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Sequence and Expression of a cDNA Encoding Both Pituitary Adenylate Cyclase Activating Polypeptide and Growth Hormone-Releasing Hormone-like Peptide in Channel Catfish (*Ictalurus punctatus*)

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In nonmammalian vertebrates, pituitary adenylate cyclase activating polypeptide (PACAP) and a putative growth hormone-releasing hormone (GHRH-like peptide) are encoded by a single mRNA transcript. Both PACAP and GHRH have been implicated in the control of fish growth. Although the gene encoding PACAP and GHRH-like peptide (GHRHLP) has been cloned in other fishes, characterization of this gene in the commercially important channel catfish (*Ictalurus punctatus*) has not been previously reported. In this study, the GHRHLP/PACAP cDNA was cloned from channel catfish hypothalamic tissue and a brain cDNA library. Two cDNA variants of the GHRHLP/PACAP precursor gene were identified as a result of alternative splicing, a long form encoding both PACAP and GHRHLP and a short form encoding only PACAP. Both the long and the short forms of the GHRHLP/PACAP precursor cDNA were identified in channel catfish brain, pituitary, fat, gastrointestinal tract, ovary, testes, and muscle by RT-PCR detection. This study is the first to demonstrate mRNA expression of this gene in fat or skeletal muscle of fish. By characterizing the GHRHLP/PACAP gene and its distribution in channel catfish, we have developed essential tools to investigate the roles of these peptides in the regulation of catfish growth.

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Although significant strides have been made in several areas of channel catfish (*Ictalurus punctatus*) physiology, the mechanisms controlling growth have received little attention. A better understanding of these processes in catfish could be gained through the identification and characterization of genes involved in their regulation. Two neuropeptides that are likely involved in the regulation of fish growth are growth hormone-releasing hormone (GHRH) and pituitary adenylate cyclase activating polypeptide (PACAP). Analogs of these peptides have been demonstrated to cause a release of growth hormone *in vitro* in goldfish and salmon pituitary cell cultures (Vaughan *et al.*, 1992; Parker *et al.*, 1997). PACAP and a GHRH-like peptide (GHRHLP) are encoded on the same gene in nonmammalian vertebrates such as fish and birds (Parker *et al.*, 1993; McRory *et al.*, 1997), whereas in mammals PACAP and GHRH are encoded by separate genes on separate chromosomes (Hosoya *et al.*, 1992; Perez Jurado *et al.*, 1994). A gene encoding only GHRH or GHRHLP in nonmammalian vertebrates has not yet been identified. In salmon (Parker *et al.*, 1997), Thai catfish (McRory *et al.*, 1995), chicken (McRory *et al.*, 1997), and frog (Alexandre *et al.*, 2000), however, a

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shortened precursor cDNA encoding only for PACAP has been identified.

PACAP was first isolated by its stimulatory action on adenylate cyclase activity in rat pituitary cells (Miyata *et al.*, 1989). Two biologically active forms derived from the same precursor, PACAP-38 and PACAP-27, have been identified (Miyata *et al.*, 1989, 1990). PACAP-38 is a 38-amino acid polypeptide, whereas PACAP-27 is a truncated form containing only the first 27 amino acids of PACAP-38. PACAP immunoreactivity is widely distributed throughout the central nervous system (Köves *et al.*, 1994) and peripheral tissues (Arimura *et al.*, 1991). In fish, PACAP mRNA expression has been demonstrated in brain, pituitary, spinal cord, gastrointestinal tract, ovary, testes, kidney, liver, heart, gill (Wong *et al.*, 2000), and eye (Fradinger and Sherwood, 2000). Consistent with the wide range of tissue distribution, PACAP is considered a pleiotropic neuropeptide with various functions (Arimura, 1998; Wong *et al.*, 2000). Although PACAP does fulfill most of the requirements of a hypophysiotropic hormone, neither consistent stimulation of adenohyphysial hormone secretion nor suppression of *in vivo* pituitary hormone release by the blocking of endogenous PACAP action have been demonstrated.

GHRH was originally isolated from human pancreatic tumors associated with acromegaly based on its ability to stimulate growth hormone (GH) release (Guillemin *et al.*, 1982; Rivier *et al.*, 1982) and was later isolated and characterized in human hypothalamic tissue (Ling *et al.*, 1984). A hypophysiotropic hormone, GHRH interacts with somatostatin (SRIF) to regulate GH release from somatotroph cells in the anterior pituitary through the dual antagonistic regulation of adenylate cyclase and Ca^{2+} influx (Harvey, 1991). Peripherally, GHRH activity has been observed in the ovary and testes of rats (Pescovitz *et al.*, 1990; Bagnato *et al.*, 1992).

