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Ghrelin gene in cichlid fish is modulated by sex and development

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Abstract

We have identified the complementary and genomic DNA sequences of the novel growth hormone-releasing peptide, ghrelin, in the cichlid fish (tilapia *Oreochromis niloticus*). The tilapia ghrelin precursor cDNA was 855 bp long, consisting of 119 bp of 5′-untranslated region, 324 bp open reading frame, and 412 bp of 3′-untranslated region. The serine residues at positions 2 and 3 of the "active core" (GSSF) of the ghrelin mature peptide are conserved between tilapia and the mammalian species. The tilapia ghrelin gene has four exons and three introns and resembles the structure of other known ghrelin genes. RT-PCR analysis revealed ghrelin mRNA predominantly expressed in the stomach but absent in the brain, pituitary, heart, kidney, ovary, and testis. Real-time PCR analysis showed an age/body size dependent increase in gastric ghrelin, which stagnated at 7 cm body size (onset of maturation). Ghrelin mRNA levels were unchanged in food-deprived sexually mature animals but were significantly higher in females compared to males. The present study shows that the structure of ghrelin peptide is highly conserved, and the reported differences in somatic and gonadal growth in tilapia could be a consequence of age- and sex-related synthesis of gastric ghrelin.

Keywords: GHRH; Growth hormone; Maturation; Sex differentiation; Starvation; Cloning; Tilapia; *Oreochromis*

Ghrelin is a 28 amino acid growth hormone-releasing peptide; recently discovered as an endogenous ligand for growth hormone-secretagogue receptor [1]. Ghrelin mRNA expression has been detected mainly in the stomach and shows low levels in the hypothalamus, pituitary, kidney, and placenta [1–4]. Evidence in mammals suggests that, in addition to regulating growth hormone release, ghrelin produced in the stomach has a variety of regulatory actions in the brain and the periphery, which include energy balance [3,4], regulation of gastrointestinal motility [5], and feeding behavior [6–8]. Only recently, ghrelin has been identified in the bullfrog, chicken, and the goldfish [8–10], hence, little is known about its role and regulatory mechanism in non-mammalian vertebrates.

In our previous study we showed that a synthetic growth hormone-releasing peptide (KP-102) can stimulate growth hormone release in the cichlid fish (tilapia *Oreochromis*) and speculated the presence of "ghrelin-like" molecule in this species [11]. The aim of this study was to identify the genomic cDNA structure of ghrelin in tilapia; to detect the distribution of ghrelin in various tissues; and to examine endogenous ghrelin levels during starvation, development, and sex differences using real-time quantitative PCR.

Materials and methods

Animals. Male and female tilapia *Oreochromis niloticus* were maintained in freshwater aquaria at 27 ± 1 °C under a simulated natural photoregime (10 h of light, 14 h of darkness). The fish were fed commercial fish chow once daily. Fish were anesthetized in tricainemethane sulfonate (MS-222; Sigma, St. Louis, MO) before decapitation and dissection of the stomach and other tissues. Experimental procedures in the present study were performed under the guidelines of the Animal Care Committee of Nippon Medical School.

Cloning of complementary DNA. Total RNA was extracted using ISOGEN reagent (Nippon gene, Tokyo, Japan) from the stomach of an adult tilapia. Subsequently poly(A)$^+$ RNA was purified with Oligotex-dT30 "Super" (Takara, Shiga, Japan). The poly(A)$^+$ RNA was reverse transcribed with oligo(dT)-primer (G1; see Table 1 for primer sequences of ghrelin G1–G13 and β-actin B1–B4) and SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). To isolate the 3′-end of the tilapia ghrelin cDNA, an adapter primer (G2) and a degenerated primer (G3) were designed on the basis of eel ghrelin (GenBank Accession No.: AB062427) and goldfish ghrelin (GenBank Accession No.:

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Table 1
Primers used for complementary and genomic DNA cloning and RT-PCR

