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Development of an enzyme-linked immunosorbent assay for the measurement of plasma growth hormone (GH) levels in channel catfish (*Ictalurus punctatus*): assessment of environmental salinity and GH secretogogues on plasma GH levels

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Abstract

We report the development of a sensitive, and specific, competitive, antigen-capture enzyme-linked immunosorbent assay for the measurement of channel catfish (*Ictalurus punctatus*) growth hormone (cfGH). The detection limit of the assay (90% binding) was 2.0 ng/ml and the ED_{50} value (standard curve range 150–0.59 ng/ml) was 67.3 ng/ml. Recovery of cfGH-spiked plasma samples was determined to be 102%. Dose–response inhibition curves using serially diluted pituitary homogenates and plasma samples consistently showed parallelism with the standard curves using purified cfGH. The GH antibody (rabbit anti-catfish GH) specificity was demonstrated in competitive binding curves employing heterologous hormones and purified channel catfish prolactin (cfPRL). These studies show that there was no significant (0.006%) binding of cfPRL (competitive inhibition of cfGH binding), or heterologous hormones, within the working range of the assay. To physiologically validate the assay, catfish were injected (100 μg/g body weight, 3 injections every 5 days) with either bovine $GHRH_{1-29}$-amide or the synthetic hexapeptide GHRP-2 (KP-102: D-Ala-D-β-Nal-Ala-Trp-D-Phe-Lys-NH_2) suspended in corn oil. Following the last injection, half of the animals were sampled for plasma and the remaining transferred from fresh water (FW) to 12 ppt seawater (BW: brackish water). Twenty-four hours after transfer to BW, animals were again sampled for plasma. Plasma GH levels were significantly ($p < 0.001$) elevated in all the BW groups (control, KP-102, and bGHRH), compared with the FW (fresh water) groups. In addition, plasma GH levels were significantly ($p < 0.001$) elevated by treatment with either of the GH secretogogues, KP-102 or bGHRH. Our findings demonstrate that two regulatory mechanisms of GH elevation, one which is seen in euryhaline teleosts (salinity-induced GH levels) and another, which has been recently described in teleosts (GHRP-induced GH levels), are present in the stenohaline channel catfish.

Keywords: Channel catfish; Growth hormone; ELISA; GHRP-2; GHRH; Salinity

1. Introduction

In teleosts, the pituitary hormones, growth hormone (GH) and prolactin (PRL) possess actions central to the maintenance of growth and salt and water balance (Chen et al., 1994; Hirano, 1986; Rubin and Specker, 1992; Sakamoto et al., 1993). The growth-promoting, osmoregulatory and metabolic roles of GH and PRL have been studied in many teleosts; however, relatively little is known about their function in the channel catfish.

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The channel catfish, *Ictalurus punctatus*, is the most extensively cultured food-fish species in North America and plays a considerable role in sport fisheries across the United States. Despite their economic significance, there is a lack of information on the endocrine variations accompanying growth, development and environmental adaptation in this teleost. Attempts to bolster production in the catfish industry have heretofore been limited to traditional methods. In this regard, attempts to promote the growth of catfish using steroids have not been successful (Davis et al., 2000; Gannam and Lovell, 1991; Simone, 1990), and the use of steroids (androgens) to produce all male (viable) catfish have not been entirely successful or easily achieved (Davis et al., 1990, 1992, 2000; Galvez et al., 1995; Goudie et al., 1983). Furthermore, studies (Silverstein et al., 2000; Wilson et al., 1988) that have utilized exogenous GH to promote growth in channel catfish have reported actions (increases in percentage of fat, decreases in percentage of protein, and decreased feed efficiency) not typically associated with GH treatment in other teleosts. These difficulties are at least partially related to the lack of basic information regarding the endocrine regulation of growth and development in this species.

Recent work by Tang et al. (2001) showed that GH and PRL mRNA levels in the pituitary vary in relation to changes in environmental salinity, developmental stage, and sex of the channel catfish. Specifically, Tang et al. (2001) showed that exposure of channel catfish to brackish water resulted in an increase in pituitary GH mRNA, suggesting that GH may possess some hypoosmoregulatory actions. However, recent work by Eckert et al. (2001) could not demonstrate any hypoosmoregulatory actions of GH in channel catfish injected with heterologous (ovine) GH prior to exposure to brackish water. Although the reason for these discordant observations is not clear, it is evident that the actions of GH in growth, osmoregulation, and development of channel catfish are not well understood and thus require further study.