Both PACAP and GHRH belong to the vasoactive intestinal polypeptide (VIP)–glucagon–secretin family. PACAP is the most highly conserved member of this family. Remarkably, conservation during the evolution from protochordate to mammals has yielded a 96% amino acid sequence identity between mammalian and tunicate PACAP-27 (McRory and Sherwood, 1997). In contrast, GHRH is only moderately conserved. Including translated GHRHLP cDNA sequences, peptide lengths vary from 42 to 46 amino acids with only 31% identity between Thai catfish and human sequences (McRory *et al.*, 1995). Thai catfish GHRHLP shares a 50.0% sequence identity with PACAP-related peptide (PRP), which is located 5′ upstream of the PACAP coding region on the mammalian precursor, as is GHRHLP in the nonmammalian PACAP precursor. Human PRP has only a 38% sequence identity with GHRH (Ohkubo *et al.*, 1992). Gene duplication is thought to have played an important role in the evolution of the glucagon superfamily. It has been suggested that the mammalian prepro-GHRH and prepro-PACAP arose from duplication of an ancestral gene with subsequent exon loss and amino acid substitution (Parker *et al.*, 1997).

As an early step in the elucidation of the mechanisms controlling growth in channel catfish, we have isolated the cDNA encoding both GHRHLP and PACAP from channel catfish hypothalamic tissue and a brain cDNA library. The distribution of these growth-potentiating peptides was studied with RT-PCR to determine tissue mRNA expression. As a result of alternative splicing, two populations of GHRHLP/PACAP precursor cDNAs, a long form encoding both PACAP and GHRHLP and a short form encoding only PACAP, were identified in channel catfish brain, pituitary, fat, gastrointestinal tract, ovary, testes, and muscle. Phylogenetic comparisons, with an emphasis on known GHRHLP/PACAP sequences of teleost fishes, are presented.

MATERIALS AND METHODS

Isolation of GHRHLP/PACAP Precursor cDNA from Catfish Hypothalamus

Total RNA was extracted from juvenile USDA 103 strain channel catfish hypothalamic tissue with Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's instructions. First-strand cDNA was synthesized from 1 μg total RNA. Twelve microliters of total RNA and 1 μl oligo$(dT)_{15}$ (0.5 μg/μl) were incubated at 70° for 5 min and placed on ice. The reaction mixture was brought to a volume of 25 μl with 1× M-MLV RT buffer, 2 mM dNTPs, 20 units rRNasin (Promega, Madison, WI),

and 200 units of M-MLV Reverse Transcriptase (Promega), incubated at 42° for 1 h and at 94° for 5 min, and then diluted to a final volume of 100 μl. The first-strand cDNA was then used as a template for PCR with primers designed according to the consensus nucleotide sequences of salmon (Parker *et al.*, 1993) and Thai catfish (McRory *et al.*, 1997) GHRHLP/PACAP cDNA sequences: FOR1, GAGAGAAGAGCCGAAACGCATGCAGA and REV1, TCGCTTTGACAGTGGCTCTGAGTCCT. PCR was conducted at an annealing temperature of 57° and cycled 35 times. The FOR1/REV1 primer set was designed to amplify the GHRHLP coding region and avoid selecting for a shortened precursor gene containing only the PACAP coding region. A separate PCR was conducted with primers FOR2 (CTCATCTATGGGATCTTAATGCGCTA) and REV2 (CTGTCTGTACCTTCTTCCCAGCACTG), which were designed 5′ and 3′ outside the putative GHRHLP coding region.

Rapid amplification of cDNA ends (RACE) was conducted with the SMART RACE cDNA amplification kit (Clontech, Palo Alto, CA). RACE-ready first-strand cDNA was synthesized from 300 ng of hypothalamic total RNA. Two separate 3′-RACE reactions were conducted with primers FOR1 and FOR 2. PCR products that showed distinct bands by agarose gel electrophoresis were TA-cloned into the TOPO TA cloning vector pCR2.1 (Invitrogen, Carlsbad, CA). White colonies were screened by PCR for an insert of the correct size with primer sets FOR1/REV1 and FOR2/REV2. Positive clones were grown overnight and sequenced with M13 forward and reverse primers as described below.

Library Construction and Sequencing of Clones

A channel catfish brain cDNA library was directionally cloned into pSport1 vector (Life Technologies, Rockville, MD) following the protocol for the SuperScript Plasmid cDNA Library kit. Brain tissue was obtained from USDA 103 channel catfish. After transformation into DH5α cells, plasmid DNA was isolated by a modified alkaline lysis with Procipitate reagent (Ligochem, Fairfield, NJ) and 0.45-μm hydrophobic filter plates (Millipore, Bedford, MA). Sequences were determined on an ABI 3700 automated sequencer (P. E. Biosystems, Foster City, CA).

