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Growth hormone (GH) treatment acts on the endocrine and autocrine/paracrine GH/IGF-axis and on TNF-α expression in bony fish pituitary and immune organs

N. Shved[a,b], G. Berishvili[a], P. Mazel[a], J.-F. Baroiller[c], Elisabeth Eppler[a,*]

[a] Research Group Neuro-endocrine-immune Interactions, Institute of Anatomy, University of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland
[b] Center for Evolutionary Medicine, Institute of Anatomy, University of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland
[c] CIRAD-EMVT UPR20, Aquaculture Unit, Campus International de Baillarguet, F-34398 Montpellier, France

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ABSTRACT

There exist indications that the growth hormone (GH)/insulin-like growth factor (IGF) axis may play a role in fish immune regulation, and that interactions occur via tumour necrosis factor (TNF)-α at least in mammals, but no systematic data exist on potential changes in GH, IGF-I, IGF-II, GH receptor (GHR) and TNF-α expression after GH treatment. Thus, we investigated in the Nile tilapia the influence of GH injections by real-time qPCR at different levels of the GH/IGF-axis (brain, pituitary, peripheral organs) with special emphasis on the immune organs head kidney and spleen. Endocrine IGF-I served as positive control for GH treatment efficiency. Basal TNF-α gene expression was detected in all organs investigated with the expression being most pronounced in brain. Two consecutive intraperitoneal injections of bream GH elevated liver IGF-I mRNA and plasma IGF-I concentration. Also liver IGF-II mRNA and TNF-α were increased while the GHR was downregulated. In brain, no change occurred in the expression levels of all genes investigated. GH gene expression was exclusively detected in the pituitary where the GH injections elevated both GH and IGF-I gene expression. In the head kidney, GH upregulated IGF-I mRNA to an even higher extent than liver IGF-I while IGF-II and GHR gene expressions were not affected. Also in the spleen, no change occurred in GHR mRNA, however, IGF-I and IGF-II mRNAs were increased. In correlation, *in situ* hybridisation showed a markedly higher amount of IGF-I mRNA in head kidney and spleen after GH injection. In both immune tissues, TNF-α gene expression showed a trend to decrease after GH treatment. The stimulation of IGF-I and also partially of IGF-II expression in the fish immune organs by GH indicates a local role of the IGFs in immune organ regulation while the differential changes in TNF-α support the in mammals postulated interactions with the GH/IGF-axis which demand for further investigations.

1. Introduction

Increasing knowledge is achieved that the neuroendocrine and the immune system interact in manifold ways in fish like in mammals. Especially the growth hormone (GH)/insulin-like growth factor (IGF) system has for several decades been proposed to interact with the immune system. In mammals, functional significance of GH for the immune system is indicated by the presence of the GH receptor (GHR) on lymphocytes [1,2] and of IGF-I in immune tissues including macrophages, dendritic cells and endothelial cells of human lymph node [3].

In general, GH is considered as a stimulator of the immune system in mammals like in bony fish [1,2,4–7] but reports from growth-deficient children as well as from kachectic patients treated with GH indicate a role also in adverse effects including septic shock, uncontrolled infections and depression due to enhanced production of cytokines, especially tumour necrosis factor (TNF)-α [8,9]. Even lowered IGF-I levels were measured in children during sepsis and septic shock [10] and in non-survivors from septic shock compared to survivors [11]. In both studies, high GH serum levels have been measured which were attributed to stimulated GH release via the feedback mechanism induced by low IGF-serum levels [12]. Similarly, in cultured rat hepatocytes low IGF-I levels in the presence of high GH levels were observed but were ascribed to GH resistance due to the suppression of hepatic GHR synthesis by interleukin (IL)-1β and TNF-α [13] which points to a role for pro-inflammatory cytokines in the regulation of the whole endocrine

* Corresponding author. Tel.: +41 44 635 5333; fax: +41 44 635 5702.
E-mail address: eppler@anatom.uzh.ch (E. Eppler).

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GH/IGF-I axis at least in mammals. In brief, there is general agreement in mammals and fish that IGF-I is mainly produced in the liver with GH as the major stimulus for its synthesis and release into the circulation and that the serum IGF-I concentration influences GH release from the anterior pituitary via a negative feedback mechanism by specific inhibition of GH gene transcription and secretion. This is termed the endocrine GH/IGF-I route [14–16]. However, there exist indications at least in bony fish that IGF-I exerts local effects on the GH cells at the level of the pituitary in addition to the well-established endocrine feedback loop [17–19]. Only few and contradictory data are available about effects of exogenous GH on GH expression in the anterior pituitary, and these have been exclusively achieved after life-long GH-overexpression in transgenic fish [20–22] but no data are available after short-term stimulation with GH.

In fish immune system, exogenous administration of GH enhanced non-specific activities of leukocytes [23] and phagocytes [24] and activated rainbow trout and gilthead sea bream phagocytes *in vitro* [25] and *in vivo* [26,27]. Furthermore, GH stimulated respiratory burst in vibriosis-furunculosis-vaccinated rainbow trout [28] and plasma lysozyme activity in rainbow trout and channel catfish [29,30]. The stimulatory effects of GH on fish immune functions may be mediated via IGF-I as indicated by the presence of IGF-I gene and peptide in immune tissues [22,31–35], where in tilapia, it has been localised to interrenal cells of the head kidney [31] and to head kidney and spleen leukocytes [22,35].

