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Neuroendocrine Regulation of Growth Hormone Secretion and Body Growth in Carp: A Review[1]

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Abstract

Growth hormone (GH) secretion in carp is stimulated by a number of neuropeptides (e.g., GH-releasing factor, gonadotropin-releasing hormone, thyrotropin-releasing hormone, etc.) and neurotransmitters (e.g., dopamine). Sex steroids potentiate the responsiveness of the somatotrophs to neuroendocrine factors. Somatostatin is the primary inhibitory control of basal and stimulated GH release. Norepinephrine and serotonin also inhibit GH release. Carp GH, a 188-amino acid peptide with five cysteine residues, controls the rate of body growth. Treatment with a superactive agonist analog of luteinizing hormone releasing hormone (LHRH) by intraperitoneal injection, esophageal intubation or feeding is effective in stimulating GH release and body growth of grass carp *Ctenopharyngodon idella*. Injection or feeding with dopamine receptor agonists (e.g., apomorphine) also stimulates GH relase and body growth of grass carp. Treatment with recombinant fish GH by injection or feeding significantly increased serum GH levels and growth rate of grass carp. Treatment with LHRH-A, dopamine D-1 receptor agonists or exogeneous recombinant GH by feeding constitutes a practical approach to accelerate the growth of cultured carp.

Introduction

In carp, the growth hormone (GH) has been cloned and sequenced in common carp *Cyprinus carpio* (Chao et al. 1989; Korn et al. 1989), grass carp *Ctenopharyngodon idella*, silver carp *Hypophthalmichthys molitrix*, and bighead carp *Aristichthys nobilis* (Chang et al. 1992). The GHs of these cyprinid species all contain 188 amino acids with five cysteine residues as opposed to four residues found in other species of fish and tetrapods. Except for Cys^{123}, the other four cysteines of cyprinid GHs could be aligned at positions corresponding to those of other species (Chang et al. 1992). The homology between the GHs of these four cyprinid species is very high, up to 94% (Koren et al. 1989). Such a high degree of GH homology has also been observed among various species within the same order of fish (82-100% identity); but there is greater diversity between orders (49-68% identity). Fish GHs are even more different from tetrapod GHs (37-58% identity) (Chang et al. 1992).

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The growth-promoting effect of exogenously-administered GH in common carp (Fine et al. 1993) and other teleosts is well established, and endogenous GH is the principal hormone which regulates somatic growth in teleosts. GH stimulates growth through direct action on some tissues, as well as by stimulating production of insulin-like growth factors (IGF). The liver has a high number of GH receptors and is the primary target organ. However, there is no precise relationship between blood GH levels and the rate of somatic growth; and it is increasingly clear that a variety of components within the GH-growth axis interact to determine growth rate in teleosts (Peter and Marchant 1995).

Neuroendocrine Regulation of GH Secretion

The neuroendocrine regulation of GH secretion in goldfish and other carp is multifactorial, with a balance of stimulatory and inhibitory inputs to somtotrophs (Peter and Marchant 1995).

Stimulators of GH Secretion

The GH-releasing factor (GRF) characterized from common carp hypothalamic extracts is a 45-amino acid peptide with 45% amino acid homology to mammalian GRFs (Vaughan et al. 1992). Synthetic common carp GRF is a potent stimulator of GH release from perifused fragments of the goldfish pituitary, and for increasing serum GH levels in goldfish following intraperitoneal injection (Vaughan et al. 1992).

Mammalian GnRH (mGnRH) and sGnRH, and their superactive analogs, stimulate GH secretion in goldfish both *in vivo* and *in vitro* (Marchant et al. 1989; Habibi et al. 1992), common carp *in vitro* (Lin et al. 1993, 1994a), and grass carp *in vivo* (Lin et al. 1994a). In structure-activity studies on native GnRH peptides and their analogs, sGnRH tended to be more potent than mGnRH and cGnRH-II in stimulating GH release from perifused goldfish (Habibi et al. 1992) and common carp (Lin et al. 1994a) pituitary fragments. sGnRH-A (D-Arg^6, Pro^9-NEt-sGnRH) also showed a significantly greater effect than LHRH-A (D-Ala^6, Pro^9-NEt-LHRH) in stimulating GH release from perifused common carp pituitary fragments (Lin et al. 1994a, 1995a). This suggests that development of a superactive GnRH analog that preferentially releases GH may be feasible.

Sex steroids, in particular estradiol, play a role in the seasonal changes in circulating GH levels in goldfish and common carp. Implantation of estradiol, but not testosterone, increases serum GH levels in female goldfish and common carp (Trudeau et al. 1992; Lin et al. 1995a).

Studies of goldfish both *in vivo* and *in vitro* by Chang et al. (1990) and Wong et al. (1992, 1993a) have provided evidence that dopamine functions as a GH-releasing factor. Using a combination of dopamine receptor agonist and antagonist drugs, they have demonstrated that D-1 receptors are specific to this stimulatory effect on GH release. Apomorphine (APO), the dopamine receptor agonist, stimulates GH release from common carp perifused pituitary fragments (Lin et al. 1993a, 1994a). Intraperitoneal

injection of APO in grass carp fingerlings significantly increased serum GH levels (Lin et al. 1994a). Treatment of goldfish by intraperitoneal injection with APO or by addition of APO to the food increases serum GH levels and growth rates (Wong et al. 1993b, 1993c). Intraperitoneal injection of dopamine and APO consistently stimulated GH release in a dose-dependent manner during gonadal development and maturation of common carp; the maximal GH level was observed in fish in the gonadal recrudescing stage (Lin et al. 1994b). Overall, these data indicate that dopamine plays an important role in the stimulation of GH release in carp.