Gene	Primer sequence
Ghrelin	G1: 5′-GGCCACGCGTCGACTAGTAC(T)$_{17}$-3′
	G2: 5′-GGCCACGCGTCGACTAGTAC-3′
	G3: 5′-WGGYWCCAGCTTCYTSAGCC-3′
	G4: 5′-CACAGTCTCTGATCTTCAACG-3′
	G5: 5′-GATCTTCAACGAGGAGATGGTC-3′
	G6: 5′-GGCCACGCGTCGACTAGTACGG GIIGGGIIGGGIIG-3′
	G7: 5′-GCAGCTCTACAATGTAGTCCG-3′
	G8: 5′-GGCCACGCGTCGACTAGTAC-3′
	G9: 5′-AGATCCATATCAGAATCAGATG-3′
	G10: 5′-GAGCCAAATCAAGCCAATGAG-3′
	G11: 5′-GTATAAGGCATACTCGAATCC-3′
	G12: 5′-TTGTGGTGCAAGTCAACCAGTG-3′
	G13: 5′-TCTGCTCTTAAAGTGACGCCAAT-3′
β-Actin	B1: 5′-CACAGTGCCCATCTACGAG-3′
	B2: 5′-CCATCTCCTGCTCGAAGTC-3′
	B3: 5′-CCTGACAGAGCGTGGCTACTC-3′
	B4: 5′-TCTCTTTGATGTCACGCACGAT-3′

Mixed bases: W, A or T; Y, C or T; S, C or G; I, Inosine.

AF454389) sequences. PCR was carried out using a thermal cycler (Gene Amp PCR system 9700, Perkin–Elmer (PE) Applied Biosystems, Foster City, CA). The conditions for PCR were 10 min at 94 °C, followed by 35 cycles of denaturation (94 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 30 s), followed by a final extension at 72 °C for 7 min. PCR products were separated by 1% agarose gel electrophoresis and the desired PCR products were then subcloned into pGEM-T easy vector system (Promega, Madison, WI). Plasmid DNA containing the DNA insert was purified with QIAprep Miniprep Kit (Qiagen, Hilden, Germany). Both strands of cloned DNA were sequenced in opposite directions using SP6 and T7 sequencing primers that flank the inserted DNA. DNA sequence analyses were carried out using ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems).

The 5′-end of the cDNA sequence was analyzed using a commercial kit for rapid amplification of cDNA ends (RACE) system (Version 2.0, Invitrogen). First strand cDNA was synthesized from poly(A)$^+$ RNA using SuperScript II reverse transcriptase and gene specific primer (G4). The 3′-end tailing of the cDNA with dCTP was performed using terminal deoxynucleotidyl transferase. Tailing products were amplified by PCR using nested primer (G5) and 5′-RACE Abridged Anchor Primer (G6). The resultant PCR products were purified with SUPREC-02 (Takara) and subjected to second round PCR using nested primer (G7) and Abridged Universal Amplification Primer (G8). The PCR products were cloned and sequenced as described above.

Cloning of genomic DNA. Genomic DNA was isolated from muscle tissue using PUREGENE DNA isolation kit (Gentra Systems, Minneapolis, MN). DNA was then amplified by two rounds of PCR using primers G7 and G9, and nested primers G10 and G11. The amplified products were subcloned and sequenced as described above.

Phylogenetic analysis. The sequences of ghrelin mature peptide from different animal species were aligned to generate a phylogenetic tree using the Neighbor-Joining GENETYX-MAC 8.0 software program (Genetyx, Tokyo, Japan). Full species names and GenBank Accession Nos. of the species used to generate the tree are as follows: eel (*Anguilla japonica*): AB062427, goldfish (*Carassius auratus*): AF454389, bullfrog (*Rana catesbeiana*): AB058510, chicken (*Gallus gallus*): AB075215, mouse (*Mus musculus*): AB035701, gerbil (*Meriones unguiculatus*): AF442491, rat (*Rattus norvegicus*): AB029433, pig (*Sus scrofa*): AF308930, and human (*Homo sapiens*): AB029434.

