General and Comparative Endocrinology 168 (2010) 377–387

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

The serotonin subtype 1A receptor regulates cortisol secretion in the Gulf toadfish, *Opsanus beta*

Lea R. Medeiros *, Edward M. Mager, Martin Grosell, M. Danielle McDonald

Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL 33149-1098, USA

ARTICLE INFO

Article history:
Received 28 September 2009
Revised 26 February 2010
Accepted 12 May 2010
Available online 19 May 2010

Keywords:
Hypothalamic–pituitary–interrenal axis
Hypothalamic–pituitary–adrenal axis
5-Hydroxytryptamine
5-HT_{1A}
8-OH-DPAT
HTR1A

ABSTRACT

It is well established that serotonin (5-HT; 5-hydroxytryptamine) plays a role in mammalian regulation of the hypothalamic–pituitary–adrenal (HPA) axis via the 5-HT receptor subtype 1A (5-HT_{1A}). To date, there has not been a comprehensive investigation of the molecular, pharmacological and physiological aspects of the 5-HT_{1A} receptor and its role in the activation of the hypothalamic–pituitary–interrenal (HPI) axis in teleost fish. The 5-HT_{1A} receptor of the Gulf toadfish (*Opsanus beta*) was cloned and sequenced, showing 67.5% amino acid similarity to the human homologue. The 5-HT_{1A} receptor was distributed throughout the brain, with the whole brain containing significantly higher levels of 5-HT_{1A} mRNA compared to all other tissues and the midbrain/diencephalon region containing significantly higher levels of transcript than any other brain region. Substantial levels of transcript were also found in the pituitary, while very low levels were in the kidney that contains the interrenal cells. *Xenopus* oocytes injected with toadfish 5-HT_{1A} receptor cRNA displayed significantly higher binding of [^{3}H]5-HT that was abolished by the mammalian 5-HT_{1A} receptor agonist, 8-OH-DPAT, indicating a conserved binding site of the toadfish 5-HT_{1A} receptor and a high specificity for the agonist. Supporting this, binding of [^{3}H]5-HT was not affected by the mammalian 5-HT_{1B} receptor agonist, 5-nonyloxytryptamine, the 5-HT_7 receptor antagonist, SB269970, or the 5-HT_2 receptor agonist, α-methylserotonin. Confirming these molecular and pharmacological findings, intravenous injection of 8-OH-DPAT stimulated the HPI axis to cause a 2-fold increase in circulating levels of cortisol. The present study of the 5-HT_{1A} receptor in a single teleost species illustrates the high conservation of this 5-HT receptor amongst vertebrates.

1. Introduction

The monoamine neurotransmitter, serotonin (5-HT; 5-hydroxytryptamine), affects many systems in the body, mediating the physiological processes that regulate anger, mood, sleep, appetite, and even learning (Fuller, 1990; Uphouse, 1997; Barnes and Sharp, 1999). Serotonergic activation of the hypothalamic–pituitary–adrenal (HPA) axis, an important component of the mammalian stress response, results in an increase in levels of corticotropin-releasing hormone (CRH) from the hypothalamus and stimulates the secretion of adrenocorticotropic-releasing hormone (ACTH) from the pituitary, which then activates the secretion of glucocorticoids, such as cortisol, from the adrenal gland (Calogero et al., 1990). In addition to the indirect stimulation of gluococorticoid secretion via CRH and ACTH (Calogero et al., 1990), 5-HT also activates the release of cortisol directly by acting on the adrenal gland; however, this increase is thought to be independent of the HPA axis with 5-HT acting as a local paracrine factor (Alper, 1990). Reciprocally, it has been shown that brain 5-HT synthesis and turnover is greatly impacted by stressful events (Bliss et al., 1972), which, in turn, will feedback on the HPA axis. Thus, brain 5-HT plays an integral role in a complex neuroendocrine loop serving to maintain homeostasis and promote acclimation during physiological and/or environmental challenges. It was not until the last few decades that the connection between the HPA axis, 5-HT and the 5-HT receptor subtype 1A (5-HT_{1A}) was made (Lorens and Van de Kar, 1987; Fuller, 1992).

