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Effects of Human Insulin-Like Growth Factor-I on Release of Growth Hormone by Rainbow Trout (*Oncorhynchus mykiss*) Pituitary Cells

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ABSTRACT A recombinant human IGF-I (rhIGF-I) was applied to primary cultures of rainbow trout pituitary cells. In wells containing 3×10^4 and 6×10^4 cells/well, rhIGF-I inhibited basal GH release both in short (6 h) and long (12 and 24 h) exposures. The decline in GH release was dose-dependent over the range of 0.01 and 100 mM. The combination of rhIGF-I and low concentrations of synthetic somatostatin$_{14}$ (SRIF) enhanced the inhibitory effect of rhIGF-I in an additive manner. Any appreciable effect of rhIGF-I on PRL release was not evidenced. To our knowledge, this report demonstrates for the first time the participation of IGFs on the inhibitory component of fish GH regulation.

In fish as in other vertebrate species, the release of growth hormone (GH) appears to be regulated by two hypothalamic neuropeptides, the inhibitory somatostatin (SRIF) (Fryer et al., '79; Cook and Peter, '84, '86; Marchant et al., '87; Luo et al., '90; Le Bail et al., '91) and the stimulatory growth hormone-releasing factor (GRF) (Luo and Mckeown, '89; Luo et al., '90; Le Bail et al., '91). There are, however, other metabolic factors that interact at the central level and contribute to establishing the control of pituitary GH release. Thus a variety of in vivo (Abe et al., '83; Tannenbaum et al., '83) and in vitro (Berelowitz et al., '81; Brazeau et al., '82; Goodyer et al., '84; Yamashita and Melmed, '86; Lamberts et al., '89) systems have shown that the insulin-like growth factors (IGFs), defined as the mediators of the somatogenic action of GH (Daughaday et al., '72), inhibit GH release by mammalian pituitary cells.

Serum IGF-I activity has been detected in rainbow trout (*Oncorhynchus mykiss*) (Daughaday et al., '85), Atlantic salmon (*Salmo salar*) (Lindahl et al., '85), tilapia (*Oreochromus mossambicus*) (Drakenberg et al., '89), and sea bream (*Sparus aurata*) (Funkenstein et al., '89) using radioimmunoassays (RIAs) or placental radioreceptor assays (RRAs) for mammalian IGF-I. Cao et al. ('89) have obtained the cDNA sequence encoding preproIGF-I from coho salmon (*Oncorhynchus kisutch*), evidencing that the primary structure of IGF-I is highly conserved in relation to its mammalian homologues. In addition, it has been observed that IGFs can mediate the stimulatory action of GH on the sulfate uptake by trout (Komourdjian and Idler, '78) and Japanese eel (Duan and Hirano, '90; Duan and Inui, '90) cartilage. It has also been demonstrated that GH treatment increases IGF-I mRNA levels in coho salmon and serum IGF-I immunoreactivity in sea bream (Funkenstein et al., '89). However, whether IGFs participate on the regulation of fish GH release remains unexplored. The present study demonstrates a selective and dose-dependent IGF-I inhibition of basal GH secretion by pituitary cells of rainbow trout, delineating the participation of IGFs on the negative feedback loop of GH release.

MATERIALS AND METHODS

Animals. Studies were carried out on 1-year-old (90–130 g weight) rainbow trout (purchased from the INRA Gournay fish farm, France) kept at 15°C in a recirculating water system under a 15 h light: 9 h dark photoperiod.

Test agents. Recombinant human IGF-I (rhIGF-I) was kindly provided by Ciba-Geigy. Synthetic somatostatin$_{14}$ (SRIF) was offered generously by Sanofi.

Cell culture. Pituitary glands from 200 immature fish were removed, submitted to collagenase dispersion, and cultured according to the method of Weil et al. ('86). After 3 days of culture in 96-well

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plates (NUNC) at 18°C, pituitary cells were washed twice with RPMI medium (GIBCO Laboratories), containing Hepes (20 mM) and $NaHCO_3$ (9 mM) (pH = 7.5; osmotic pressure = 300 mOsm/Kg). Subsequently, pituitary cells were reincubated in this medium with or without test agents (rhIGF-I, SRIF). Test incubations lasted from 6 to 24 h. At the end of incubation time, well plates were centrifuged at 200 g × 10 min, the medium was removed, diluted (1:100) in hormone-assay buffer containing BSA (1%), and frozen for later homologous salmon GH (Le Bail et al., '91) and salmon prolactin (Prunet et al., '85) RIAs. A nonparametric test (Kolmogorof-Smirnov) was used for statistical evaluation of cell culture results.

