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Ghrelin decreases food intake in juvenile rainbow trout (*Oncorhynchus mykiss*) through the central anorexigenic corticotropin-releasing factor system

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ABSTRACT

Ghrelin stimulates pituitary growth hormone (GH) release, and has a key role in the regulation of food intake and adiposity in vertebrates. To investigate the central effect of native rainbow trout ghrelin (rtghrelin) on food intake in rainbow trout, as well as its possible mode of action, four groups of fish received a single injection into the third brain ventricle (i.c.v. injection): (1) control group (physiological saline) (2) ghrelin-treated group (2.0 ng rtghrelin g bwt^{-1}), (3) group given the corticotropin-releasing hormone receptor antagonist α-helical CRF 9–41 (ahCRF) (4.0 ng g bwt^{-1}) and (4) group receiving the same dose of both ghrelin and ahCRF. Food intake was assessed 1 h after treatment. In addition, the presence of the GHS-R (the ghrelin receptor) in the rainbow trout CNS was examined with Western blot. To investigate peripheral effects of ghrelin, rainbow trout received an intraperitoneal cholesterol-based implant with or without rtghrelin, and daily food intake was measured during 14 days. Weight and length were measured at the start and termination of the experiment and specific growth rates were calculated. Mesenteric fat stores, muscle and liver lipid content were analysed after the treatment period. Central ghrelin injections decreased food intake compared with controls, and treatment with ahCRF abolished the ghrelin-effect. Western blot analysis of the GHS-R revealed a single band at around 60 kDa in pituitary, hypothalamus, brain and stomach. Long-term peripheral ghrelin treatment decreased daily food intake compared with controls. This was reflected in a ghrelin-induced decrease in weight growth rate ($p < 0.06$). There was no effect of ghrelin on plasma GH levels or tissue fat stores. The conclusion from this study is that the GHS-R is indicated in the CNS in rainbow trout and that ghrelin may act there as an anorexigenic hormone, through a CRF-mediated pathway. Elevated peripheral ghrelin levels also seem to lead to decreased feed intake in the longer term.

1. Introduction

Ghrelin was first identified in 1999 as the endogenous ligand to an "orphan" receptor, the growth hormone secretagogue-receptor (GHS-R), and thus a potent stimulator of pituitary GH release in rat and human (Kojima et al., 1999). The receptor, also termed the ghrelin receptor (Davenport et al., 2005), has been identified, both at the gene transcript and protein level, in hypothalamic appetite centers as well as in various peripheral tissues in several species (see Kaiya et al. (2008b) for review) *e.g.*, black seabream (*Acanthopagrus schlegeli*) (Chan and Cheng, 2004) rainbow trout (*Oncorhynchus mykiss*) (Kaiya et al., 2009), chicken (Geelissen et al., 2003) and rat (Guan et al., 1997; Mondal et al., 2005; Shuto et al., 2001).

Ghrelin is mainly produced in the stomach of vertebrates and exists in two major forms; the biologically active form having a well conserved fatty acid modification (octanoylation) at the third amino acid, and a non-active form without this modification (Kaiya et al., 2008b; Kojima and Kangawa, 2005). The past few years, the ghrelin gene has been cloned in several fish species, including goldfish (*Carassius auratus*), eel (*Anguilla japonica*), Mozambique and Nile tilapia (*Oreochromis mossambicus and Oreochromis niloticus*), channel catfish (*Ictalurus punctatus*), Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*) rainbow trout and sea bass (*Dicentrarchus labrax*), (Kaiya et al., 2003a,b,c, 2005; Murashita et al., 2009; Parhar et al., 2003; Terova et al., 2008; Unniappan et al., 2002; Xu and Volkoff, 2009).

