Dual mode of cortisol action on GH/IGF-I/IGF binding proteins in the tilapia, *Oreochromis mossambicus*

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Abstract

Glucocorticoids are known to impede somatic growth in a wide range of vertebrates. In order to clarify the mechanisms through which they may act in an advanced teleost fish, we examined the effects of cortisol administration on the growth hormone (GH)/insulin-like growth factor-I (IGF-I)/IGF-binding protein (IGFBP) system in the tilapia (*Oreochromis mossambicus*). In a short-term experiment, fish were injected intraperitoneally with cortisol (2 or 10 µg/g), and killed at 2, 4, 8 and 24 h after the injection. In a longer-term experiment, fish were killed 24 and 48 h after cortisol injection (2, 10 and 50 µg/g). Cortisol at doses of 2 and 10 µg/g significantly increased IGFBPs of four different sizes (24, 28, 30, and 32 kDa) in the plasma within 2 h without altering plasma levels of IGF-I or GH. On the other hand, cortisol at doses of

10 and 50 µg/g significantly reduced plasma IGF-I levels after 24 and 48 h. IGF-I mRNA levels in the liver were also significantly reduced by cortisol at doses of 10 and 50 µg/g after 48 h, suggesting that a decrease in plasma IGF-I levels is mediated through the attenuation of IGF-I gene expression in the liver. In contrast, no significant change was observed in plasma or pituitary contents of GH at any time point examined, which would appear to indicate that cortisol reduces IGF sensitivity to GH (GH-resistance). These results clearly indicate that cortisol induces a rapid increase in plasma IGFBPs and a more delayed decrease in IGF-I production. The dual mode of cortisol action may contribute to the inhibitory influence of cortisol on somatic growth in teleosts.

Journal of Endocrinology (2003) **178**, 91–99

Introduction

Growth in vertebrates is controlled to a large degree by the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis. The regulation of growth by the GH/IGF-I axis also seems to be well conserved in teleosts (Duan 1997). As in mammals, the liver appears to be the primary production site of IGF-I, while locally produced IGF-I in extrahepatic tissues seems to play an autocrine or paracrine role in teleosts (Duan 1997, Kajimura *et al.* 2001). Biological activities of IGFs are modulated by a set of specific IGF-binding proteins (IGFBPs), and are mediated by specific receptors on the cell surface. Six distinct forms of IGFBPs, referred to as IGFBP-1 through IGFBP-6 with equal or greater affinities for IGFs than IGF receptors, have been identified in humans and other mammalian species. These IGF/IGFBP complexes prolong the residence time of IGFs in the circulation and control the action of IGFs by regulating availability of free IGFs to the target tissue receptors, a mechanism that is thought to prevent potential hypoglycemia (Collett-Solberg & Cohen 2000, Kelley *et al.* 2002). In teleosts, at least three different forms of

IGFBP, a 40–50 kDa and two smaller IGFBPs (24–31 kDa), have been constantly observed in the circulation (Park *et al.* 2000, Shimizu *et al.* 2000, Bauchat *et al.* 2001, Kelley *et al.* 2001, 2002). Recently, the cDNA of IGFBP-1 and -2 from zebrafish and IGFBP-3 from tilapia have been cloned (Duan *et al.* 1999, Cheng *et al.* 2002, Maures & Duan 2002).

Exogenous glucocorticoids are known to retard somatic growth (Loeb 1976). In mammals, glucocorticoids reduce IGF-I levels in the circulation and IGF-I gene expression both directly and indirectly by blocking GH-induced IGF-I gene expression through the attenuation of GH receptor synthesis (McCarthy *et al.* 1990, Unterman *et al.* 1993, King & Carter-Su 1995, Jux *et al.* 1998, Delany *et al.* 2001). Glucocorticoids also modulate the production of IGFBPs, increasing IGFBP-1 and -2, and decreasing IGFBP-3, which presumably lead to inhibitory effects on IGF-I's action (Unterman *et al.* 1993, Okazaki *et al.* 1994, Rodgers *et al.* 1995b, Conover *et al.* 1996). An inhibition of somatic growth after administration of cortisol, a major corticosteroid secreted by the interrenal gland in teleost fish, has been reported in rainbow trout and channel

catfish (Davis *et al.* 1985, Barton *et al.* 1987). However, the mechanism underlying this growth-inhibitory effect remains unknown.

