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The Review of the Effect of Growth Hormone on Immune System, Metabolism and Osmoregulation of Fish

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Abstract: The aim of the present review is to summarize the knowledge on the neuroendocrine control of Growth hormone (GH). The biological actions of growth hormone are pleiotropic, including growth promotion, energy mobilization, gonadal development, appetite, social behavior, osmoregulation and control of immune system. Accordingly, the regulatory network for GH is complex and includes many endocrine and environmental factors. Growth hormone, a member of the family of hormones as prolactin, promotes acclimation to seawater in several teleost fish, at least in part through the action of insulin-like growth factor I. In branchial epithelia, development and differentiation of the seawater-type chloride cell is regulated by GH, IGF-I, and cortisol. Control of salinity acclimation in teleosts by prolactin and growth hormone primarily involves regulation of cell proliferation, apoptosis, and differentiation. The interaction of these hormones with corticosteroids.is very important and should be represent. The present review investigate the role of GH\insulin-like growth factor(IGF-1) axis in the immune system of teleost fish. in some euryhaline fish the activation of immune functions, observed during acclimation that are associated with the osmoregulatory action of GH, are discussed (Yada, 2007).

Key words: Growth hormone, Immune system, osmoregulation, corticosteroid

INTRODUCTION

Growth hormone (GH) is a pluripotent hormone produced by the pituitary gland in teleosts as in other vertebrates. Ligand binding induces receptor dimerization producing an active trimeric complex (for review, see Pérez-SJnchez *et al*., 2002). GH has been sequenced and/or the protein isolated from scores of teleosts, various immunoassays established, and a number of GH transgenic fish strains established. Over the last two decades, many aspects of GH physiology have been the subject of intense research in fish such as the salmonids, cyprinids, and sparids. In fish, GH have effect in many different function and participates in almost all major physiological processes in the body including the regulation of ionic and lipid, protein, osmotic balance, and carbohydrate metabolism, skeletal and soft tissue growth, reproduction and immune function. the biological actions of GH are not restricted to growth promotion; but also they include energy mobilization, gonad development, appetite, and social behavior. Similarly, the regulatory network for GH is highly intricate, including many endocrine and environmental factors appropriate for the diverse physiological circumstances in which GH is involved. Recent studies have indicated that GH affects several aspects of behaviour, including appetite, foraging behavior, aggression, and predator avoidance, which in turn has ecological consequences (Bj.rnsson *et al*., 2004; Pérez-Sanchez, 2000; Peter and Marchant, 1995). There are many information of Gh action in fish but the most of its action still is needed to be investigated. Most of the authors claim that most/all GH s are indirect, based on an outdated mammalian view where the pituitary/hepatic GH/IGF-I system was seen as an "axis" with IGF-I mediating the physiological action of GH (Bj.rnsson *et al*., 2004; Butler and Le Roith, 2001). The wide tissue distribution of IGF-I producing cells (Reinecke *et al*., 1997) and IGF-I receptors (IGF-1R) (Radaelli *et al*., 2003a), together with the extensive tissue distribution of GH-receptors (Pérez-SJnchez *et al*., 2002) makes it a truly challenging task to provide unequivocal data on how GH mediates its actions at the cellular level. In this context, the cloning of the teleostean GH-receptors has been a major break-through. For more understanding the action and molecular information of GH in the cells, since 2001 when the GHR was first cloned in goldfish (Lee *et al*., 2001) and turbot (Calduch-Giner *et al*., 2001), it has been cloned in a rapidly growing number of teleost species, 15 at the latest count. Such research into the temporal and spatial distribution of the receptor, receptor sub-types and intracellular signaling pathways will hopefully help explain how the pluripotent actions of GH are mediated, but at the moment, very little is

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known about the signaling mechanisms of the various forms of fish GH-Rs. Further fields of research would include aspects such as hormone-receptor interaction, receptor-mediated turn-over of the hormone affecting the GH clearance rate, as well as the likely dual role of the receptor molecule to also act as a GH-binding protein in plasma.