Sequence Analysis

DNA sequences were analyzed with the BLASTn program available from the NCBI internet website (http://www.ncbi.nlm.nih.gov). Protein translations were done and analyzed with the same source and the programs BLASTx or BLASTp. Significant similarities were assumed at a *P* level of less than 0.0001. Multiple alignments of cDNA and amino acid sequences were performed with the programs CLUSTAL and ALIGN, respectively (http://workbench.sdsc.edu). Phylogenetic analysis was conducted with the GrowTree program (GCG v. 10.1; Genetics Computer Group, Madison, WI) with UPGMA (unweighted pair group method using arithmetic averages), Kimura protein distance correction, and the blosum62 scoring matrix.

Tissue Expression and Distribution

Brain and peripheral tissue expression and distribution of the channel catfish GHRHLP/PACAP precursor mRNAs were examined by RT-PCR with primers FOR2 and REV2. First-strand cDNA was prepared from total RNA isolated from the various tissues of juvenile USDA 103 channel catfish as described above for hypothalamic tissue. PCR conditions were 35 cycles of 94° for 30 s, 57° for 45 s, and 72° for 1 min, followed by a final extension at 72° for 10 min. For an internal control, RT-PCR was performed at the same time with a primer for channel catfish α-tubulin (αTUB-F, TCTCCATCCACGTCGGCCAG; αTUB-R, TAAGTGCCCGTGCGAACCTC). PCR products were separated on 1.2% agarose gels containing ethidium bromide and viewed under ultraviolet light with the NucleoVision imaging system and Gel Expert v.3.5 software (Nucleotech, San Mateo, CA).

RESULTS

Isolation of Channel Catfish GHRHLP/PACAP Precursor cDNA

A single cDNA fragment of 159 bp was isolated from channel catfish hypothalamic tissue with primers FOR1 and REV1 (Figs. 1 and 2). This fragment shared a 91% nucleotide sequence identity with Thai catfish,

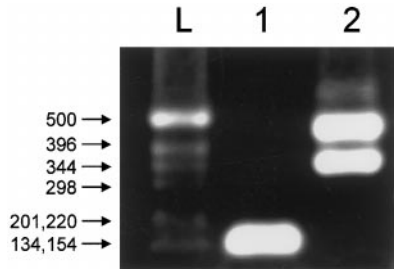

FIG. 1. Results of RT-PCR with two unique primer sets to detect GHRHLP/PACAP mRNA in the hypothalamus of channel catfish. Lane L, ladder; lane 1, primer set FOR1/REV1; lane 2, primer set FOR2/REV2.

Clarias gariepinus, GHRHLP/PACAP precursor cDNA. Two fragments of 351 and 456 bp were isolated with primers FOR2 and REV2, respectively (Figs. 1 and 2). These fragments share a 95% sequence identity with the corresponding region in Thai catfish. The two products isolated with primers FOR2 and REV2 differed only in their deletion or retention of exon 4, respectively. Two clones containing the complete GHRHLP/PACAP precursor cDNA, except for deletion of exon 4 in both clones, were randomly sequenced from the brain cDNA library. Two clones containing exon 4 and one clone with exon 4 deleted were amplified from hypothalamic cDNA with 3′-RACE and sequenced.

The complete cDNA sequence of the channel catfish GHRHLP/PACAP precursor (Fig. 2) in our study was composed of 375 bp of 5′-untranslated region that contained a compound microsatellite (CT-CA) that was polymorphic in USDA 103 strain channel catfish. The coding region covered nucleotides 376–900 and shared an 85% nucleotide and an 86% amino acid sequence identity with the coding region of Thai catfish GHRHLP/PACAP precursor cDNA. The channel catfish precursor cDNA contained a signal peptide from 376–435 bp (aa 1–20), GHRHLP from 625–759 bp (aa 84–128), and PACAP from 765–879 bp (aa 131–168). The 3′-untranslated region contained another potentially polymorphic compound microsatellite (GC-GA) that started within the stop codon. The consensus polyadenylation signal was found at positions 1058–1063 of the reported cDNA sequence. The intron–exon organization appears to be conserved relative to that of other nonmammalian vertebrates, since the organization of exon 4 insertion or deletion is consistent with the exon phase of other species (Parker *et al.,* 1997; McRory *et al.,* 1997; Alexandre *et al.,* 2000).