Among more than 22 000 species of teleosts, the tilapia is one of the most advanced and also one of the most important aquaculture species in the world (Naylor *et al.* 2000). Studies on the mechanisms of cortisol-induced growth retardation will thus provide useful information of both a basic and an applied nature. Our goal in the present study is to evaluate the influence of exogenous cortisol on the GH/IGF-I/IGFBPs system in the tilapia, *Oreochromis mossambicus*. To clarify the mechanism underlying the inhibitory effects of cortisol on somatic growth, we examined *in vivo* effects on plasma levels of GH, IGF-I, IGFBPs, and mRNA levels of IGF-I in the liver.

Materials and Methods

Fish

Male tilapia (*Oreochromis mossambicus*), weighing 40–80 g, were reared in circular 700 liter tanks in fresh water under natural photoperiod at the Hawaii Institute of Marine Biology, University of Hawaii. They were fed twice daily with ProForm (Agro Pacific, Chilliwack, BC, Canada), approximately 2% of body weight per day. Water temperature was maintained at 24 ± 2 °C. The following experiments were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, University of Hawaii.

Experimental protocols

The effects of cortisol on the GH/IGF-I/IGFBPs system were examined in two different experiments. In a short-term experiment, cortisol (Sigma, St Louis, MO, USA), dissolved in soybean oil (2 and 10 mg/ml), was injected intraperitoneally at doses of 2 and 10 µg/g ($n=8-10$). Doses of cortisol were selected according to our previous study (Eckert *et al.* 2001) and our preliminary experiments using different doses of cortisol at different sampling intervals. Control fish received soybean oil only (0.1 ml/100 g). Samples were collected from separated groups of fish at 2, 4, 8 and 24 h post injection. To minimize the effect of handling stress on plasma cortisol, fish were anesthetized in 2-phenoxyethanol at a dose of 2 ml/l (Auperin *et al.* 1997), and blood samples were quickly taken from the caudal vasculature using a needle and syringe treated with ammonium heparin (Sigma, 200 U/ml). Plasma was separated by centrifugation and stored at -20 °C until radioimmunoassays and Western ligand blot assays were performed.

In a longer-term experiment, cortisol was injected at doses of 2, 10 and 50 µg/g. At 24 and 48 h post injection, plasma was obtained as described above and stored at

-20 °C until analyses. Subsequently, fish were decapitated, and the liver and pituitary were sampled, frozen in liquid nitrogen and stored at -80 °C. Plasma glucose was measured using a glucose (glucose oxidase) assay kit (Sigma).

Radioimmunoassays

For IGF-I RIA, plasma was first extracted with acid-ethanol following the procedures described by Shimizu *et al.* (2000), and total IGF-I was measured by homologous RIA as described by Kajimura *et al.* (2001). Plasma levels of GH and prolactin (PRL188; PRL) were measured by homologous RIA according to Ayson *et al.* (1993). Pituitaries were sonicated individually in ice-cold phosphate-buffered saline. Pituitary contents of GH and PRL were measured by RIA as described above. Plasma cortisol levels were measured using a cortisol assay kit (ICN Biomedicals, Costa Mesa, CA, USA).

Western ligand blot assay for IGFBPs

Four microliters plasma were diluted 1:4 with 10% glycerol, 0.75% Tris-OH, and 2% SDS and subjected to SDS-PAGE in a 12.5% separating gel/4% stacking gel. After SDS-PAGE, gels were placed in a transfer buffer containing 0.58% Tris-OH, 0.29% glycine, 0.038% SDS, and 20% methanol. An electrotransfer apparatus (Hoefer Scientific Instruments, San Francisco, CA, USA) was used to transfer proteins onto nitrocellulose membranes (Bio-Rad, Richmond, CA, USA) overnight at 100 mA constant current. The membranes were washed in Tris-buffer saline solution (TBS: 20 mM Tris-OH, 500 mM NaCl, pH 7.5) with 0.1% Tween-20 for 5 min, and then placed in blocking solution (0.1% Tween-20 and 1% BSA in TBS) for 6 h. Subsequently, the membranes were incubated overnight with 150 000 c.p.m./ml 125 I-human (h) IGF-I (Amersham, Piscataway, NJ, USA) at 4 °C. After the incubation, the membranes were washed three times with TBS to reduce background, allowed to air dry, then exposed to Bio-Max X-ray film (Eastman Kodak, Rochester, NY, USA) with intensifying screen for 3 days at -80 °C. For the quantification of IGFBPs, the autoradiographs were scanned and analyzed with an image analysis software program (NIH image, NIH, Bethesda, MD, USA). Values were expressed as optical densitometric units (ODU).