Within the CNS, 5-HT binds to several different types of 5-HT receptors (HTRs), located both pre- and postsynaptically. To date, seven families of HTRs have been identified (5-HT_1–5-HT_7), with a total of 14 subtypes having been characterized (Hoyer et al., 2002). All families are G protein-coupled receptors, with the exception of 5-HT_3, which is a ligand-gated ion channel. The 5-HT_1 family inhibits the formation of cyclic AMP (cAMP), whereas 5-$HT_{4,6,7}$ families stimulate the production of cAMP. The 5-HT_2 family communicates via the second messenger phospholipase C, while the mechanism whereby the 5-HT_5 family acts remains a little obscure (see Hoyer et al., 2002). While all of these receptors have a specific and important purpose, the 5-HT_{1A} receptor is one of the most

* Corresponding author. Address: Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL 33149-1098, USA. Fax: +1 305 421 4600.
E-mail address: lmedeiros@rsmas.miami.edu (L.R. Medeiros).

0016-6480/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2010.05.004

abundant subtypes of 5-HT receptors in the mammalian brain (Albert et al., 1990) and, being the first HTR cloned (Kobilka et al., 1987), has been the most studied. The G-protein-coupled receptor employs a G_i/G_0 transduction system that primarily decreases adenylate cyclase formation and/or increases K^+ conductance (Barnes and Sharp, 1999). These mechanisms are postulated to inhibit firing of the postsynaptic cell, and it has been observed that 5-HT, mediated by the 5-HT_{1A} receptor, exerts a predominantly inhibitory effect on neuron firing rate in many areas of the brain (Clark et al., 1987; Araneda and Andrade, 1991; Kow et al., 1992). There are two populations of 5-HT_{1A} receptors: somatodendritic autoreceptors located mainly in the rostral raphe nucleus region of the medulla and postsynaptic receptors, located in the projection areas of the raphe nuclei, such as the telencephalon and hypothalamic/pituitary region. The somatodendritic 5-HT_{1A} autoreceptors regulate the release of 5-HT by the presynaptic neuron, ultimately affecting the stimulation of 5-HT_{1A} receptors on the postsynaptic cell and thus the activation of the 5-HT_{1A} neuron projection areas in the brain (Lanfumey and Hamon, 2000; Albert and Lemonde, 2004). Agonists of mammalian 5-HT_{1A} receptors, such as 8-hydroxy-2-(di-n-propylamino) tetraline (8-OH-DPAT), have been found to elevate plasma corticosteroid levels in mammals (Dinan, 1996). Depending on the location of receptor, this endpoint can be achieved in several different ways, *i.e.*, when applied directly to the rat hypothalamus, 8-OH-DPAT stimulates the release of CRH, though when applied directly to the pituitary, ACTH is released (Calogero et al., 1990). There is also evidence for the presence of a negative feedback loop whereby increased circulating levels of cortisol inhibit 5-HT_{1A} receptor activity (Zhong and Ciaranello, 1995) in addition to directly inhibiting the release of ACTH and CRH (Canny et al., 1989; Delbende et al., 1992).

Work done on teleost fish investigating the interaction between 5-HT and the teleost homologue of the HPA axis, the hypothalamic–pituitary–interrenal (HPI) axis, has mostly focused on how these systems work together during social interactions (Winberg and Nilsson, 1993; Larson et al., 2003; Clotfelter et al., 2007). Social stressors, such as subordination, elevate brain 5-HT activity as indicated by brain 5-hydroxyindoleacetic acid (5-HIAA; the major 5-HT metabolite) concentrations and 5-HIAA:5-HT ratios (Winberg and Nilsson, 1993). At the same time, socially subordinate fish display elevated plasma cortisol levels (Ejike and Schreck, 1980) and increased interrenal cell size, suggesting a chronic activation of the HPI axis (Noakes and Leatherland, 1977). Work done on the brain of Arctic Charr (*Salvelinus alpinus*) suggested that, like in mammals, there are a multitude of 5-HT receptors in teleost fish to carry out the actions of 5-HT (Winberg and Nilsson, 1996). Furthermore, similar to its action in mammals, the 5-HT_{1A} receptor agonist, 8-OH-DPAT, has been shown to stimulate the release of cortisol in the rainbow trout (*Oncorhynchus mykiss*, Winberg et al., 1997), with a binding affinity (K_i) similar to what is measured in mammals (Zifa and Fillion, 1992).