Alternative Splicing and Tissue Distribution

Two RT-PCR products were isolated in brain (Fig. 3) and specific peripheral tissues (Fig. 4) with primers FOR2 and REV2. Sequence analysis showed that the two bands represent two differently processed mRNA transcripts, resulting in a long and a short GHRHLP/PACAP precursor cDNA in which 105 bp are missing from the shortened sequence. This deletion corresponds to the excision of exon 4 in the salmon GHRHLP/PACAP precursor primary mRNA transcript (Parker *et al.,* 1997). GHRHLP/PACAP precursor mRNA expression and alternative splicing were detected in channel catfish hypothalamus, telencephalon, optic tectum, corpus cerebellum, and myelencephalon within the brain and pituitary (Fig. 3). Peripherally, bands corresponding to both the long and the short precursor primary mRNA transcripts were observed in fat, gastrointestinal tract, muscle, ovary, and testes (Fig. 4).

DISCUSSION

In this study, two cDNA variants of the GHRHLP/PACAP precursor gene in channel catfish were characterized, only one of which encodes both PACAP and a second peptide with high sequence identity to GHRHLP of fish, frog, and chicken (Table 1). Comparison of the channel catfish PACAP sequence (1–38) reveals an 89.5% identity with human PACAP (Table 2); however, channel catfish GHRHLP shares only a 31.1% identity to human GHRH (Table 1). Structural organization of the channel catfish GHRHLP/PACAP precursor gene was determined by comparison to salmon and Thai catfish cDNA sequences (Parker *et al.,* 1993; McRory *et al.,* 1995). The insertion or deletion of exon 4 in the channel catfish gene is consistent with the exon phase in salmon; however, in Thai catfish alternate splicing was not detected (McRory *et al.,* 1995). As observed with salmon, exon 4 deletion from the channel catfish precursor mRNA transcript does not change the frame of the downstream amino acid sequence of PACAP. The majority of the GHRHLP se-

