Association of cocaine- and amphetamine-regulated transcript (CART) messenger RNA level, food intake, and growth in channel catfish

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A B S T R A C T

Cocaine-and Amphetamine-Regulated Transcript (CART) is a potent hypothalamic anorectic peptide in mammals and fish. We hypothesized that increased food intake is associated with changes in expression of CART mRNA within the brain of channel catfish. Objectives were to clone the CART gene, examine tissue CART mRNA distribution, and changes in the amount of CART mRNA in relation to changes in food intake in channel catfish. Our results showed that channel catfish CART was highly similar to those of other fish species, particularly in the biologically active portion of the peptide. Expression of CART mRNA was detected in the brain and testis but not in other somatic tissues. Thirty days of fasting decreased (P<0.05) the amount of CART mRNA within the brain of channel catfish, while refeeding for 15 days restored its amount to a level similar to the fed control. In a separate 7 week feeding study, CART mRNA expression was lower in fish that consumed more food and gained more weight (P<0.05). These results suggest that CART is involved in regulation of food intake in channel catfish, similarly as it has been reported in other fish and mammals.

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1. Introduction

Regulation of food intake and maintenance of nutrient homeostasis in mammals and fish involve regulation of hypothalamic orexigenic and anorectic neurotransmitter expression by various endocrine systems including the somatotropic axis (Renaville et al., 2002), hypothalamic–pituitary–adrenal axis, and leptin system (Kalra et al., 1999; Erlanson-Albertsson, 2005; Stanley et al., 2005). Within the hypothalamus, these neurotransmitters interact with each other to regulate food intake (Kalra et al., 1999; Erlanson-Albertsson, 2003; Ellacott et al., 2004; Erlanson-Albertsson, 2005; Volkoff et al., 2005).

One of the neurotransmitters involved in regulation of food intake is Cocaine-and Amphetamine-Regulated Transcript (CART) (Kristensen et al., 1998; Adams et al., 1999; Kalra et al., 1999; Kuhar et al., 2000; Murphy, 2005). In rodents and fish, CART is a potent anorectic hypothalamic peptide (Kristensen et al., 1998; Volkkof et al., 2000; Stanley et al., 2001; Volkoff et al., 2001). Central administration of CART inhibits food intake in mice (Kristensen et al., 1998), rats (Stanley et al., 2001), and fish (Volkoff et al., 2000; Volkoff et al., 2001). In mice, CART also inhibits neuropeptide-Y (NPY)-induced food intake (Kristensen et al., 1998). Hypothalamic expression of CART in mice and fish is regulated by food intake. Food deprivation decreases expression of CART within the hypothalamus in fish (Volkkof et al., 2001; Volkkof et al., 2005; Volkkof, 2006; Kehoe et al., 2007). Expression of CART decreased within arcuate nucleus of the rat hypothalamus after food deprivation (Kristensen et al., 1998). In addition, leptin, an anorectic hormone produced by adipose tissue, increases hypothalamic expression of CART in mice (Kristensen et al., 1998) and goldfish (Volkkof et al., 2001).

We have previously shown that faster growth in channel catfish was associated with increased insulin-like growth factor (IGF) II mRNA expression in muscles (Peterson et al., 2008). Moreover, catfish with faster growth generally consume more feed than lines of slower growing fish (Silverstein et al., 1999; Peterson et al., 2008), which agrees with the general notion that increased production of IGF is caused by increased food intake (Jones et al., 1995). However, the mechanisms that cause an increase in food intake are unknown. Peterson et al. (2008) showed that hypothalamic expression of NPY mRNA was similar between fast and slow growing fish. This suggests that the increase in feed intake in fast growing fish is not caused by increased amount of NPY. It is possible that expression of other orexigenic peptides such as orexin, melanin-concentrating hormone, and Agouti-related peptide are elevated in fast growing fish. It is also possible that decreased amounts of hypothalamic anorectic peptides such as CART may contribute to increased feed intake as well. Therefore, we hypothesized that expression of the CART gene is negatively correlated with feed intake and growth in channel catfish. The objectives of this study were to clone the channel catfish CART.
gene, characterize tissue distribution of CART mRNA, and determine changes in the amount of CART mRNA in relationship to changes in feed intake of catfish.

