Homologous growth hormone (GH) binding in gilthead sea bream (Sparus aurata). Effect of fasting and refeeding on hepatic GH binding and plasma somatomedin-like immunoreactivity

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Homologous growth hormone (GH) binding in gilthead sea bream (*Sparus aurata*). Effect of fasting and refeeding on hepatic GH-binding and plasma somatomedin-like immunoreactivity

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Specific binding of gilthead sea bream growth hormone (sbGH) to liver membrane preparations was a time and temperature dependent process, and was saturable by increasing amounts of membrane proteins. Scatchard analysis evidenced a single class of high-affinity and low-capacity binding sites. Ovine prolactin, recombinant tilapia prolactin, carp gonadotropin and chinook salmon gonadotropin did not compete for the ^{125}I-sbGH binding sites, while recombinant trout GH, bovine GH and human GH displaced iodinated sbGH in a dose dependent-manner. IGF-I-like immunoreactivity was detected after acidification of plasma and removal of IGF-I binding activity. A parallel displacement to the rhIGF-I standard was observed with extracted plasma samples. Free and total hepatic GH-binding decreased during long-term starvation (3–9 weeks), returning to control values during the refeeding period. Plasma IGF-I-like immunoreactivity showed a similar trend. To our knowledge, this is the first report that indicates a coordinated regulation of GH-binding and plasma somatomedin-like activity in a typical marine fish.

Key words: growth hormone; insulin-like growth factors; fasting; gilthead sea bream; teleost.

I. INTRODUCTION

The importance of growth hormone (GH) in supporting animal growth has been known for a long time. The original concept implies that GH stimulates the systemic release from the liver of insulin-like growth factor-I (IGF-I) which, in turn, stimulates bone growth (Salmon & Daughaday, 1957). In addition, GH induces the production of IGF-I in many tissues other than liver, initiating local growth by paracrine or autocrine mechanisms (Holly & Wass, 1989). A further complication is that IGFs are bound to specific carrier proteins, which exert an important modulatory action on systemic and local IGF activity (Baxter, 1988). All this has been defined primarily in mammalian systems, but there is evidence supporting a physiological role of IGFs on the growth-promoting effect of GH in other vertebrates.

In fish, several reports indicate that the liver is the most important target tissue for the direct action of GH (Fryer, 1979; Gray *et al.*, 1990; Hirano, 1991; Yao *et al.*, 1991; Sakamoto & Hirano, 1991; Ng *et al.*, 1992). GH treatment increases hepatic IGF-I mRNA (Cao *et al.*, 1989) and serum IGF-I immunoreactivity (Funkenstein *et al.*, 1989; Ng *et al.*, 1991). A close relationship between GH

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pulses and plasma IGF-I-like immunoreactivity has been observed in rainbow trout *Oncorhynchus mykiss* Walbaum (Niu *et al.*, 1993). In addition, it has been demonstrated that human IGF-I inhibits basal GH secretion by pituitary cells of rainbow trout (Pérez-Sánchez *et al.*, 1992), delineating the participation of somatomedins on the negative feedback loop of GH release. Further studies indicate that IGFs mediate the stimulatory action of GH on the sulphate uptake by trout (Komourdjian & Idler, 1978) and Japanese eel *Anguilla japonica* Sohlegel (Duan & Hirano, 1990; Duan & Inui, 1990) cartilage. Recent studies in long-jawed mudsucker *Gillichthys mirabilis* Cooper (Gray & Kelley, 1991) and coho salmon *Oncorhynchus kisutch* Walbaum (McCormick *et al.*, 1992) indicate that short-term inanition decreases this sulphate uptake, which is enhanced by bovine IGF-I. Moreover, it has been demonstrated that long-term fasting reduces not only hepatic IGF-I mRNA but also hepatic GH-binding in Japanese eel (Duan & Hirano, 1992) and coho salmon (Gray *et al.*, 1992), respectively. The aim of this work was to examine the co-ordinate regulation of GH-binding and plasma IGF-I activity in a typical marine fish, such as gilthead sea bream *Sparus aurata* L., which is successfully cultured in the Mediterranean area. For this purpose, we have developed a homologous GH radioreceptor assay and validated a double antibody-radioimmunoassay for human IGF-I.

II. MATERIALS AND METHODS

HORMONES AND REAGENTS

Gilthead sea bream GH (sbGH) was purified from pituitary extracts according to the isolation procedure used for chinook salmon *Oncorhynchus tshawytscha* Walbaum GH (sGH) (Le Bail *et al.*, 1989). Electrophoretic characterization of sbGH evidenced two major bands which corresponded to the monomeric forms of sGH. Rabbit antibodies against sbGH immunostained somatotrope cells of the proximal pars distalis of gilthead sea bream pituitary (Le Bail *et al.*, 1993). Recombinant human IGF-I (rhIGF-I) was kindly provided by Dr K. Müller (Ciba-Geigy, Basel, Switzerland). The rabbit antiserum (UB3-189) against human IGF-I was prepared by L. Underwood and J. J. Van Wyk (University of North Carolina at Chapel Hill, Chapel Hill, NC, U.S.A.) and made available through the National Hormone and Pituitary Distribution Program (Baltimore, MD, U.S.A.). This rabbit antiserum does not show appreciable cross-reaction with insulin and IGF-II. Human GH (hGH), bovine GH (bGH) and ovine prolactin (oPRL) were obtained from the National Hormone and Pituitary Distribution Program. Chinook salmon gonadotropin (sGtH) and carp *Cyprinus carpio* L. gonadotropin (cGtH) were generously provided by Dr B. Breton (Laboratoire Physiologie des Poissons, Rennes, France). Recombinant tilapia *Oreochromis* sp. prolactin (rtiPRL) and recombinant trout GH (rtGH) were generously provided by Dr J. Smal (Eurogentec, Liège, Belgium).