RNA extraction and RNase protection assay (RPA)

Total RNA was extracted individually from the liver using an RNA extraction solution (TRI-Reagent; MRC, Cincinnati, OH, USA) according to the manufacturer's instructions. IGF-I mRNA levels in the liver were quantified by RNase protection assay using the RPA III kit

Figure 1 Short-term effects of cortisol on plasma glucose (A), IGF-I (B), and GH (C). Vertical bars represent means $\pm$ S.E.M. ($n=8-10$). * $P < 0.05$, ** $P < 0.01$: significantly different from the control (oil, open bars).

(Ambion, Austin, TX, USA) with minor modifications as described by Kajimura *et al.* (2001).

Briefly, a plasmid containing tilapia IGF-I cDNA was linearized by XbaI (Promega, Madison, WI, USA), and transcribed with T7-polymerase to give a ^{32}P -cRNA probe with [α - ^{32}P]uridine 5-triphosphate (ICN Bio-medicals). A plasmid of tilapia β -actin was linearized by EcoRI, and a labeled cRNA probe was generated with SP6-polymerase. All of the cRNA probes were prepared using a MAXIscript transcription kit (Ambion). Twenty micrograms total liver RNA were hybridized with 2.0×10^4 c.p.m. of each of the ^{32}P -cRNA probes in 10 μl

Figure 2 IGF-BPs in the tilapia plasma identified by Western ligand blot assay. Plasma samples were subjected to SDS-PAGE and proteins were transferred onto nitrocellulose membranes. The membranes were incubated overnight with 150 000 c.p.m./ml ^{125}I -human IGF-I at 4 °C. After the incubation, the membranes were washed with TBS buffer, followed by exposure to Bio-Max X-ray film with an intensifying screen for 3 days at -80 °C. Marker, molecular marker; Intact, intact tilapia plasma; 1, control; 2, tilapia plasma after injection of 2 µg/g cortisol; 3, tilapia plasma after injection of 10 µg/g cortisol.

hybridization buffer overnight at 56 °C. Subsequently, samples were digested by RNase T1 (20 U/tube) for 1 h at 37 °C, followed by precipitation, and then resuspended in gel-loading buffer. The protected fragments were fractionated through a 5% acrylamide and 8 M urea gel. The gel was dried for 2 h at 80 °C by a gel dryer (Bio-Rad), and the relative density of each band was determined by exposing the gel to Phosphor-Imager screens (Amersham) and then quantified using an image analysis software program (Amersham). IGF-I mRNA levels were expressed as the relative values normalized by β -actin.

Statistical analysis

Significance of differences was analyzed by one-way or two-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD), using Stat View software (SAS Institute, Cary, NC, USA). Plasma levels of IGF-BPs were first log-transformed, and then applied to two-way ANOVA followed by PLSD or to correlation analysis. Levels of correlation were determined by calculating the correlation coefficient (r). Significance was accepted as $P < 0.05$.

Results

Short-term effects

As shown in Fig. 1, plasma glucose increased significantly within 2 h after injection of cortisol (2 and 10 µg/g), and

Figure 3 Short-term effects of cortisol on plasma levels of 24-kDa IGFBP (A), 28-kDa IGFBP (B), 30-kDa IGFBP (C), 32-kDa IGFBP (D), 40-kDa IGFBP (E), and 42-kDa IGFBP (F). Values are expressed as optical densitometric units (ODU). Vertical bars represent means $\pm$ S.E.M. ($n=8-10$). * $P < 0.05$, ** $P < 0.01$: significantly different from the control (oil, open bars). † $P < 0.05$, †† $P < 0.01$: significantly different from the initial value (time 0). ND, non-detectable.

a significant increase was still observed after 24 h. A significant reduction in plasma IGF-I was observed 24 h after injection of 10 µg/g cortisol, although no significant change was seen at other time points. Cortisol at a dose of 2 µg/g had no effect on plasma IGF-I at any time point. There was no significant difference in plasma GH at any of the time points examined.