While molecular evidence for the presence of 5-HT_{1A} receptors in fish (Yamaguchi and Brenner, 1997; Wang and Tsai, 2006; Airhart et al., 2007) exist and studies have provided pharmacological evidence for a role for 5-HT_{1A} in teleosts (Winberg et al., 1997; Clotfelter et al., 2007; Smith and Combs, 2008) no single study has reported molecular, pharmacological and physiological characterization of the teleost 5-HT_{1A} receptor. Thus, previous to this study there has not been an extensive investigation that has determined conclusively that the 5-HT_{1A} receptor in fish is pharmacologically similar to that found in mammals and that it plays a role in the activation of the HPI axis. Furthermore, the distribution of 5-HT_{1A} transcript within the HPI axis has not been investigated in teleost fish, although it can be hypothesized based on mammalian studies that most of the 5-HT_{1A} transcript would be found in the pituitary gland (Chalmers and Watson, 1991; Lopez et al., 1998; Drevets et al., 2007; Kumar and Mann, 2007). Preliminary pharmacological evidence on the Gulf toadfish, *Opsanus beta*, a benthic, marine teleost fish suggested that a 5-HT_{1A}-like receptor may not be involved in the regulation of the HPI axis, as fish injected with 8-OH-DPAT failed to show an increase in plasma cortisol levels that exceeded the increase measured in saline-injected controls (McDonald and Walsh, 2004). However, it was speculated in that study that the high circulating cortisol levels experienced typically by cannulated toadfish ($200–400 \times 10^{-9}$ g ml^{-1}) may have reduced the sensitivity of the 5-HT_{1A} receptor, making it difficult to further stimulate the HPI axis in these fish. We hypothesize now that the 5-HT_{1A} receptor is indeed present in toadfish, but perhaps a higher dose of 8-OH-DPAT is necessary to overcome this potential desensitization. Thus, the objectives of this study were to examine the existence of the 5-HT_{1A} receptor in the Gulf toadfish, and specifically determine its distribution within the HPI axis, and at the same time provide functional evidence linking the toadfish 5-HT_{1A} receptor to the activation of the HPI axis. To do this, we first determined the full length nucleotide sequence of the toadfish 5-HT_{1A} receptor, we then evaluated the distribution of 5-HT_{1A} receptor transcript levels within different tissues, including those of the HPI axis. Lastly, we set out to provide pharmacological and functional evidence linking the 5-HT_{1A} receptor to HPI axis regulation.

2. Materials and methods

2.1. Experimental animals

Gulf toadfish (*O. beta*) were captured by roller trawl used by commercial shrimpers in Biscayne Bay, Florida in the summer of 2007, after which they were then immediately transferred to the laboratory where they were held for up to one month. Fish were treated with a dose of malachite green (final concentration 0.05 mg l^{-1}) in formalin (15 mg l^{-1}) (AquaVet) on the day of transfer to the laboratory in order to prevent infection by the ciliate *Cryptocaryon irritans* (Stoskopf, 1993). The fish were kept in 50-l glass aquaria with flowing, aerated seawater at a temperature of 24–29 °C and were fed weekly with squid.

2.2. Experimental protocol

2.2.1. RNA extractions

Tissues were excised from toadfish that had been held for one week in uncrowded conditions in outdoor 6000 l tanks seeded with the seagrass, *Thalassia testudinum* which emulates the natural environment of Gulf toadfish (Serafy et al., 1997). Toadfish were over-anesthetized with MS222 (3 g l^{-1}) and tissues were collected terminally, frozen immediately in liquid N_2 and stored at −80 °C. Total RNA was isolated from tissues following the protocol provided with the Trizol reagent (Invitrogen). Total RNA was subsequently treated with DNAse I to remove potential residual genomic DNA according to the protocol provided with the Turbo-DNA-free kit (Ambion).

2.2.2. PCR and 5′ and 3′ rapid amplification of cDNA ends (RACE)

Toadfish poly(A) RNA was extracted from the total RNA using the PolyATract mRNA Isolation System III (Promega) for use in RACE reactions. cDNA was synthesized with Oligo(dT) primers from 1 μg of DNase I-treated total RNA according to the protocol provided with the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). An initial fragment of 779 bp was cloned from toadfish brain cDNA using degenerate primers (Table 1) designed using alignments of other teleosts. Reactions were performed using GoTaq DNA polymerase (Promega) and the following cycling conditions: 94 °C for 30 s, a temperature gradient of 50–70 °C for