To begin addressing these problems, a better understanding of the role of endogenous (native) GH in the growth physiology of the channel catfish is needed. To achieve this, it is necessary to develop a method to measure GH in the channel catfish. The predominant method for measuring GH and PRL levels in teleosts is via radioimmunoassay(s) (RIA) which is highly sensitive and specific (Swanson, 1994). Despite the strengths of the RIA, there are disadvantages such as the use of radioisotope, the attendant short half-life and decay of labeled tracers and the disposal of radioactive waste. Alternatives to the use of RIAs for hormone measurement in teleosts include non-isotopic methods such as the enzyme-linked immunosorbent assay (ELISA) and time-resolved fluoroimmunometric assays (TR-FIA) (e.g., Cheung et al., 2002; Farbridge and Leatherland, 1991; Holland et al., 1998; Kah et al., 1994; Manaños et al., 1997; Small, 2002; Yamada et al., 2002). Despite some drawbacks (the need for greater amounts of purified ligand and lower sensitivity) to the ELISA approach, it can be useful in cases where the polypeptide cannot be conveniently labeled with a radioactive tracer and where sensitivity is not an issue. While the TR-FIA approach is extremely sensitive, this approach is not as generally accessible owing to the high cost of reagents and specialized equipment. To date, there is no assay available to measure GH levels in the channel catfish. The development of an assay will accelerate studies aimed at elucidating the function of GH in this teleost. In the present study, we report the development of an ELISA for measurement of plasma GH levels and the effects of environmental salinity and GH secretogogues on plasma GH levels in channel catfish.

2. Materials and methods

2.1. Animals

Channel catfish fry were obtained from Long View Ranch (Wetumka, OK) and reared in 6000-liter tanks supplied with fresh water ($24 \pm 2\,^{\circ}C$) and held under natural photoperiod at the Hawaii Institute of Marine Biology, University of Hawaii. The animals were fed to satiation twice daily with Purina Trout Chow and used in the injection studies described below. Channel catfish used for establishment of plasma pools and pituitary homogenates used for assay development were obtained from the State of Kentucky, Frankfort (Pfeiffer) fish hatchery.

2.2. Hormone isolation, sources, and antibody generation for use in ELISA validation

Native channel catfish (cf)GH and (cf)PRL were isolated from whole pituitaries by gel-filtration and ion-exchange chromatography followed by reversed-phase HPLC purification as previously described (Watanabe et al., 1992). Additionally, the purity of the isolated cfGH and cfPRL was assessed by SDS–PAGE and Western blot analysis and the identities of the immunoreactive proteins (cfGH and cfPRL), identified by Western blot analysis, were also confirmed by structural analysis (Watanabe et al., 1992). Antibodies against catfish GH were raised in two rabbits. The antigen (50 μg) was dissolved in 100 μl of distilled water, diluted to 1 ml with 0.9% NaCl and emulsified with complete Freund's adjuvant (1 ml). Each rabbit received five subdermal injections at 3 week intervals. Test bleedings, for measurement of anti-catfish GH serum titers, were done 2 weeks after the third and fourth injections. The rabbits were bled 2 weeks after the final injection and serum

lyophilized. Sources for hormones, used in the assay development are as follows: catfish GH and PRL were obtained as described above, salmon GH (Lot No. IJH-GHB1, GroPep Pty, North Adelaide, South Australia, Australia), recombinant eel GH (Professors T. Hirano, Ocean Research Institute, University of Tokyo, Japan and T. Chen, Department of Molecular and Cell Biology, University of Connecticut, Lot No. E-21-15), tilapia hormones (GH and PRLs: Professor H. Bern, Department of Integrative Biology, U.C. Berkeley), bovine GH (USDA Animal Hormone Program, Lot No. USDA-bGH-B-1), ovine prolactin (NIDDK-NHPP, Lot No. AFP10692C), and placental lactogen (Dr. R.J. Collier, Monsanto Animal Agriculture Group, St. Louis, MO, no lot number) were obtained from the aforementioned sources.

2.3. Pituitary homogenates

For demonstration of competitive binding, pituitary homogenates from various teleosts were prepared by sonicating whole pituitaries suspended in 100 μl of PBST buffer (0.01 M $NaPO_4$, 0.15 M NaCl, and 0.05% Tween 20, pH 7.4) on ice for 15 s using a Branson Sonifier (Model 250, Danbury, CT). Following sonication, volume was brought up to 1 ml with PBST and this volume was considered to be at a concentration of 1×.