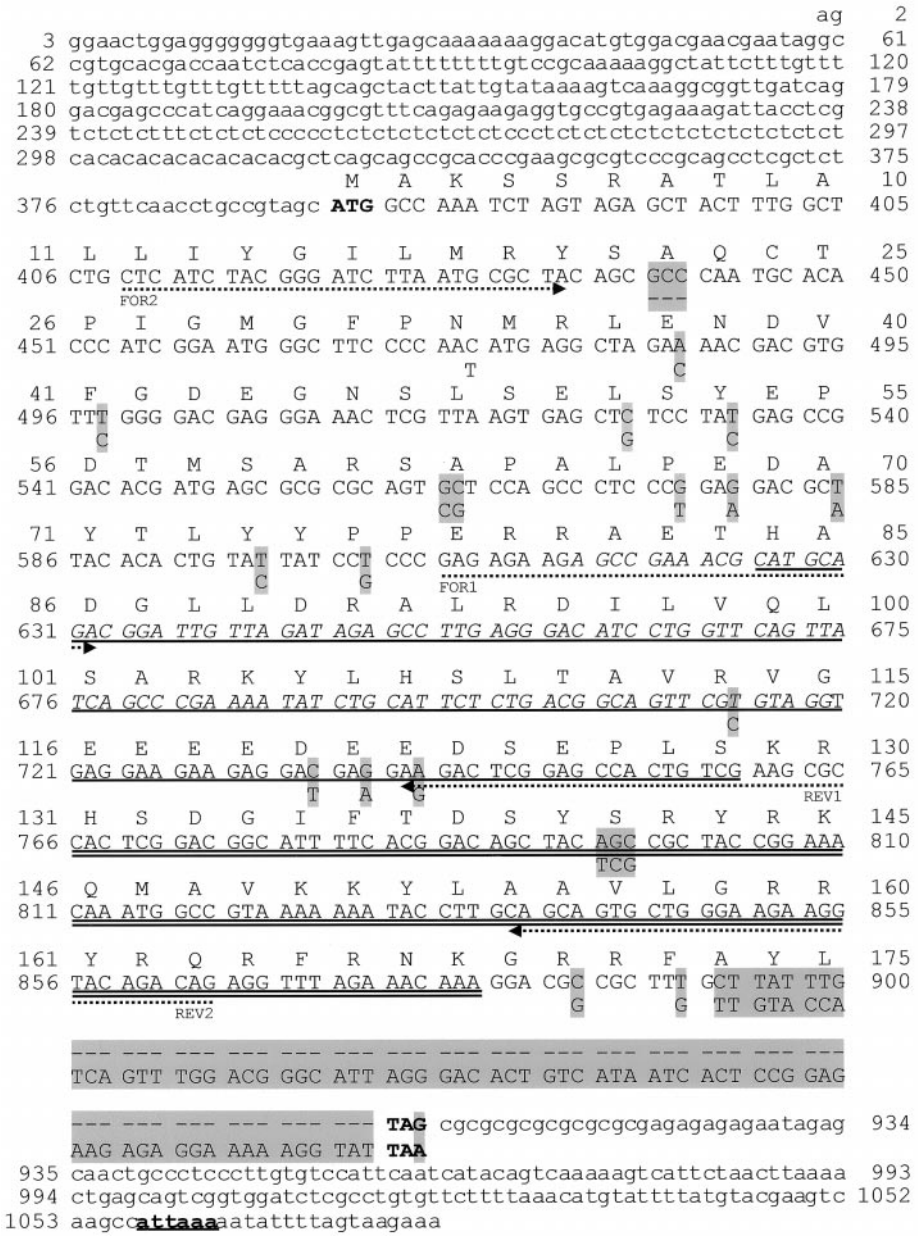

FIG. 2. Nucleotide sequence of the channel catfish GHRHLP/PACAP precursor cDNA (GenBank Accession No. AF321243) and deduced amino acid sequence. Arrows (→) denote PCR primer locations. The sequence encoding GHRHLP is singly underlined and the sequence encoding PACAP is doubly underlined. A potential polyadenylation signal is underlined in boldface. Alternate splicing results in the deletion of nucleotides 615–719 (italics). Shading indicates nucleotide differences and dashes (−) indicate nucleotide gaps within the coding region as compared to Thai catfish GHRHLP/PACAP precursor cDNA (X79078).

quence is encoded on exon 4, with residues 33–45 being encoded on exon 5. Similar to the GHRHLP/PACAP precursor in nonmammalian vertebrates, the mammalian PACAP precursor gene is composed of 5 exons, with the PACAP coding region located on exon 5. In mammals, however, GHRH is encoded by a separate gene on a separate chromosome (Hosoya *et al.*, 1992; Perez Jurado *et al.*, 1994), and the sequence of PRP is encoded on exon 4 of the PACAP precursor.

The sequence of PACAP is highly conserved throughout evolution, as evidenced by the remarkable 96% identity between mammalian and tunicate

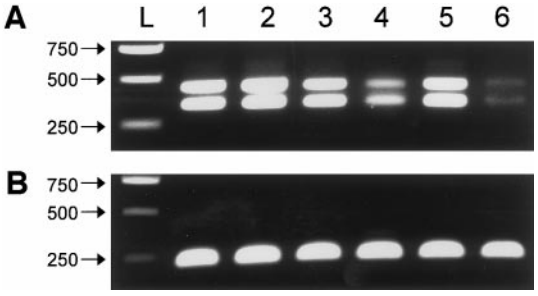

FIG. 3. (A) Expression of GHRHLP/PACAP mRNA in the channel catfish brain and pituitary as detected by RT-PCR with primer set FOR2/REV2. Lane L, ladder; lane 1, hypothalamus; lane 2, telencephalon; lane 3, optic tectum; lane 4, corpus cerebellum; lane 5, myelencephalon; lane 6, pituitary. Double bands were expressed throughout the brain and pituitary. (B) RT-PCR products resulting from primers made against channel catfish α-tubulin with the same cDNA as that in A.

PACAP-27 (McRory and Sherwood, 1997). The sequence of GHRHLP is only somewhat conserved among fish, frog, and chicken (Table 1). When GHRHLP is compared to mammalian GHRH, sequence identity drops below 40%; however, when the first 29 amino acid residues of channel catfish GHRHLP are compared to PRP, a 29-amino acid peptide located in the same position of the mammalian PACAP precursor, sequence identity reaches 50% or greater (Table 1).

A comparison of the channel catfish GHRHLP/PACAP precursor cDNA sequence with that of Thai catfish demonstrates a high degree of homology between these distant relatives. Channel catfish (Order: Siluriformes; Family: Ictaluridae) share an 83% sequence identity with the 5′ untranslated region and an 85% identity with the coding region of Thai catfish (Order: Siluriformes; Family: Clariidae). The 3′ untranslated regions, however, share no sequence identity between these two catfishes. At the amino acid level, the deduced sequences for the channel and Thai catfish coding regions are 86% identical; however, despite the differences at the nucleic acid level, there is a remarkable 100% conservation of amino acids comprising GHRHLP and PACAP for these two catfishes. When the deduced amino acid sequence of the channel catfish coding region is compared with that known for teleost fishes outside of the order Siluriformes, identity is considerably less: sockeye salmon (Order: Salmoniformes), 59%; zebrafish (Order: Cypriniformes), 65% (Fig. 5). Of the four species presented in Fig. 5, alternate splicing has been observed only in the channel catfish and salmon mRNA transcripts. It is also interesting to note that the Thai catfish open reading frame is extended by an additional 21 amino acids as compared to the other three teleost species.