In line with the gut-brain distribution of the hormone and its receptor, ghrelin has an instrumental role in the regulation food intake and energy balance in various vertebrate groups (Kaiya et al., 2008a,b; Kojima and Kangawa, 2005). In mammals, ghrelin generally promotes food intake, body weight gain and adiposity through central and peripheral modes of action (Choi et al., 2003; Druce et al., 2005, 2006; Tschop et al., 2000; Wren et al., 2000, 2001a,b). In avian species, the effects of ghrelin treatment on food

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intake depend on dose, species, and route of administration (Furuse et al., 2001; Geelissen et al., 2006; Kaiya et al., 2007; Saito et al., 2002, 2005; Shousha et al., 2005). Central ghrelin injections suppress food intake (Furuse et al., 2001; Saito et al., 2002, 2005; Shousha et al., 2005). This effect may be mediated via the central anorexigenic corticotropin-releasing factor (CRF) system as shown in neonatal chicks (Saito et al., 2005). In goldfish, both systemic and central ghrelin treatments increase feeding by stimulating hypothalamic orexigenic peptides, such as neuropeptide Y (NPY) and orexin (Miura et al., 2006, 2007; Unniappan et al., 2002, 2004). This is in line with the current mammalian model (Chen et al., 2004; Kamegai et al., 2001; Toshinai et al., 2003). Also, long-term peripheral ghrelin treatment in Mozambique tilapia increases food intake, and hence body weight gain and hepatic fat deposition (Riley et al., 2005). In rainbow trout, a single intraperitoneal (i.p.) injection of rainbow trout ghrelin (rtghrelin) did not influence food intake when measured for up to 12 h post-injection (Jönsson et al., 2007). Similar administration of rat ghrelin increased food consumption when measured after 2 and 5 h in rainbow trout (Shepherd et al., 2007). More long-term peripheral, as well as CNS studies on the effects of ghrelin on food intake in salmonid species are lacking.

Salmonids are interesting models for the study of energy balance regulation, both due to their importance in aquaculture and their seasonal and life-stage dependent changes in appetite, and hence growth and energy deposition (Kadri et al., 1997; Metcalfe et al., 1986; Metcalfe and Thorpe, 1992). The aim of this study was to further examine the potential role of native ghrelin in food intake regulation in juvenile rainbow trout. Two experiments were carried out: (1) a short-term experiment where fish received a single intracerebroventricular (i.c.v.) injection of rtghrelin and food intake for each fish was assessed 1 h post-injection. The possible involvement of CRF in mediating the effects of ghrelin on food intake at the central level was examined using the CRF receptor antagonist α-helical CRF 9–41 (ahCRF). To further clarify whether the CNS is a target tissue for ghrelin the presence of the GHS-R in the brain was examined with Western blot, and (2) a 2-week treatment study using rtghrelin i.p. implants and measurement of individual food intake along with swimming activity, growth rate, plasma GH levels and tissue fat content.

2. Materials and methods

2.1. Experiment 1: Effects of centrally administered rtghrelin and ahCRF on food intake

2.1.1. Fish, holding and acclimatization conditions

Fish with an average weight of 130 g were transported from a local hatchery (Anten AB) to the experimental facilities at the Department of Zoology at the University of Gothenburg. After the transportation, the fish were kept in indoor tanks with aerated re-circulating fresh water, and allowed to acclimatize for at least 2 weeks before the experiment started. The temperature was kept at 11 °C, and a 12 h light, 12 h dark (12L:12D) photoperiod regime was maintained. Fish were hand-fed 1.5% bwt day^{-1} during this period. The fish were observed during feeding and were seen to feed actively prior to the start of the experiments. Five days prior to the experimental day, six fish (one fish at a time) were randomly netted, anesthetized in 2-phenoxyethanol (0.04%) and body weights and lengths were recorded. Each individual was then transferred to a separate 50-l glass experimental aquarium that was covered with black plastic to prevent disturbance. The aquaria had aerated re-circulating fresh water, photoperiod was set at 12L:12D, and water temperature was kept at 11 °C. The fish were allowed to acclimatize to this environment for 5 days and during this period the fish were fed once daily (2.5% bwt day^{-1}). During this acclimatization period a daily control was made that the fish were feeding and that they were taking pellets from the bottom. Fish that had not fed and lost weight during the acclimatization period were not further used in the injection experiment that was conducted on day 6. The experimental procedure was repeated nine times, giving a total of 54 fish in the experiment.

