Insulin-like growth factor-I stimulates gonadotrophin production from eel pituitary cells: a possible metabolic signal for induction of puberty

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Abstract

Insulin-like growth factor (IGF)-I has been suggested as a potential signal linking growth and puberty in mammals. Using the juvenile European eel as a model, we employed a long-term, serum-free primary culture of pituitary cells to study the direct effect of IGF-I on gonadotrophin (GtH-II=LH) production. IGF-I increased both cell content and release of GtH-II in a time- and dose-dependent manner. IGF-I and IGF-II had similar potencies but insulin was 100-fold less effective, suggesting the implication of an IGF type 1 receptor. Other growth and metabolic factors, such as basic fibroblast growth factor and thyroid hormones, had no effect on GtH-II production. IGF-I did not significantly increase the number of GtH-II immunoreactive cells, indicating that its stimulatory effect

on GtH-II production does not result from gonadotroph proliferation. Comparison of IGF-I and somatostatin (SRIF-14) effects showed that both factors inhibited growth hormone (GH) release but only IGF-I stimulated GtH-II production by eel pituitary cells. This indicates that the effect of IGF-I on gonadotrophs is not mediated by the reduction of GH released by somatotrophs into the culture medium. This study demonstrates a specific stimulatory effect of IGF-I on eel GtH-II production, played out directly at the pituitary level. These data obtained in a primitive teleost suggest that the role of IGF-I as a link between body growth and puberty may have been established early in the evolution of vertebrates.

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Introduction

In mammals, data suggest that the initiation and progression of the pubertal process may be influenced by metabolic factors of peripheral origin (see reviews by Ojeda & Urbanski 1988, Plant 1988). Experimental observations first carried out in the rat showed a minimal body size or fat content was involved in the onset of puberty (Kennedy & Mitra 1963, Frisch & McArthur 1974, Frisch *et al.* 1977). Clinical observations fit this hypothesis, as children with delayed body growth usually also present with delayed sexual development (e.g. Stanhope *et al.* 1987), and growth hormone (GH) treatment in prepubertal children presents a risk of inducing precocious puberty (Colle & Broussin 1993). Moreover, recent data from transgenic rats expressing the GH gene show precocious puberty in these animals (Ikeda *et al.* 1994).

In fish, as in mammals, a positive relationship between body growth and age at first reproduction ('puberty') has also been observed. For instance, results obtained from various strains of salmonids indicate that early puberty depends on a high growth rate (review: Le Bail 1988). In male Masu salmon (*Oncorhynchus masu*), the precocious

puberty that occurs in some under-yearling fish can be related to a higher body growth rate (Amano *et al.* 1997).

Recent studies in mammals suggest that insulin-like growth factor (IGF)-I, a growth factor mainly produced by the liver under the stimulatory control of GH (see reviews by Froesch *et al.* 1985, Cohick & Clemmons 1993) could be a good candidate for a link between somatic development and pubertal activation of reproductive function. Indeed, circulating levels of IGF-I increase strikingly during puberty in rodents (Handelsman *et al.* 1987, Takahashi *et al.* 1997), ruminants (Roberts *et al.* 1990) and primates (Copeland *et al.* 1982, 1985), including humans (Luna *et al.* 1983, Anders *et al.* 1994).

However, few data are available concerning the possible mechanisms of IGF-I action in activating puberty. Some studies suggest that besides its autocrine/paracrine stimulatory effects on gonadal function (review: Giudice 1992), IGF-I could play a key role in the onset of puberty by acting centrally on the hypothalamo-pituitary-gonadotrophic axis. Hiney *et al.* (1996) reported that, at the time of puberty in the rat, the increase in circulating IGF-I levels is accompanied by an increased synthesis of IGF-I receptors in the median eminence. The same

authors also showed that intraventricular administration of IGF-I increased plasma luteinizing hormone (LH) levels and advanced the onset of puberty. *In vitro* data also showed that, in the rat, IGF-I could stimulate gonadotrophin-releasing hormone (GnRH) release from the median eminence (Hiney *et al.* 1991), and release of gonadotrophins (LH and follicle stimulating hormone, FSH) from pituitary cells (Kanematsu *et al.* 1991, Soldani *et al.* 1994, 1995).