Figure 2 shows representative images obtained from Western ligand blot assays. Rat serum contains several IGFBPs of 40–50 kDa (IGFBP-3), 30–31 kDa (IGFBP-1, -2, and/or -5), and 28 kDa (IGFBP-4). In the tilapia plasma, four different major bands were constantly de-

tected around 24, 30, 40, and 42 kDa, and two minor bands at around 28 and 32 kDa appeared after injection of cortisol, in accordance with the reports in other teleost species (Fukazawa *et al.* 1995, Park *et al.* 2000, Shimizu *et al.* 2000, Bauchat *et al.* 2001, Kelley *et al.* 2001). We were not able to detect either a smaller form of IGFBP (20 kDa) as reported in tilapia (Park *et al.* 2000) or larger forms of IGFBPs as found in striped bass (>85 kDa) (Fukazawa *et al.* 1995) and in coho salmon (>76 kDa) (Shimizu *et al.* 2000).

Figure 3 shows the effects of cortisol on plasma levels of IGFBPs. Significant increases in 24-kDa IGFBP from

control levels were observed 2 and 4 h after 2 and 10 µg/g cortisol injection, and 8 h after 10 µg/g cortisol. No significant effect was observed after 24 h. Cortisol injection significantly increased 28-kDa IGFBP after 2 h at a dose of 2 µg/g, and after 4 and 8 h at a dose of 10 µg/g. Similarly, 2 and 10 µg/g cortisol augmented plasma 30-kDa IGFBP levels significantly after 2 h, and the maximum increase was seen at a dose of 10 µg/g after 4 h. The effect was still significant after 8 h, but no significant difference was seen after 24 h. Significant increases in 32-kDa IGFBP were found 4 h after 10 µg/g cortisol, and 8 h after 2 and 10 µg/g cortisol injection.

By contrast, there were significant decreases in 40-kDa IGFBP from control levels after 2 h at a dose of 2 µg/g cortisol, and after 4 h at a dose of 10 µg/g. Compared with initial levels, there were significant decreases after 2 and 4 h at doses of 2 and 10 µg/g cortisol, after 8 h in all groups and after 24 h at a dose of 10 µg/g cortisol. A similar trend was observed in 42-kDa IGFBP. Significant decreases from the control were seen after 2 h at a dose of 2 and 10 µg/g, and after 8 h at a dose of 10 µg/g cortisol. There was a significant decrease from initial levels after 2 h at doses of 2 and 10 µg/g, after 4 and 8 h in all groups and after 24 h at a dose of 10 µg/g cortisol.

Correlation analyses revealed a significant ($P < 0.01$) inverse correlation between 40- and 42-kDa IGFBPs and plasma cortisol ($r = -0.44$ and -0.41 respectively) and also between 40- and 42-kDa IGFBPs and plasma glucose ($r = -0.39$ and -0.52 respectively). By contrast, 24-, 28-, 30-, and 32-kDa IGFBPs were positively correlated ($P < 0.01$) with plasma cortisol ($r = 0.43, 0.46, 0.46$, and 0.47 respectively) and with plasma glucose ($r = 0.68, 0.66, 0.69$, and 0.59 respectively). No significant correlation was found between any of the IGFBPs and plasma IGF-I, GH, and PRL.

Longer-term effects

A significant increase in plasma glucose was observed in the fish injected with cortisol at doses of 2, 10, and 50 µg/g after 24 and 48 h. On the other hand, plasma IGF-I levels were significantly reduced at doses of 10 and 50 µg/g cortisol after 24 and 48 h. No effect was observed on plasma GH at any concentration throughout the time course examined (Fig. 4).

Table 1 shows the effects of cortisol on pituitary contents of GH and PRL. No significant change was seen in GH content. Cortisol at doses of 10 and 50 µg/g significantly reduced pituitary content of PRL after 24 h. No significant effect was observed after 48 h.

In contrast with the short-term experiment, we found no significant change in any of the three forms of IGFBPs among the treatment groups in the long-term experiment (data not shown).