Structure of the GH Family of Proteins:

This family of hormones includes GH, prolactin (PRL), chorionic somatomammotropin or placental lactogen (PL), and fish somatolactin (SL), all of which likely have arisen from a common ancestor (Niall *et al*., 1971; Miller and Eberhardt, 1983; Ono *et al*., 1990; Walker *et al*., 1991). Members of the GH family have an approximate molecular mass of 22,000 (22kDa), with approximately 200 amino acids, including two (GH, PL) or three (PRL) disulfide internal bonds. In teleost fish, it have been investigated and showed approximately the same molecular mass, but more variable in size than vertebrate. GH is a single polypeptide protein of 21-23kDa (Cavari *et al*., 1994; Martinez-Barbera *et al*., 1994; Sciara *et al*., 2006). The cysteine residues involved in the disulfide bonds are highly conserved in all members of the family, suggesting an important role in biological actions. In this regard, it was found that the large disulfide loop is essential for growth-promoting activity (Chen *et al*., 1992). The fish hormone exhibits typical GH features, such as four cysteine residues, capable of forming two disulfide bonds that are assumed to contribute to the tertiary structure of the hormone molecule and a site for *N*-linked glycosylation (Cavari *et al*., 1994; Law *et al*., 1996; Marins *et al*., 2003; Clements *et al*., 2004; Sciara *et al*., 2006). In ostariophysan fish, GH sequences contain an unpaired cysteine residue with unknown functional significance (Ho *et al*., 1991; Hong and Schartl, 1993; Cavari *et al*., 1994; Law *et al*., 1996; Anathy *et al*., 2001; Clements *et al*., 2004). As mentioned above, it is believed that all members of the family arose from a common ancestral gene (Niall *et al*., 1971; Amemiya *et al*., 1999). The available data suggest that a first gene duplication was the origin of the divergence of PRL gene and a precursor of GH/PL gene during early vertebrate evolution (Miller and Eberhardt, 1983). A second gene duplication, that occurred earlier than the separation of bony fish lineages from the lineages that originate tetrapods, might have lead to the occurrence of SL in fish, including lungfish (May *et al*., 1999). Presumably, the occurrence of SL is derived from either PRL gene duplication or independently from the duplication of the ancestral form (Wallis, 1992; May *et al*., 1999) and less likely from GH gene. It is also possible that these three genes arose during the two rounds of genome duplication that took place during chordate evolution (Forsyth and Wallis, 2002). In mammals, further gene duplication of GH or PRL genes gave origin to placental members of the family (Walker *et al*., 1991; Forsyth and Wallis, 2002). The structure of GH gene in fish shows higher variability when compared to tetrapods. In fish, GH gene includes either five exons and four introns (similar to mammalian and avian species) as found in members of superorder Ostariophysi or six exons and five introns as found in species of superorder Prota acanthopterigii and Acanthopterigii (Venkatesh and Brenner, 1997; Almuly *et al*., 2000).

GHR Receptors: Mechanism of Action:

GH initiates biological actions by binding to GHR present on the cell membrane of target tissues. However GHR are also present in many tissues including muscle, adipose tissue, bone, kidney and numerous others (Kopchick and Andry, 2000).

To understand the mechanism of GHR will help to illustrate the effect of this hormone in different function in body of fish.The growth hormone receptor (GHR) is the key regulator of postnatal growth and has important actions on metabolism, reproductive, gastrointestinal, cardiovascular, hepto-biliary, and renal systems (Lichanska & Waters, in press). The growth hormone receptor is expressed as a monomer which forms a ligand-receptor complex made up of one growth hormone (GH) molecule and two GHRs (GH:2GHR). One of the receptors binds with a strong affinity to site 1 of the GH followed by the weaker site 2 binding to the second receptor (Fig. 2).

Several authors suggested different models of the activation of class I cytokine receptors for example ligand-induced receptor dimerisation, ligand-induced receptor aggregation, and a ligand-induced receptor conformational change that results in signal transduction. The textbook model for GHR activation posits that GH induces receptor dimerisation resulting in JAK/STAT signalling by transactivation of JAKs (Waters *et al*., 2006). More recently, studies using full length GHR have revealed that the receptor is found as a dimer on the surface of the cell in the absence of GH(Brown *et al*., 2005; Gent *et al*., 2002). This has led to a paradigm shift whereby most current evidence supports a model of GH binding to a constitutively homodimerised GHR which causes a repositioning of the Intera Cellular Domain (ICDs), resulting in the activation of associated tyrosine kinases and signal transduction (Fig. 2). Rotation of individual GHR molecules in relation to their

dimerised partner is an important critical component in the structural changes induced by GH binding as illustrated by recent structural and biochemical studies from our laboratory (Brown *et al*., 2005) and the modelling of binding of an agonist monoclonal antibody to the GHR ECD (Wan *et al*., 2003). However, other GH induced structural changes in the dimerised GHR are also likely to play significant roles in the production of effective GHR signalling (Fig. 2). This current model of signalling is not unique to GHR as other recent studies have suggested a similar activation model for the closely related erythropoietin receptor (Lu *et al*., 2006).