A strong molecular and functional conservation of IGF-I is observed among all classes of vertebrates (review: Upton *et al.* 1997). In teleosts, as in mammals, IGF-I is mainly produced by the liver under the control of GH (Funkenstein *et al.* 1990, Shambloott *et al.* 1995, Duguay *et al.* 1996) and plays, with GH itself, an important role in regulating body growth (see reviews by Chan & Steiner 1994, Plisetskaya *et al.* 1994). Some data also suggest that IGF-I may participate in the direct regulation of the gonads in teleosts (Duguay *et al.* 1992, Duan *et al.* 1993, Gutiérrez *et al.* 1993, Kagawa *et al.* 1995, and review by Le Gac *et al.* 1993), as it does in mammals (Hansson *et al.* 1989, Monniaux & Pisselet 1992, and review by Giudice 1992). As to its central actions, an inhibitory effect on GH secretion was demonstrated in the rainbow trout (*Oncorhynchus mykiss*) (Pérez-Sánchez *et al.* 1992, Blaise *et al.* 1995) and in the European eel (*Anguilla anguilla*) (Rousseau *et al.* 1998), indicating a classical negative feedback on the somatotrophic axis, as in mammals (Berelowitz *et al.* 1981, Yamashita & Melmed 1986). However, no data are yet available on the possible actions of IGF-I on the gonadotrophic axis in non-mammalian vertebrates.

In the present study, we looked for the possible direct pituitary effect of IGF-I on the production of type II gonadotrophin (GtH-II, teleost gonadotrophin homologous to tetrapod LH: Quérat 1994, Swanson & Dittman 1997) in the European eel. This animal model is particularly appropriate for studying mechanisms of experimental stimulation of puberty. First, it has a striking life cycle with a long period of juvenile growth (yellow stage). Secondly, sexual development is still blocked at a prepubertal stage even when the eels begin their reproductive migration (silver stage) (reviews: Dufour *et al.* 1983b, Dufour 1994). Thirdly, the phylogenetical position of the eel, a member of the group of Elopomorphs considered to be close to the origin of teleost evolution, may provide information on ancestral regulations in vertebrates (Lauder & Liem 1983, Roberts *et al.* 1989).

In order to study the direct pituitary effect of IGF-I on eel GtH-II production, we used a long-term, serum-free system of primary cultures of eel pituitary cells that we have recently developed (Huang *et al.* 1997). Comparisons of the effects of other growth and hormonal factors on GtH-II, as well as on GH in the same culture system, were also performed to test the specificity of IGF-I action. Preliminary results have been presented at the Third

International Symposium on Research for Aquaculture (Huang *et al.* 1998).

Materials and Methods

Animals

Several batches of juvenile (yellow stage) female eels (*Anguilla anguilla* L.), caught during spring and summer in north or west France, were transferred to the laboratory and kept in running aerated freshwater for short periods (from one to a few weeks) until used. Their body weights were between 100 and 200 g, and their gonadosomatic indexes were below 0.7%.

Hormones

Human recombinant insulin-like growth factor-I (IGF-I, somatomedin C), human recombinant insulin-like growth factor-II (IGF-II) and bovine recombinant basic fibroblast growth factor (bFGF) were obtained from Boehringer Mannheim (Meylan, France). Somatostatin (SRIH-14), human recombinant insulin and thyroid hormones (tri-iodothyronine (T_3) and thyroxine (T_4)) were obtained from Sigma (St Quentin-Fallavier, France). Stock solutions (10^{-3} or 10^{-4} M) were prepared in pure sterile water (Sigma) for IGF-I, IGF-II, bFGF and SRIH-14, in 0.1 M acetic acid for insulin and in 0.1 M NaOH for T_3 and T_4 . Stock solutions were aliquoted and kept at -20°C ; further dilutions were prepared in culture medium just before addition to the wells.