2. Materials and methods

2.1. Cloning of channel catfish CART gene and screening of catfish BAC library

The CART transcript sequences from Danio rerio (GenBank Accession Number: NC007136 and NC007130) and Tetraodon nigroviridis (Ensembl Accession number: GSTEMG00031914001) were aligned, and degenerate primers were designed in regions of high DNA sequence similarity (Table 1). Products were amplified from the catfish genomic DNA by PCR. The reaction mixture (10 μl total) consisted of 50 ng DNA, 10 nM forward and reverse primers, 5 nM dNTP mix, 50 nM MgCl2, 1 U Taq polymerase (Promega Corporation, Madison, WI), and 1 μl 10× reaction buffer (Promega). The thermal cycle program used consisted of 35 cycles at 95 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min, with final extension at 72 °C for 10 min. Amplified product was ligated into pCR II-TOPO vector (Invitrogen Corp., Carlsbad, CA), and sequenced using fluorescent dye terminators on an ABI 3100 Prism Genetic Analyzer (Applied Biosystem, Foster City, CA). Cart-specific primers (Table 1) were then designed for PCR-based screening of a BAC library (Quinino et al., 2003). The CART gene sequence was obtained by direct sequencing of a BAC clone using previously established methods (Waldieuser et al., 2003).

2.2. Genotype analysis

A dinucleotide (GT) microsatellite repeat in the 3′ non-translated region was utilized for genotype analysis. Allelic polymorphism was determined in commercial catfish by fluorescent fragment sizing on an ABI 3100 Genetic Analyzer (Waldieuser et al., 2003). Mendelian inheritance of polymorphic alleles in two reference families permitted placement of the CART gene on the catfish genetic map (Waldieuser et al., 2001).

2.3. Tissue distribution of CART mRNA

Mature catfish were maintained in an earthen pond. Tissue samples were collected from reproductively mature male channel catfish (approximately 3 yr old, n = 3). Brain, heart, spleen, kidney, and testis were collected from each fish after euthanasia by overdose (0.3 g/l) with tricainemethane sulfonate (MS–222; Western Chemical Inc., Ferndale, WA). Additionally, ovary samples were collected from three sexually mature female catfish after euthanasia. Tissue samples (approximately 100 mg) were placed in 1 ml of TRI Reagent (Molecular Research Center, Inc. Cincinnati, OH), flash frozen in liquid nitrogen, and stored at −80 °C until RNA isolation.

Total RNA was isolated using TRI Reagent according to the manufacturer’s instruction. After isolation, RNA was treated with a commercially available RQ1 DNase I (DNA-free, Ambion, Inc., Austin, TX) according to the manufacturer’s instruction. The RNA was quantified by measuring absorbance at 260 nm using a NanoDrop ND–1000 spectrophotometer (NanoDrop Technologies, Rockland, DE). The RNA was stored at −80 °C until CDNA synthesis. The CDNA was synthesized by reverse transcribing 1 μg total RNA template using the iScript cDNA synthesis kit (BioRad, Hercules, CA) according to the manufacturer’s instruction. The cDNA was quantified by UV absorbance and stored at −20 °C until use.

2.4. The effect of fasting on CART mRNA expression

Juvenile USDA103 strain catfish were obtained from natural pond spawns. Three families from the USDA103 strain were pooled for use in the growth experiment. The fish were maintained in 76-L tanks throughout the experiment. The aquaria were supplied with flow-through well water (1.0 l/min) and continuous aeration. Water temperature averaged 26.6 ± 0.2 °C and light:dark cycle was set at 14 h: 10 h. Water quality (pH ~ 8.5 and dissolved oxygen levels ~5.0 mg/l) and flow rates were similar between tanks. One hundred catfish (average body weight 73.4 ± 2.2 g) were randomly assigned to either fed control or restricted feeding with five replicates each (10 fish/tank). Fish assigned to the fed control received feed daily, whereas those assigned to the restricted feeding treatment did not receive any feed. On day 30 of the experiment, one-half (n = 50, 25 per treatment) of fish were euthanized for sample collection. The remaining fish in the two treatments received additional 15 days of daily feeding and were euthanized. Fish were fed once daily with a commercial catfish diet (Farmland Industries, Inc., Kansas City, MO) to visual satiety providing all the feed the fish would consume in 15 min. No mortalities were observed throughout the study. Studies were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, United States Department of Agriculture/Agriculture Research Service Catfish Genetics Research Unit.