2.4. Assay procedure

Once an optimum ligand (cfGH) standard range and primary antibody (rabbit anti-catfish GH) dilution were identified from preliminary experiments, assays were performed to determine the following: (1) GH antibody specificity, using heterologous pituitary hormones and purified cfPRL, in competitive binding assays, (2) parallelism between plasma and pituitary homogenates of channel catfish and other teleosts (see below), and (3) determination of plasma GH levels in channel catfish from our in vivo studies. Immunoplates (96-well Costar high binding immunoplates, Acton, MA) were coated with 100 μl of 250 ng/ml of ligand in carbonate buffer (0.05 M sodium carbonate, pH 9.6), with additional wells for non-specific binding (NSB) coated with 100 μl of 250 ng/ml of BSA (Bovine Serum Albumin, RIA-Grade, Sigma Chemical, St. Louis, MO) in carbonate buffer and incubated overnight, without shaking, at 4 °C. The next day plates were dried on paper towels, and then all wells were blocked with the addition of 200 μl of 5% NGS (Normal Goat Serum, Sigma Chemical, St. Louis, MO) in PBST (0.01 M $NaPO_4$, 0.15 M NaCl, and 0.05% Tween 20, pH 7.4) overnight at 4 °C without shaking.

Each hormone standard dilution (150 ng/ml serially diluted 1:2 to a final dilution of 0.58 ng/ml) was made in assay buffer (1% BSA in PBST). Plasma samples (unknowns) and plasma pools were diluted 1:4 (13.75 μl of plasma + 41.25 μl assay buffer) with sufficient volume for triplicate analyses. Total volume for a single replicate was 110 or 330 μl for triplicate analyses. The hormone dilutions and plasma dilutions were then mixed 1:1 with 2% NGS in PBST with primary antibody (Rabbit anti-cfGH, Lot GH-1) at a dilution of 1:10,000. Total binding (Bound-zero: Bo) references were prepared (110 μl per replicate) using a 1:1 mixture of assay buffer (1% BSA in PBST) and 2% NGS in PBST with primary antibody (1:10,000 dilution) to determine the maximum binding capacity of the assay. Similarly, non-specific binding (NSB) references were prepared (110 μl per replicate) using a 1:1 mixture of assay buffer (1% BSA in PBST) and 2% NGS in PBST without primary antibody. Everything was prepared in 1.5 ml centrifuge tubes and incubated at 37 °C for 90 min with shaking.

After incubation of the standards, NSB, Bo, and plasma tubes, 100 μl aliquots were pipetted into individual wells (in triplicate) of the plate and incubated at 37 °C for 90 min. Following incubation, the plate was washed three times with PBST. The plate was subsequently coated with 100 μl per well of goat anti-rabbit-horseradish peroxidase (GAR-HRP, Bio-Rad Laboratories, Hercules, CA) secondary antibody conjugate diluted 1:5000 in PBST and incubated at 37 °C for 90 min. The plate was washed again and 100 μl per well of OPD (*o*-phenylenediamine dihydrochloride and hydrogen peroxide, Sigma Chemical, St. Louis, MO) was added, developed at room temperature and read at 450 nm using a Molecular Devices (Sunnyvale, CA) plate reader.

Under these conditions, we examined antibody specificity using heterologous plasma, pituitary homogenate, and purified homologous and heterologous hormones. For the pituitary and plasma dilutions, tissues were used from the following animals: catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*), goldfish (*Carassius auratus*), carp (*Cyprinus carpio*), hybrid striped bass (*Morone saxatilis* × *Morone chryosops*), and yellow perch (*Perca flavescens*). For the pituitary dilutions, the following dilutions were used 1:10, 1:100, 1:1000, and 1:10,000, whereas the following dilutions of 1:1, 1:2, 1:4, 1:8, and 1:16 were used for the plasma dilution series. To determine antibody specificity, the following hormones were used in the direct, competitive, antigen-capture ELISA: catfish growth hormone (cfGH), cf prolactin (cfPRL), tilapia (ti)GH, $tiPRL_{177}$ (variant form), $tiPRL_{188}$, salmon (s)GH, eel (e)GH, ovine (o)PRL, and bovine (b)PL (placental lactogen) at 1, 10, 100, and a 1000 ng/ml.