Primary culture of eel pituitary cells and in vitro treatments

Dispersion of eel pituitary cells was performed using an enzymatic and mechanical procedure as previously described by Montero *et al.* (1996). About 100 eels were used for each dispersion. Cells were cultured on poly-L-lysine precoated plates (96 wells/plate; Costar, Cambridge, MA, USA) at a density of 62 500 cells/well in serum-free culture medium (M199 with Earle's salt, sodium bicarbonate, 100 U/ml penicillin, 100 µg/ml streptomycin, 250 ng/ml fungizone; Gibco, Cergy-Pontoise, France), in a CO_2 incubator (NAPCO, ATGC, Noisy-Le Grand, France) at 18°C , as described by Huang *et al.* (1997). Treatments were started after 24 h of preculture. Media and treatments were renewed every 3 days for up to 15 days. Cultures were stopped before addition of hormones (day 0) or at various times of culture up to 15 days. GtH-II and GH contents in cells were assayed after submitting cells to osmotic shock and two repeated cycles of freezing and thawing (Huang *et al.* 1997, Rousseau *et al.* 1998). Replicates of 6 wells were used for each treatment. Collected media and cell extracts were kept frozen (-20°C) until radioimmunoassay (RIA).

Figure 1 Time course of the effect of IGF-I (10^{-8} M) on eel GtH-II cell content and release. Cells were cultured in serum-free medium (see Materials and Methods). Cultures were stopped on day 0 (before IGF-I addition) and at various times up to 15 days to measure GtH-II cell content. GtH-II release is calculated as cumulated release up to day 15, the medium and treatment being renewed every 3 days. Results are means $\pm$ S.E.M. ($n=6$ wells/group; 62 500 cells/wells). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs controls at the same time point; ^a $P<0.05$, ^b $P<0.01$ vs controls at day 0 (variance analysis).

Radioimmunoassays

GtH-II and GH from intracellular content and culture media were assayed in duplicate using respectively an RIA for carp GtH-II β subunit validated for eel GtH-II (Dufour *et al.* 1983a) and an homologous RIA for eel GH (Marchelidon *et al.* 1996). Total GtH-II or GH released into the culture medium was calculated by cumulating GtH-II or GH released every 3 days. GtH-II stimulation and GH inhibition in treated wells were calculated as a percentage of mean content or release by control wells in the same experiment.

Immunocytochemistry

Immunocytochemical characterization of GtH-II cells was performed as previously described (Montero *et al.* 1996). Dispersed pituitary cells were cultured on poly-L-lysine precoated coverslips (Nunc, Naperville, IL, USA) placed in 24-well plates (Costar) at a density of 150 000 cells/well. Cells were cultured for 12 days in the presence or absence of 10^{-7} M IGF-I. Media and treatment were renewed every 3 days. After 12 days, cells were fixed in 2% paraformaldehyde in 0.05 M sodium phosphate buffer and 0.15 M NaCl for 2 h at room temperature. Non-specific sites were saturated by incubation with 10% normal goat serum (Biosys, Compiègne, France) for 1 h at room temperature. Primary antibody (rabbit antiserum to carp GtH-II, the same as for the RIA; dilution 1/5000) was incubated overnight at 4 °C. Secondary antibody (goat

antiserum to rabbit immunoglobulins coupled to peroxidase; Biosys; dilution 1/100) was incubated for 1 h at 25 °C. Peroxidase activity was revealed with a solution of 0.003% 4-chloro-1-naphthol (Sigma) and 0.03% hydrogen peroxide in 0.05 M Tris-buffer. Three independent experiments were performed. Counting of total cell number per well area and of GtH-II immunoreactive cells (ir-GtH-II cells) was performed using an image analyser LEITZ DMR (Germany).

Statistical analysis

Each experiment was repeated in 3 independent studies (performed on different cell preparations). Results of representative experiments are shown. For each experiment, replicates of 6 wells were used for each treatment and values are given as means $\pm$ S.E.M. Homogeneity of variance was assessed by Bartlett's test, and data from different groups were compared by one-way analysis of variance followed by Student-Newman-Keuls multiple comparisons test.