GHR Structure:

The GHR extracellular domain (ECD) consists of two fibronectin type III _ sandwich domains connected by a short flexible linker that are described by(Brooks *et al*.,2008) and showed in Fig. 1. These two domains are connected to a rigid single pass helical transmembrane domain via a flexible linker. The intracellular domain (ICD) comprises Box 1 and Box 2 motifs, which bind the tyrosine kinase JAK2, and several tyrosine residues that are substrates for phosphorylation by JAK2 and so become binding sites for SH2 domain proteins. The actions of GH are initiated by binding to membrane- bound GH receptors (GHRs) located on target tissues. GHRs are members of the type I cytokine receptor family (Waters, 1999; Butler and Le Roith, 2001) that include prolactin receptor, interleukin receptor and colony stimulation factor receptor, erythropoietin receptor, and leptin receptor (Waters, 1999). It consists of a single transmembrane protein with extracellular, transmembrane, and cytoplasmatic domains. The extracellular part is arranged in two domains that form a sandwich of two antiparallel-sheets in a fashion similar to the fibronectin III domainsm (Waters, 1999), which contains the hormone-binding sites. Gover*set al*., 1999 described it in this way that the cytoplasmatic domain presents two conserved sequences or boxes important for signal transduction. Box1 represents a binding site for Janus kinase 2 (JAK 2) essential for most of the functions. On the other hand, Box 2 seems to be involved in the internalization process.

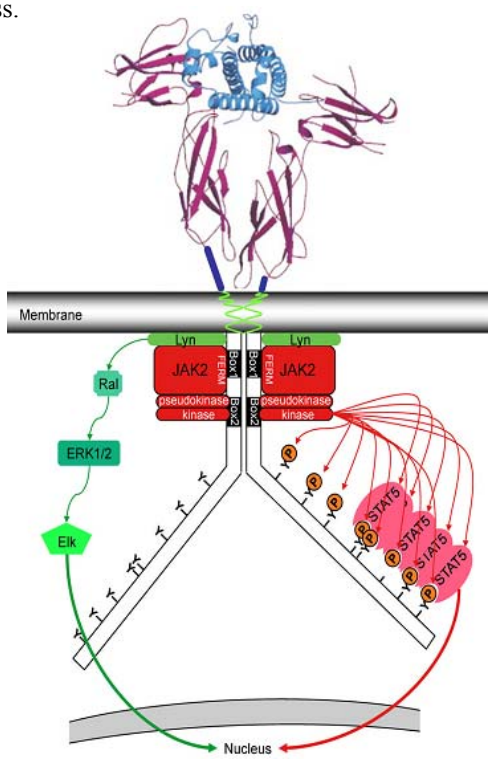

Fig. 1: Structure of the GH bound GHR and activated signaling pathways. Dimerised GHR ECD bound to GH (crystal structure shown as a ribbon diagram; GHR ECD magenta, GH blue) is depicted above the membrane and the ICD is illustrated below the membrane connected via the TMD. Tyrosine kinases JAK2 (domains labeled in white) and Lyn are shown bound to the ICD with their signaling pathways illustrated (JAK2 red arrows, Lyn green arrows).Cited by Brooks *et al*.,2008.

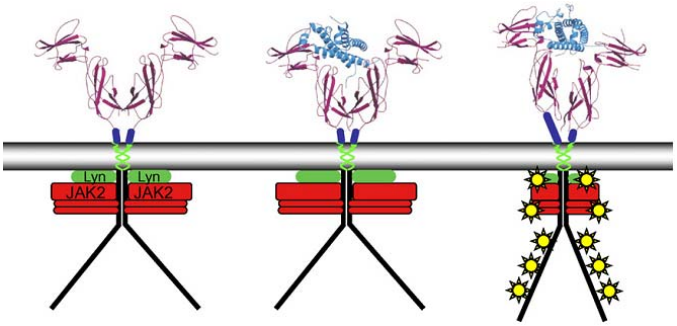

Fig. 2: Model for GHR activation. GH binding to a constitutive receptor dimer results in structural changes including relative rotation of receptor subunits in the homodimer, producing realignment of JAK2 and Lyn kinases bound to the membrane proximal sequence below the cell membrane. Appropriately aligned kinases are then able to activate each other by transphosphorylation, initiating signaling cascades (highlighted by star-shaped symbols). Cited by Brooks *et al*., 2008.