2.5. In vivo experiments

Prior to the study, animals were netted, anesthetized with bicarbonate-buffered MS-222 (100 mg/liter) and transferred to oval 60-liter tanks ($n = 20$/tank) supplied with flow-through fresh water. Animals were allowed to

acclimate to these conditions for 7 days prior to the study. During this period, animals were fed once daily to satiety; food was withheld 24 h prior to the experiment as well as prior to injections. Animals were given intraperitoneal injections of vehicle (corn oil) alone or vehicle containing suspended GH secretogogues KP-102 (D-Ala-D-β-Nal-Ala-Trp-D-Phe-Lys-NH_2) (Kaken Pharmaceuticals, Tokyo, Japan) or bGHRH_{1-29}-amide (Sigma Chemical, St. Louis, MO) at a dose of 100 ng/g bw. Injections were given every 5 days for 15 days. Injection volume was 5 μl/g body weight for all groups.

Five days after the last injection, half of the animals were sampled for plasma. The remaining animals were then subjected to an increase in salinity. For the first 24 h, the remaining animals were held in 8 ppt seawater (SW) by diluting natural SW with fresh municipal water (FW) over the course of 4–6 h. Salinity was measured using a temperature-compensated refractometer. By 48 h, salinity was increased from 8 to 12 ppt over the course of 4–6 h. Animals were sampled for plasma 24 h following the final adjustment to 12 ppt SW (BW: brackish water).

2.6. Blood sampling

For sampling, animals from each group (control, GHRH, and GHRP) were anesthetized in bicarbonate-buffered MS-222 (100 mg/liter) and blood was withdrawn by caudal puncture using heparinized syringes. Plasma was separated by centrifugation and stored at −80 °C for hormone analyses. Plasma levels of catfish GH were determined using the ELISA described herein. Values are reported in nanograms per milliliter (ng/ml) plus or minus the standard error of the mean (SEM).

2.7. Statistics

Differences among groups were evaluated by two-way analysis of variance (two-way ANOVA) with salinity and treatment as independent variables (main effects) (Minitab Statistical Software Package, State College, PA). Where significant differences occurred ($p < 0.05$) with the main effects, comparisons between group means were performed using Fisher's Least Significant Test (FPLSD) for predetermined pairwise comparisons (Steele and Torrie, 1980). All values (where indicated) are presented as means plus or minus the standard error of the mean (SEM).

3. Results

3.1. Parameters and specificity of the ELISA

A coating concentration of 250 ng/ml (100 μl/well), a primary antibody dilution of 1:10,000 (1:20,000 final dilution), a 1:4 dilution of plasma (41.25 μl for triplicate measurements), and a secondary antibody dilution of 1:5000 (goat-anti-rabbit IgG horseradish peroxidase conjugate) were determined to be the optimal conditions for this direct, competitive antigen-capture ELISA.

The intraassay coefficient of variation was determined from multiple plasma pools from a single assay, whereas the interassay coefficient of variation was determined from the ED_{50} value from standard curves and plasma pool values obtained from separate assays. For assessment of intraassay coefficient of variation (CV), repeated determinations ($n = 21$) of plasma pool samples within the same assay gave a mean value of 18.1 ± 0.31 ng/ml and a CV of 7.7%. For assessment of the interassay coefficient of variation, mean ED_{50} values from separate assay standard curves ($n = 18$) were determined to be 67.3 ± 1.61 ng/ml with a CV of 9.8%. Plasma pool values obtained from separate assays ($n = 9$ assays) were also used to determine the interassay coefficient of variation and showed a mean pool value of 16.5 ± 0.56 ng/ml and a variation of 10.2%. The upper and lower detection limits of the assay, defined as the 35% binding (minimum binding in this assay) and 90% binding, are 161.9 ± 5.55 and 2.0 ± 0.08 ng/ml, respectively. While we have conservatively identified 2.0 ng/ml as our lower detection limit, we consistently found that our lowest standard of 0.58 ng/ml exhibited $96.4 \pm 0.5\%$ binding, which is lower than Bo (100% binding). Collectively, the assay parameters, calculated as ED_{35} (minimum assay binding), ED_{50}, ED_{80}, and ED_{90} from several individual assays ($n = 18$) are 161.9 ng/ml (CV = 14.1%), 67.3 ng/ml (CV = 9.8%), 7.1 ng/ml (CV = 20.2%), and 2.0 ng/ml (CV = 17.7%), respectively.