Detection of ghrelin mRNA in different tissues by RT-PCR. Total RNAs from pituitary, stomach, eye, gill, heart, liver, spleen, kidney, intestine, ovary, testis, muscle, and various brain areas, including olfactory bulbs, telencephalon, hypothalamus, and posterior brain, were extracted from fresh tissues of an adult fish using ISOGEN reagent. Final RNA concentrations were determined by optical density readings at 260 nm. One microgram of total RNAs was reverse transcribed into first strand cDNA with oligo(dT)-adapter primer G1 using SuperScript II reverse transcriptase. PCR amplification was performed with primers G5 and G12 under the same conditions mentioned above. PCR products were separated by 1% agarose gel electrophoresis.

As a negative control, PCRs were performed in the absence of cDNA to examine the cross-contamination of samples. For internal control of the reverse-transcription step, PCR amplification was carried out with a pair of primers, B1 and B2 (Table 1), design based on the sequence of tilapia β-actin (GenBank Accession No.: AB037865).

Experiment 1: ghrelin mRNA during development. To examine the change in ghrelin mRNA expression during development, fish were grouped according to their standard lengths and body weights (~3 cm group: 3.1 ± 0.77 cm, 1.04 ± 0.64 g, $n = 20$ undefined sex; ~7 cm group: 6.9 ± 0.2 cm, 9.65 ± 1.06 g, $n = 8$ males; and ~27 cm group: 27.8 ± 0.76 cm, 777.67 ± 130.6 g, $n = 3$ males). To examine possible sex differences in ghrelin expression, stomachs from equal size males (standard length: 12.8 ± 0.2 cm; body weight: 71.0 ± 3.6 g, $n = 5$) and females (standard length: 12.3 ± 0.7 cm; body weight: 64.3 ± 12.8 g, $n = 5$), were collected for analysis. In all groups, total RNA was extracted from the stomach and processed for real-time quantitative PCR, as described below.

Experiment 2: ghrelin mRNA during food deprivation. Three experimental groups, each containing 5–6 fish (standard length: 10.3 ± 0.4 cm; body weight: 34.4 ± 2.5 g), were acclimatized to a regular feeding routine. An approximately 0.7% bw ration of pellets per fish was given at a regular feeding time to which the fish had been adapted (7 days), before food deprivation. Group 1 (controls): fish were fed a normal daily ration through the 21 day duration of the experiment. Group 2 (unfed): fish were fed for the first 7 days and were deprived food for the next 7 days. Group 3 (refed): fish were fed for the first 7 days, were deprived food for the next 7 days, and were fed for the following 7 days. In all groups, total RNA was extracted from the stomach and processed for real-time quantitative PCR, as described below.

Real-time quantitative PCR for ghrelin mRNA. Total RNA was extracted from the stomach of all fish in experiments 1 and 2 using ISOGEN reagent. Five micrograms of total RNA was treated with RQ I RNase-Free DNase (Promega). The first strand cDNA synthesis was primed using oligo(dT)-adapter primer G1 with SuperScript II reverse transcriptase. PCR amplification was performed with primers G10 and G13 and 1× SYBR Green PCR Master Mix (PE Applied Biosystems) in the ABI PRISM 7700 Sequence Detection System (TaqMan PCR, PE Applied Biosystems). Primers B3 and B4 were used to amplify the β-actin gene in tilapia (Table 1). The reaction volume was 25 μl in MicroAmp optical tubes (PE Applied Biosystem). The ghrelin mRNA levels were estimated in absolute or relative copy numbers against β-actin mRNA. This was achieved by establishing a linear amplification curve from serial dilutions (from 10 fg to 10 pg) of a plasmid DNA carrying β-actin gene of tilapia. The number of target copies in each sample was interpolated from its detection threshold (C_T) value using the plasmid DNA. Amplicon size and reaction specificity were confirmed by 1% agarose gel electrophoresis. Statistical analysis was performed using Fisher's PLSD test.

Results

Tilapia ghrelin cDNA sequence

Degenerate primers were designed based on relatively conserved regions among known ghrelins (Table 1).