To determine the phylogenetic relationship of teleostean GHRHLP/PACAP with that of other vertebrates, amino acids 63 to 175 were compared among channel catfish, Thai catfish, sockeye salmon, zebrafish, chicken, and frog sequences with the GrowTree program. Analogous sequence from tunicate (*Chelyosoma productum*) GHRHLP/PACAP, human PACAP, and human GHRH were included in the phylogenetic analysis to illustrate the possible evolution of PACAP and GHRH-like peptides. The resulting phylogenetic tree (Fig. 6) showed that channel catfish GHRHLP/PACAP is more closely related to that of the Thai catfish than to that of the other species; however, each of the teleost GHRHLP/PACAP sequences were more closely related to human PACAP and tunicate GHRHLP/PACAP than to human GHRH.

It is hypothesized that duplication of a gene encoding both GHRHLP and PACAP late in evolution re-

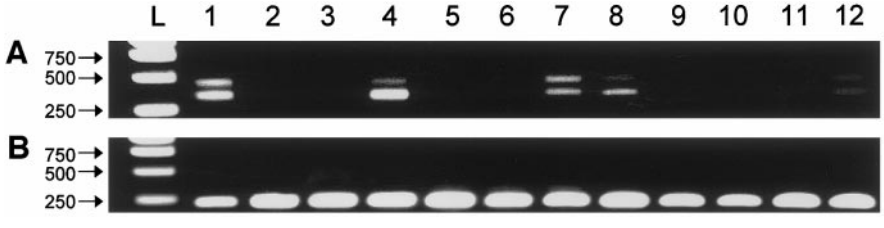

FIG. 4. (A) RT-PCR products from peripheral tissues of channel catfish amplified with primer set FOR2/REV2 to detect GHRHLP/PACAP mRNA expression. Lane L, ladder; lane 1, fat; lane 2, gill; lane 3, heart; lane 4, intestine; lane 5, kidney; lane 6, liver; lane 7, muscle; lane 8, ovary; lane 9, pancreas; lane 10, skin; lane 11, spleen; lane 12, testes. Double bands were expressed in fat, intestine, muscle, ovary, and testes. (B) RT-PCR products resulting from primers made against channel catfish α-tubulin with the same cDNA as that in A.

TABLE 1
Homology of Channel Catfish Growth Hormone-Releasing Hormone-like Peptide (GHRHLP) to Published Sequences

	Identity (%)	Similarity (%)	References
GHRH-like peptide			
Thai catfish	100	100	McRory *et al.* (1995)
Goldfish	82.2	88.9	Leung *et al.* (1999)
Salmon	62.2	73.3	Parker *et al.* (1993)
Carp	60.0	75.6	Vaughan *et al.* (1992)
Frog	58.7	78.3	Alexandre *et al.* (2000)
Zebrafish	57.8	75.6	Fradinger and Sherwood (2000)
Chicken	56.5	71.8	McRory *et al.* (1997)
PACAP-related peptide			
Sheep	53.3	74.0	Kimura *et al.* (1990)
Human	50.0	74.1	Kimura *et al.* (1990)
Mouse	50.0	74.1	Yamamoto *et al.* (1998)
Rat	50.0	74.1	Miyata *et al.* (1989)
GHRH			
Rat	35.6	62.2	Mayo *et al.* (1985b)
Sheep	33.3	66.7	Brazeau *et al.* (1984)
Pig	31.1	68.9	Bohlen *et al.* (1983)
Human	31.1	66.7	Mayo *et al.* (1985a)
Cow	31.1	66.7	Esch *et al.* (1983)
Hamster	31.1	66.7	Ono *et al.* (1994)
Mouse	31.1	60.0	Suhr *et al.* (1989)

sulted in separate mammalian prepro-GHRH and prepro-PACAP genes (Parker *et al.,* 1997). Evidence supporting this hypothesis includes the isolation of a native peptide from the hypothalamus of carp with structural similarity to members of the glucagon–secretin superfamily which stimulates GH release from goldfish pituitary cells in a dose-dependent manor (Vaughan *et al.,* 1992). This carp GHRHLP shares a 91% identity with the proposed salmon GHRHLP, and data supporting a separate prepro-GHRH gene in nonmammalian species is lacking. However, Parker *et al.* (1997) were unable to demonstrate consistent GH stimulation in salmon pituitary cell cultures with the salmon GHRHLP, suggesting the possibility of a more effective salmon GHRHLP that has yet to be identified. Parker *et al.* (1997) were able to demonstrate PACAP-stimulated GH release *in vitro,* suggesting that PACAP may play a significant role in salmon GH release, whether directly or indirectly. Whereas the native carp GHRHLP sequence has been available for nearly a decade, characterization of a cDNA encoding carp GHRHLP has not been forthcoming.