The effects of cortisol on IGF-I gene expression were examined in the liver, as a primary source of IGF-I in the

Figure 4 Longer-term effects of cortisol on plasma glucose (A), IGF-I (B), and GH (C). Vertical bars represent means $\pm$ S.E.M. ($n = 8-10$). *** $P < 0.01$: significantly different from the control (oil, open bars).

circulation (Fig. 5). In accordance with the inhibitory effects of cortisol on plasma IGF-I, IGF-I mRNA levels in the liver were reduced by cortisol at doses of 10 and 50 µg/g after 48 h. Maximum inhibition (47%) was caused by 50 µg/g cortisol.

Discussion

The present study was aimed at examining the effects of cortisol administration on the GH/IGF-I/IGFBP system in the tilapia. In agreement with well-known gluconeogenic actions of cortisol, plasma glucose increased within

Table 1 Longer-term effects of cortisol on pituitary contents of GH and PRL. Data are expressed as µg/pituitary. Values represent means ± S.E.M. (n=8–10)

Hormone	Cortisol dose (µg/g)	Time after injection		
		0 (initial)	24 h	48 h
GH	0 (oil)	8.71 ± 1.35	6.99 ± 0.75	7.92 ± 0.83
	2	8.44 ± 1.81	8.18 ± 0.92	8.53 ± 1.42
	10	8.17 ± 0.86	7.64 ± 0.90	9.02 ± 0.77
	50	8.34 ± 1.75	8.55 ± 1.43	6.88 ± 0.32
PRL	0 (oil)	7.61 ± 1.34	7.82 ± 2.08	6.77 ± 1.83
	2	7.33 ± 1.38	4.25 ± 1.51	5.17 ± 1.37
	10	8.05 ± 2.00	3.47 ± 0.81*	5.30 ± 0.41
	50	7.54 ± 2.67	3.51 ± 0.56**	4.07 ± 0.42

*P<0.05, **P<0.01: significantly different from the control (0 dose).

Figure 5 Longer-term effects of cortisol on mRNA levels of IGF-I in the liver. Vertical bars represent means ± S.E.M. (n=8–10). *P<0.05, **P<0.01: significantly different from the control (oil, open bars).

2 h after cortisol injection, an effect that lasted for at least 48 h. Although no change was seen in plasma levels of GH or IGF-I until 8 h, we found significant increases in two major IGFBPs at 24 and 30 kDa and two minor bands at 28 and 32 kDa within 2 h after injection of 2 and 10 µg/g cortisol, and the maximum effect was observed after 4 h. Kelley *et al.* (2001) demonstrated in the jack mackerel (*Trachurus symmetricus*) that confinement with handling stress for 60 min increased 24- and 30-kDa IGFBPs in the circulation, concomitant with an increase in plasma cortisol. They also reported that catabolic status brought on by a 20-day fasting or by insulin-dependent diabetes mellitus induced by isletectomy increased 24- and 30-kDa IGFBPs in the circulation. These results indicate a strong relationship between elevated cortisol and these IGFBPs in fish. In

accordance with these results, we found in the tilapia that handling stress induced an increase in plasma cortisol up to 400–500 ng/ml, and a twofold increase in plasma glucose within 15 min. Significant increases were also seen in the four different sizes of IGFBPs at 24, 28, 30 and 32 kDa within 2 h, whereas there was no change in plasma GH or IGF-I (S Kajimura, T Hirano & E G Grau, unpublished observation).

In mammals, IGFBP-1 production is up-regulated by glucocorticoids and down-regulated by insulin, which acts as a growth inhibitor by preventing IGFs from reaching their presumed target receptors (Unterman *et al.* 1993, Rodgers *et al.* 1995a,b, Conover *et al.* 1996). IGFBP-2 is also known to increase under catabolic conditions including prolonged fasting and insulin-dependent diabetes mellitus (Unterman *et al.* 1993, Rodgers *et al.* 1995a,b, Collett-Solberg & Cohen 2000). In teleosts, Maures & Duan (2002) recently cloned cDNA of zebrafish IGFBP-1 with its predicted molecular size of 25 kDa, and demonstrated that its gene expression was stimulated by 2 weeks of fasting and by hypoxia. The same investigators cloned cDNA of zebrafish IGFBP-2 with its apparent molecular size of 31 kDa (Duan *et al.* 1999). They proposed IGFBP-2 as a growth inhibitor based on its inhibitory effects on DNA synthesis and physiological changes under catabolic condition as in mammals. According to Bauchat *et al.* (2001), 30-kDa IGFBP isolated from the rainbow trout has an inhibitory effect on DNA synthesis, with a high homology with mammalian IGFBP-1 and -4. Based on similarity in molecular sizes as well as responsiveness to cortisol, the two IGFBPs (30 and 32 kDa) may correspond to mammalian IGFBP-1 and -2. The other two IGFBPs (24 and 28 kDa) are likely to be comparable to mammalian IGFBP-4 judging from the molecular sizes, which has non-glycosylated (24 kDa) and glycosylated (28 kDa) forms. As far as biological functions are concerned, IGFBP-1 has inhibitory effects on IGF action by sequestering IGFs in the extracellular environment (Collett-Solberg & Cohen 2000, Kelley *et al.* 2002).

