Growth hormone secretion from the Arctic charr (*Salvelinus alpinus*) pituitary gland in vitro: effects of somatostatin-14, insulin-like growth factor-I, and nutritional status

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Abstract

This study investigated the influence of nutritional status on the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis in Arctic charr (*Salvelinus alpinus*). The objectives were to study the regulation of GH secretion in vitro by somatostatin-14 (SRIF) and hIGF-I, and to determine whether pituitary sensitivity to these factors is dependent upon nutritional status. Arctic charr were fed at three different ration levels (0, 0.35, and 0.70% BW d⁻¹), and pituitary glands were harvested at 1, 2, and 5 weeks for in vitro study. Both SRIF and hIGF-I inhibited GH secretion from Arctic charr pituitary tissue in long-term (18 h) static hemipituitary culture, as well as after acute exposure in a pituitary fragment perifusion system. This response appeared to be dose-dependent for SRIF in static culture over the range of 0.01–1 nM, but not for hIGF-I. The acute inhibitory action of hIGF-I on GH release in the perifusion system suggests an action that is initially independent of any effects on GH gene expression or protein synthesis. Nutritional status did not affect the sensitivity of Arctic charr pituitary tissue to either SRIF or hIGF-I in vitro, indicating that changes in abundance of pituitary SRIF or IGF-I receptors may not explain the alterations in plasma GH levels found during dietary restriction.

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1. Introduction

The regulation of growth hormone (GH) secretion in fishes is multifactorial, with inputs from both neuroendocrine and circulating sources. Several members of the somatostatin family of peptides have been identified as inhibitors of GH secretion, and this function, as well as peptide structure, has been highly conserved across the vertebrates (Lin et al., 1998). Somatostatin-14 (SRIF) has been conserved with identical primary structure in all vertebrates, and the inhibition of GH release by this peptide has been confirmed in several teleost species (Holloway et al., 1997; Lin and Peter, 2001). Insulin-like growth factor-I (IGF-I), the mediator of many of the actions of GH, is another inhibitor of GH secretion, providing negative feedback on pituitary GH secretion in many vertebrate species. This function has also been confirmed in several fish species (Blaise et al., 1995; Fruchtman et al., 2000, 2002; Huang et al., 1997; Pérez-Sánchez et al., 1992; Rousseau et al., 1997).

It is unclear from the studies in fishes whether the inhibition of GH secretion by IGF-I in vitro is a result of an instantaneous change in the rate of hormone secretion, changes in GH gene expression, or a combination of these possibilities. Striped bass hybrid (*Morone
saxatilis x M. chrysops) somatotroph GH content and protein synthesis are reduced after 18–20 h of exposure of to IGF-I (Fruchtman et al., 2000), and tilapia (Oreochromis mossambicus) pituitary glands treated with IGF-I for 24 h have reduced levels of GH mRNA, although GH secretion does not differ significantly after 4 h (Kajimura et al., 2002). Conversely, injection of IGF-I into catheterized rainbow trout (Oncorhynchus mykiss) resulted in rapid (<60 min) reductions in plasma GH concentrations (Blaise et al., 1995). In rat pituitary cells, IGF-I inhibits GH secretion and reduces levels of GH mRNA expression by somatotrophs in vitro (Niiori et al., 1994). It has been demonstrated that hepatic GH-binding and GH-receptor (Pérez-Sánchez et al., 1994a,b) mRNA expression is reduced during dietary restriction (Straus, 1993), and a reduced plasma IGF-I concentration (Blaise et al., 1995) has been observed in relation to nutritional and hormonal status. The changes in plasma GH concentrations related to nutritional status could be explained by altered pituitary sensitivity to factors that inhibit GH secretion, such as SRIF or IGF-I. Pituitary sensitivity to these factors depends on the abundance of SRIF or IGF-I receptors, and changes in the abundance of these receptors has been observed in relation to nutritional and hormonal status of fish (Pesek and Sheridan, 1996; Planas et al., 2000). Sensitivity to hypothalamic factors that stimulate GH secretion may also be important, however, GHRH does not appear to be as potent in salmonid fishes (Holloway and Leatherland, 1998).

The purpose of the present study was to investigate the effects of SRIF and IGF-I on GH secretion from Arctic charr pituitary glands in vitro, and to determine if nutritional status has an influence on pituitary sensitivity to these factors. Static hemipituitary gland culture was used to study changes in GH secretion after long-term exposure to peptides, whereas pituitary gland perfusion was used for acute exposures.