Characterization of the channel catfish precursor revealed that PACAP is processed in a manner similar to that of all species studied to date. Channel catfish PACAP is preceded by a dibasic amino acid enzyme processing site (Lys-Arg) and is followed by a Gly-Arg-Arg processing site which would yield a 38-amino-acid peptide with an amidated C terminus. Processing at a second amidation site within the PACAP sequence would result in the 27-amino-acid PACAP. As in salmon and Thai catfish, processing of the GHRHLP is less clear. In salmon, the GHRHLP peptide sequence is preceded by a single arginine cleavage site; however, a dibasic cleavage site 3 amino acids upstream does exist (Parker *et al.*, 1997). A dibasic site also preceds the Thai catfish sequence 3 amino acids upstream from the suggested site of cleavage, which McRory *et al.* (1995) suggest to be a threonine. In fish, frog, and chicken, the GHRHLP sequence is followed by a dibasic cleavage site, resulting in a free C terminus. Organization of the channel catfish gene is very similar to that of the Thai catfish. Channel catfish GHRHLP is preceded by a threonine with a dibasic Arg-Arg site 3 amino acids upstream and followed by a Lys-Arg site at the C terminus. Of interest is the processing of rat PACAP. In the rat, cleavage at three dibasic sites initially generates a large intermediate PRP precursor and PACAP-38. Cleavage at a single arginine, which might correspond to Arg^{113} in channel catfish, followed by carboxypeptidase hydrolysis, generates rat PRP (Rouillé *et al.,* 1995). Similar processing of the catfish GHRHLP/PACAP precursor would

TABLE 2
Homology of Channel Catfish Pituitary Adenylate Cyclase Activating Polypeptide to Published Sequences

	Identity (%)	Similarity (%)	References
Thai catfish	100	100	McRory *et al.* (1995)
Salmon	94.7	100	Parker *et al.* (1993)
Goldfish	92.1	94.7	Leung *et al.* (1999)
Frog	89.5	100	Alexandre *et al.* (2000)
Human	89.5	97.4	Kimura *et al.* (1990)
Sheep	89.5	97.4	Kimura *et al.* (1990)
Rat	89.5	97.4	Miyata *et al.* (1989)
Mouse	89.5	97.4	Yamamoto *et al.* (1998)
Zebrafish	86.8	100	Fradinger and Sherwood (2000)
Chicken	86.8	94.7	McRory *et al.* (1997)

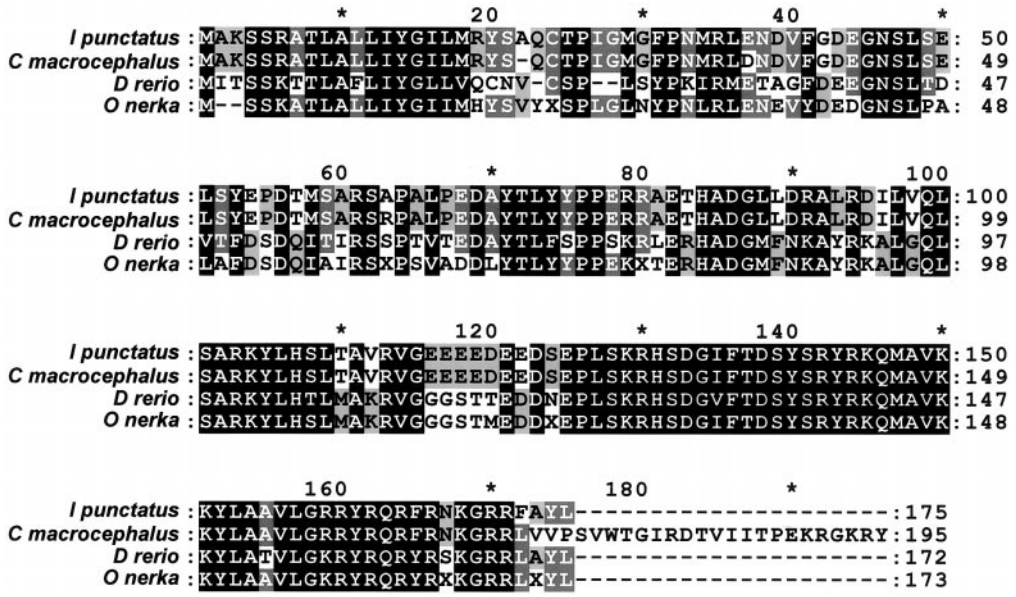

FIG. 5. Amino acid alignment of channel catfish (*I. punctatus*), Thai catfish (*C. macrocephalus*), zebrafish (*D. rerio*), and sockeye salmon (*O. nerka*) GHRHLP/PACAP open reading frames. Identities and similarities are shaded and gaps are indicated by dashes (−).