IODINATION

sbGH and rhIGF-I were iodinated by the chloramine T method (Greenwood *et al.*, 1963) with Martal's (1972) modification. Unreacted iodide was separated from labelled proteins by gel filtration in a Sephadex G-25 column (PD10, Pharmacia). Both ^{125}I-sbGH and ^{125}I-rhIGF-I were stable for about 2 months, stored in glycerol (1 : 1) at − 20° C.

MEMBRANE PREPARATION

Liver, brain, muscle and visceral fat tissues were placed in ice-cold extraction buffer (20 mM Tris–HCl, 5 mM $MgCl_2$, 0·1% sodium azide, pH=7·5). Each tissue was homogenized using a Polytron homogenizer. The homogenate was passed through a cheesecloth and was further homogenized with a glass Teflon homogenizer. The final homogenate was centrifuged at 3000 *g* for 15 min. The pellet was suspended with the extraction buffer and centrifuged again at 3000 *g*. The pellet was kept to test its GH binding. Protein concentration was determined by a Bio-Rad protein assay kit, using bovine serum albumin (BSA) as standard.

DISSOCIATION PROCEDURE AND GH-BINDING ASSAY

To determine total binding sites, bound GH was dissociated from its receptor by exposing membranes to 4 M $MgCl_2$ as described by Kelly *et al.* (1979) and Maiter *et al.* (1988). Free binding sites were determined with untreated membrane preparations. To verify that 4 M $MgCl_2$ treatment for 10 min was efficient for the removal of endogeneous GH, hepatic membranes from gilthead sea bream were saturated with unlabelled sbGH (200 ng ml^{-1} or 50 ng ml^{-1}). The 3000 *g* pellet from these membranes was then treated with $MgCl_2$ and tested for binding of ^{125}I-sbGH. The decrease in specific binding following preincubation with sbGH was restored by the dissociation procedure (Table I).

For binding studies, membrane preparations and labelled sbGH in assay buffer containing 0·5% BSA, 0·25 mg ml^{-1} of trypsin inhibitor and 0·5 mM ascorbic acid were incubated for 14 h at 15° C in a final volume of 300 µl. The assay was terminated by adding 3 ml ice-cold extracted buffer. Bound and free radioactivity were separated by centrifugation (3000 *g* for 15 min) and the pellets were counted in a Packard gamma counter. Total binding was measured in the absence of unlabelled hormone and non-specific binding was taken as the ^{125}I remaining in the presence of 2 µg unlabelled rtGH. Specific binding was determined by subtracting the non-specific binding from the total binding.

IGF-I RADIOIMMUNOASSAY

Anti-human IGF-I serum (100 µl) diluted (1 : 2500) in RIA buffer (50 mM Tris–HCl pH=7·5, containing 10 mM $MgCl_2$, 0·05% sodium azide, 0·1% Triton X-100 and 1% BSA) was added to 100 µl plasma aliquots after each acid treatment and dilution, which is described later. After 24 h of incubation at room temperature, 100 µl of buffered rhIGF-1 (15 000 cpm) were added to each tube and incubation continued for 24 h. Precipitation of the antibody-bound peptide was made by addition of 100 µl of diluted serum of goat anti-rabbit γ globulin in 50 mM Tris–HCl (pH=7·5), containing 10 mM $MgCl_2$, 0·05% sodium azide and 7·5% polyethylene glycol. The incubation was prolonged for 24 h at room temperature and the assay was terminated by adding 3 ml of 50 mM Tris–HCl (pH=7·5), containing 10 mM $MgCl_2$ and 0·05% sodium azide. The tubes were then centrifuged for 45 min at 3000 *g*. The supernatant was decanted and the radioactivity in the bound fraction was determined in a gamma counter. A significant displacement of ^{125}I-labelled rhIGF-I occurred at 30 pg ligand with 1% non-specific binding and 40% of specific bound count at zero dose/total count.

ACID GEL FILTRATION

The extraction was performed according to the procedure of Zapf *et al.* (1980). Plasma samples of 1 ml were acidified with acetic acid to a final concentration of 0·5 M and incubated for 1 h at 4° C to dissociate IGFs from IGF-binding proteins. The acidified plasma was then chromatographed on Sephadex G-75 using a glass column (7 × 500 mm), calibrated before use with dextran blue, albumin, ovalbumin, chymotrypsinogen A and labelled rhIGF-I. The samples were eluted with 1 M acetic acid containing 150 mM NaCl and 0·1% BSA at a flow rate of 8 ml/h. Fractions of 300 µl were collected and neutralized (1 : 5) with Tris base (200 mM, pH=9). The supernatant was diluted in RIA buffer before further assays.