Amplification of the tilapia stomach cDNA with these primers yielded the cDNA fragment. The full-length cDNA for ghrelin was subsequently isolated by 5′- and 3′-RACE. The nucleotide and deduced amino acid sequences for ghrelin are shown in Figs. 1 and 2.

The tilapia ghrelin precursor cDNA consists of 855 bp, comprising a 5′-untranslated region (119 bp), an open reading frame (324 bp), and a 3′-untranslated region (412 bp), including the possible polyadenylation signal (AATAAA) (Figs. 1 and 2). The deduced amino acid sequence shows that tilapia ghrelin is part of a 107 amino acid precursor. Potential signal peptide cleavage site occurs in the precursor after amino acid residue 26 (alanine) (Figs. 2 and 3). In the open reading frame, we have identified potential cAMP- and cGMP-dependent protein kinase (PK) phosphorylation site at amino acid positions 4–7 (KRNT); PKC phosphorylation sites at amino acid positions 34–36 (SQK), 43–45 (SSR), and 74–76 (TLR); tyrosine kinase phosphorylation site at amino acid position 76–83 (RAEDLADY), and N-myristoylation site at amino acid position 96–101 (GNTETA) (Figs. 1 and 2).

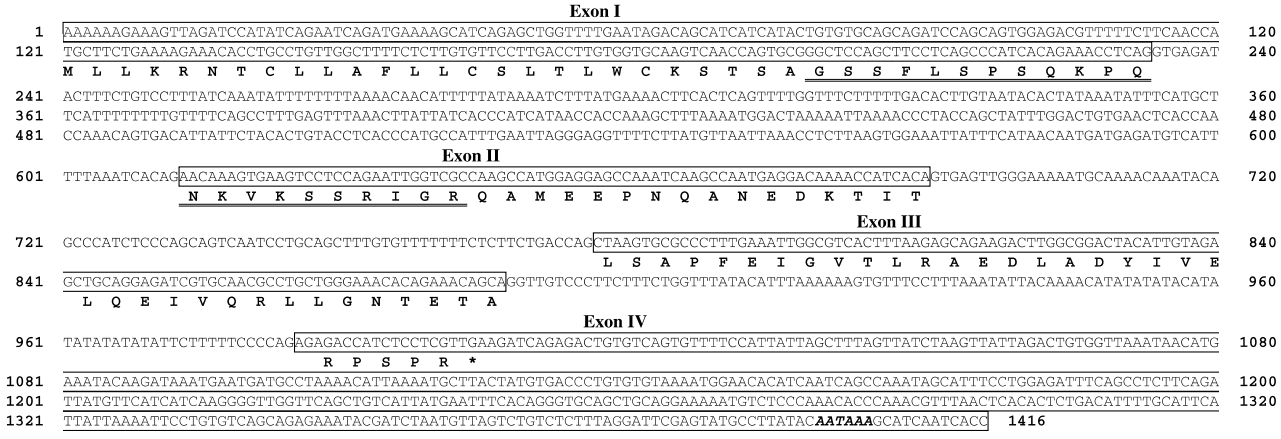

Fig. 1. Nucleotide and deduced amino acid sequences of the tilapia ghrelin gene. The tilapia ghrelin cDNA contains 855 bp sequence. Prepro-ghrelin is composed of 107 amino acids. The four exons of ghrelin are boxed and the 22 amino acid mature ghrelin peptide is double underlined. The AATAAA sequence in bold italic letters indicates the polyadenylation signal. (See GenBank Accession No. AB104859.)