Intraperitoneal injections of thyrotropin-releasing hormone (TRH) in goldfish stimulate an increase in serum GH levels (Cook and Peter 1984). THR is highly potent in stimulating GH release from goldfish perifused pituitary fragments; pituitaries from sexually mature fish have a greater sensitivity to TRH than pituitaries from sexually regressed fish (Trudeau et al. 1992). Common carp pituitary is also highly responsive to TRH *in vitro* (Lin et al. 1993b).

Neuropeptide Y (NPY), a 36-amino acid peptide, is potent in stimulating GH release in goldfish (Peng et al. 1990, 1993). The stimulation of GH release by NPY involves two components, a direct action on pituitary cells, and stimulation of GnRH release from neurosecretory terminals in the pituitary.

The sulfated form of cholecystokinin (CCK-8s) is highly effective in stimulating GH release from perifused goldfish pituitary fragments (Himick et al. 1993). Preliminary data presented by Himik et al. (1993) indicate that bombesin also stimulates GH release from goldfish pituitary *in vitro*.

Inhibitors of GH Release

Somatostatin-14 (SRIF-14) inhibits basal GH levels both *in vivo* (Cook and Peter 1984) and *in vitro* (Marchant et al. 1987) in goldfish. SRIF-14 is also a potent inhibitor of stimulated GH release *in vitro* in goldfish (Marchant et al. 1989; Peng et al. 1993; Wong et al. 1993a, 1993b) and in common carp (Lin et al. 1993a), as well as *in vivo* in goldfish (Wong et al. 1993b). Obviously, SRIF-14 is the primary inhibitory control of basal and stimulated GH release in fish.

Both norepinephrine (NE) and serotonin (5-HT) have inhibitory action on GH release *in vitro* in goldfish (Peter et al. 1990; Somoza and Peter 1991; Wong 1993), and the action is directly on somatotrophs (Wong 1993). Intraperitoneal injection of NE also suppresses serum GH levels in goldfish (Chang et al. 1985) and grass carp (Lin et al. 1994a). The inhibitory actions of NE are similar to SRIF-14 in that it can completely suppress the stimulatory actions of sGnRH and dopamine on GH *in vitro* (Wong 1993). These results indicate that the neurotransmitter NE and 5-HT functions as an inhibitor for GH release in carp.

Effects of Recombinant Fish GH on Growth Rate

Exogenous GH can be administered to fish via a number of routes to accelerate body growth. The uptake of GH from the gastrointestinal tract of fish has also been reviewed (McLean and Donaldson 1990; Sire and Vernier 1992). It is clear that biologically active proteins and peptides can be absorbed intact from the gastrointestinal tract into the blood of fish. Recent advances in recombinant DNA technology provide the means to produce GH on a scale suitable for commercial application. We have compared the effectiveness of injection, oral administration by intubation and by feeding of recombinant tuna GH (r-tGH) in stimulating growth rate of juvenile grass carp (Lin et al. 1995b). Intraperitoneal injection of 0.1 or 1.0 $ug \cdot g^{-1}$ body weight r-tGH every week for 4 weeks resulted in a highly significant increase in growth rate in both body weight and body length, as well as an increase in condition factor and serum GH levels. Fish received treatment every week for 6 weeks with 1.0 or 10 $ug \cdot g^{-1}$ body weight r-tGH by esophageal intubation, or received r-tGH in the diet (15 $ug \cdot g^{-1}$ diet) for 6 weeks. The result was a highly significant increase in the growth rate in weight and length, as well as an increase in condition factor and serum GH level. These results suggest that there is a promising potential in the approach of feeding to administer recombinant fish GH on a large-scale to accelerate growth rate in cultured fish.

Use of Neuroendocrine Factors to Stimulate Body Growth

GH secretion is apparently regulated by several neuroendocrine stimulators working together in concert, and these peptides and amines can also be absorbed intact from the gastrointestinal tract into the blood of fish. Accordingly, addition of a combination of suitable neuroendocrine factors to food may be a cost-effective means to stimulate growth rates in the cultured carp and a wide range of species. Intraperitoneal injection or feeding treatment of APO, a dopamine receptor agonist, stimulates growth rates of goldfish (Wong et al. 1993b, 1993c). Treatment with a superactive agonist analog of GnRH by multiple intraperitoneal injection is also effective in stimulating growth rates of goldfish (Marchant et al. 1989) and common carp (Lin et al. 1995a). Administration of LHRH-A in the diet (1 or 10 $ug \cdot g^{-1}$ diet) for 5 weeks resulted in a highly significant increase in the growth rate of juvenile grass carp (Lin et al. 1995b). These results demonstrate that oral administration of neuroendocrine regulatory factors, with a GnRH agonist (LHRH-A or sGnRH-A) either alone or in combination with the dopamine D-1 agonist, may be a viable way to increase the growth rate of cultured carp by promoting the release of endogenous GH from the pituitary.

In the near future, after further experiments, we hope to develop and refine a dietary formulation of growth enhancing neuroendocrine factors, mainly by using a combination of dopamine D-1 and GnRH agonists, to enhance body growth and to shorten the time to market of cultured fish.

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