Multiple dose–response inhibition curves for channel catfish (cf)GH, using cfGH and other purified hormones (see Fig. 1), revealed no cross-reactivity with other hormones, particularly catfish prolactin (cfPRL). In this regard, cfPRL weakly inhibited cfGH binding only at the highest concentration (1000 ng/ml) tested and comparison of the ED_{50} values from cfGH and cfPRL dose–response inhibition curves show a cross-reactivity of 0.006%. Serial dilutions of teleost pituitary homogenates revealed displacement curves that paralleled the cfGH standard curve (Fig. 2). Specifically, catfish pituitary homogenate paralleled the cfGH standard curve. In addition, pituitary homogenates from taxonomically primitive teleosts (trout, carp, and goldfish) exhibited some parallelism to the cfGH standard curve, but pituitary homogenate from a neo-teleost (hybrid striped bass) exhibited little parallelism to the cfGH standard curve. To assess the effects of plasma components on assay performance, plasma dilution series were performed (Fig. 3). Serial dilutions of catfish and rainbow trout plasma showed inhibition curves that were parallel to the cfGH standard curve; however, serially diluted

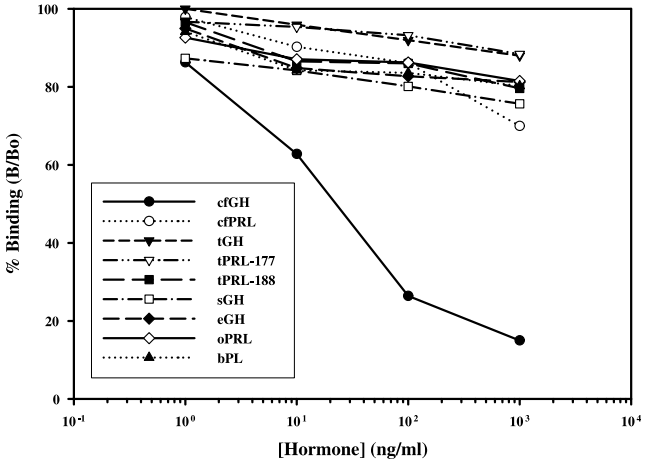

Fig. 1. Dose–response inhibition curves to determine specificity of channel catfish primary GH antibody (Lot No. GH1). Inhibition of channel catfish GH (cfGH) binding, in this competitive ELISA, was tested against homologous channel catfish prolactin (cfPRL) and heterologous hormones including tilapia growth hormone (tGH), variant tilapia prolactin ($tPRL_{177}$), tilapia prolactin ($tPRL_{188}$), recombinant salmon growth hormone (sGH), recombinant eel growth hormone (eGH), ovine prolactin (oPRL), and bovine placental lactogen (bPL). Each point is the mean of triplicate determinations and units are in nanograms/milliliter (ng/ml).

tilapia (Neoteleostei: *Oreochromis mossambicus*) plasma did not. In addition, we assessed the recovery of cfGH that was added in increasing concentrations to 43.75 µl of hypophysectomized catfish plasma (for triplicate analyses). Regression analysis between the amount of hormone added and that recovered showed a regression coefficient (r) of 0.997. Furthermore, comparison of the ED_{50} values from spiked plasma samples (65.8 ± 2.9 ng/ml, $n = 4$) and standard curves (67.2 ± 2.1 ng/ml, $n = 18$) show a recovery of hormone from spiked samples to be $102 \pm 0.5\%$, which is not significantly different from an expected value of 100%.

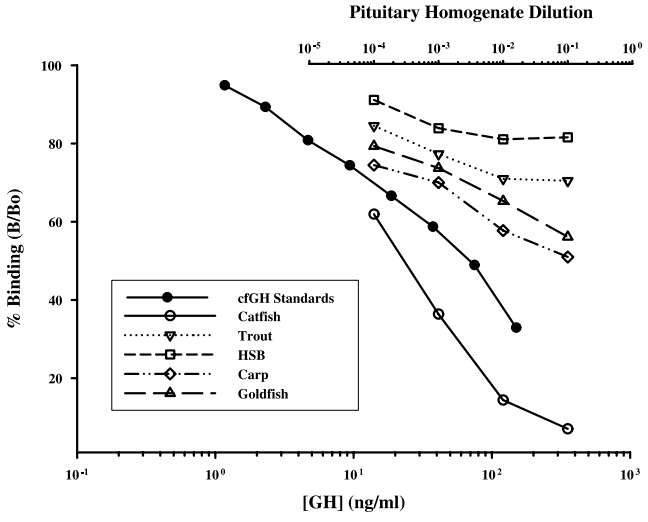

Fig. 2. Dose–response inhibition curves for purified cfGH standards and serial dilutions of pituitary homogenates from channel catfish and other teleosts. Each point is the mean of triplicate determinations and units are in nanograms/milliliter (ng/ml).