Insulin-like Growth Factor I:

During the growth period mainly in summer time in temperate rejoin change will occure in blood biochemical profile. IGF-I increases in blood during the growing season in temperate fishes showing seasonal growth (Mingarro *et al*., 2002), and is stimulated by increased temperature (Beckman *et al*., 1998) and day length (McCormick *et al*., 2000). Furthermore, treatment of fish with IGF-I implants stimulates growth (McCormick *et al*., 1992). IGF-I in fish has been associated not only with growth, but also several physiological effecting e.g. with metabolism (Castillo *et al*., 2004), development (Greene and Chen, 1999b; Pozios *et al*., 2001), reproduction (Maestro *et al*., 1997; Weber and Sullivan, 2000), and osmoregulation in seawater (McCormick, 2001). IGF-I exerts its effects on cells through binding to the IGF-I receptor (IGF-1R), which binds IGF-II in a similar manner in zebra fish (Mendez *et al*., 2001; Pozios *et al*., 2001) but not so well in rainbow trout (Loir and Le Gac, 1994). IGF-I function in fish is controlled variously by regulation of IGF-I production, IGF receptor function, signaling pathways and cross-talk, and modulation by systemic and local production of IGFBPs. A full understanding of IGF-I function in fish requires more work in all of these areas, in a variety of physiological processes, such as growth, metabolism, reproduction, osmoregulation, and in fish species with various life history and ecological types(Reinecke *et al*.,2005).

Insulin-like Growth Factor II:

Parallel investigation with IGF-I mRNA have been with study of IGF-II mRNA in bony and it have been detected both in liver and in numerous other organs, such as brain, eye, gills, heart, gastrointestinal tract, pancreatic islets, kidney, skeletal muscle, spleen, and male and female gonads (Ayson *et al*., 2002; Caelers *et al*., 2004; Vong *et al*., 2003). Previously, the IGF type 2 receptor was found only in mammals and it was generally believed to be absent from non-mammalian vertebrates (for review, see Reinecke and Collet, 1998). indicating that IGF-II, like IGF-I, could act not only as a growth factor but also as a metabolic hormone. More study by several authors proved that in bony fish both the IGF-I gene and IGF-II gene are controlled by GH in all organs (Shamblott *et al*., 1995; Tse *et al*., 2002; Vong *et al*., 2003). This makes bony fish quite unique because in other vertebrate classes, GH most likely regulates only the expression of the IGF-I gene (for review, see Reinecke and Collet, 1998). Whether the above results indicate a particular impact of IGF-II in fish remains to be clarified (Reinecke *et al*.,2005). Subsequently, the potential changes in circulating IGF-II during development or under different physiological conditions, including nutritional status, smoltification, and temperature, and varying GH levels should be investigated and correlated to alterations in the expression of the IGF-II gene.

Growth Hormone in Fish Osmoregulation:

Growth hormone has also been shown to have a role in teleost osmoregulation, in addition to its growth promoting role. Smith (1956) was the first to observe that growth hormone treatment could increase the capacity of fish (brown trout, *Salmo trutta*) to tolerate exposure to seawater. It was later determined that this effect was due to the capacity of this hormone to increase the number and size of gill chloridecells, Na+,K+-ATPase, and the Na+,K+,2Cl‹ cotransporter (NKCC), ion transporters involved in salt secretion(McCormick, 2001; Pelis and McCormick, 2001). The effect of GH on salinity tolerance and differentiation of salt secretory