Results

Long-term kinetics of the effects of IGF-I on GtH-II cell content and release (Fig. 1)

In control wells, a time-dependent increase in GtH-II cell content was observed. As compared with the initial

Figure 2 Dose-dependent effect of IGF-I on GtH-II cell content. Cultures were stopped on day 12 after addition (or not, controls) of various doses of IGF-I, to measure GtH-II cell content by RIA. Media and treatment were renewed every 3 days. Results are expressed as percentage of mean value of GtH-II cell content in controls. Values are given as means $\pm$ S.E.M. ($n=6$ wells/group; 62 500 cells/wells). ** $P<0.01$, *** $P<0.001$ vs controls (variance analysis).

content (day 0: 0.57 ± 0.06 ng/62 500 cells), GtH-II cell content was significantly increased at day 12 (2.37 times compared with day 0, $P<0.05$) and at day 15 (4.3 times compared with day 0, $P<0.01$). Addition of IGF-I (10^{-8} M) strongly increased GtH-II cell content, which reached a value at day 15 (5.84 ± 0.28 ng/62 500 cells) 10-fold higher than the initial GtH-II cell content ($P<0.001$). As compared with control cells at the same culture time, the stimulatory effect of 10^{-8} M IGF-I on GtH-II cell content was time-dependent (135% $P>0.05$; 157% $P<0.01$; 207% $P<0.01$; 240%, $P<0.001$, for 6, 9, 12 and 15 days of cell treatment respectively).

GtH-II release was low in control and IGF-I-treated wells, representing only a small percentage of the final cell content over 15 days of culture. A significant increase in cumulated GtH-II released was observed after 9 days of IGF-I treatment compared with control wells ($P<0.05$). Cumulated GtH-II released over 15 days of culture reached 0.40 ± 0.02 ng/62 500 cells in IGF-I-treated wells versus 0.08 ± 0.02 ng/62 500 cells in control wells ($P<0.01$).

Dose-dependent effect of IGF-I on GtH-II cell content (Fig. 2)

The effects of various doses of IGF-I were tested over 12 days of treatment. IGF-I showed a dose-dependent stimulatory effect on GtH-II cell content, with a significant

effect at 10^{-11} M ($P<0.01$). The highest concentration tested (10^{-7} M) gave a maximal stimulation of 480% ($P<0.001$). The EC_{50} was 10^{-10} M.

Comparative effects of IGF-I, IGF-II, insulin and bFGF on GtH-II cell content (Fig. 3)

The effects of various factors belonging to the insulin-like superfamily (IGF-I, IGF-II and insulin) and of another type of growth factor family (bFGF) were compared over 12 days of treatment. GtH-II cell content was increased in a dose-dependent manner by IGF-I and IGF-II, with a similar maximal stimulation (423 and 445% respectively) at the highest dose tested (10^{-7} M). Insulin was less potent, with a significant effect at 10^{-7} M ($P<0.001$), equivalent to that produced by 10^{-9} M IGF-I or IGF-II. In contrast, no significant effect was observed with bFGF at any dose tested.

Comparative effects of IGF-I and thyroid hormones on GtH-II cell content (Fig. 4)

The effects of thyroid hormones (T_3 and T_4) on GtH-II cell content were tested at various doses, and compared with the effect of IGF-I over 12 days of treatment. Neither T_3 nor T_4 had any significant effects on GtH-II cell

Figure 3 Effects of various doses of IGF-I, IGF-II, insulin and bFGF on GtH-II cell content. Cultures were stopped on day 12 after addition (or not, controls) of various doses of IGF-I, IGF-II, insulin or bFGF, to measure GtH-II cell content by RIA. Media and treatments were renewed every 3 days. Results are expressed as percentage of mean value of GtH-II cell content in controls. Values are given as means $\pm$ S.E.M. ($n=6$ wells/group; 62 500 cells/wells). ** $P<0.01$, *** $P<0.001$ vs controls (variance analysis).

Figure 4 Effects of various doses of IGF-I, T_3 and T_4 on GtH-II cell content. Cultures were stopped on day 12 after addition (or not, controls) of various doses of IGF-I, T_3 or T_4 , to measure GtH-II cell content by RIA. Media and treatments were renewed every 3 days. Results are expressed as percentage of mean value of GtH-II cell content in controls. Values are given as means $\pm$ S.E.M. ($n=6$ wells/group; 62 500 cells/wells). *** $P<0.001$ vs controls (variance analysis).

content at any dose tested (from 10^{-9} to 10^{-6} M). In contrast, IGF-I significantly increased GtH-II cell content in a dose-dependent manner, in the same experimental conditions.