RESULTS

In wells containing 3×10^4 and 6×10^4 cells/well, the secretion of GH into the medium increased progressively during the 24-h time course of incubations (Fig. 1). In both density conditions, addition of rhIGF-I (10 nM) to the medium caused a significant decrease in basal GH secretion both in short (6 h) and long (12 and 24 h) exposures. As rhIGF-I may exert its action on GH secretion by a nonselective suppression of pituitary hormone release, we also measured the levels of PRL into the culture medium. The results showed that rhIGF-I (10 nM) did not alter basal PRL secretion by trout pituitary cells (Fig. 2).

Figure 3A shows that the 6-h treatment with rhIGF-I inhibits basal GH secretion (3×10^4 cells/well) in a dose-dependent fashion. This decline in GH secretion was significant when cultured cells were exposed to concentrations of 1 nM ($P < 0.05$) and higher ($P < 0.01$). The maximal inhibitory concentration (100–300 nM) inhibited GH release by 50%. A significant suppressive effect of SRIF on GH release was also observed when cultured cells were exposed to concentrations of 0.03 nM ($P < 0.05$). The maximal SRIF concentration (100 nM) achieved an inhibitory value of 80%. ($P < 0.01$). This suppressive effect was more powerful than that exerted by rhIGF-I at equimolar concentrations. Figure 3B shows that the combination of rhIGF-I (1-10-100 nM) and low concentrations of SRIF (0.1 nM) enhanced the inhibitory effect of rhIGF-I in a strictly additive manner.

DISCUSSION

The present study demonstrates IGF inhibition of GH release by trout pituitary cells maintained in culture. Experiments were conducted on day 3 after initial plating, when the majority of cells ($3–6 \times 10^4$ cells/well) appeared firmly attached to wells as Weil et al. ('86) previously described. In these

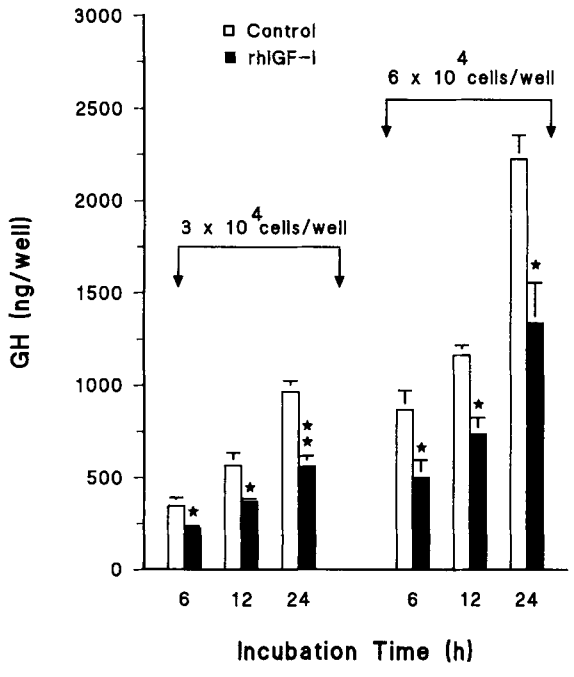

Fig. 1. Effects of cell density and duration of incubation on GH release in response to rhIGF-I (10 nM) by cultured rainbow trout pituitary cells. Each value represents the mean and standard error of three or four replicates. ($P < 0.05$), ($P < 0.01$); significant differences in relation control values.

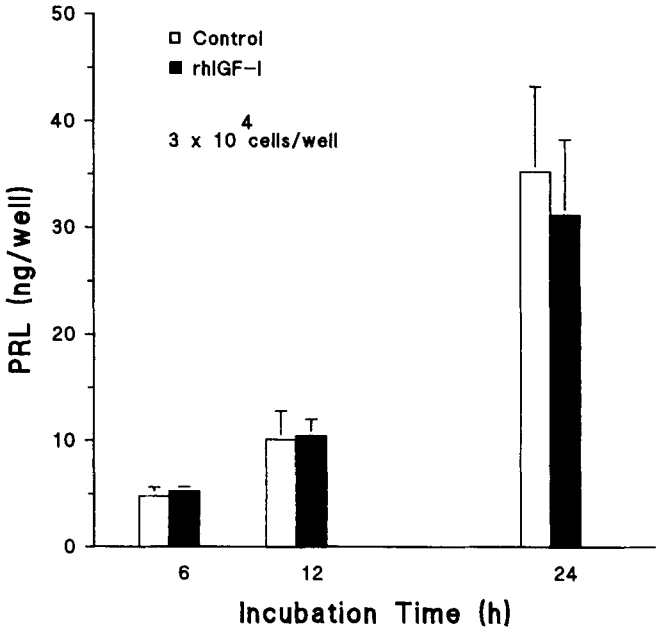

Fig. 2. Effect of duration of incubation on PRL release in response to rhIGF-I (10 nM) by cultured rainbow trout pituitary cells. Each value represents the mean and standard error of three or four replicates.