2. Materials and methods

2.1. Animals

All experimental protocols used in this study were approved by the University of Guelph Animal Care Committee. Arctic charr (Salvelinus alpinus) of the Labrador strain were obtained from the Alma Aquaculture Research Station (Alma, Ont.). Fish had been maintained outdoors in 10 m circular tanks, with a water temperature of 8.5 °C, and were fed a standard commercial diet (Martin Mills, Elmira, Ont.) using demand feeders.

2.2. Experimental design

Approximately 900 male and female Arctic charr (average weight = 397.9 g) were randomly distributed among nine 2 m tanks with a target initial stocking density of 40 km m⁻³. The tanks were located indoors under a natural photoperiod. Water flow rates were adjusted to 30 L min⁻¹; water temperature was 8.5 °C; dissolved oxygen was monitored regularly and remained within normal limits for the duration of the study (8.5–10 mg L⁻¹).

After three days, the fish in all tanks were fed the same commercial diet at 0.70% BW d⁻¹ via automatic belt feeders. This was maintained for a period of 12 weeks during which growth and feed conversion efficiency were monitored. At the beginning of the study (March 11 2002) the tanks were randomly assigned to one of three ration groups (0, 0.35, or 0.70% BW d⁻¹; 3 tanks/ration level). The 0.70% BW d⁻¹ ration was selected as a full ration as the apparent feed conversion efficiencies observed during the 12 week acclimation period indicated that a portion of this ration remained uneaten in some tanks (data not shown). The experimental ration levels were maintained for a period of five weeks.

A sample of 12 fish was taken from each tank after 1, 2, and 5 weeks. Fish were removed from the tanks in the morning prior to feeding (8:30–10:30) and euthanized with MS-222 (>125 mg L⁻¹). Pituitary glands were removed from each fish and placed in ice-cold 80%
HBSS (Gibco-BRL, Canadian Life Technologies, Burlington, Ont.; with 4.17 mM NaHCO₃, 0.1% BSA, pH 7.5, diluted to 80%).

2.3. Static hemipituitary gland culture

Pituitary glands were dissected along the sagittal axis into two equal parts. The hemipituitaries were then rinsed in 80% HBSS, placed individually into single wells of 24-well tissue culture plates (Corning Costar, Corning, NY), and cultured for 1 h in 1 ml of 80% HBSS at 10°C on a shaker platform. The medium was then replaced with 1 ml M199 (Sigma Chemical, St. Louis, MO; with 4.17 mM NaHCO₃, 25 mM Hapes, 0.1% BSA, 0.7 mM L-glutamine, 100 µg ml⁻¹ streptomycin, and 100,000 U L⁻¹ penicillin-G, pH 7.5) and incubated for 4 h at 10°C to establish basal release. The medium was then replaced with either fresh M199 (control halves) or with fresh M199 containing the test substance (treatment halves). Treatments consisted of recombinant human IGF-I (GroPep, Adelaide, Australia) or SRIF-14 (Sigma Chemical) at concentrations of 0.01 or 1 nM. After an 18 h incubation, the medium was removed and stored at −20°C prior to hormone assay.

2.4. Pituitary gland fragment perifusion

The pituitary fragment perifusion procedure is based upon techniques developed for use with goldfish (Marchant et al., 1987; Marchant and Peter, 1989), which were subsequently modified and validated for use with rainbow trout (Holloway and Leatherland, 1997). Pituitary glands were cut into fragments, rinsed with 80% HBSS, and three fragmented glands were incubated in each perifusion chamber. The pituitary fragments were perifused with M199 for 5–6 h at a rate of 5 ml h⁻¹, after which the rate of flow was increased to 15 ml h⁻¹. Sample collection began 2 h after this increase in flow rate.

For the pituitary fragment perifusion validation experiment, 10 min fractions were collected for 7 h, then 5 min fractions were collected for 1 h prior to, and following, treatment. Forskolin (10 µM; Sigma Chemical) was solubilized in DMSO so that the final concentration of DMSO in the test pulse was 0.008%. For pituitary glands collected from fish in the ration level experiment, the collection of 5 min fractions began 2 h prior to the first treatment pulse (hIGF-I or SRIF-14; 0.01 and 1 nM). Four culture chambers were used for each ration treatment group and pituitary glands from each group were exposed to all treatment and dose combinations, with an elapsed time between treatment pulses of 90 to 120 min. To minimize any effect of previous treatment on the results, each incubation chamber within a treatment group received doses in a different order. Fractions were stored at −20°C prior to hormone assay.