All fish were anesthetized with 0.1 g/l MS–222 and weighed and sampled on d 0, 30, and 45. On days of sampling, fish were euthanized with an overdose (0.3 g/l) of MS–222 and the brain excised from 15 fish per treatment (three fish/tank). The brain from each fish was placed in 1 ml TRI Reagent, flash frozen in liquid nitrogen, and stored at −80 °C until RNA isolation. Total RNA was isolated, and cDNA was synthesized as described above.

2.5. The effect of fast growth vs. slow growth on CART mRNA in the hypothalamus of catfish

Hypothalami were obtained from fast and slow growing catfish families that was described previously (Peterson et al., 2008). Briefly, fast and slow growing catfish families were grown for 7 wks and multiple tissues were taken from the fastest and the slowest growing families for analysis. For the current study, CART mRNA was examined in the hypothalamus of the fastest growing (3/tank) and slowest growing (3/tank) catfish families (2 families; 15 fish/family).

Table 1

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The hypothalamus was dissected from the brain of each fish and was placed in 1 ml TRI Reagent, flash frozen in liquid nitrogen, and stored at −80 °C until RNA isolation. Total RNA was isolated, and cDNA was synthesized as described above.

2.6. Real-time RT-PCR

The amount of CART mRNA was measured using a real-time RT-PCR protocol described previously (Peterson et al., 2005a; Peterson et al., 2005b). A dual-labeled probe for CART mRNA was designed to target a region at the junction of exons 2 and 3 (Table 1). The amount of CART mRNA was assayed using 400 ng cDNA, 20 μM of forward and reverse primers, and 2.5 μM of dual-labeled probe. The amount of CART mRNA (calculated as copy number) was normalized against the amount of alpha-tubulin mRNA (Peterson et al., 2005a; Peterson et al., 2005b).

2.7. Statistical analysis

Statistical analyses were conducted using the mixed model procedure of the Statistical Analysis System (SAS, Version 9.1 software) followed by a Duncan’s multiple range test. Normalized gene expression data passed Levene’s test for homogeneity of variance. For the fasting study, CART mRNA levels and weight gain

Fig. 1. Channel catfish CART gene sequence. Deduced amino acid sequence is denoted above nucleotide sequence, and immature CART peptide sequence is in bold. Microsatellite sequence in 3′ untranslated region is italic. Primers and probe used for quantitative real-time PCR are denoted by single and double underlines, respectively. The asterisk (*) denotes the stop codon.

Fig. 2. Amino acid sequence alignment of vertebrate CART. Bases identical to the catfish sequence are denoted by a dot, and gaps are denoted by a dash. Vertebrate sequences are the same as in Table 2. A hatched bar indicates the biologically active portion of CART peptide.
were subjected to one-way analyses of variance (ANOVA) with treatment as a fixed effect and tank within treatment as a random effect. For the growth rate study, CART mRNA level was analyzed by one-way ANOVA using family lines designated based on growth rate as described previously (Peterson et al., 2008) as a fixed effect. Tank served as the experimental unit for each variable measured for both analyses. Differences between treatments and family were considered significantly different at *P*<0.05.

3. Results

3.1. Cloning of the channel catfish CART gene

A partial channel catfish CART gene fragment containing exons 2 and 3 was amplified from genomic DNA using primers based on sequence homology between the zebrafish and pufferfish CART genes. Based on sequence similarity to CART in other species, this fragment was subsequently used to design catfish-specific primers for identification of two BAC clones, GY028M17 and GY030A19. Because both clones overlapped on contig 216 of the BAC physical map (Quiniou et al., 2007), only GY030A19 was further sequenced by primer walking (Waldbieser et al., 2003). The resulting channel catfish CART gene consisted of three exons and two introns with an open reading frame (Fig. 2). These sequences of the catfish CART gene fragment containing exons 2 and 3 was amplified from genomic DNA using primers based on sequence homology between the zebrafish and pufferfish CART genes.