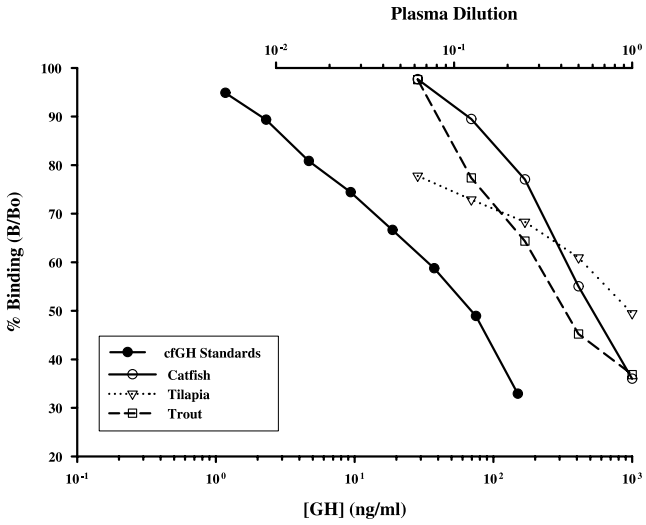

Fig. 3. Dose–response inhibition curves for purified cfGH standards and serially diluted plasma from channel catfish and other teleosts. Each point is the mean of triplicate determinations and units are in nanograms/milliliter (ng/ml).

3.2. In vivo studies

We examined plasma GH levels in channel catfish that were exposed to different salinities as well as treated with two GH secretogogues (KP-102 and bGHRH) that we have previously shown to be effective in tilapia, *O. mossambicus* (Shepherd et al., 2000). There were signif-

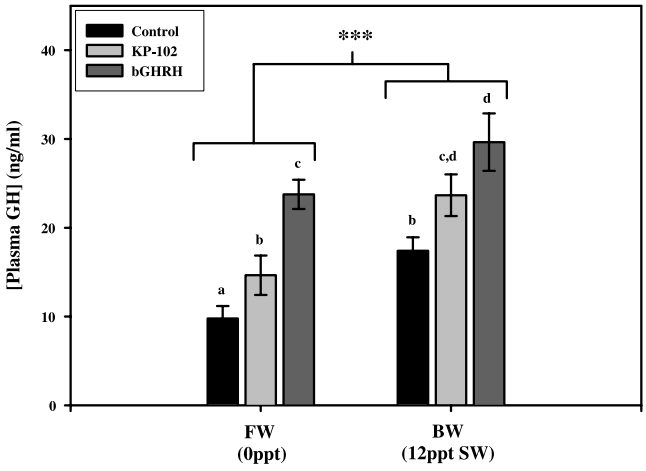

Fig. 4. Effects of intraperitoneal injection (100 ng/g bw) of KP-102, bGHRH-amide, and vehicle (corn oil) on plasma GH levels in channel catfish held in FW (0 ppt) and BW (12 ppt seawater). Values are means ± SEM ($n = 7$–10/group). ***$p < 0.001$ compared with the brackish water (12 ppt) groups (two-way ANOVA). Groups with different subscripts are significantly ($p < 0.05$) different (Fisher's Protected Least Significant Difference Test).

icant main effects of salinity ($p < 0.001$) and secretogogue treatment ($p < 0.001$) on plasma GH levels. Pairwise comparisons revealed significantly ($p < 0.005$) higher levels of plasma GH in the BW (brackish water) control group as compared with the FW (fresh water) control group (Fig. 4). In both salinities, KP-102 treatment significantly ($p < 0.05$) elevated plasma GH levels, with significantly higher ($p < 0.05$) levels occurring in the BW KP-102 group as compared with the FW KP-102 group. Similarly, bGHRH treatment, in both salinities, resulted in significant ($p < 0.05$) elevations in plasma GH levels, with the highest levels occurring in the BW bGHRH group (Fig. 4).