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Signal peptide
Tilapia      1  M-LLKRNTCLLAFLLCSLTLWCKSTSA  26
Eel          1  MRQMKRTAYII-LLVCVLALWMDSVQA  26
Goldfish     1  M-PLRRRASHMFVLLCALSLCVESVKG  26
Chicken      1  M--FLRVILLGILLLSIL-GTETA-LA  23
Human        1  M--PSPGTVCSLLLLGML-WLDLA-MA  23
Rat          1  M--VSSATICSLLLLSML-WMDMA-MA  23
Bullfrog     1  M-NFGKAAIFGVVLFC-L-LWTEGAQA  24
                *           *   *

Ghrelin
Tilapia     27  GSSFLSPSQKPQNK-VKSSRIG-R----  48
Eel         27  GSSFLSPSQRPQGKDKKPPRVG------  48
Goldfish    27  GTSFLSPAQKPQ-GRRPPRMGRR-----  48
Chicken     24  GSSFLSPTYKNIQQQKDTRKPTARLH--  49
Human       24  GSSFLSPEHQRVQQRKESKKPPAKLQPR  51
Rat         24  GSSFLSPEHQKAQQRKESKKPPAKLQPR  51
Bullfrog    25  GLTFLSPADMQKIAERQSQNKLRHGNMN  52
                *  ****

C-terminal peptide
Tilapia     49  ----QA-ME-----EPNQANED-KTITLSAPFEIG-VTLRAEDLADYIVELQEIVQRL--LGNTETAERPSPR  107
Eel         49  RRDSDGILDLF-M-RPPLQDEDIRHITFNTPFEIG-ITMTEELFQQYGEVMQKIMQDL--LMDTPAKE-----  111
Goldfish    49  -DVAEPEIPV-IKEDDQFMMSAPFELSVSLSEAEYEKYGPVLQKVL-VNLLG-DSPLEF--------------  103
Chicken     50  ----RRGTESFW-DTDE-TEGEDDNNSVDIKFNVPFEIGVKITEREYQEYGQALEKMLQDILAENAEETQTKS  116
Human       52  ------ALAG-WLRPEDGGQAEGAEDELEVRFNAPFDVGIKLSGVQYQQHSQALGKFLQDILWEEAKEAPADK  117
Rat         52  ------ALEG-WLHPEDRGQAEEAEEELEIRFNAPFDVGIKLSGAQYQQHGRALGKFLQDILWEEVKEAPANK  117
Bullfrog    53  ------RRGV-EDDLAGEEIGVTFPLDMKMTQEQFQKQRAAVQDFLYSSLLSLGSVQDTEDKNENPQSQ----  114
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Fig. 2. Alignment of amino acid sequence of tilapia ghrelin precursor with those of other species. Asterisks indicate the bases that are identical in all species. Amino acid sequences are available from the DDBJ/EMBL/GenBank databases (Accession No. AB062427 for eel; Accession No. AF454389 for goldfish; Accession No. AB075215 for chicken; Accession No. AB029434 for human; Accession No. AB029433 for rat; and Accession No. AB058510 for bullfrog).

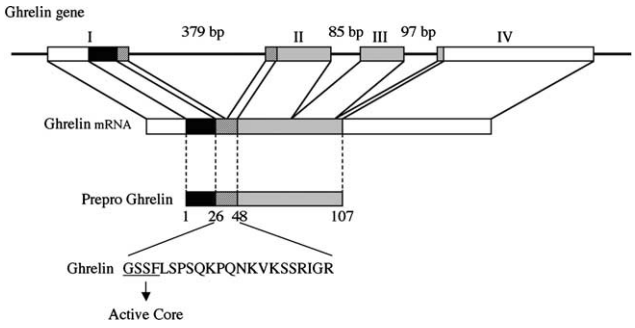

Fig. 3. Schematic diagram illustrating the intron/exon organization of the tilapia ghrelin gene. Open boxes, 5′- and 3′-untranslated regions; black boxes, signal peptide coding region; hatched boxes, ghrelin coding region; and grey boxes, C-terminal peptide coding region. The thick horizontal lines are the introns, 379 bp (intron 1), 85 bp (intron 2), and 97 bp (intron 3). The prepro-ghrelin is composed of 107 amino acids. The sequence of the deduced 22 amino acid ghrelin mature peptide is shown below and the active core is underlined.