Based on sequence similarity to CART in other species, this fragment was subsequently used to design catfish-specific primers for identification of two BAC clones, GY028M17 and GY030A19. Because both clones overlapped on contig 216 of the BAC physical map (Quiniou et al., 2007), only GY030A19 was further sequenced by primer walking (Waldbieser et al., 2003). The resulting channel catfish CART gene consisted of three exons and two introns with an open reading frame of 357 bp (Fig. 1). Intron/exon junctions were conserved with other vertebrates.

Full-length predicted catfish CART peptide was aligned against CART from other fish species, Xeopus tropicalis, and mammals (Fig. 2). The sequence of the catfish CART peptide was more similar to CART peptide in other fish species than to mammalian CART peptide sequences (Table 2). Differences in peptide sequence were found in the N-terminus of the CART peptide preceding the biologically active portion (Fig. 2). The level of similarity for all species was higher at the biologically active portion of CART peptide (Fig. 2).

3.2. Genotype analysis

Genotype analysis of 47 commercial catfish revealed 7 alleles at 168 bp (14% frequency), 170 bp (14%), 172 bp (14%), 174 bp (22%), 176 bp (28%), 178 bp (6%), and 182 bp (2%). Average level of heterozygosity in the population was 81%. Allelic polymorphism in two reference families permitted localization of the CART gene, thus BAC clone 30A19, on linkage group U1 (Waldbieser et al., 2001). Placement of CART/GY030A19 on this linkage group was supported by complete linkage (no recombination) with BAC clone GY098E07, and both clones were located on the same contig (contig 2016) of the physical map.

### Table 2

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### Table Notes

GenBank accession numbers for CART peptides: Zebrafish II—NP_001017570; Common carp—AL410908; Zebrafish I—XP_685429; Common carp—AL410907; Atlantic cod—AAZ294720; Lake trout—ABJ20509; Xeopus tropicalis—NP_001072571; Mouse—NP_001074962; Rat—NP_058806; Human—NP_004282.

3.3. Expression of CART mRNA

Tissue distribution of CART mRNA in mature male channel catfish was examined using real-time RT-PCR. Levels of CART mRNA were highest in the brain followed by testis of catfish but not in heart, spleen, ovary, or kidney (Fig. 3).

The initial average weight of all fish for the fasting study was 73.4±2.2 g. By day 30, fed and restricted fish weighed 105.5±2.2 g and 65.2±3.8 g, respectively. After 15 days of refeeding, the fed group weighed 133.5±7.9 g, while the restricted group weighed 83.4±4.1 g. The amount of CART mRNA in the brain was 2.5 fold greater in the catfish fed daily compared to restricted catfish on day 30 (*P*<0.05, Fig. 4A). After 15 days of refeeding, levels of CART mRNA in the brain of fed-restricted catfish increased to the level observed in continually fed catfish (Fig. 4B).

Growth characteristics and feed consumption between fast and slow growing families of catfish were described elsewhere (Peterson et al., 2008). The fish selected for faster growth were, on average, 80.7 g heavier and consumed 97.1 g more feed compared to those with slow growth at the end of the seven-week trial (Peterson et al., 2008). For this study, we compared the amount of CART mRNA in the
hypothalamic region of fish with the fastest and slowest growth rate. Within the hypothalamus, CART mRNA levels were lower in the families of catfish with fast growth rate compared to those with slow growth rate \((P<0.05, \text{Fig. 5})\).