TABLE I. Dissociation of sbGH bound to liver binding sites of gilthead sea bream and rebinding of ^{125}I-sbGH

Preincubation	Treatment	^{125}I-sbGH specific binding	
		B/T × 100	Percentage of control
Assay buffer (control)	Untreated	14·1 ± 0·5	100
Assay buffer	4 M $MgCl_2$	18·2 ± 0·8	129
+sbGH (50 ng ml^{-1})	Untreated	4·2 ± 0·3	29·7
+sbGH (50 ng ml^{-1})	4 M $MgCl_2$	17·2 ± 1·5	121·9
+sbGH (200 ng ml^{-1})	Untreated	0	0
+sbGH (200 ng ml^{-1})	4 M $MgCl_2$	18·4 ± 0·9	130·9

Note, values are expressed as mean ± S.E.M. (n=3). The specific binding is shown as a percentage of the total radioactivity bound to 200 µg of membrane proteins.

SEPHADEX CM-25 EXTRACTION

Plasma samples of 250 µl were acidified with 250 µl of 1 M acetic acid containing 150 mM NaCl and incubated for 1 h at 4° C. IGFs were then extracted according to the procedure of Niu *et al.* (1993). Briefly, 500 µl of Sephadex CM-25 in acetic acid solution (1 : 1, v/v) were added to acidified plasma. The tubes were incubated for 30 min at room temperature and the supernatant was decanted after centrifugation at 3000 *g* for 15 min. The precipitate was incubated with 2 ml of 10 mM acetic acid (pH=3) for 30 min at room temperature to remove IGF carrier proteins. The tubes were centrifuged and the precipitate washed three times (750 µl) in Tris base (200 mM, pH=9). The supernatants were then pooled and diluted in RIA buffer before further assays.

FASTING

In order to determine the effects of long-term fasting on GH-binding and plasma IGF-I immunoreactivity, two groups (n=70) of 1-yr-old gilthead sea bream were acclimated in separate 500-l fibreglass tanks for a period of 3 weeks. Following the acclimation period, food was withheld for one group during 9 weeks with a subsequent 2-week period of refeeding. At 0, 7, 21, 63 and 77 days after the beginning of the experiment, 10 animals from fed and fasted fish were sampled. Blood was collected from the caudal vessels into heparinized tubes and centrifuged at 3000 *g* for 20 min; plasma was stored at −40° C until analysis. Liver membranes were immediately prepared and stored at −40° C in the extraction buffer diluted in glycerol (1 : 1). Just before use, the frozen suspensions were washed and diluted with assay buffer to appropriate concentration.

STATISTICS

B/Bo values derived from serial dilutions of hormone preparations and plasma samples were converted to logits; the slopes were calculated for each set of points and then compared to sbGH or rhIGF-I standard curve by analysis of covariance. The slopes were considered to be significantly different if $P<0·05$. One-way analysis of variance followed by Duncan's multiple range-test was used to evaluate differences between fed and fasted groups during each sampling time, as well as within each group over time.

III. RESULTS

GH binding to liver membrane preparations was time and temperature-dependent (Fig. 1). At 4° C, the specific binding of labelled sbGH to 250 µg of

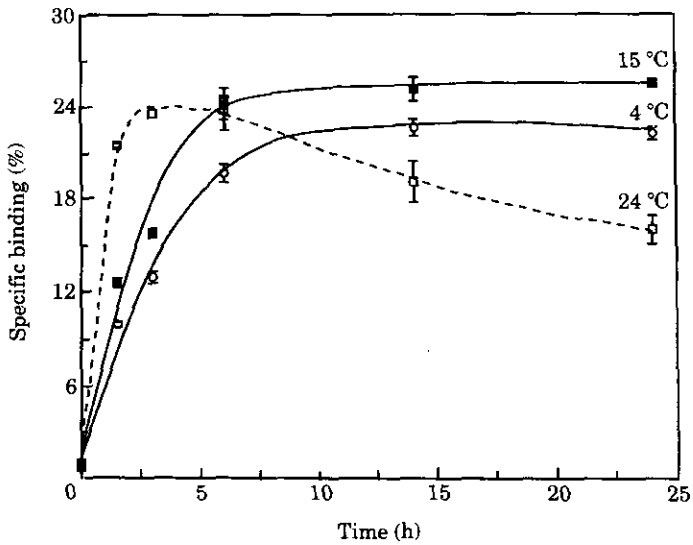


FIG. 1. Effect of time and temperature on specific binding of ^{125}I-sbGH (24 000 cpm) to gilthead sea bream liver membranes. Each point represents the mean ± S.E.M. of triplicate determinations.

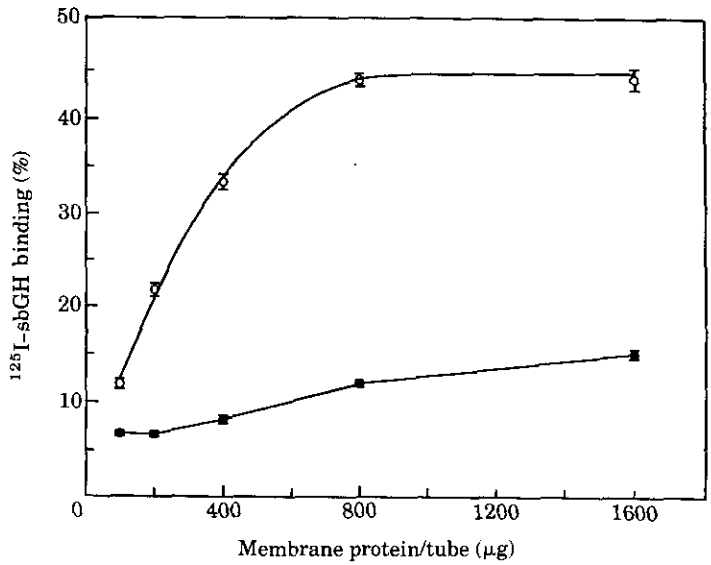


FIG. 2. Effect of the amount of liver membrane proteins on specific (○) and non-specific (■) binding of ^{125}I-sbGH (25 000 cpm). Each point represents the mean ± S.E.M. of triplicate determinations.

