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Characterization of two channel catfish, *Ictalurus punctatus*, glucocorticoid receptors and expression following an acute stressor

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ABSTRACT

Two channel catfish glucocorticoid receptor genes, ipGR1 (NR3C1_1) and ipGR2 (NR3C1_2) were partially characterized. Identification and analysis of the genomic organization of two channel catfish glucocorticoid (GC) receptors (GRs) revealed differences in the lengths of exons 1 and 2 and the addition of an extra 27-bp exon inserted after exon 2 in the GR1 gene, yielding a 9-aa insert in the receptor protein. Sequence of the 9-aa insert in ipGR1 (WRARQNTHG) is unique compared to other teleost fish GRs. Amino acid sequence alignment of the two channel catfish GRs, revealed 55% sequence identity between them, with a high degree of sequence conservation (82%) in the DNA binding and ligand binding domains. Real-time PCR indicated that ipGR1 and ipGR2 were expressed in all tissues evaluated. Channel catfish GR1 was predominantly expressed in the gills, nearly 25-fold higher than in the liver. GR1 expression was higher than GR2 expression in gills, intestine, head kidney and heart ($P < 0.05$). Channel catfish hepatic GR1 mRNA expression was significantly ($P < 0.05$) increased from pre-stress expression 30 min following removal of the acute stressor. After 30 min of stress and during the 2 h recovery period, ipGR1 mRNA expression was higher relative to ipGR2 expression. Hepatic ipGR2 expression was not affected ($P > 0.05$) by the acute stress event. The present study adds to the growing body of information on GR evolution and function and further demonstrates the unique regulation of the GC/GR system in teleost fish.

1. Introduction

Glucocorticoids (GCs) are well conserved throughout vertebrate evolution and are involved in numerous physiological processes, not the least of which is the stress response. In teleost fish, cortisol is accepted as the primary glucocorticoid produced in response to stressful stimuli. Cortisol acts upon the cells by way of binding to nuclear glucocorticoid receptors (GR). In this way, the GC/GR system mediates the response to stress through modulation of metabolic, cardiovascular and immune function (Sapolsky et al., 2000; Stratakis and Chrousos, 1995).

In 2003, two different genes encoding GRs were observed in two teleost fishes, the rainbow trout (*Oncorhynchus mykiss*; Bury et al., 2003) and Burtons' mouthbrooder (*Haplochromis burtoni*; Greenwood et al., 2003). Since then, several species of teleost fish have been observed to possess two different GR genes (Li et al., 2012; Stolte et al., 2006). Interestingly, only one zebrafish (*Danio rerio*) GR has been identified to date (Alsop and Vijayan, 2008; Schaaf et al., 2009; Stolte et al., 2006). Within those species having two GR genes, a high degree of amino acid sequence identity is reported (Li et al., 2012; Stolte et al., 2006), with the highest sequence identity being in the C-terminal portion of the receptor (≥ 80%; Stolte et al., 2006).

Nomenclature of the two teleost GRs has not always been consistent (O'Connor et al., 2013). It is now accepted that GR1, also referred to as Nuclear Receptor Subfamily 3 Group C Member 1_1 (NR3C1_1), has an additional exon resulting in a nine amino acid insert in the DNA binding domain, and GR2 (NR3C1_2) does not (Maruska and Fernald, 2010; O'Connor et al., 2013). This insert, located between two zinc fingers, appears to be unique to teleost fishes and sets it apart from other vertebrates (Takeo et al., 1996). For many of the species studied to date, a WRARQNTDG insert is present; however, a WRARQNTVC insert has been reported for tetraodon (*T. nigroviridis*; Stolte et al., 2006).

While both GR genes are co-expressed in a variety of tissue types (Bury et al., 2003), the functional roles for the two teleost GRs appear to differ and have yet to be defined. Functional differences are supported by differential expression across tissue types (Kim et al., 2011; Li et al., 2012; Stolte et al., 2006) and different affinities for glucorticoids (Bury et al., 2003; Greenwood et al., 2003). Transactivation studies in rainbow trout indicate differences in affinity for cortisol between the two GRs, with lower concentrations of cortisol required to induce transactivation in GR2 (Bury et al., 2003). As such, it is proposed that

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Table 1
Nucleotide sequences of PCR primers and probes specific to channel catfish (*Ictalurus punctatus*) used to assay gene expression by real-time PCR.

Gene	Primer	Sequence (5′ − 3′)	Amplicon length (bp)	PCR Efficiency (%)
GR1	Sense	ACCTCAATGTTCCTGTCA	101	99.1
	Anti-sense	TAGCCTCTCCTCTCACTT		
GR2	Sense	TATCACCATCAATTTACCATCC	75	99.7
	Anti-sense	AAGTCCTTCCCAACCATT		
18S[a]	Sense	GGATTGACAGATTGATAGC	101	97.5
	Anti-sense	TCGTTCGTTATCGGAATT		

[a] 18S ribosomal RNA (GenBank Accession #AF021880)

cortisol preferentially binds GR2 when stress levels are low and binds both GR1 and GR2 during periods of intense stress (Bury et al., 2003). Indeed, increased GR1 and GR2 gene expression have been reported following exposure to an acute stressor in rainbow trout (López-Patiño et al., 2014). The separation of function is further suggested in response to different exogenous ligands and physiological states (Bury et al., 2003; Bury and Sturm, 2007; Pavlidis et al., 2011; Prunet et al., 2006).

The present study was conducted to identify and partially characterize channel catfish glucocorticoid receptors. The hypothesis was that two genes encoding separate channel catfish GRs exist having differential tissue expression patterns and elevated hepatic gene expression following an acute stressor. As such, the objectives were to isolate DNA encoding channel catfish glucocorticoid receptors and to examine tissue distribution and the effects of acute stress on hepatic GRs mRNA expression.