Tilapia ghrelin genomic sequence

Tilapia ghrelin gene has four exons and three introns (Figs. 1 and 3). Exon 1 (233 bp) contains the 5′-untranslated region (UTR) and the region encoding the first 12 amino acids of the mature peptide. Exon 2 (78 bp) encodes the remaining portions of the mature peptide and a part of the C-terminal peptide. Exon 3 (111 bp) encodes a part of the C-terminal peptide. Exon 4 (432 bp) encodes the terminal region of the C-terminal peptide and the 3′ UTR. The introns are 379 bp (intron 1), 85 bp (intron 2), and 97 bp (intron 3) in length (GenBank Accession No. AB104859).

Phylogenetic analysis

Alignment of the amino acid sequence of tilapia ghrelin precursor with those of other species is shown in Fig. 2. The amino acid sequence identity among the species was calculated using the open reading frame. Tilapia ghrelin had the highest sequence homology with teleost (goldfish: 55%; eel: 72%) and avian species (chicken: 52%) but lower homology with amphibians and mammals (bullfrog: 41%; rat, mouse, gerbil, and human: 47%) (Figs. 2 and 4).

A phylogenetic tree generated by the Neighbor-Joining method showed that the vertebrate ghrelins identified to date fell into four distinct lineage groups (Fig. 4). One lineage included the teleost (tilapia, goldfish, and eel), whereas the amphibians (bullfrog), birds (chicken), and the mammals (rat, mouse, gerbil, and human) formed the second, third, and the fourth lineage.

Tissue distribution of ghrelin mRNA

RT-PCR of RNA with specific primers recognizing the coding region of the tilapia ghrelin cDNA resulted in a single band of expected size of 275 bp (Fig. 5). Ghrelin mRNA was detected in the stomach but not in the olfactory bulb, telencephalon, hypothalamus, midbrain, posterior brain, pituitary, eye, gill, heart, liver, spleen, kidney, intestine, ovary, testis, and muscle (Fig. 5). No products were detected from the negative control. PCR amplification of the cDNA samples using a specific primer set of tilapia β-actin (B1 and B2, Table 1), used as an internal control for the RT step, revealed a band of 200 bp. The β-actin mRNA was detected in all tissues examined, verifying the quality of the mRNA and DNA samples (Fig. 5).

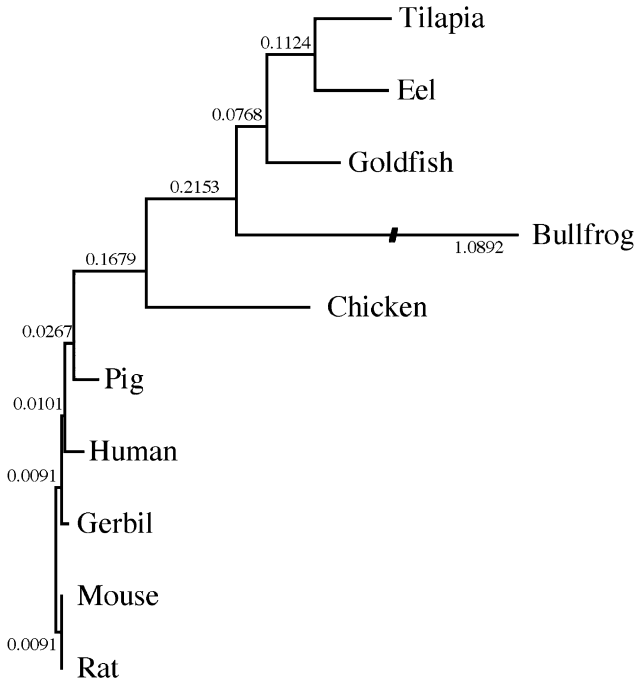

Fig. 4. Phylogenic tree was generated by Neighbor-Joining analysis using the GENETYX-MAC 8.0 software program. Percent identities of mature tilapia ghrelin to other ghrelins are 72% for eel, 55% for goldfish, 52% for chicken, 47% for gerbil, human, mouse, pig, and rat, and 41% for bullfrog. The following ghrelin amino acid sequences were used in this phylogenetic analysis: eel (GenBank Accession No. AB062427), goldfish (AF454389), human (AB029434), rat (AB029433), bullfrog (AB058510), chicken (AB075215), mouse (AB035701), gerbil (AF442491), and pig (AF308930).