membrane proteins increased from 10% after 1·5 h to 22% after 14 h, when it appeared to reach equilibrium. At 15° C, the specific binding increased from 12% after 1·5 h to 24% after 6 h, and then reached a steady state (14–24 h). At 24° C, the specific binding was at maximum (22%) after 3 h, but it declined gradually after 6 h of incubation. Thus, further assays were performed at 15° C for 15–20 h.

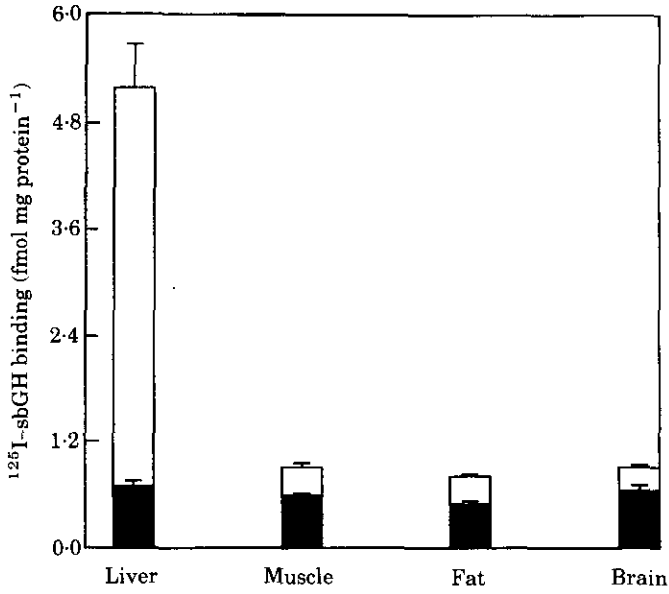


FIG. 3. Total (□) and non-specific (■) binding of ^{125}I-sbGH (30 000 cpm) to membranes from gilthead sea bream organs. Each histogram represents the mean ± S.E.M. of five determinations.

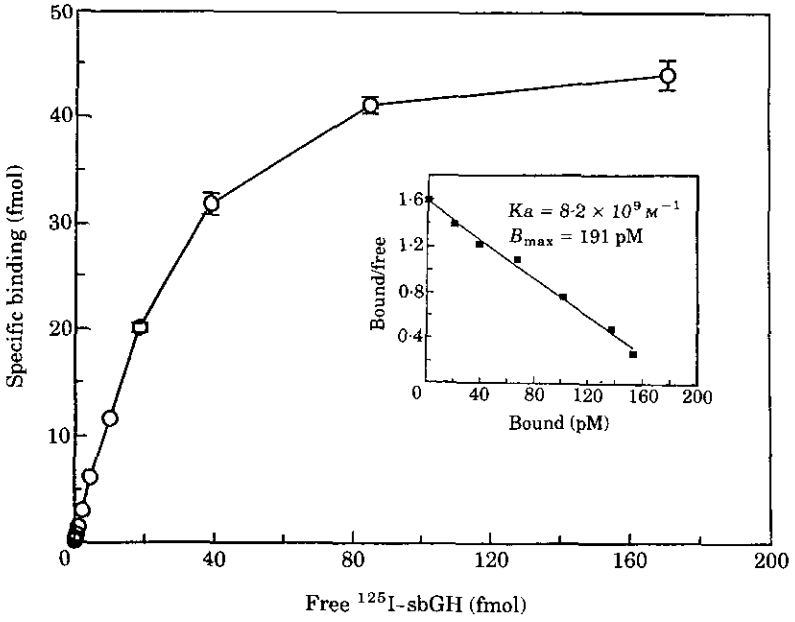


FIG. 4. Saturable specific binding of ^{125}I-sbGH to gilthead sea bream liver membrane proteins (250 μg). Each point represents the mean ± S.E.M. of triplicate determinations. The insert represents the derived Scatchard plot.

The specific binding of ^{125}I-sbGH increased with the amount of membrane proteins added, achieving a plateau that demonstrated that up to 45% of iodinated sbGH can be specifically bound to somatogenic binding sites (Fig. 2).

TABLE II. Binding affinity (Ka) and binding capacity (B_{max}) of treated ($MgCl_2$) and untreated liver membrane preparations

	Ka (M^{-1})	B_{max} (fmol mg protein^{-1})
Untreated	$6{\cdot}8 \pm 0{\cdot}7 \times 10^9$	$150{\cdot}5 \pm 15{\cdot}3$
4 M $MgCl_2$	$7{\cdot}1 \pm 1{\cdot}1 \times 10^9$	$195{\cdot}8 \pm 20{\cdot}2$

Note, values are expressed as mean ± S.E.M. of three–four separate determinations.