The importance of the GH/IGF-I system during infection in fish was emphasized by dropped IGF-I gene expression in liver and kidney of sea bream during vibriosis [36] and, along with down-regulated GHR gene expression, in head kidney of *Enteromyxum leei*-infected gilthead sea bream while non-infected individuals showed increased IGF-I and GHR levels. The latter finding was designated as advantage of the immune cells to combat the parasite [37]. Similar to the above mentioned clinical observations, lower IGF-I serum levels – accompanied by downregulated GHR mRNA - were found in channel catfish infected with *Edwardsiella ictaluri* and in confinement stress-exposed gilthead sea bream [30,38]. In the latter study [38] hepatic IGF-II and TNF-α gene expressions were also dropped. So far, IGF-II generally seems to behave similar to IGF-I, but its role is still enigmatic [39] and differential actions have been recently observed in fish physiology [40]. Potential interactions of GH and IGF-I with pro-inflammatory cytokines such as TNF-α have been suggested for a long time in mammals, where human and murine myeloid cells, particularly macrophages, have been found to produce IGF-I under stimulatory control of cytokines including TNF-α [41,42]. Vice versa, also IGF-I was found to directly stimulate murine monocyte and macrophage TNF-α production [43].

However, not all interactions seem to be stimulatory with respect to the above mentioned suppressed GHR synthesis by IL-1β and TNF-α in rat hepatocyte culture [13]. In accordance with this, most recently a defective GH response to exercise was postulated to be associated with increased blood interleukins levels and pain severity in fibromyalgia patients [44].

There exist first hints that, vice versa, IGF-I (and also IGF-II) counteract some effects induced by TNF-α especially in the brain such as depression and sickness behaviour syndrome, e.g. [45,46]. In addition, a local role for TNF-α is postulated at the level of the pituitary [47,48], namely in adenoma pathogenesis [49] and in direct interaction with the somatotrophs [50]. Thus, very scarce data on interactions between the GH/IGF-I axis and cytokines also at the level of brain and pituitary exist but they point to important autocrine and paracrine effects at least in mammals.

Even less is known on potential stimulatory and inhibitory actions between the physiological state, growth factors and cytokines in fish, e.g. [5,6,51,52] in general and especially with respect to interactions between the GH/IGF-I axis and TNF-α which is involved in numerous physiological and pathological immune responses [53,54]. So far, flounder TNF-α mRNA was induced in a GH-RH-treated embryonic cell line indicating that indirect GH-RH effects were mediated through GH release but in primary pituitary cell culture TNF-α mRNA was decreased indicating that its expression might be negatively regulated by GH-RH [55]. This recent study supports the bidirectional communication between neuroendocrine and immune system and underlines the necessity for a better understanding of these complex processes.

The aim of the present study was to quantify changes in GH, GHR, IGF-I, IGF-II as representatives of the GH/IGF-axis and TNF-α expression at different levels (brain, pituitary, peripheral organs, especially the immune organs spleen and head kidney) by acute challenge with GH. To assure the efficiency of our treatment protocol with bream GH, the endocrine IGF-I route was investigated by measuring liver IGF-I gene expression using real-time qPCR and plasma IGF-I levels with a fish specific IGF-I radioimmunoassay. Hepatic IGF-II and GHR expressions were also determined. Most pronounced treatment effects on IGF-I expression in head kidney and spleen were visualized by *in situ* hybridisation with tilapia IGF-I specific probes. Since only few systematic data exist on the expression levels of TNF-α in different fish organs, especially in tilapia, and no data on GH effects towards TNF-α in fish at all, we also determined basal expression levels for this cytokine in brain, pituitary, liver, head kidney and spleen and GH treatment effects by real-time qPCR. As a model fish, we have chosen the Nile tilapia which is intensively reared world-wide in aquaculture.

2. Material and methods

2.1. Fish culture and GH treatment

Balanced populations of tilapia (*Oreochromis niloticus*) were kept in the rearing structures of Cemagref, CIRAD, Montpellier and fed ad libitum with a commercial salmonid food. At 165 DPF 18 fish (36.8 ± 8.1 g, 12.9 ± 0.6 cm) were injected intraperitoneally with 2 μg/g body weight (bw) recombinant bream (*Acanthopagrus butcheri*) GH (GroPep, Adelaide, Australia) in sterile distilled water as recently described by our group as efficient GH treatment mode [56]. 14 controls (36.9 ± 8.7 g, 13.3 ± 1.0 cm) were mock-injected with sterile water. Injections were repeated the following day and samples were taken 6 h after the second injection. For sampling, the fish were anaesthetized with 2-phenoxy-ethanol (Sigma) added to the water, weight and length were measured and tissue specimens were excised. The principles of animal care and specific national laws were followed.

2.2. Blood sampling and radioimmunoassay (RIA)

Blood was collected from the caudal vein using a heparinized 1 ml syringe and centrifuged for 15 min at 4 °C at 10,000 rpm. Plasma was removed and stored at −20 °C. Plasma IGF-I levels were determined in undiluted samples by RIA after SepPak C18 chromatography (Waters Corp., Milford, MA, USA), as described earlier for mammals [57]. In brief, 0.15 ml PBS containing 0.2% human serum albumin (HSA), pH 7.4, was added to 0.1 ml plasma. All samples were acid-treated and run over SepPak C18 cartridges (Immunonuclear, Stillwater, MN, USA). After reconstitution with 1 ml PBS/0.2% HSA, fish plasma samples were assayed as previously described [58,59]: at 3 different dilutions (1:5, 1:10, 1:20) in 0.2 ml of samples or standards (fish IGF-I from GroPep, Adelaide, Australia) and 0.1 ml of IGF-I antiserum (final dilution 1:20000, GroPep) were preincubated for 24 h at 4 °C. 25000–35000 cpm of