4. Discussion

The direct competitive, antigen-capture ELISA, developed for measuring plasma GH levels in channel catfish, shows that environmental salinity and GH secretogogues effectively elevate plasma GH levels in this teleost. Specificity and validity of this ELISA system was demonstrated in four conventional ways: First, the channel catfish GH antiserum was tested for cross-reactivity against purified native channel catfish PRL as well as heterologous hormones showing that the anti-cfGH antibody did not bind purified cfPRL or other heterologous hormones. Second, parallelism between serially diluted pituitary homogenates from channel catfish, and other teleosts, was established. Third, parallelism between serially diluted plasma from channel catfish, and other teleosts, was also examined. Last, this ELISA system was physiologically validated using approaches (salinity challenge and GH secretogogues), shown to be effective in other teleosts, to alter endogenous GH levels in the channel catfish.

To our knowledge, this is only the second ELISA-based assay system that has been developed to measure plasma GH levels in a teleost. The first assay was a non-competitive ELISA developed by Farbridge and Leatherland (1991) to measure plasma GH in teleosts of the genus *Oncorhynchus*. More recently, however, Cheung et al. (2002) reported the development of a specific and sensitive ELISA for goldfish PRL. The assay described herein is specific to channel catfish growth hormone (cfGH) as no significant cross-reactivity to other vertebrate pituitary hormones, including purified channel catfish prolactin (cfPRL), was observed within the working range of this ELISA. Furthermore, our recovery and plasma dilution series experiments indicate that spiked (cfGH) plasma samples and competitive displacement by non-spiked, serially diluted, plasma samples yielded a recovery of 102% and parallelism with our cfGH standard curves, respectively. These results suggest the presence of little or no interference of plasma components in this assay system. A similar parallelism was observed using pituitary homogenates from channel catfish and other teleosts. The competitive displacement seen by rainbow trout (and other primitive teleosts) plasma and pituitary homogenates is not unexpected in light of the fact that anti-salmon GH and PRL antibodies were used by Watanabe et al. (1992) to purify and identify cfGH and cfPRL. Furthermore, given the structural similarity (70%) of a more primitive teleost (e.g., carp) GH (Watanabe et al., 1992) to that of cfGH, it is not surprising that some competitive inhibition was seen by pituitary homogenates from the more primitive teleosts (carp, goldfish, and trout). By contrast, pituitary homogenates and plasma samples from neo-teleosts (hybrid striped bass and tilapia, respectively), which have growth hormone molecules that are structurally more dissimilar to cfGH than the aforementioned teleosts, exhibited little or no competitive inhibition or parallelism. As expected, we detected minimal cross-reactivity (0.006%) of our anti-cfGH antibody to purified cfPRL in competitive binding assays which is not surprising given the low sequence identity (27%) between cfGH and cfPRL (Watanabe et al., 1992).

The sensitivity of the assay, defined as 90% binding, was determined to be 2.0 ng/ml and is well below levels observed in unknown plasma samples as well as plasma pool samples. Precision (interassay CV < 16%) of the assay was greatest within the detection range ($ED_{70} = 17.2$ ng/ml to $ED_{50} = 67.3$ ng/ml) of the assay system and accuracy (intraassay CV < 10.2%) of the assay system is within acceptable parameters. Indeed, assay performance characteristics of the catfish GH ELISA reported herein are comparable to those reported for teleost GnRH and the goldfish PRL ELISAs (Cheung et al., 2002; Holland et al., 1998; Kah et al., 1994; Manaños et al., 1997). Levels of plasma GH in catfish from this study were consistently above the detection limits of the ELISA assay described herein. Specifically, mean levels for plasma GH ranged from 8 ng/ml in catfish held in fresh water (FW) to 30 ng/ml in catfish held in brackish water (BW) and injected with bGHRH. While we have seen significant differences in the levels of plasma GH from channel catfish of various genetic strains (B. Shepherd, unpublished observations), the values reported herein are comparable to those (~1–11 ng/ml) seen in tilapia (*O. mossambicus*) injected with these same secretogogues (Shepherd et al., 2000), but approximately 1–5 fold higher than levels reported in the African catfish (*Clarias gariepinus*) (Lescroart et al., 1996, 1997, 1998). Overall, however, values are well within the reported range of plasma GH levels seen in primitive teleosts such as grass carp (30–480 ng/ml) (Zhang et al., 1994), goldfish (75–200 ng/ml) (Zou et al., 1997), rainbow trout (~0.5–75 ng/ml) (Farbridge and Leatherland, 1991, 1992; Gomez et al., 1996; Kakizawa et al., 1995; Sakamoto et al., 1990) and more advanced teleosts such as the tilapia hybrid (*Oreochromis*

niloticus × *Oreochromis aureus*: ~4–40 ng/ml) (Melamed et al., 1995a,b), sea bream (~2.5–17.5 ng/ml) (Mingarro et al., 2002) and Atlantic halibut (~5–50 ng/ml) (Einarsdottir et al., 2002).