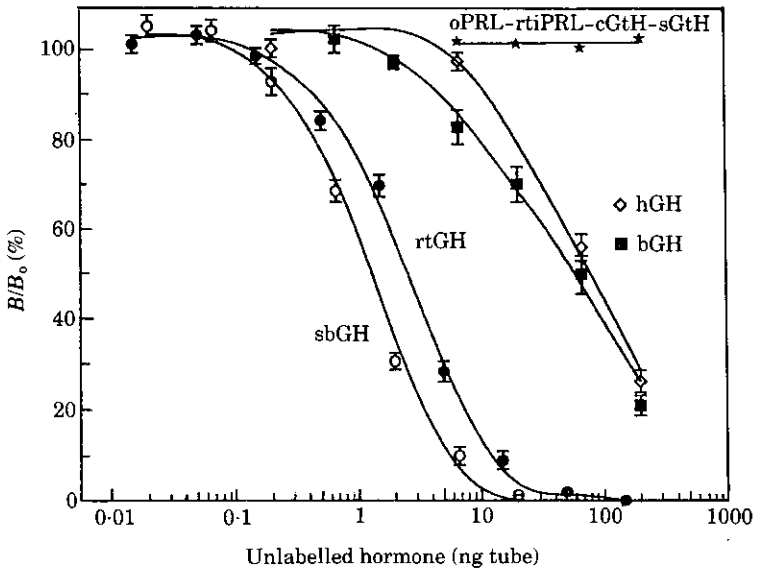


FIG. 5. Competitive displacement of ^{125}I-sbGH (30 000 cpm) from gilthead sea bream liver membrane proteins (100 µg). Gilthead sea bream (sbGH); recombinant trout GH (rtGH); human GH (hGH); bovine GH (bGH); ovine prolactin (oPRL); recombinant tilapia prolactin (rtiPRL); carp gonadotropin (cGtH); chinook salmon gonadotropin (sGtH). Each value represents the mean ± S.E.M. of four replicates.

The non-specific binding ranged between 7 and 15% of total counts added. Appreciable binding was detected in brain, muscle and visceral fat membrane preparations. However, the amount of specific binding was markedly lower than in liver ones (Fig. 3).

Scatchard analyses were carried out by incubating a fixed amount of liver membranes with increased amounts of labelled sbGH (Fig. 4). Scatchard plots were always linear, denoting the presence of a single class of high affinity and low capacity binding sites. Table II summarizes sbGH-binding characteristics in treated ($MgCl_2$) and untreated membrane preparations.

Competitive studies were performed incubating liver membranes with a fixed amount of iodinated sbGH and increasing amounts of unlabelled hormone preparations (Fig. 5). oPRL, rtiPRL, cGtH and sGtH did not displace ^{125}I-sbGH from the binding sites at any of the concentrations tested. hGH and bGH displaced iodinated sbGH in a dose dependent-manner, though the inhibitory

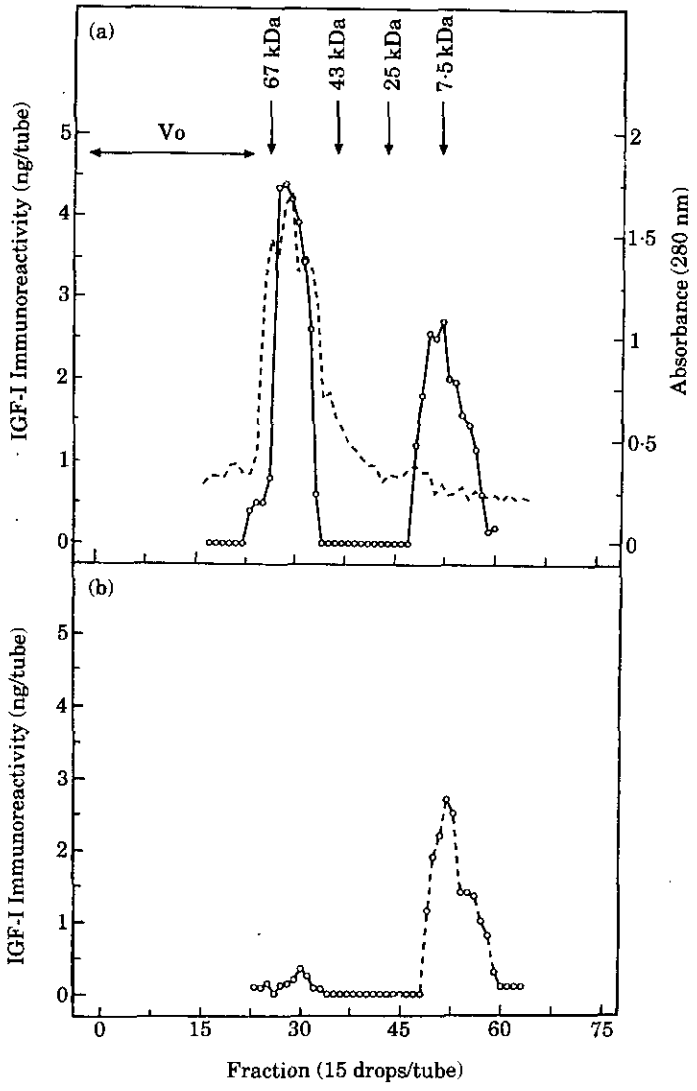


FIG. 6. Acid gel filtration of gilthead sea bream plasma on Sephadex G-75 column before (a) and after CM-25 extraction (b). The column effluent was monitored by IGF-I immunoreactivity (—○—). The protein profile was plotted on the same graph as optical density (– –). Vo, Void volume. Arrows indicate molecular weight markers, including albumin (67 kDa), ovalbumin (43 kDa), chymotrypsinogen A (25 kDa) and ^{125}I-rhIGF-I (7·5 kDa).

potency appear to be 20–40-fold lower than that of sbGH. In contrast, the inhibitory potency of rtGH was two-fold lower than that of sbGH.