4. Discussion

In this study, we showed that CART genomic DNA and predicted CART amino acid sequences in channel catfish are highly similar to sequences found in other fish species and mammals. The nucleotide sequence of CART mRNA and amino acid sequence of CART peptide have been described in many mammalian species including humans (Douglass et al., 1996), rodents (Douglass et al., 1995; Adams et al., 1999; Kuhr et al., 2000), and cattle (Kobayashi et al., 2004), as well as in some fish species (Volkoff et al., 2001; Kehoe et al., 2007). The amino acid sequence of CART peptide is highly similar among different mammalian (Adams et al., 1999; Kuhr et al., 2000) and fish species (Volkoff et al., 2001; Kehoe et al., 2007). The sequence conservation is especially high in the biologically active portion of the CART peptide, which is commonly referred to as CART (55–102). Results of our studies showed that catfish CART nucleotide and amino acid sequences were highly similar to those of other fish species and mammalian CART, particularly in the biologically active portion. The key feature that is crucial for biological activity of CART peptide is formation of the fold-structure through cystine knots (Ludvigsen et al., 2001). This fold within the biologically active portion of CART peptide is formed through three disulfide bridges and found in many small peptides consisting of 25 to 50 amino acids (Ludvigsen et al., 2001), and reduction of these disulfide bridges results in the loss of biological activity (Kristensen et al., 1998). All cystine residues within the biologically active region of the peptide are conserved among all CART peptides, suggesting that, regardless of the species, formation of the fold is crucial for biological function of CART peptide.

Two CART transcripts are found in goldfish (Volkoff et al., 2001). These transcripts are highly similar to each other and have similar tissue distributions but different response to changes in food intake and leptin treatment (Volkoff et al., 2001). In our study, CART mRNA expression was detected in the brain and testis but not in kidney, heart, and spleen, which is similar to the tissue distribution of CART mRNA in Atlantic Cod (Kehoe et al., 2007) and goldfish CART I and II mRNA (Volkoff et al., 2001). Based on the amino acid sequence similarity, catfish CART peptide is more similar to goldfish CART II peptide. However, changes in CART mRNA expression in response to feed intake in our study strongly suggest that the transcript we cloned is analogous to goldfish CART I peptide. This discrepancy may be the result of deletion of a duplicated gene during evolution (Postlethwait, 2007). During evolution, the common ancestor of fish may have had multiple copies of CART gene because of the whole genome duplication (Delah et al., 2006; Postlethwait, 2007). As fish species diverged, some genes were lost in some lineages of fish species but preserved in others (Postlethwait, 2007). It is possible that catfish may have retained only one copy of CART gene as it diverged during evolution, and the gene that was retained was homologous to the goldfish CART II gene. Whether the second CART transcript exists in channel catfish remains unclear. Our repeated attempts to clone the second CART transcript were unsuccessful.

In our study, we showed that CART mRNA is expressed in the brain and testis of channel catfish. Gonadal expression of CART mRNA was found in goldfish (Volkoff et al., 2001) and Atlantic cod (Kehoe et al., 2007). In rodents, expression of CART mRNA and peptide is restricted to the central nervous system and peripheral nerves that innervate somatic tissues (Dun et al., 2000; Murphy et al., 2000; Ekblad et al., 2003). Other than fish, the only species reported to express CART mRNA and peptide in reproductive tissues is cattle, where CART mRNA and peptide are expressed in the oocyte and granulosa cells within the ovary (Kobayashi et al., 2004). In cattle, expression of CART mRNA is negatively associated with the follicle’s ability to produce estradiol (Kobayashi et al., 2004) and CART (55–102) inhibits production of estradiol by cultured granulosa cells (Kobayashi et al., 2004; Sen et al., 2007). Expression of CART mRNA in fish gonads suggests that CART peptide may play a role in reproduction in fish. Compared to other studies (Volkoff et al., 2001; Kehoe et al., 2007), we did not detect CART mRNA expression in the ovary of channel catfish. This may suggest that CART peptide plays the role in male reproduction but not in female reproduction in channel catfish. The role of CART peptide in regulation of fish gonadal function remains unclear and needs to be investigated further.

In addition to gonads, CART mRNA is expressed in the gills and kidney of goldfish (Volkoff et al., 2001). However, expression of CART mRNA was not detected in these tissues in Atlantic cod (Kehoe et al., 2007). Our study also showed that CART mRNA was not expressed in the kidney of the channel catfish. Differences in CART mRNA expression among these fish species may be associated with differences in physiological adaptability of these fish including salinity tolerance. Along with its role in regulation of gonadal function, a role for CART peptide in the regulation of fish kidney function is unknown and warrants further investigation.