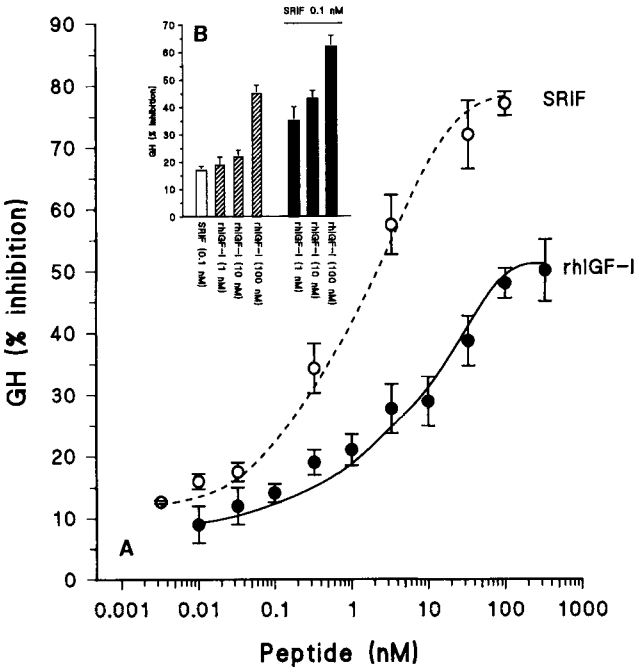

Fig. 3. **A.** Dose-dependent curve of the effect of rhIGF-I and SRIF on secretion of GH by cultured rainbow trout pituitary cells (3×10^4 cells/well) after 6-h incubation period. **B.** The inset shows the effect on GH release of combined concentration of rhIGF-I and SRIF. Each value represents the mean and standard error of four or five replicates.

culture conditions, rhIGF-I inhibited basal trout GH release both in short and long exposures, in a similar manner as it has been reported for basal and GRF-stimulated rat GH release (Yamashita and Melmed, '86). Brazeau et al. ('82) have also reported that a highly purified mammalian IGF-I decreases rat GRF-stimulated GH release in both 4- and 24-h treatments, whereas Berelowitz and coworkers ('81) only found a chronic effect. Conversely, Goodyer et al. ('84) reported that a semipurified preparation of IGF peptides decreases both basal and theophylline-stimulated GH release after a short exposure, but only extremely high concentrations are effective at 24 h. Therefore, it appears clear that IGF-I can act directly on the pituitary to inhibit GH release, but the dynamics of IGF-I inhibition are contradictory probably due to differences in culture conditions.

Our results also indicate that the inhibition of trout GH release by a mammalian IGF-I occurs in a dose-dependent manner. Differences in IGF sequences between mammalian and fish species (see Cao et al., '89) can explain that the dose of half maximum effect (3 nM) is relatively higher than those reported in primary cultures of rat pituitary cells. Although at equimolar concentrations, the maximum inhibitory effect is comparable (Yamashita and Melmed, '86) and even higher (Lamberts et al, '89) than those observed in homologous mammalian systems. This fact supports the view that the IGF-I inhibition of trout GH release is of a physiological nature rather than a pharmacological one. In contrast, the inhibition of GH secretion, with no effect on PRL release, suggests that IGF action is not due to a nonspecific inhibition of pituitary hormone release. Earlier studies have evidenced the suitability of our culture system to inhibit basal PRL release (Le Goff, '90). Nevertheless, taking into account that the amount of PRL secreted in this and other pituitary fish culture systems (Suzuki et al., '87, '91) is markedly lower than the one of GH, we cannot exclude the possibility that the inhibition of PRL by IGF preparations is more difficult to evidence than the GH one. To make things more confusing, controversial results have been reported in previous mammalian studies. Yasmahita and Melmed ('86) and Berelowitz and coworkers ('81) did not show any effect of IGFs on PRL release, whereas Goodyer et al. ('84) found an inhibitory action of a human blood IGF-I preparation on rat PRL release. However, as it has been suggested by Lamberts et al. ('89), this fact might be due to impurities of IGF preparation.

From a functional point of view, it is interesting to denote that our results also corroborate a SRIF dose-dependent suppression of fish GH release, evidencing an additive action of SRIF and rhIGF-I when both peptides were incubated on the same wells. Furthermore, as expected from the results obtained in mammals (see Lamberts et al., '89), SRIF inhibition of GH release is more powerful than that reported for a comparable amount of IGF-I, probably due to the fact that IGF-I inhibits preferentially the release of newly synthesized GH, whereas SRIF acts on both stored and recently synthesized hormone (Sheppard et al. '86).

From all these results, we can conclude that IGFs can act as an inhibitory component of fish GH regulation. This finding, together with the earlier observation that GH treatment increases IGF-I activity in fish species (Cao et al., '89; Funkenstein et al., '89), delineates the participation of IGFs on the negative feedback loop of GH release, in a similar manner as it has been reported for higher vertebrates.

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