Similarly, IGFBP-2 and -4 have inhibitory effects on IGF action locally. Therefore, the rapid increases in these IGFBPs induced by cortisol presumably inhibit IGF-I action, which contributes to the cortisol-induced growth retardation.

Among the tilapia IGFBPs, 40- and 42-kDa IGFBP were most prominent in the plasma before the injection, and these may be the fish counterpart of mammalian IGFBP-3, which is known to be glycosylated with a molecular weight between 40 and 44 kDa (Zapf *et al.* 1989). As with mammalian IGFBP-3, >40-kDa IGFBP was up-regulated by GH treatment (Park *et al.* 2000, Shimizu *et al.* 2000). Cheng *et al.* (2002) also found that mRNA levels of IGFBP-3 were increased by GH administration in the tilapia. In support of our findings, IGFBP-3 in the circulation and its gene expression were reduced by glucocorticoids in mammals (Unterman *et al.* 1993, Okazaki *et al.* 1994, Rodgers *et al.* 1995b). In our study, however, 40-kDa and 42-kDa IGFBP showed declines in all fish including control groups after injection, which is probably due to the stress of the injection.

Correlation analysis for the six IGFBPs with plasma levels of cortisol and glucose revealed two clearly different patterns. The two larger IGFBPs (40 and 42 kDa) showed inverse correlations with plasma cortisol and glucose, suggesting their anabolic characteristics. Kelley *et al.* (2002) also reported a marked reduction in >40-kDa IGFBP in the golden-mantled ground squirrel, *Spermophilus lateralis*, during hibernation, a catabolic situation. Consistent with these results, 40- to 50-kDa IGFBPs showed a positive correlation with growth activity in terms of cartilage ³⁵S-proteoglycan synthesis in several teleost species (Kelley *et al.* 2002), further supporting the contention that the 40-kDa and 42-kDa IGFBPs in the tilapia are candidates for mammalian IGFBP-3. On the other hand, the four smaller IGFBPs (IGFBP-1-, -2-, and -4-like proteins) showed positive correlations with plasma cortisol and glucose, suggesting their catabolic characteristics. However, identification of each IGFBP in comparison with mammalian counterparts awaits analyses of their DNA or protein sequences and of their biological functions in fishes.

In the first short-term experiment, a significant reduction in plasma IGF-I was observed 24 h after injection of 10 µg/g cortisol. As expected, plasma glucose was increased by all doses of cortisol in the longer-term experiment, and significant effects were observed up to 48 h. A significant reduction in plasma IGF-I by cortisol at doses of 10 and 50 µg/g was also seen after 24 h and lasted for 48 h. Furthermore, plasma IGF-I showed a significant inverse correlation with plasma cortisol after 24 h ($r = -0.55$, $P < 0.001$) and 48 h ($r = -0.47$, $P < 0.001$). We also found that cortisol reduced IGF-I mRNA levels in the liver after 48 h at doses of 10 and 50 µg/g. This is in agreement with previous findings in mammals in which glucocorticoids suppress IGF-I synthesis and gene expression *in vivo*

(Unterman *et al.* 1993, Rodgers *et al.* 1994, 1995b) and *in vitro* (McCarthy *et al.* 1990, Jux *et al.* 1998, Delany *et al.* 2001). Inasmuch as the liver is the primary source of IGF-I in teleosts and in mammals (Duan 1997, Kajimura *et al.* 2001), cortisol may reduce IGF-I levels in the circulation through the attenuation of IGF-I gene expression in the liver. Longer exposure to high levels of glucocorticoids seems to be required for the inhibitory effects on IGF-I production, which probably leads to growth retardation (McCarthy *et al.* 1990, Unterman *et al.* 1993). We have previously reported that plasma IGF-I levels in the tilapia showed a significant positive correlation with somatic growth, indicating that IGF-I is a good index for assessing somatic growth (Kajimura *et al.* 2001, Uchida *et al.* 2003). Therefore, this reduction in plasma IGF-I levels may also be a contributor to the growth retardation observed after cortisol administration in two teleost species (Davis *et al.* 1985, Barton *et al.* 1987).