2. Materials and methods

2.1. Channel catfish

All channel catfish described in the following experiments were of the NWAC103 commercial strain maintained under standard conditions at the USDA-ARS Warmwater Aquaculture Research Unit, Stoneville, MS following accepted standards of animal care and use approved by the Institutional Animal Care and Use Committee (IACUC) according to USDA-ARS policies and procedures.

2.2. ipGR1 and ipGR2 genes

We used the two known receptor sequences from *Onchorhynchus mykiss* (GR1, CAA90937, Ducouret et al., 1995; GR2, AAR87479, Bury et al., 2003) to screen ESTs from *Ictalurus punctatus* (Wang et al., 2010, https://naldc.nal.usda.gov/download/40309/PDF) using tblastn (http://www.ncbi.nlm.nih.gov; Altschul et al., 1990). The blast identified three DNA sequences which were used to screen a third generation long read genome assembly of the CCBL1 fish (Quiniou, unpublished; Quiniou et al., 2003; Quiniou et al., 2007). Two genome contigs containing the ipGR genes were identified. Primers were designed for the start and the stop codon. PCR products obtained from pooled cDNA of three juvenile channel catfish were used to deduce the intron-exon structure from the genomic sequence.

2.3. Tissue expression

Total RNA of the gills, intestine, head kidney, trunk kidney, heart, spleen, muscle, and liver, was extracted separately from three ~250 g fish after euthanizing them in 300 mg/L aerated bath of MS-222 (TRICAINE-S®; Western Chemical incorporated®, Ferndale, Washington) buffered with sodium bicarbonate. Tissues were flash frozen in liquid nitrogen then stored at − 80 °C until RNA isolation, cDNA conversion and real-time qPCR.

2.4. ipGR mRNA expression and plasma cortisol levels following acute stress

The effect of an acute stressor on catfish glucocorticoid receptor expression in the liver was evaluated relative to a cortisol stress response. Eighteen 76-L aquaria, as part of a recirculating aquaculture system, were each stocked with 8 fish each weighing approximately 120 g. The fish were acclimated to their new environment for 2-wk. while being fed a commercial catfish feed to satiety once daily in the morning. The fish were maintained under a 12 h light and 12 h dark photoperiod. Temperature was maintained at 25 °C, and other water quality parameters (ammonia, nitrite, pH, alkalinity, and hardness) were within optimal catfish rearing parameters. At time zero, blood and liver samples were collected from three fish per tank from three separate tanks. For blood collection, fish were sedated in 100 mg/L aerated bath of buffered MS-222. All blood samples were collected within 5 min of sedation ventrally from the caudal vasculature using 1 mL heparinized syringes, dispensed into 1.5 mL centrifuge tubes, and stored on ice until centrifuged (3000*g*, 10 min, 4 °C) to separate plasma. Plasma

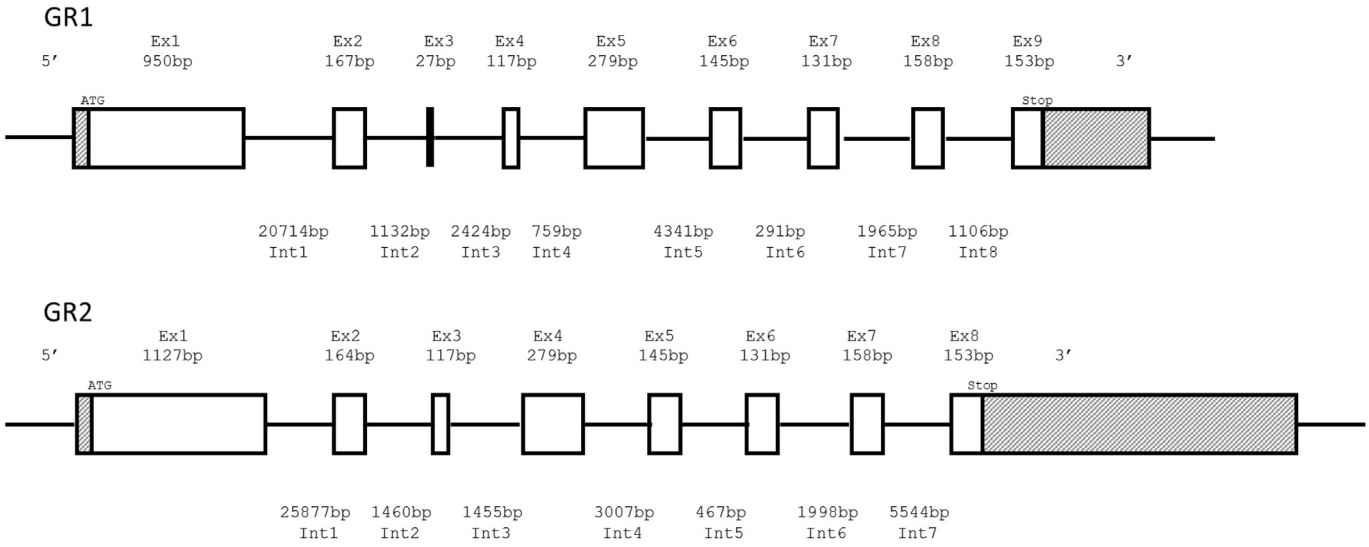

Fig. 1. Genomic organization of the channel catfish (*Ictalurus punctatus*) GR1 and GR2 genes. The boxes represent coding exons with exon (ex) length, in base pairs (bp), indicated above. Intron (Int) positions are indicated by straight lines with length indicated below.