mechanisms is not restricted to salmonids, as this effect has been found in two other euryhaline species, tilapia and killifish (Sakamoto *et al*., 1997; Mancera and McCormick, 1999). Some of the actions of growth hormone are through insulin-like growth factor I (IGF-I). Exogenous treatment of IGF-I has been found to increase the salinity tolerance of rainbow trout, Atlantic salmon, and killifish (Mancera and McCormick, 1998). In brown trout, long term IGF-I treatment can increase the number of gill chloride cells and Na+,K+-ATPase activity concurrent with increased salt secretory capacity (Seidelin *et al*., 1999). In addition to the effects of exogenous hormones treatments, changes in pituitary gene expression, secretion, circulating levels, and metabolic clearance rate of growth hormone also provides evidence for the osmoregulatory actions of growth hormone in several euryhaline species widely separated in the evolution (Sakamoto *et al*., 1993). Plasma GH levels have also been found to increase in stenohaline catfish following exposure to 12 ppt seawater (Drennon *et al*., 2003). It was proved by Shepherd *et al*. (2005) that the circulating levels of IGF-I increases following exposure of rainbow trout to seawater. IGF-I has been found specifically in gill chloride cells whose number and/or size are stimulated by growth hormone (Sakamoto *et al*., 2001). Growth hormone has also been detected in osmoregulatory organs and may be acting in an autocrine or paracrine manner in these tissues (Sakamoto *et al*., 2005a,b; Yang *et al*., 1999). The GH/IGF-I axis is also important in the preparatory physiological adaptations that comprise the parr-smolt transformation of anadromous salmonids (Sakamoto and McCormick,2006). This transformation includes a number of changes that are adaptive for seawater entry, including increased salinity tolerance. To date a relatively small number of teleosts have been examined for the physiological impact of the GH/IGF-I axis on osmoregulation. Exogenous treatments have been found to affect most salmonids, tilapia, and killifish(Sakamoto and McCormick,2006). Evidence from circulating hormones, local production, and from salmonids provides convincing evidence for endocrine and paracrine actions of the GH/IGF-I axis, but there is relatively little information in this area from other teleosts. However, there is no apparent effect of exogenous GH on several osmoregulatory parameters in the gilthead sea bream (*Sparus auratus*) (Mancera *et al*., 2002), and osmoregulatory effects on the another sea bream, *Sparus sarba*, are not consistent with a seawater acclimating impact (Kelly *et al*., 1999). Pituitary GH and liver IGF-I mRNA levels in sea bream were lower after exposure to both hyper- and hyposaline conditions (Deane and Woo, 2004). Similarly, GH may not play an osmoregulatory role in the eel (Sakamoto *et al*., 1993). Sea bream and eel have marine origins or a limited capacity to hyperosmoregulate. Species variation linked to different limitations in ion regulatory capacity and/or strategies for ion regulation may affect whether and to what extent the GH/IGF-I axis is involved in osmoregulation.

Actions of Growth Hormone on Osmoregulation and Carbohydrate Metabolism:

GH may also influence acclimation to seawater indirectly through effects on energy metabolism. Seawater acclimation is a highly energetic process, undoubtedly associated with major changes in plasma metabolite levels and repartitioning of energy reserves, as well as with changes in plasma osmotic pressure and ion concentration during seawater adaptation. Based on several investigation, it was found several hormones levels increased during osmotic acclimation including cortisol, GH, and prolactin have been associated with the presence of ion regulation and with seawater acclimation in fish. In addition, these hormones have been shown to play a role in the mobilization of energy substrates in fish (Leung *et al*., 1991; Sheridan, 1986). When considering GH, it remains to be determined if the reported changes in GH concentration are associated with seawater adaptation per se or with concomitant alterations in plasma metabolite levels.

In mammals, the effect of GH on carbohydrate and lipid metabolism can be divided into acute insulin-like and chronic anti insulin-like activities (Davidson, 1987). The rapid and transient insulin-like of GH include increase in glucose utilization and decrease of blood glucose in hypophysectomized rats. The chronic anti insulin-like effects of GH occur only after a time lag of several hours after GH administration and include inhibition of glucose utilization and stimulation of lipolysis. GH is believed to posses similar functions in fish (Bj.rnsson, 1997).

In fish, GH seems to have lipolytic, diabetogenic, and protein-anabolic effects. Thus, GH has been found to be lipolytic in goldfish (Minick and Chavin, 1970), rainbow trout (Leather land and Nuti, 1981; O'Connor *et al*., 1993), kokanee salmon (McKeown *et al*., 1975), and coho salmon (Sheridan, 1986). As for protein metabolism, GH effects appear to be anabolic through increased protein synthesis (Foster *et al*., 1991). Moreover, an increased oxygen consumption has been reported after GH treatment (Herbert *et al*., 2001; Seddiki *et al*., 1995) further supporting the notion that GH has anabolic effects on protein turnover for example GH treatment reduces glycogen synthetase activity and decreased hepatic glycogen levels in tilapia (Leung *et al*., 1991) and, in line with this observation, this treatment also induces hyperglycemia in several fish species (Sweeting *et al*., 1985; Leung *et al*., 1991). In freshwater (FW) adapted rainbow trout, GH increases plasma

glucose levels, decreases the glycolytic potential and the capacity to export glucose from liver; and also affects carbohydrate metabolism of gills, kidney and brain (Sangiao-Alvarellos *et al*., 2005b).