By contrast, there was no change in plasma or pituitary levels of GH at any dose of cortisol at any time point examined. Auperin *et al.* (1997) reported that confinement stress for 1 h reduced plasma GH levels, accompanied by an increase in plasma cortisol levels in the Nile tilapia (*O. niloticus*). According to Nishioka *et al.* (1985), however, cortisol increased GH release from the organ-cultured pituitary of the Mozambique tilapia. The reason for these inconsistent results remains unclear. In any event, the reduction of IGF-I synthesis without altering plasma GH levels suggests that the sensitivity of IGF-I production to GH is reduced by cortisol (GH-resistance). Rodgers *et al.* (1994) reported that the adrenocorticotropin-adrenal cortical axis was responsible for GH-resistance by demonstrating that hypophysectomy or adrenalectomy restores the responsiveness to GH in rats with insulin-dependent diabetes mellitus. King and Carter-Su (1995) showed that dexamethasone induced GH-resistance by down-regulation of GH binding in fibroblasts. According to Jux *et al.* (1998), dexamethasone also impaired GH-stimulated growth by suppressing local IGF-I production and expression of the GH- and IGF-I-receptor in cultured rat chondrocytes. The phenomenon of 'GH-resistance' is also observed in fasted fish. In our previous study (Uchida *et al.* 2003), 2 weeks of fasting in the tilapia caused significant reductions in plasma IGF-I and hepatic IGF-I mRNA levels, while plasma GH levels were unaffected. There is a close temporal relationship between the fall in plasma IGF-I concentrations and a similar drop in the number of hepatic GH binding sites during fasting and refeeding in the gilthead seabream (Pérez-Sánchez *et al.* 1995). A recent study has shown that fasting attenuated GH-receptor signaling of Janus kinase 2 (JAK2) and the signal transducer and activator of transcription 5 (STAT5) pathway, but that adrenalectomy failed to prevent the alternation of the JAK-STAT pathway induced by fasting in rat liver (Beauloye *et al.* 2002). These findings suggest that a reduction in the hepatic GH binding capacity might be

one of the mechanisms responsible for the fasting or cortisol-induced decline in IGF-I. Therefore, our current research is aimed at elucidating the effects of cortisol on sensitivity to GH in the tilapia.

In conclusion, the present study demonstrated, for the first time in teleosts, that cortisol administration rapidly increased several forms of IGFBPs in the circulation within 2 h without affecting total levels of plasma IGF-I, presumably resulting in the inhibitory effects on IGF-I action. A reduction in total plasma IGF-I was observed 24 and 48 h after cortisol administration, which was accompanied by the attenuation of the IGF-I gene expression in the liver. The dual mode of cortisol action on the GH/IGF-I/IGFBP system, a rapid increase in IGFBPs and a slow decrease in total levels of IGF-I, may contribute to the inhibitory effects of cortisol on somatic growth in teleosts.

Acknowledgements

We are grateful to Dr NH Richman III, Mr SK Shimoda, and Ms C Ball, Hawaii Institute of Marine Biology, University of Hawaii, for their encouragement and support. We are grateful to Prof. MH Stetson, University of Delaware, and Prof. C Duan, University of Michigan, for their invaluable comments and suggestions. This work was funded in part by grants from the US Department of Agriculture (#983506644), the National Science Foundation (IBN 01-33714) and also from the University of Hawaii Sea Grant College Program (NOAA project R/AQ-62, Institutional Grant No. NA86RG0041, UHIIH-SEAGRANT-JC-01-26). The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies. S K was also supported by the Prince Akihito Scholarship Foundation.

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Received 25 February 2003

Accepted 18 March 2003

Made available online as an Accepted Preprint
27 March 2003