Effect of IGF-I on the number of GtH-II immunoreactive cells

The effect of 10^{-7} M IGF-I on GtH-II cell number was tested over 12 days of culture. Gonadotrophs were

Figure 5 Comparative effects of IGF-I and SRIH-14 on (a) GtH-II cell content and (b) GH release. Cultures were stopped on day 12 after addition (or not, controls) of various doses of IGF-I or SRIH-14, to measure GtH-II cell content by RIA. Media and treatments were renewed every 3 days. Results are expressed as percentage of mean value of GtH-II cell content in controls. Total GH released over 12 days of culture was expressed as a percentage of the mean value of total GH released in control wells. Values are given as means $\pm$ S.E.M. ($n=6$ wells/group; 62 500 cells/wells). ** $P<0.01$, *** $P<0.001$ vs controls (variance analysis).

identified by immunocytochemistry. Counting of total pituitary cells per well area showed no differences between control and IGF-I-treated wells. Immunoreactive GtH-II cells were found to represent $4.42 \pm 0.61\%$ of total cells in control wells and $5.33 \pm 0.58\%$ in IGF-I-treated wells, indicating no significant effect of IGF-I on GtH-II cell number ($P>0.05$, Student's t -test; 3 independent experiments, ≥ 800 cells counted/treatment/experiment). In the same experiments, we assessed that IGF-I stimulated GtH-II production.

Comparative effects of IGF-I and SRIH-14 on GtH-II cell content and GH release (Fig. 5)

In order to address the question whether the stimulatory effect of IGF-I on GtH-II could be mediated by its inhibitory effect on GH release, we compared the effects of IGF-I and SRIH-14, both factors previously shown to inhibit GH release (Rousseau *et al.* 1998). Various doses of SRIH-14 and IGF-I were tested over 12 days of culture. Both factors dose-dependently inhibited GH release, with

maximal inhibitory effects of 85% at 10^{-9} M IGF-I and 90% at 10^{-8} M SRIH (Fig. 5b). In contrast, only IGF-I showed a dose-dependent stimulatory effect on GtH-II cell content, SRIH having no significant effect on GtH-II cell content at any dose tested (from 10^{-10} to 10^{-7} M) (Fig. 5a).

Discussion

In this series of experiments we used long-term, serum-free primary cultures of eel pituitary cells to investigate the effect of IGF-I on GtH-II production. This model has already proved useful for studying pituitary function in defined conditions, in particular for analysing the regulation of GtH-II (Huang *et al.* 1997) and GH production (Rousseau *et al.* 1998).

A significant increase in GtH-II cell content, as well as a slight but significant release of GtH-II, were observed in control cells over 2 weeks of culture. This confirms previous observations by Huang *et al.* (1997) that eel gonadotrophs can sustain the production of GtH-II over the long-term in serum-free culture conditions. A similar result was recently obtained when investigating GH production by eel somatotrophs in culture (Rousseau *et al.* 1998). The serum-free conditions are particularly suitable for the study of the specific effects of growth factors on hormone production by pituitary cells, because no interference from other exogenous hormonal or growth factors present in sera can occur.

The present results show that IGF-I increased GtH-II cell content in a time- and dose-dependent manner. At 12 days of culture, its effect was significant at a dose of 10^{-11} M and reached a maximum (300 to 500% of stimulation) at 10^{-7} M. IGF-I also significantly stimulated GtH-II release. The increases in both cell content and release indicate a stimulation of GtH-II production. In the same culture conditions, we have previously demonstrated a stimulatory effect of androgens, but not of oestradiol, on GtH-II production (Huang *et al.* 1997). Further studies will be aimed at investigating the possible interactions between IGF-I and sex steroids on GtH-II production.