In waters of low salinity or FW environments, pituitary expression of PRL mRNA (Martin *et al*., 1999) and plasma levels of PRL (Auperin *et al*., 1995) increase to produce various osmoregulatory changes (Manzon, 2002). In addition, acclimation to hypoosmotic media induces changes in energy metabolism (Sangiao-Alvarellos *et al*., 2003b, 2005c). Surprisingly, very few investigations have examined the effects of PRL on the energy metabolism of osmoregulatory (Leena and Oommen, 2000) and non-osmoregulatory organs of teleosts. The few studies available regarding metabolic effects of PRL in fish only address effects on lipid metabolism (Sheridan, 1986; Leena and Oommen, 2000, 2001; Leena *et al*., 2001), and limited results on carbohydrate metabolism are available (Mancera *et al*., 2002). During osmotic acclimation, the gilthead sea bream (Sparus auratus) shows osmoregulatory (Mancera *et al*., 1993a; 2002) and metabolic changes (Sangiao-Alvarellos *et al*., 2003b, 2005c). In this species, adaptation to brackish water or lower salinity activates PRL cells (Mancera *et al*., 1993b) whereas acclimation to hyperosmotic salinity enhances GH cells (Mancera *et al*., 1995). In fish, PRL controls water and electrolyte balance (especially in FW conditions), growth and development. Moreover, its pleiotropic character is illustrated by its effects on metabolism, behaviour, reproduction and immunoregulation (Hirano, 1986; Manzon, 2002). Indeed, PRL receptors were demonstrated in gills, kidney, gut, skin, and liver of several fish species (Prunet and Auperin, 1995; Manzon, 2002).

In contrast to the preceding, the effects of GH on carbohydrate metabolism in fish are less well studied. This lack of information also applies to the different tissues involved in carbohydrate metabolism since the only studies performed to date were those carried out in liver (Farbridge and Leather land, 1988; Leung *et al*., 1991). Considering (1) the presence of receptors for GH in tissues like gills, brain, and kidney (Leibush *et al*., 1996; Sakamoto and Hirano, 1991), (2) the increased levels of plasma GH during osmotic acclimation in teleosts (McCormick, 2001), and (3) the existence of changes in carbohydrate metabolism of osmoregulatory and non-osmoregulatory tissues during osmotic acclimation (Sangiao-Alvarellos *et al*., 2003a,b; Soengas *et al*., 1995a,b), a possible involvement of GH on carbohydrate metabolism cannot be discarded to occur, and has not been thoroughly assessed to date in teleost fish.

Effects of GH on the Fish Immune Functions:

Advances in molecular approaches in immunology prove that fish possesses a complex network of immune system similar to that of mammals, characterized by the different types and subtypes of leucocytes, immunoglobulin (Ig), complement factors, a major histocompatibility complex, T cell receptors and cytokine molecules (Manning, 1994; Secombes *et al*., 1998; Yada and Nakanishi, 2002; Bird *et al*., 2006). *et al*., 1998; Yada and Nakanishi, 2002). In euryhaline fishes, an increase in circulating growth hormone (GH) level during a course of adaptation from fresh water to seawater is correlated with the activation of immune function (Marc *et al*., 1995; Yada *et al*., 2001a, 2002; Pettersen *et al*., 2003; Dominguez *et al*., 2004, 2005). In these fishes, GH is known to increase seawater adaptability through differentiation of salt secretory mechanisms (McCormick, 2001; Sakamoto and McCormick, 2006). In higher vertebrates, it is now apparent that somatogenic hormones including GH exercise important stimulatory regulation of the immune system (Clark, 1997; Dorshkind and Horseman, 2000; Venters *et al*., 2001; Jeay *et al*., 2002). The earliest report of the regulation of the fish immune system by hypophyseal hormones including GH was published by Rasquin in 1951. Administration of carp pituitary extract into a characin fish (*Astyanax mexicanus*) resulted in an increased number of leucocytes in the head kidney and the spleen. In sailfin molly (*Poecilia latipinna*), however, the administration of mammalian GH had no effect on the number of leucocyte in peripheral blood (Ball and Hawkins, 1976). These seemingly inconsistent results may stem from the use of heterologous hormones. non-specific cytotoxic activity in leucocytes isolated from the kidney, spleen and peripheral blood of rainbow trout following the *in vivo* administration of chum salmon (*O. keta*) GH, which is identical with trout GH. Subsequent studies of modulation of the fish immune system by GH have focused mainly on its actions on the phagocytosis. GH has been found to increase particle ingestion by fish leucocytes.

