canis familiaris*) and ferrets (*Mustela putorius*) (Tan et al., 2002), rhesus monkeys (*Macaca mulatta*) (Fried et al., 2002) and humans (Sailer et al., 2001). The only evidence that MCH-R2 is involved in energy homeostasis is that MCH-R2 is present is human adipose tissue (Hill et al., 2001) and mediates the differentiation of preadipocytes into mature cells when exposed to MCH (Yang et al., 2009).

Fish appear to have at least two MCH-Rs. In goldfish, MCH-Rs (MCH-R1 and MCH-R2) are present in the brain and are postulated to mediate the central effects of MCH (Mizusawa et al., 2009). In goldfish, both MCH-Rs are also present in several peripheral tissues, including skin, where MCH-Rs might mediate color changes, as well as intestine and fat tissue, where they might regulate appetite and energy homeostasis (Mizusawa et al., 2009). Three MCH-Rs (MCH-R1a, MCH-R1b and MCH-2R) have been identified in zebrafish (Logan et al., 2003). MCH-R1a and MCH-R1b are thought to be a product of the teleost-derived whole genome duplication (WGD) event as they are both orthologues of human and mouse MCH-R1. When the MCH-R1 gene is knocked down in zebrafish, melanosome dispersal is impaired (Richardson et al., 2008), indicating a role for MCH-R1 in mediating color changes. In barfin flounder, MCH-R2 is present throughout the body and appears to mediate widespread MCH effects, including the induction of melanosome dispersal (Takahashi et al., 2007; Mizusawa et al., 2009). No studies have ever examined the function of fish MCH receptors with respect to energy homeostasis.

Winter flounder (*Pseudopleuronectes americanus*) is a bottom-dwelling flatfish inhabiting the shores of Newfoundland. These fish are readily available year round and represent an interesting model to study seasonal feeding behaviour as they undergo a period of fasting during the winter months. Surprisingly, this fasting occurs before spawning, at a time when animals increase their gonadosomatic index (GSI, or gonadal weight/body weight ratio) (Stoner et al., 1999).

In this study, cDNAs for two variants of MCH (MCH and MCH2) and MCH-Rs (MCH-R1 and MCH-R2) were isolated in winter flounder. To further typify the transcripts encoding these peptides and their receptors, we examined their central nervous system – including pituitary – and peripheral tissue distributions. In order to assess a possible role of MCH peptides in the regulation of feeding in winter flounder, the effects of fasting on the brain mRNA expression of both peptides and receptors were assessed.

2. Materials and methods

2.1. Animals

Winter flounder brain tissue used for cloning was sampled from 3 to 4 male and female wild fish collected by scuba divers along the shore of St. John's (Logy Bay, Newfoundland and Labrador, Canada). After collection, fish were kept in 2 m × 2 m flow through tanks at the Ocean Sciences Centre (OSC, Memorial University of Newfoundland, St. John's, NL, Canada). Fish were kept under natural photoperiod and temperature conditions (12 °C) and fed frozen herring to satiety two or three times a week at the same time of the day (10:00).

Fish for the food deprivation experiment were obtained by seining and held at the Bonne Bay Marine Station (BBMS; Norris Point, NL, Canada). Fish (five per tank) were maintained in four white 0.5 m × 0.5 m flow through tanks with a sandy substrate to imitate their natural environment, at ambient water temperatures and lighting (see below). Males and females were used with an approximate 50:50 ratio in each treatment. Fish were fed cut up frozen squid every 2–3 days at the same time of the day (21:00) due to scheduling constraints. On the sampling day, fish were fed 1 h prior to sampling. Weights were obtained before the start of experiment and during sampling. Gonad and liver weights were measured for calculation of GSI and hepatosomatic index (HSI). All experiments were conducted in accordance with the principles found in the Canadian Council on Animal Care guide.

2.2. Food deprivation experimental design

Winter flounder ($n = 20$; average weight of 115.59 ± 22.67 g) were acclimated for two weeks in four tanks (five fish per tank) under natural photoperiod and an average water temperature of 10 °C (July 6th to July 30th 2009). The same feeding regimen was used as previously described (Section 2.1). Following acclimation, two tanks continued to be fed (controls), while two tanks were food deprived for 10 days. Duplicate tanks were used to account for any tank effect. Following experimentation, fish were sacrificed with an overdose (100 mg/L) of tricaine methanesulfonate (Syndel Laboratories, Vancouver, British Columbia, Canada), and brains were dissected and stored in RNAlater (Qiagen Inc., Mississauga, Ontario, Canada) at −20 °C until further processing.