This study provides the first demonstration of a stimulatory effect of IGF-I on gonadotroph activity in teleosts. Indeed, Blaise *et al.* (1997) found a permissive effect of 48-h preincubation with IGF-I on GnRH-stimulated GH release *in vitro* by somatotrophs in the rainbow trout (*Oncorhynchus mykiss*), but they did not observe any significant effect of IGF-I on basal or on GnRH-stimulated GtH-II release by gonadotrophs. In mammals some data demonstrate a positive effect of IGF-I on basal or GnRH-stimulated gonadotrophin release *in vitro*. In the rat, IGF-I was shown to increase basal release of FSH and LH by pituitary cells from mature females and to enhance basal as well as GnRH-stimulated LH release by pituitary cells from immature male rats (Soldani *et al.* 1994). In

addition, IGF-I was shown to stimulate the secretion of FSH, but not LH, by dispersed human anterior pituitary adenomas (Atkin *et al.* 1993). In pituitary cell cultures from mature female rats, measurement of cell content indicated that neither the production of LH nor FSH was significantly stimulated by IGF-I after 72 h of treatment (Kanematsu *et al.* 1991). The model of juvenile eel gonadotrophs is particularly useful for displaying the stimulatory action of regulatory factors on GtH-II production (Huang *et al.* 1997). First, gonadotrophs from juvenile eels exhibit low initial GtH-II cell content and release (Montero *et al.* 1996). Secondly, gonadotrophs can sustain their activity for up to 3 weeks of culture in serum-free conditions (Huang *et al.* 1997), allowing long-term studies of the action of regulatory factors. It is the combination of these characteristics that has allowed this first experimental demonstration of a striking positive effect of IGF-I on GtH production.

In this study we used mammalian (human recombinant) IGF-I, since a high degree of molecular conservation has been shown for IGF-I from teleosts to mammals (Cao *et al.* 1989, and review by Upton *et al.* 1997). Moreover, mammalian IGF-I has been shown to be biologically active in the eel in two experimental situations. On the one hand, bovine recombinant IGF-I stimulated *in vivo* sulphate uptake by branchial cartilage in the Japanese eel (*Anguilla japonica*) (Duan & Hirano 1990, 1992), and on the other hand, we recently showed that human recombinant IGF-I strongly inhibited GH release from European eel pituitary cells *in vitro* (Rousseau *et al.* 1998).

In order to test the specificity of IGF-I action, we compared the effects of three members of the insulin-like superfamily, human recombinant IGF-I, IGF-II and insulin. IGF-I and IGF-II had similar capacities (same dose-dependences and maximal effects) in increasing eel GtH-II cell content. In contrast, insulin was at least 100 times less potent. This agrees with the demonstration, in the trout pituitary, of binding sites having the same high affinity for IGF-I and IGF-II and a lower affinity for insulin (Blaise *et al.* 1995). According to the classification in mammals (see review by Jones & Clemmons 1995), these binding properties are characteristic of the type 1 IGF receptor. The recent cloning of IGF type 1 receptor in two teleosts, trout (*Oncorhynchus mykiss*) and turbot (*Scophthalmus maximus*), showed that, like IGFs, IGF type 1 receptor polypeptide sequences are highly conserved (>90% identity) among vertebrates (Elies *et al.* 1996). The specificity of IGF action was further demonstrated by the lack of effect of bFGF, a growth factor unrelated to the insulin-like superfamily. We also compared the effect of thyroid hormones (T_4 and T_3) known to play a major role in the control of development, growth and metabolism in vertebrates (see review by McNabb 1994). Neither T_3 nor T_4 had any significant effect on GtH-II cell content at any dose tested (from 10^{-9} to 10^{-6} M). This further emphasizes the specificity of the stimulatory effect of IGF-I on gonadotrophs.

As IGF-I is a known mitogenic factor involved in cell growth (review: Cohick & Clemmons 1993), it was conceivable that the stimulatory effect on GtH-II production could result from proliferation of gonadotrophs. However, counting immunoreactive gonadotrophs after 12 days of culture did not show any significant difference between control cells and cells treated with a maximal dose of IGF-I (10^{-7} M). In mammals, similar data have been obtained indicating that IGF-I does not stimulate proliferation of gonadotrophs *in vitro* (rat: Tilemans *et al.* 1991, Soldani *et al.* 1994; mice: Takahashi *et al.* 1997). This suggests that the stimulatory effect of IGF-I on eel GtH-II production results from increased GtH-II production per cell rather than from gonadotroph proliferation. Our experimental observations support this finding as the immunoreactive intensity of gonadotrophs appeared stronger in IGF-treated cells than in control cells (data not shown).