2.1.2. Experimental design (experiment 1)

Starting at 1000 h on day 6, each fish was rapidly netted, anaesthetized in 2-phenoxyethanol (0.04% v/w), weighed, and placed on a wet towel on a PVC-board to secure its body position. A free-hand injection into the third brain ventricle was carried out using a 30 g microlance needle (0.3 mm) coupled to a 10 μl Hamilton syringe with an 18P cannula (De Pedro et al., 1993; Jönsson et al., 2003). Injections were done between the two optic tecta, which are visible from outside, in line with the base of the eyes, at a depth of 3 mm below the skull. Injection volume was 1 μl. Four treatment groups were included: (1) control group (vehicle, physiological saline), (2) ghrelin-treated group (2.0 ng rtghrelin g bwt^{-1}, octanoylated 23-amino acid form, synthesized by Peptide Institute Inc., Osaka, Japan, see Kaiya et al. (2003a)), (3) group given a corticotropin-releasing hormone receptor antagonist (ahCRF) (4 ng g bwt^{-1}, α-helical CRF 9–41 μl^{-1}; SIGMA), and (4) group receiving the same doses of both ghrelin and ahCRF concurrently. To minimize leakage from the injection site, 15 s were allowed to elapse before the needle was withdrawn. Prior dissection of 50 fish i.c.v.-injected with methylene blue indicated a 90% success rate for delivery to the third ventricle (Jönsson et al., 2003). The effect of the i.c.v. injection *per se* on feeding and swimming activity was also analysed prior to the study, showing no difference between sham-injected and non-injected fish ($p > 0.5$ for both swimming and food intake, data not shown).

The ghrelin dose used (2 ng rtGhr g bw^{-1}) was based on a pilot study, see Fig. 1 for description.

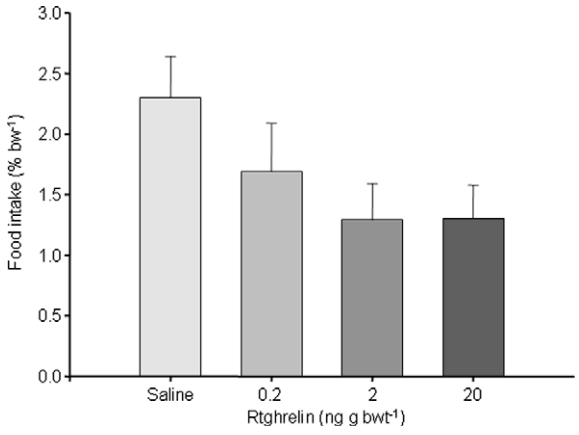

Fig. 1. Data on the effects of rtghrelin on food intake in rainbow trout, obtained in a pilot study which preceded experiment 1. Four groups of juvenile rainbow trout were injected into the third ventricle of the brain with saline (control) and three different doses of rtghrelin, respectively. Food intake was then measured according to the same protocol as used in experiment 1 (see Materials and Methods section under Experiment 1). The pilot experiment had to be terminated early, when only 9–10 fish per group had been injected and studied. This was due to an onset of a fungal infection among the fish in the holding tank, awaiting treatment. None of the fish already used in the experiment were affected. Although not statistically significant due to a low number of fish, a dose-dependent decrease in total food intake 1 h after the rtghrelin injection was indicated ($p = 0.09$, $n = 37$). The data indicate that a maximal response to rtghrelin was similar at 2 and 20 ng g bwt^{-1}. Therefore, the 2 ng g bwt^{-1} rtghrelin dose was chosen for experiment 1. There was no indication of an effect of ghrelin treatment on swimming behaviour ($p = 0.73$), and thus, this was not assessed in experiment 1.