Available online at www.sciencedirect.com
ScienceDirect
Hormones and Behavior 54 (2008) 506–513
Hormones and Behavior
www.elsevier.com/locate/yhbeh

Disruption of seasonality in growth hormone-transgenic coho salmon (*Oncorhynchus kisutch*) and the role of cholecystokinin in seasonal feeding behavior

Mare Lõhmus *, Peter A. Raven, L. Fredrik Sundström, Robert H. Devlin

Fisheries and Oceans Canada, Centre for Aquaculture and Environmental Research, 4160 Marine Drive, West Vancouver, V7V 1N6, BC Canada

Received 28 November 2007; revised 11 February 2008; accepted 11 February 2008
Available online 10 March 2008

Abstract

Seasonal variation in daily food intake is a well-documented phenomenon in many organisms including wild-type coho salmon where the appetite is noticeably reduced during periods of decreased day length and low water temperature. This reduction may in part be explained by altered production of cholecystokinin (CCK) and growth hormone (GH). CCK is a hormone produced in the brain and gut that mediates a feeling of satiety and thus has an inhibitory effect on food intake and foraging behaviour. Growth hormone (GH) enhances feeding behaviour and consequently growth, but its production is reduced during winter. The objectives of this study were: first, to compare the seasonal feeding behaviour of wild and GH-transgenic coho salmon; second, to determine the behavioural effect of blocking the action of CCK (by using devazepide) on the seasonal food intake; and third, to measure CCK expression in brain and gut tissues between the two genotypes across seasons. We found that, in contrast to wild salmon, food intake in transgenic salmon was not reduced during winter indicating that seasonal control of appetite regulation has been disrupted by constitutive production of GH in transgenic animals. Blocking of CCK increased food intake in both genotypes in all seasons. The increase was stronger in wild genotypes than transgenic fish; however blocking CCK in wild-type fish in winter did not elevate appetites to levels observed in the summer. The response to devazepide was generally faster in transgenic than in wild salmon with more rapid effects observed during summer than during winter, possibly due to a higher temperature in summer. Overall, a seasonal effect on CCK mRNA levels was observed in telencephalon with levels during winter being higher compared to the summer in wild fish, but with no seasonal effect in transgenic fish. No differences in seasonal CCK expression were found in hypothalamus. Higher levels of CCK were detected in the gut of both genotypes in winter compared to summer. Thus, CCK appears to mediate food intake among seasons in both wild-type and GH-transgenic salmon, and an altered CCK regulation may be responsible at least in part for the seasonal regulation of food intake.

Keywords: Cholecystokinin; Seasonal effects; Transgenic coho salmon

Introduction

Feeding behaviour in animals is influenced by both intrinsic and extrinsic factors including body energy reserves, stress and social interactions (Bernier, 2006; Boujard et al., 2000; Dallman et al., 2004; Konturek et al., 2004; Lõhmus and Sundström, 2004; Øverli et al., 1998). Additionally, feeding frequency and duration can be strongly influenced by environmental elements such as temperature and photoperiod that change seasonally in non-tropical regions (Brett, 1979). Such seasonal variation is reflected by rhythmic patterns of many biochemical processes, physiological functions and behaviours in nature, including daily food intake and growth in many organisms including fish (Björnsson, 1997; Brett, 1979; Lincoln and Richardson, 1998; Rhind et al., 2001). Periods of increased daylight are generally associated with warmer weather, greater food availability, and stimulated appetite which is partly mediated by endocrine and neural factors that influence seasonal levels of circulating growth hormone (GH) secreted by the pituitary gland (Figueroa et al., 2005; Pierce et al., 2001; Webster et al., 1996).

GH is a principal regulator of somatic growth in salmonids and its secretion is influenced by both neural and endocrine signals (Björnsson, 1997). Major metabolic effects of GH include

* Corresponding author.
E-mail address: mare.lohmus@zool.gu.se (M. Lõhmus).

0018-506X/$ - see front matter © 2008 Published by Elsevier Inc.
doi:10.1016/j.yhbeh.2008.02.010

the stimulation of lipid mobilization and protein accretion, but GH has also been shown to increase feeding behaviour in fish (Björnsson et al., 2002). It is not known whether GH affects the central nervous system directly or indirectly through metabolic or downstream endocrine effects (Björnsson, 1997; Jönsson et al., 2003). GH may pass through the blood-brain barrier and stimulate its own receptor in the CNS affecting neuroendocrine secretion of appetite regulating peptides such as neuropeptide Y, bombesin, and cholecystokinin (Johansson et al., 2005).

Circulating levels of GH change with season and are correlated with altered feeding behaviour (Björnsson, 1997). Melatonin, a hormone secreted from the pineal gland in response to darkness is one of the main regulators of organisms' circadian rhythms and has been shown to have a direct negative effect on GH secretion in many different organisms, including teleost fish (Boeuf and Falcon, 2001; Falcon et al., 2003; Lincoln and Richardson, 1998; Zeman et al., 1999). Thus, it is conceivable that the decreased appetite of salmonids during the short day period is partly induced by the suppressive effects of melatonin on GH production.