Activation of phagocytic activity were proved by *in vitro*, investigation by (Sakai *et al*., 1996a; Calduch-Giner *et al*., 1997; Narnaware *et al*., 1997). *In vivo* and *in vitro* administration of GH enhanced superoxide anion production as a killing mechanism following phagocytosis (Sakai *et al*., 1995, 1996a; Mu˚oz *et al*., 1998; Yada *et al*., 2001a, 2002). GH regulates not only phagocytosis but also humoral defenses in fish. Haemolytic activity of serum, which involves the complement system, was increased in rainbow trout by administration of exogenous GH (Sakai *et al*., 1996c). On the other hand, studies on the possible of GH on antibody production or specific immune function in fish are limited. Administration of homologous GH had no effect on the circulating levels of Ig in trout (Yada *et al*., 2001a). In rainbow trout, significant amounts of GH-

receptor mRNA were detected in several tissues including spleen by real-time PCR (Very *et al*., 2005). These observations support the direct action of GH in modulation of immune function in fish.

GH Expression in the Fish Immune System:

As the signal molecules in the immune system, hormones produced in lymphoid tissues and cells may be categorized as cytokines. The GH gene is expressed in mammalian lymphoid organs, and GH is thought to act in a paracrine manner (Clark, 1997; Kooijman *et al*., 2000; Venters *et al*., 2001). In trout, GH gene expression in the immune system has been repeatedly reported in lymphoid tissues, although its distribution varies (Calduch-Giner and Pérez-Sánchez, 1999; Mori and Devlin, 1999; Yang *et al*., 1999 Yada *et al*., 2001b). Peripheral expression of GH mRNA is also observed in the immune system of rainbow trout and Mozambique tilapia, whereas GH mRNA was not detected in lymphoid tissues and cells of channel catfish (Yada and Azuma, 2002; Yada *et al*., 2001b, 2002). This difference in tissue distribution of GH may be related to variation in the degree of endocrine regulation of the immune function among fish species.

GH/IGF-I Axis in the Fish Immune System:

As in higher vertebrates, the growth-promoting actions of GH in the fish are mediated to a large extent by IGF-I (Duan, 1998). In mammalian species, IGF-I is also known to be an important factor that stimulates many aspects of the immune system, including lymphocytes proliferation, antibody production, phagocytosis and natural killer cell activity (Clark, 1997; Dorshkind and Horseman, 2000; Venters *et al*., 2001). Coinciding with the reports in mammals, IGF-I possesses immunomodulatory functions also in fish. Fig. 1 shows that proliferation of tilapia head-kidney leucocytes was stimulated by salmon IGF-I. The stimulatory effect of IGF-I was also examined in the superoxide production in phagocytic leucocytes (Fig. 2). A short-term treatment with IGF-I produced a significant elevation of superoxide production in tilapia head-kidney leucocytes, probably independent of stimulatory effect of IGF-I on mitosis. The expression of IGF-I gene has been detected by RT PCR in lymphoid tissues of several species of fish (Duguay *et al*., 1992, 1996; Shamblott and Chen, 1993; Loyng-Cueni *et al*., 1998; Yada *et al*., 2002). In euryhaline fish, IGF-I is also an important regulator for hydromineral balance, and GH directly stimulates IGF-I gene expression in gills, a major osmoregulatory organ during seawater acclimation (Sakamoto and McCormick, 2006). Gills are not only the respiratory and osmoregulatory organs but also the place of the trapping and processing of antigens in fish (Zapata *et al*., 1994). The GH induced expression of IGF-I in fish gills may modulate the induction of immune responses following the penetration or engulfment of pathogens via the gills. Taken together, these findings indicate that the GH/IGF-I axis plays an important regulatory role in the teleost fish immune system. Further investigations are needed to clarify the role of GH/ IGF-I axis in the fish immune system in different species under different environmental condition.

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