The chromatographic profile of gilthead sea bream plasma following acidification and separation over a Sephadex G-75 column is shown in Fig. 6(a). Two immunoreactive peaks were detected by IGF-I RIA. Peak I showed an apparent molecular weight of 50–60 kDa. Peak II showed an apparent molecular weight of 7–8 kDa. IGF-I immunoreactivity after Sephadex CM-25 extraction was also characterized. The extract was concentrated in ultrafree-MC filters (Millipore) with a 5000 molecular weight limit, and then chromatographed over a Sephadex

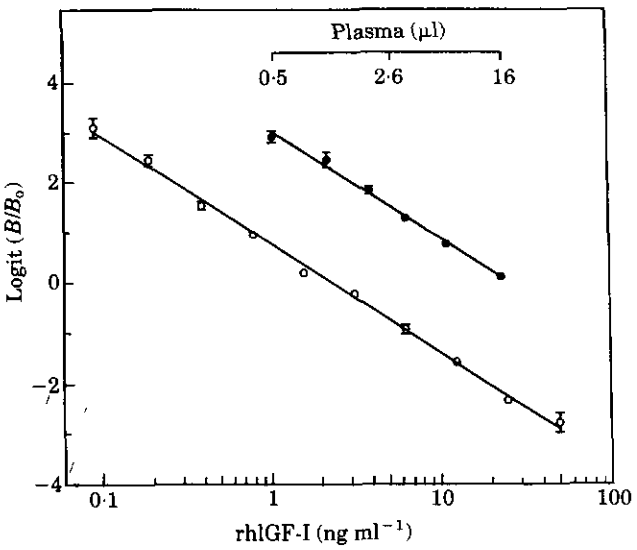


FIG. 7. Parallelism between serial dilutions of gilthead sea bream plasma (●) and rhIGF-I standard (○) measured by radioimmunoassay. Plasma IGF-I-like peptides were extracted by Sephadex CM-25. Each value represents the mean ± S.E.M. of four replicates.

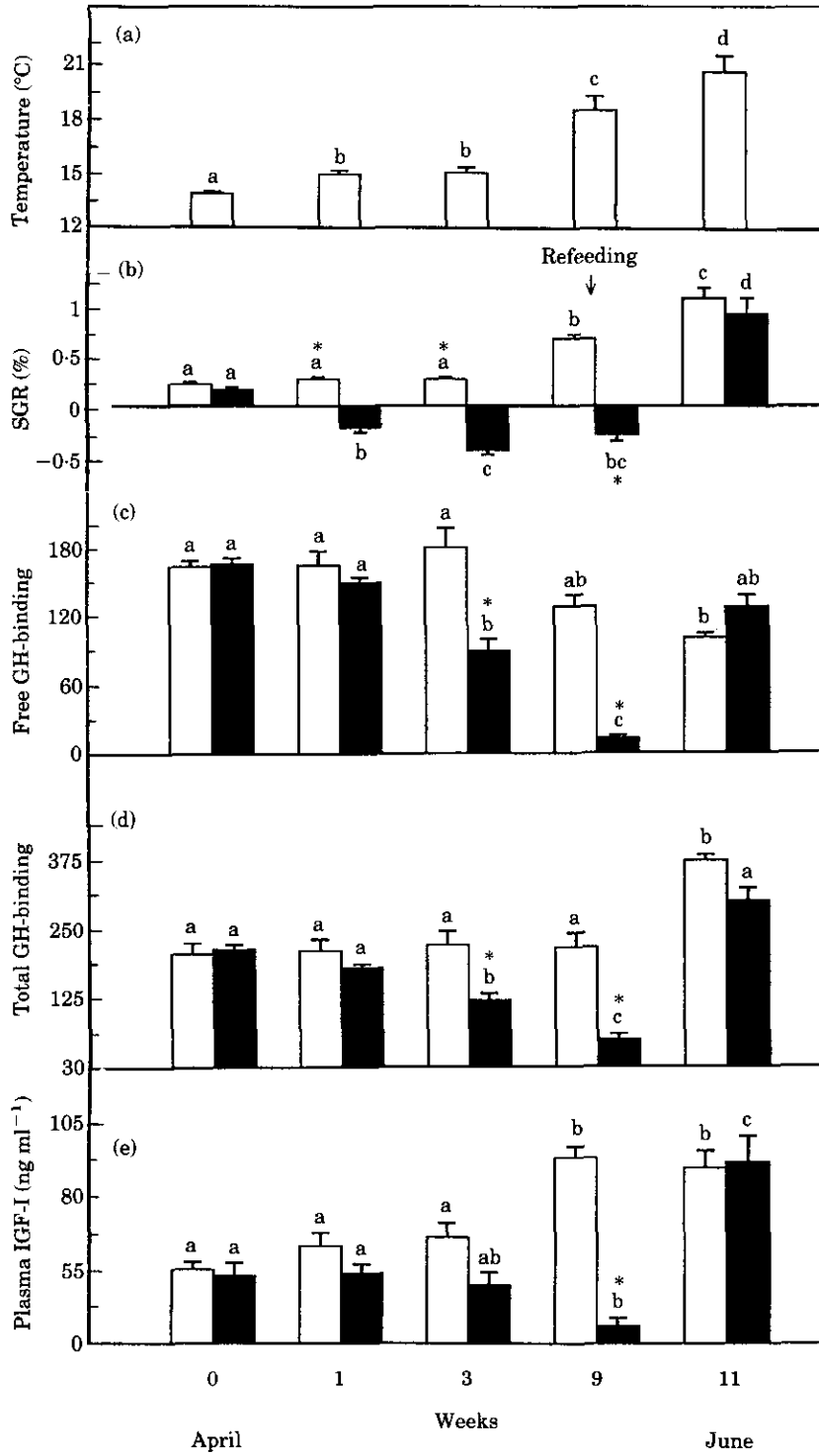
G-75 column (acid-gel filtration). Peak I was absent, without a significant loss of IGF-I immunoreactivity in the region of the free tracer peak [Fig. 6(b)], whose displacement curve was parallel to the standard curve in the IGF-I radioimmunoassay (Fig. 7). The recovery of rhIGF-I added to non-extracted gilthead sea bream plasma ranged between 85 and 95%.