Hatchery-reared transgenic coho salmon constitutively expressing GH possess elevated plasma GH and have daily increased specific growth rates relative to wild type (Devlin et al., 2004, 1994; Mori and Devlin, 1999; Oakes et al., 2007; Raven et al., 2006). The production of GH in non-pituitary tissues in transgenic salmon leads to suppressed production of endogenous GH in the pituitary through negative feedback regulation, which may at least in part uncouple such fish from brain-regulated GH production (Mori and Devlin, 1999). The increased growth rate of transgenic fish is associated with a strong enhancement of appetite and food intake relative to wild-type (Devlin et al., 1999; Oakes et al., 2007; Raven et al., 2006), and results in salmon of the same age having very different sizes compared to wild-type fish (Devlin et al., 2004, 1994). GH-transgenic salmon are also more active and more willing to take risks which leads to an increased competitive foraging ability but decreased predator awareness and survival in the presence of predators (Abrahams and Sutterlin, 1999; Devlin et al., 1999; Sundström et al., 2003, 2004). How the increased production of GH by various non-pituitary tissues affects the normal seasonal changes in foraging behaviour that is presumably adaptive in wild conspecifics, has not yet been investigated.

Although GH may act directly to affecting appetite (Jönsson and Björnsson, 2002), it also acts as a mediator influencing several hormones and peptides (Björnsson, 1997). Cholecystokinin (CCK) is a hormone with appetite satiating effects which in mammals is mediated by CCK_A and CCK_B receptors in the gut and in the brain (Gelineau and Boujard, 2001). However in fish it has been suggested that only one ancestral type of CCK receptor is expressed (Vigna, 2000). In the digestive tract, CCK stimulates gallbladder motility and pancreatic enzyme secretion and delays gastric emptying. Manipulations of the signalling of CCK by use of CCK inhibitors have been explored in attempts to introduce a long-term increase in food intake in domesticated animals and to overcome the adaptive seasonal decrease in food intake during winter (Pekas, 1991; Pekas and Trout, 1990; Rhind et al., 2001; Trout et al., 1989). The results from these studies vary, showing an effect of CCK in some species but not others, suggesting a difference in the physiological role of CCK among species. Inhibition of CCK signalling with a receptor antagonist has been shown to enhance food intake in rainbow trout (Gelineau and Boujard, 2001). Thus, even though CCK seems to be an important component in reducing appetite, the role of CCK in mediating seasonal changes in meal size and pattern is still poorly understood and not investigated in fish.

The present study aimed to investigate the presence of seasonal changes in feeding behaviour in wild and GH-transgenic coho salmon. We also examined the role of CCK to better understand the function of this appetite regulating system in food intake across different seasons. We hypothesized that the effect of CCK inhibition on food intake would be greater in wild-type fish than in transgenic, as the former have generally suppressed appetites compared to the GH-transgenic conspecifics. We also anticipated the general effect of CCK inhibition to be more obvious during winter when food intake is normally suppressed, compared with the summer when the feeding activity should be closer to a physiological maximum in both genotypes. In addition, we investigated winter and summer CCK mRNA levels in hypothalamus and telencephalon, where feeding regulation may be occurring, and that have been revealed to express relatively high amount of CCK (Peyon et al., 1999). Hypothalamus and telencephalon are also high density binding sites for CCK in the fish brain (Canosa and Peter, 2004; Himick et al., 1996; Raven, 2006). As CCK is largely produced by the small intestine, we also measured CCK mRNA levels in the gut. We hypothesized that if CCK is involved in the seasonal control of food intake, the expression of this hormone is expected to be elevated in winter to mediate suppression of feeding behaviour in wild-type fish and, to a lesser extent, in transgenic salmon.

Methods

Study site and species

The study was conducted at the DFO/UBC Centre for Aquaculture and Environmental Research, West Vancouver, Canada which houses a contained aquatic facility designed to prevent the escape of transgenic fish. Coho salmon of wild genotype were the offspring of wild caught parents from the Chehalis River, BC, Canada, and were reared under hatchery conditions from fertilization. Transgenic coho salmon were originally produced by microinjecting eggs from wild parents with the gene construct OnMTGH1 that contains a metallothionein-B promoter driving the over-expression of the type-I GH gene from sockeye salmon (*Oncorhynchus nerka*) (Devlin et al., 1994). The transgenic strain utilized (M77) was maintained through crosses with wild salmon and thus contain on average the same genetic background as the wild-type fish except for the presence of the OnMTGH1 transgene. Experimental transgenic fish were produced by crossing wild caught females from the Chehalis River with transgenic males reared at the experimental facility, and then rearing their offspring under the same hatchery conditions as wild genotype fish. To produce fish that were comparable in size, the same amount of food that satiated wild genotype salmon was provided to the transgenic fish (pair feeding). This feeding regimen was maintained from first feeding through the first (winter, 10 months of age), second (spring, 12 months) and third (summer, 15 months) experimental periods. This approach eliminates age effects when studying transgenic and non-transgenic fish at the same developmental stages, but necessarily introduces the variable of ration restriction of the transgenic strain.

Assessment of feeding behaviour

Transgenic and wild-type salmon (16 individuals of each genotype in three different seasons) were placed singly into 30 L plastic tanks with a transparent front wall. The fish were held in natural daylight conditions without any