2.5. Assays

For measurement of GH secreted in vitro, a non-competitive enzyme-linked immunosorbent assay (ELISA) developed for oncorhynchid species (Farbridge and Leatherland, 1991), modified by Holloway and Leatherland (1997), and validated for use in Arctic charr (Cameron et al., 2002), was used.

2.6. Statistics

The percent of control GH released into the culture medium following treatment for 18 h was compared to the percent of control GH released into medium during basal (pre-treatment) release period using a paired t test. A 2-way ANOVA was used for comparison of percent GH release between different doses and ration levels over the 18 h culture.

For pituitary fragment perifusion data, the average concentration of GH in the 10 fractions prior to a pulse of test substance was normalized to 100%. Each fraction following the pulse was then calculated as a percentage of this prepulse value for comparison. This correction was necessary because of the variation in GH release from individual pituitary glands in culture. The percent of control GH released in each five minute fraction during perifusion following administration of test substance was compared to prepulse values using a Student’s t test. The average maximal inhibition observed within 60 min, as well as the average of the first 10 fractions following a pulse, were compared to the prepulse (100%) using a Student’s t test. In all cases in which perifusion data failed tests of normality, a Mann–Whitney rank-sum test was used. For all statistical tests, a p < 0.05 was considered significant.

3. Results

3.1. Static hemipituitary gland culture

The total GH secreted into the medium from treated hemipituitaries is expressed as a percentage of GH secreted from the control hemipituitaries from fish sampled at 1, 2, and 5 weeks (Figs. 1A–C, respectively). With three exceptions (SRIF at 0.01 nM, and hIGF-I at 1 nM, after 1 week in fish fed the 0.70% BWd⁻¹ ration, and hIGF-I at 0.01 nM after 2 weeks in food-deprived fish), GH secretion from Arctic charr hemipituitary glands was significantly inhibited by SRIF and hIGF-I at both treatment levels (0.01 or 1 nM). However, there was no apparent effect of dietary treatment on GH suppression. There appeared to be a dose-related suppression of GH by SRIF, but no such dose-response was evident for hIGF-I. No significant differences were observed between the percent of control GH release, at any dose of SRIF or hIGF-I, when compared across the three sampling periods.
3.2. Pituitary gland fragment perifusion

Perifused Arctic charr pituitary fragments displayed a 13.8 ± 1.9% fall in GH release into the medium during the 7 h pre-experimental period (data not shown). However, no significant changes in GH secretion, relative to prepulse values, were observed when comparisons were made over a shorter period of time (1–2 h) during the pre-experimental period (1 h prior to application of the forskolin carrier, DMSO, Fig. 2A). Following the application of 0.008% DMSO in M199, small, but significant reductions in GH release were observed. However, treatment of pituitary fragments with 10 μM forskolin elicited a significant increase in GH secretion within 25 min, overriding the suppressive effects of DMSO, with a subsequent return to prepulse levels (Fig. 2B). These findings confirm the viability of the pituitary preparations and their ability to respond to secretagogues in vitro.

Figs. 3 and 4 show examples of the plots generated following exposure of Arctic charr pituitary gland fragments to SRIF and hIGF-I pulses in the perifusion experiments. The data from the full set of these plots are summarized to show the maximal inhibition of GH release from pituitary fragments (Fig. 5). With the exception of 1 nM SRIF in the 0.35 BWd⁻¹ group and 1 nM hIGF-I in the 0.70 BWd⁻¹ group after 2 weeks, both SRIF and hIGF-I significantly inhibited the release of GH in the perifusion experiment as measured by the maximal inhibition of GH release whatever the concentration, sampling date or ration level. Both secretagogues significantly suppressed GH secretion, regardless of time of sample, or of dietary treatments, although it was not always evident for both of the applied doses of the secretagogues.

4. Discussion

This study reveals that SRIF and hIGF-I inhibit GH secretion from Arctic charr pituitary tissue in vitro with similar potency in both static and perifusion culture.
systems. Changes in nutritional status over a five-week period did not result in any concomitant change in the pituitary sensitivity to either SRIF or hIGF-I, suggesting that pituitary sensitivity to these factors may not explain changes in plasma GH concentrations during fasting.

The pituitary fragment perifusion validation demonstrates that the rate of GH secretion from Arctic charr pituitary glands was stable, at least in the timescale over which prepulse values were compared to response values in these experiments. Significant differences in GH release were not observed in the absence of a secretagogue pulse, validating the argument that GH secretion was inhibited by hIGF-I and SRIF rather than any decline in somatotroph function. This experiment also demonstrated that forskolin, an activator of adenylate cyclase, was capable of stimulating GH secretion from Arctic charr pituitary glands, as has also been found previously in other fish species (Chang et al., 1996; Falcón et al., 2003; Kwong and Chang, 1997). This stimulation of GH release from pituitary fragments in the perifusion system confirms the viability of Arctic charr somatotrophs in vitro and their ability to respond to increases in intracellular cAMP.