Figure 8 shows the effect of fasting and refeeding on hepatic GH-binding and plasma IGF-I-like levels (Sephadex CM-25 extract). GH-binding affinity remained unchanged, but a progressive loss in total and free GH-binding was evidenced after 9 weeks of fasting, returning to control values after 2 weeks of refeeding. A similar trend was observed in plasma IGF-I-like immunoreactivity. Interestingly, we also observed that fed fish showed a significant increase of plasma IGF-I-like immunoreactivity in coincidence with the seasonal increase of water temperature and somatic growth. Free GH-binding decreased gradually, while total GH-binding increased significantly in the last sampling time.

IV. DISCUSSION

The present study demonstrated for the first time in a typical marine fish the presence of specific GH-binding sites with the general characteristics of hormone receptors. As expected, the liver appeared as the main source of GH-binding, corroborating the idea that the liver is an important target tissue for the direct action of GH (Mendelshon, 1988). Of particular significance is the observation that long-term fasting reduced both hepatic GH-binding and plasma IGF-I-like immunoreactivity.

Specific sbGH-binding was dependent on time, temperature and membrane concentration as it has been described for other polypeptide hormones (Cuatrecasas *et al.*, 1977). At 15° C, specific binding was maximal in 6 h, being


(a)
Temperature (°C)
21
18
15
12
(b)
Refeeding
SGR (%)
1
0·5
0
−0·5
(c)
Free GH-binding
180
120
60
0
(d)
Total GH-binding
375
250
125
30
(e)
Plasma IGF-I (ng ml⁻¹)
105
80
55
0
0
1
3
9
11
Weeks
April
June

steady at least until 24 h. In contrast, a significant decline in specific binding was observed at 24° C after 14 h of incubation, which could be due to denaturation and/or degradation of ligand and/or receptor protein. A similar phenomenon has been reported in trout (Yao *et al.*, 1991) and eel (Hirano, 1991) GH radioreceptor assays at 20 and 35° C, respectively. Tarpey & Nicoll (1985) have also reported a decrease in the specific binding of bovine GH to long-jawed mudsucker liver membranes 8 h after incubation at 37° C.

Scatchard analysis of the binding of iodinated sbGH to gilthead sea bream liver membranes consistently produced a straight-line Scatchard plot, indicating a single class of binding sites. The binding affinity and capacity are in accordance with the values reported for the homologous binding of mammalian (Gerasimo *et al.*, 1979; Hughes, 1979), avian (Leung *et al.*, 1987) and fish (Fryer, 1979; Gray *et al.*, 1990; Hirano, 1991; Yao *et al.*, 1991; Ng *et al.*, 1992) GHs to liver membrane preparations, and appear to conform to the general requirements expected in hormone receptor interactions.

Similarities in primary structure between GHs and PRLs suggest that these hormones have evolved from a common ancestral molecule (Li, 1972). In spite of this, both oPRL and rtiPRL did not demonstrate affinity for the sbGH-binding sites. Other pituitary hormones (cGtH and sGtH) did not compete with labelled sbGH for binding sites, which is not surprising given the structural and functional differences between GHs and GtHs. Conversely, rtGH, and mammalian GHs were able to displace labelled sbGH bound to hepatic binding sites, though human and bovine GHs exhibited a limited affinity for these somatogenic binding sites, in a similar manner as it has been established in tilapia (Fryer, 1979; Ng *et al.*, 1992) and salmonid (Gray *et al.*, 1990; Yao *et al.*, 1991) GH radioreceptor assays. In contrast, the inhibitory potency of rtGH was comparable to that of sbGH, which is in agreement with the observation that sbGH shows a high affinity for chinook salmon GH-binding sites (Yao *et al.*, 1991).