The results of our study demonstrated that expression of CART mRNA was inversely associated with increased food intake, which was associated with increased growth rate. One possibility for increased food intake in faster growing fish is that reduced CART in fast growing fish may have reduced inhibition of NPY action. Central infusion of CART inhibited feed intake induced by NPY in mice (Kristensen et al., 1998) and rats (Vrang et al., 1999b), suggesting that CART acts as an antagonist of NPY action. However, in these studies, antagonistic relationship between CART peptide and NPY has been demonstrated through co-infusion of CART peptide and NPY. Whether CART peptide antagonizes NPY action under physiological condition is unclear.

Reduced CART mRNA in faster growing catfish may be the result of reduced number of neurons expressing CART mRNA within the hypothalamus. In rats, CART mRNA and peptide expression was detected in a different population of neurons from those expressing NPY (Vrang et al., 1999a; Sainsbury et al., 2002). However, the possibility that reduced CART mRNA expression is the result of decreased promoter activities cannot be ignored. Whether reduced expression of CART mRNA in fast growing catfish is the result of reduced CART gene promoter activity or reduced numbers of neurons expressing CART mRNA is unclear and needs to be examined further.

Hypothalamic expression of CART mRNA and peptide in mammals is regulated by many hormones including leptin (Kristensen et al., 1998; Stanley et al., 2005) and glucocorticoid (Balkan et al., 2001). In mice, adrenalectomy decreases hypothalamic CART peptide expression (Balkan et al., 2001). Adrenalectomy-induced decrease in CART peptide expression is restored to the level observed in intact animals by glucocorticoid replacement. In fast growing catfish, blood concentrations of cortisol after stress challenge were lower than those of slow growing counterparts (Peterson et al., 2008), and results of our
current study showed that the hypothalamic expression of CART mRNA was low in fast growing fish. It is possible that lower concentrations of cortisol in fast growing fish causes lower expression of CART mRNA within the hypothalamus, and this in turn increases food intake. However, basal level of cortisol was not measured in the previous study (Peterson et al., 2008), which makes it impossible to estimate the exact relationship between hypothalamic CART mRNA expression and blood level of cortisol in fast growing catfish. The role of glucocorticoids in CART mRNA expression in fish needs to be examined in detail. In goldfish, leptin increased expression of CART mRNA (Volkoff et al., 2001), but the role of leptin in the regulation of CART mRNA expression in catfish is unknown and requires further investigation.

Decreased expression of CART mRNA in fast growing fish may suggest that CART gene can be used as a genetic marker for selecting fish for faster growth. Polymorphisms of the CART gene have been investigated in humans (del Giudice et al., 2001; Yamada et al., 2002; Jung et al., 2004; Guérardel et al., 2005; del Giudice et al., 2006) and cat (Sherman et al., 2008). A polymorphism in the putative promoter region of CART gene and a leucine-phenylalanine substitution within the N-terminal region of CART peptide were associated with obesity in humans (del Giudice et al., 2001; Yamada et al., 2002; Guérardel et al., 2005). In contrast, CART gene polymorphism identified in beef cattle were not associated with growth and carcass traits (Sherman et al., 2008). The polymorphic microsatellite sequence in the 3′ untranslated region of catfish CART will permit investigation into the possible association of CART locus polymorphisms with growth phenotypes.

In summary, increased growth rate in channel catfish is an economically important trait. Faster growth rate in channel catfish is generally associated with increased food consumption. However, the mechanisms that cause an increase in food intake in fish with faster growth are unclear. We showed that CART in channel catfish was highly similar to that of other fish species and mammals. We also showed that CART mRNA was expressed in the brain and testes of channel catfish similar to the expression pattern of CART mRNA in other fish species. Furthermore, expression of CART mRNA in the brain of channel catfish was regulated by food intake and inversely associated with growth rate of channel catfish. Further studies are required to identify polymorphisms within the CART gene to be used as a genetic marker.

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References