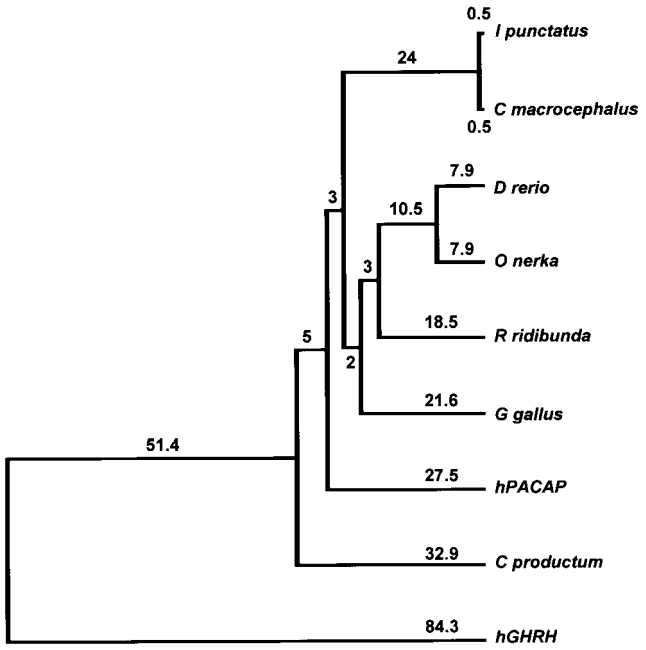

FIG. 6. Phylogenetic tree of PACAP and GHRH-like peptides. The tree was constructed with amino acids 63 to 175 with the GrowTree program with UPGMA (unweighted pair group method using arithmetic averages), Kimura protein distance correction, and the blosum62 scoring matrix. Values on the horizontal branches represent calculated substitutions per 100 amino acid residues.

yield a 32-amino-acid PRP-like peptide rather than the 45-amino-acid GHRHLP.

Consistent with the distribution in other species, where GHRHLP/PACAP precursor mRNA expression has been demonstrated throughout the central nervous system and in several peripheral tissues (Wong *et al.*, 2000), channel catfish GHRHLP/PACAP precursor expression was identified in hypothalamus, telencephalon, optic tectum, corpus cerebellum, myelencephalon, pituitary, fat, gastrointestinal tract, ovary, testes, and muscle. In the central nervous system, PACAP has been established as a neurotransmitter and a neuromodulator (Arimura, 1998), and evidence in fish suggests that it acts as a hypophysiotropic factor regulating pituitary hormone secretion (Wong *et al.*, 2000). PACAP also acts as a trophic factor in a number of peripheral tissues (Arimura, 1998). In the gastrointestinal tract, PACAP affects the secretory activity of exocrine and endocrine cells and induces concentration-dependent relaxation of gastric smooth muscles. In the gonads, PACAP operates as a local regulator of gonadal activity. The function of PACAP or GHRHLP in fat and skeletal muscle remains to be elucidated. This study is the first to demonstrate a high degree of GHRHLP/PACAP precursor expression in either fat or muscle.

The high conservation of the PACAP sequence throughout the evolutionary process allows for the unmistakable identification of the GHRHLP/PACAP precursor gene in channel catfish. The role of the GHRHLP encoded on this gene is not as clear. At the least, the existence of a separate gene encoding a potentially more potent GHRHLP cannot be ruled out. Certainly, more evidence is needed to establish the GHRHLP encoded on the PACAP precursor gene of nonmammalian vertebrates as a potent simulator of GH release. Evidence of consistent stimulation of pituitary growth hormone, isolation of the gene encoding GHRHLP in carp, and confirmation of the N-terminal sequence of salmon and catfish GHRHLP are needed. Whereas PACAP could be an important regulator of GH release in fish, consistent stimulation of GH release together with the successful suppression of *in vivo* pituitary GH release by the blocking of endogenous PACAP action has not been clearly demonstrated. Many questions concerning the evolution of the PACAP precursor remain unanswered. By characterizing the channel catfish gene and its distribution, we have developed tools to investigate the possible functions of PACAP and GHRHLP in the regulation of channel catfish fish growth and a number of other physiological processes, including the functionality of their expression in fat and skeletal muscle.

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