Thus far, information concerning IGF activity in gilthead sea bream has been limited to the study of Funkenstein and co-workers (1989). We confirmed that mammalian IGF-I antibodies can be used to detect IGF-I-like immunoreactivity in the plasma of gilthead sea bream. Serum from rainbow trout (Daughaday *et al.*, 1985), tilapia (Ng *et al.*, 1991) and channel catfish *Ictalurus punctatus* (Rafinesque) (Delahunty *et al.*, 1992) cross-react in mammalian IGF-I RIAs. However, sera from Atlantic blue fish *Pomatomus saltatrix* (L.) (Furlanetto *et al.*, 1977), Atlantic salmon *Salmo salar* L. (Lindahl *et al.*, 1985), leopard shark *Triakis semifasciata* Girarde and carp (Wilson & Hintz, 1982) do not exhibit significant cross-reactivity. These discrepancies remain unclear, though it could be due to the use of different antibodies. In addition, a suitable dissociation and removal of IGF-binding proteins (IGF-BPs) from circulating IGFs appear to

FIG. 8. Effect of fasting and refeeding on growth parameters. (a) Water temperature. (b) Weight specific growth rates. Each value represents the mean ± S.E.M. of two backs. (c) Free and (d) total hepatic GH-binding (fmol/mg protein). Each value represents the mean ± S.E.M. of four to five determinations. (e) Plasma IGF-I-like immunoreactivity. Each value represents the mean ± S.E.M. of eight to 10 determinations. Fed fish (□), starved fish (■). *$P<0{\cdot}01$; significant differences between groups at each sampling time; different letters express significant differences ($P<0{\cdot}01$) within each group throughout the experimental period (one-way analysis of variance followed by Duncan's multiple range-test).

be essential before plasma samples can be reliably assayed. The presence of IGF-BPs has been demonstrated in trout (Niu & Le Bail, 1993), channel catfish (Delahunty *et al.*, 1992), coho salmon, striped bass *Morone saxatilis* Walbaum, tilapia and long-jawed mudsucker (Kelley *et al.*, 1992). The most abundant IGF carrier has a molecular size comparable to that of mammalian IGF-BP3 (50–40 kDa). Thus, following acid gel-exclusion chromatography we detected a false immunoreactive peak in the region of 50 kDa, probably due to the presence of IGF-BPs that bind rhIGF-I tracer but are not precipitated by second antibodies in a double antibody-RIA (Nissley & Rechler, 1985). Mammalian studies support the idea that 50–40 kDa subunits are required to bind IGFs before combining with an acid-labile non-binding subunit to form a 150 kDa complex (Furlanetto, 1980; Baxter, 1986). Whether a protein homologous to an acid-labile subunit exists or not in fish species, requires further research.

The co-ordinate regulation of IGF-I synthesis and GH-binding by fasting is of considerable interest. Previous mammalian studies indicate that the number of liver GH receptors decreases during fasting (Maes *et al.*, 1983). Since GH is a positive regulator of IGF-I synthesis, this decline in GH-binding might explain, at least in part, the GH insensitivity and the decreased synthesis of IGF-I that occurs in the liver during nutritional deprivation. The most persuasive evidence is that the magnitude and kinetics of the decline in GH receptor mRNA are similar to the magnitude and kinetics of the decline in IGF-I mRNA (Straus & Takemoto, 1990). Unfortunately, the knowledge of the regulation of GH action in fish species is far from that of mammals. Using $^{35}SO_4$ incorporation by ceratobranchial cartilage as an indirect measure of somatomedin activity, it has been suggested that fasting decreases hepatic IGF-I release in trout (Komourdjian & Idler, 1978), long-jawed mudsucker (Gray & Kelley, 1991) and coho salmon (McCormick *et al.*, 1992). Moreover, Duan & Hirano (1992) have reported a significant decrease of hepatic IGF-I mRNA in starved Japanese eel. A significant decrease in free and total hepatic GH-binding has also been demonstrated in coho salmon after 3 weeks of fasting (Gray *et al.*, 1992). Similarly, we observed a temporal relationship between the decrease and increase of hepatic GH-binding and plasma IGF-I-like immunoreactivity during fasting and refeeding of gilthead sea bream. However, a significant effect of fasting and refeeding on plasma IGF-like activity has not been established in tilapia (Drakenberg *et al.*, 1989; T. B. Ng & T. C. Leung, unpublished results). This apparent discrepancy could reflect a short period of food deprivation or a low somatic growth when food deprivation was imposed. Thus, in our study, the seasonal increase of somatic growth (April–June) could contribute to amplify the difference in plasma IGF-I activity between fed and starved fish. In fed fish, we observed a significant increase of plasma IGF-I-like immunoreactivity in coincidence with a progressive decrease of free GH-binding sites. This decline was accompanied by a significant increase of total GH-binding sites in the last sampling time. This up-regulation of hepatic GH receptors could be due to the increase of plasma GH levels, which precedes the seasonal increase of somatic growth in goldfish (Marchant & Peter, 1986). In agreement with this idea, Mori and coworkers (1992) have demonstrated that a single injection of GH up-regulates hepatic GH receptors in Japanese eel.

In summary, the present paper constitutes the first report which indicates a co-ordinated regulation of GH-binding and plasma IGF activity in a typical marine fish, such as gilthead sea bream.

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