Since IGF-I also exerts an inhibitory effect on GH release in teleosts as it does in mammals, and as we used a mixed pituitary cell population, we investigated whether the stimulatory effect of IGF-I on gonadotrophs could be mediated by the reduction of GH released into the culture medium. We compared the effects of IGF-I and SRIH-14, both previously having been shown strongly to inhibit GH release by eel pituitary cells *in vitro* (Rousseau *et al.* 1998). In the present experiments, while both factors dose-dependently inhibited GH release, only IGF-I had a stimulatory effect on GtH-II cell content, SRIH-14 showing no significant effect at any dose tested (from 10^{-10} to 10^{-7} M). This demonstrates that the stimulatory effect of IGF-I on gonadotrophs is not mediated by GH. However, the possibility that some paracrine factors, possibly produced by somatotrophs or other pituitary cell types under the action of IGF-I, could be involved in the activation of gonadotrophs cannot be excluded. Indeed numerous paracrine factors have been shown to be potentially involved in intercellular communication within the anterior pituitary in mammals (review: Schwartz & Cherny 1992). In mice, IGF type 1 receptor mRNA has been detected only on pituitary somatotrophs and some corticotrophs (Takahashi *et al.* 1997). Further studies on IGF-I receptor localisation on pituitary cells in teleosts and mammals are required to determine if gonadotrophs are a direct target of IGF-I.

In the eel, IGF-I and IGF-II exhibit similar *in vitro* abilities to stimulate GtH-II production. In mammals, *in vitro* studies also revealed similar potencies of IGF-I and IGF-II on LH release (Soldani *et al.* 1994). These data suggest that both IGFs could exert potential physiological roles in the pubertal stimulation of gonadotrophins. In mammals, an endocrine role at puberty has been attributed to peripheral IGF-I (review: Thissen *et al.* 1994), since its plasma levels strongly increase at puberty (see Introduction). In fish, the production of IGF-I by the liver is under GH control, as it is in mammals. Thus IGF-I could play the same endocrine role in the two groups. However, no data are yet available concerning the variations in IGF-I

Figure 6 Role of IGF-I as a link between somatic development and induction of puberty. IGF-I, a growth factor mainly produced by the liver under the control of GH, exerts a negative feedback on GH and a stimulatory effect on GtH-II, at the pituitary cell level in the eel. GH itself has no direct action on GtH-II. Solid arrows represent the direct controls between IGF-I and pituitary hormones; broken arrows represent the physiological effects. The stimulatory effect of IGF-I on GtH-II (LH-like) makes it a good candidate for a role in the metabolic signal for the induction of the first sexual maturation (puberty).

plasma levels at puberty in fish. Much less is known about IGF-II function and regulation in both mammals and fish. In the juvenile rainbow trout (*Oncorhynchus mykiss*), Shambloot *et al.* (1995) showed a GH-dependent stimulation of both IGF-I and IGF-II messenger RNA levels in the liver, and suggested possible endocrine roles for both IGFs. However, in the gilthead seabream (*Sparus aurata*), Duguay *et al.* (1996) found a stimulatory effect of GH on hepatic IGF-I, but not IGF-II, mRNA levels.

Some data in mammals indicate that IGF-I acts on the gonadotrophic axis at the hypothalamic level by stimulating GnRH release (Hiney *et al.* 1991, 1996), and at the pituitary level by stimulating LH and FSH release (Kanematsu *et al.* 1991, Soldani *et al.* 1994, 1995). Our long-term culture system of pituitary cells from a juvenile fish allowed us to demonstrate that IGF-I not only stimulates GtH-II release but is also able strongly to increase GtH-II cell content. This direct stimulatory effect of IGF-I on GtH-II (LH-like) production may play a critical role in the pubertal stimulation of the gonadotrophic axis (Fig. 6). Future studies will aim to investigate whether the *in vitro* stimulatory effect of IGF-I on GtH-II production can also be demonstrated *in vivo*. The present data, obtained in a primitive teleost, suggest that the physiological role of IGF-I in the link between body growth and puberty may have been established early in the evolution of vertebrates.

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