Levels of GH in the plasma of channel catfish exposed to fresh water (0 ppt) and brackish water (12 ppt seawater) parallel recent findings reported by Tang et al. (2001). The latter found that transfer of channel catfish to saline water (8 ppt) resulted in significant elevations in pituitary GH mRNA levels. In this study, we observed that plasma GH levels in channel catfish transferred to BW were significantly elevated over values seen in the FW controls. These observations confirm the validity of our findings, as well as those of Tang et al. (2001), but raise the important question of the physiological actions of salinity-induced increases of cfGH in this stenohaline teleost.

Recently, Eckert et al. (2001) were unable to demonstrate any hypoosmoregulatory functions attributed to bovine GH treatment in channel catfish exposed to various concentrations of seawater. In contrast, in preliminary studies, we found that administration of bovine GH to channel catfish adapted to 12 ppt seawater had significantly elevated levels of plasma osmolality, above that of vehicle injected controls (B. Shepherd, unpublished observations), indicating a maladaptive action similar to that describe for the antagonistic actions of PRL described in salmonids during seawater adaptation (Madsen and Bern, 1992). While the questions regarding the osmoregulatory physiology of GH in channel catfish are outside the scope of this study, the possible osmoregulatory and growth-promoting actions of GH are, as yet, unclear in this teleost (Eckert et al., 2001; Silverstein et al., 2000; Tang et al., 2001; Wilson et al., 1988).

To further characterize the regulation of GH in the channel catfish, and to independently physiologically validate our cfGH ELISA, we have examined the effects of GH secretogogues on plasma GH levels. To accomplish this, we have used bovine GHRH-amide ($bGHRH_{1-29}$) and a synthetic Met-enkephalin derivative (KP-102: D-Ala-D-β-Nal-Ala-Trp-D-Phe-Lys-NH_2), also called growth hormone-releasing peptide-2 (GHRP-2), to alter endogenous GH levels. In a variety of mammalian models, GHRPs have been shown to stimulate pituitary GH release through binding to a high-affinity receptor on the pituitary and hypothalamus (Bowers, 1993, 1998). It was recently demonstrated that administration of the hexapeptide GHRP-2 (KP-102) was effective in elevating plasma GH levels in a teleost species (Tilapia: *O. mossambicus*) (Shepherd et al., 2000), thus indicating the presence of an additional regulatory axis for growth hormone release in teleosts. In a related work, Riley et al. (2002) demonstrated that (rat) Ghrelin, which is the newly discovered endogenous ligand to the GHRP receptor (Kojima et al., 1999), stimulated GH and prolactin release from tilapia pituitaries in vitro.

Our findings show that in vivo administration of KP-102 or bGHRH result in significant elevations in plasma GH levels, with the highest levels occurring in the bGHRH treated groups. These results are in accord with recent findings in tilapia (Riley et al., 2002; Shepherd et al., 2000) and suggest that this aspect of GH regulation in channel catfish is similar to other teleosts. These findings further suggest that GH secretogogues can be valuable research tools with which to further characterize the biological actions of endogenous (native) channel catfish GH, thus circumventing problems associated with the use of exogenous (heterologous) forms of GH in this teleost.

The finding that a hyperosmotic environment elevates plasma GH levels in the channel catfish, which is a stenohaline teleost, indicates that this mechanism for acclimation to environmental salinity is conserved between physiologically and taxonomically different groups of teleosts (McCormick, 1995, 2001; Sakamoto et al., 1993); however, careful thought and further study will be required to obtain meaningful insight into the significance of this conservation and whether GH possesses any osmoregulatory functions in this teleost. More importantly, the stimulatory effects of KP-102 on plasma GH levels in channel catfish further indicate that this new regulatory pathway is functionally conserved among taxonomically and physiologically distant/dissimilar vertebrate groups (Shepherd et al., 2000). The effectiveness (in vivo) of GH secretagogues in the channel catfish will enable further studies aimed at understanding the biological actions of endogenous GH in this teleost.

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