The inhibition of GH secretion by SRIF and hIGF-I from Arctic charr pituitary glands in static culture was similar to that seen in static rainbow trout pituitary cell cultures (Blaise et al., 1995; Pérez-Sánchez et al., 1992; Weil et al., 1999). The perifusion experiments in the present study revealed that there is a sustained inhibition of GH release following a treatment of Arctic charr pituitary fragments with SRIF. This response differed from that observed in goldfish at similar doses of SRIF, in which the rates of GH secretion return rather rapidly to prepulse levels (Marchant et al., 1987; Marchant and Peter, 1989). In addition, the degree of inhibition of GH release does not appear to be as great in the Arctic charr as compared to these previous studies with goldfish, suggesting a difference in species sensitivity, as was also suggested by Holloway and Leatherland (1998).

The relatively rapid decline in GH output by Arctic charr pituitary fragments exposed to hIGF-I (and SRIF) in perifusion are consistent with responses seen in rainbow trout given injections of hIGF-I in vivo (Blaise et al., 1995). The rapidity of these responses suggests that this early inhibition of GH secretion is at least initially independent of changes in pituitary GH mRNA level or protein synthesis. However, changes in rates of GH gene expression, or other genes involved in GH secretion, may also contribute to these responses after continued exposure. The apparent lack of dose dependency in some groups, which may indicate that maximal inhibition of GH secretion was achieved, may also be due to a
variation in GH output by pituitary preparations combined with a limited secretagogue dose range. The inhibition of GH release from rainbow trout pituitary cells by hIGF-I and SRIF has been demonstrated to be dose-dependent over a much wider range of doses (0.01–100 nM) (Pérez-Sánchez et al., 1992). The doses of hIGF-I used (0.01 and 1 nM; 0.076 and 7.649 ng ml\(^{-1}\), respectively) are within the physiological range reported for several salmonid species, and free IGF-I in the circulation of coho salmon has been estimated to be around 0.3% of total IGF-I (Shimizu et al., 1999). If this relationship can be extended to Arctic charr, then estimates of free IGF-I in Arctic charr plasma (unpublished results) would exceed the doses of hIGF-I employed in the in vitro portion of this study.

It is not possible to determine at which level these factors, particularly hIGF-I, inhibit GH secretion, because the pituitary gland fragments used in both the static and perfusion studies contained both hypothalamic and adenohypophysial tissue. In mammals, IGF-I can regulate GH secretion through modulation of hypothalamic factors such as SRIF and GHRH (Berelowitz et al., 1981; Sato and Frohman, 1993), and similar mechanisms may exist in fish. Thus, the responses observed in this study may include actions at the hypothalamic and pituitary (somatotroph) levels. The quantity and the integrity of the hypothalamic tissue within the pituitary fragments may account for the high individual variation in basal and secretagogue-stimulated GH release in vitro.

The total GH output by control pituitary fragments was similar for all dietary treatment groups (Cameron, 2003). This indicates that nutritional status may not affect the GH secretion potential of the somatotroph, and perhaps other mechanisms are responsible for elevations in plasma GH levels during fasting, such as changes in plasma clearance rates. Alternatively, there may be in vivo differences in the levels of factors controlling GH secretion that are lacking in the in vitro systems. This may include the changes in plasma IGF-I concentrations, but also other hormones linked to nutritional status and GH synthesis and secretion, including several neuropeptides that are involved both in food intake and GH secretion (Lin et al., 2004; Peng and Peter, 1997). Other possibilities include the gut peptide ghrelin, which in goldfish is increased during fasting and stimulates food intake (Unniappan et al., 2004), and has a stimulatory effect on GH secretion in this, and other, fish species (Kaiya et al., 2003; Riley et al., 2002; Unniappan and Peter, 2004).

In summary, the release of GH from Arctic charr pituitary tissue in vitro was inhibited by both SRIF and hIGF-I in both long-term static and short-term perfusion culture. Of particular interest was the ability of hIGF-I to inhibit GH secretion after acute exposure in a manner similar to that of SRIF. Pituitary sensitivity to the two secretagogues was not influenced by the nutritional history of the animal, suggesting that if these peptides are involved in the changes in circulating levels of GH that are observed as a result of altered nutritional status, it is not due to changes in pituitary sensitivity to these factors.

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