Ghrelin mRNA expression during development and food deprivation

Stomach ghrelin mRNA levels, measured by real-time quantitative PCR, increased significantly from 3 to 7 cm male fish, when they reached a plateau in 7, 13, and 28 cm male fish (Fisher's PLSD test, $P < 0.01$) (Fig. 6). Stomach ghrelin mRNA expression was significantly higher in females compared to males of all body sizes/ages (Fisher's PLSD test, $P < 0.1$ and $P < 0.0001$) (Fig. 6).

Real-time quantitative analysis showed no significant variations in stomach ghrelin mRNA expression in fed,

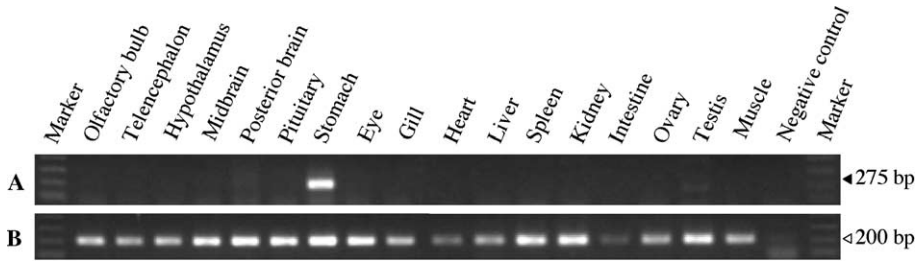

Fig. 5. Tilapia ghrelin mRNA expression in various tissues of male *O. niloticus*. (A) RT-PCR analysis. One microgram of total RNAs was reverse transcribed into first strand cDNA and 1/100 volume of the resultant cDNA was used as a template in each lane for specific amplification; (B) β-actin was amplified as internal control. Negative control, without template. Marker, 100–400 bp. Ghrelin mRNA expression was seen only in the stomach.

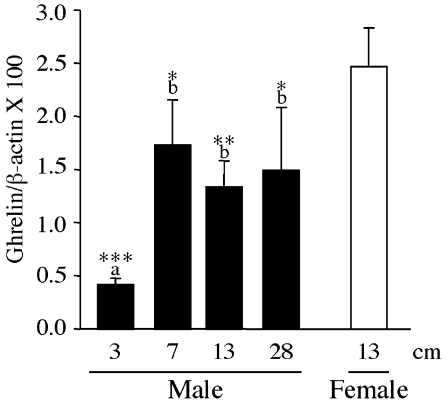

Fig. 6. Gastric expression of ghrelin mRNA in male and female tilapia of different body sizes (cm). The mRNA levels were determined by real-time PCR and the values were normalized by the content of β-actin mRNA. Values represent means ± SEM. Different alphabets on the error bars indicate significant differences between the males (Fisher's PLSD test, $P < 0.01$). Note ghrelin mRNA increased with increasing body size but stagnated at 7 cm. Asterisks, significant differences, males compared with the female group at one time point (*, $P < 0.1$; **, $P < 0.01$; ***, $P < 0.0001$ by Fisher's PLSD test).

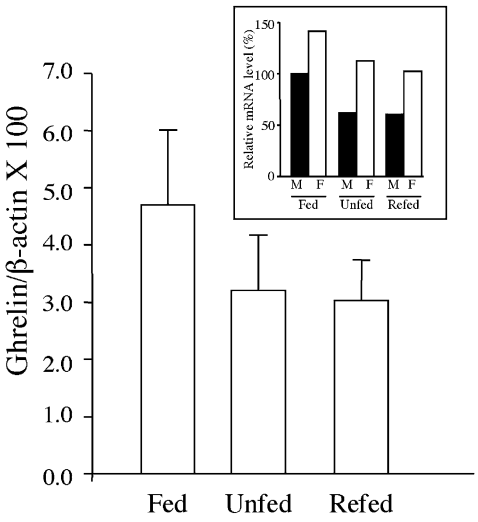

Fig. 7. Gastric ghrelin mRNA expression in male and female tilapia during food deprivation. The mRNA levels were determined by real-time PCR and the values were normalized by the content of β-actin mRNA. Values represent means ± SEM. There was no significant difference between fed, unfed, and refed group (Fisher's PLSD test, $P < 0.05$). When ghrelin mRNA was expressed as percent of fed males, about 40–50% higher values were seen in females compared to males (inset).

unfed for 7 days or refed for 7 days fish (Fisher's PLSD test, $P < 0.05$) (Fig. 7). When ghrelin mRNA was expressed as percent of fed males, about 40–50% higher values were seen in females compared to males (Fig. 7).

Discussion

Complementary and genomic DNA structure of ghrelin

Tilapia preproghrelin is about 107 amino acids, consisting of a signal peptide (26 amino acids), the mature peptide (22 amino acids), and a C-terminal peptide (59 amino acids). Comparison of amino acid sequence of the mature peptide of tilapia with known sequences of other species show a 50–70% homology between both teleost and avians, and about 40% homology between bullfrog and mammals. The C-terminal portion rather than its N-terminal end of the mature peptide has high variability. This is noteworthy because the N-terminal region is the biologically active segment of the ghrelin. The first four amino acids "GSSF," considered to be the "active core" of the ghrelin peptide in mammals [12] are conserved in tilapia but are different in the bullfrog (GLTF: [9]) and the goldfish (GTSF: [8]). The goldfish have two alternatively spliced ghrelin molecules [8] but tilapia and other vertebrates have a single ghrelin molecule because of the presence of a single cleavage site in their preproghrelin structure.

The tilapia ghrelin gene consists of four exons and three introns, and this structural organization resembles those of the goldfish and the human ghrelin gene [8,13] but differs from those of the mouse and rat ghrelin, which contains an additional non-coding exon of 19 bp in the 5′-untranslated region [14]. Furthermore, the sizes of introns in the ghrelin gene vary between animal species. Phylogenic variations in the

organization of ghrelin molecules are not unexpected because the metabolic needs of each animal species may have required the ghrelin protein to perform subtly different functions.

Expression sites and regulation of ghrelin mRNA

In tilapia, RT-PCR analysis revealed a strong signal derived from ghrelin mRNA in the stomach but no signal could be detected in other tissues. These results are consistent with the fact that the stomach is the major ghrelin-producing site in the rat, human, chicken, amphibians, and the goldfish. In addition to the stomach, lower levels of ghrelin expression have also been demonstrated in the hypothalamus, pituitary [2–4,15], intestine, kidney, and the heart [8–10]. However, our failure to observe ghrelin in the brain and peripheral tissues may be because ghrelin is either absent or present below detectable levels in these tissues.

Ghrelin has several physiological functions, which include growth hormone secretion, increased food intake, and decreased fat utilization [2–4]. Our observation of an age/size dependent increase in gastric ghrelin is in agreement with an age-dependent increase reported in rats [16–18]. The increased concentration of gastric ghrelin during development may be related to an increased growth hormone secretion, the development of the gastrointestinal tract, and/or the stimulation of appetite in order to increase food intake. It is interesting that the increase in ghrelin expression level closely relates to rapid body growth period and the onset of maturation in tilapia, which is consistent with the description postulated in the rats [18]. However, further studies are required to elucidate the relationship between ghrelin and the reproductive axis.

In tilapia, ghrelin mRNA levels were unchanged in food-deprived animals; this indicates that ghrelin expression is not influenced by nutritional state in sexually mature animals. However, significantly higher ghrelin mRNA levels were observed in females compared to males. The age- and sex-associated changes in gastric ghrelin mRNA levels could influence growth hormone secretion, which is under ghrelin regulation in tilapia [19]; [Parhar et al. unpublished results] as in other vertebrates [1–4]. Hence, the difference in somatic growth [20] and gonadal growth and/or the timing of sex differentiation between female (28 days post-fertilization) and male (50 days post-fertilization) tilapias [21,22] could be a consequence of sexually dimorphic growth hormone secretion, as in mammals [23].

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