General and Comparative Endocrinology 188 (2013) 54–59

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

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

Review

The pituitary–interrenal axis of fish: A review focusing on the lamprey and flounder

Akiyoshi Takahashi *, Yuki Kobayashi [1], Kanta Mizusawa

School of Marine Biosciences, Kitasato University, Sagamihara, Kanagawa 252-0373, Japan

ARTICLE INFO

Article history:
Available online 21 March 2013

Keywords:
Adrenocorticotropic hormone (ACTH)
Corticosteroid
Melanocortin receptor (MC receptor)
Melanocyte-stimulating hormone (MSH)
Pituitary–interrenal axis

ABSTRACT

In fish, the pituitary–interrenal axis is associated with stress response and a variety of biological processes such as metabolism, immune response, and growth. The major hormones involved in this axis are adrenocorticotropic hormone (ACTH), released from the pars distalis of the pituitary gland, and corticosteroid, released from the interrenal gland that is embedded in the head kidney in ray-finned fish. The ACTH signal, by which corticosteroid release is stimulated, is transmitted by melanocortin (MC) receptors on interrenal cells. Thus, the interaction of ACTH and MC receptors is the pivotal event for interrenal cells. Knowledge about ACTH and MC receptors in lamprey, cartilaginous fish, and ray-finned fish is available, and it suggests the pituitary–interrenal axis was established early in vertebrate evolution. Moreover, the data, including our recent results from flounders and lampreys, provide interesting features about ligand–receptor interactions. This review focuses on the characteristics of ACTH, the proopiomelanocortin gene encoding ACTH, and the MC receptor, and it is mostly based on the results of our investigations.

1. Introduction

In fish, the pituitary–interrenal axis is responsible for a variety of biological processes such as metabolism of carbohydrates, amino acids and free fatty acids; mineral balance; immune function; and growth (Wendellar Bonga, 1997). These processes are regulated by cortisol release from interrenal cells. Although many hormonal substances are associated with cortisol release, adrenocorticotropic hormone (ACTH) that is released from pituitary grand is a key stimulant. Thus, ACTH and the corresponding receptors are of utmost importance in the repertoire of functions of the pituitary–interrenal axis.

The proopiomelanocortin (POMC) gene encodes ACTH, along with melanocyte-stimulating hormone (MSH) and β-endorphin (β-END) (Takahashi and Kawauchi, 2006a,b). POMC typically consists of three regions: N-terminal N-POMC, middle ACTH, and C-terminal β-lipotropin regions. As with the tetrapod POMC, each region in lobe-finned fish possesses an MSH sequence: γ-MSH in the N-POMC region, α-MSH as an N-terminal sequence of ACTH, and β-MSH in β-lipotropin. There are three forms of α-MSH, distinguished by the presence or absence of acetyl groups at the N-terminus: α-MSH and diacetyl-α-MSH (Di-Ac-α-MSH) possess one and two acetyl groups, respectively, and desacetyl-α-MSH (Des-Ac-α-MSH) contains no acetyl groups. ACTH and MSHs are collectively termed melanocortins (MCs) because of the presence of a common His-Phe-Arg-Trp sequence. An opioid peptide, β-END, is located at the C-terminal end of β-lipotropin. POMC from the primitive group of ray-finned fish such as gar and sturgeon has a structure similar to those of tetrapods; however, the number of MSH sequences varies in other fish POMCs (Takahashi and Kawauchi, 2006a,b). In teleosts, which is a derived group of ray-finned fish, γ-MSH does not exist in the N-POMC region. In the case of cartilaginous fish, the fourth δ-MSH is present in the N-terminal region of β-lipotropin. Lampreys are intriguing because two different precursor proteins that contain ACTH and MSH separately are independently present. In spite of variation in the structure of POMC with respect to the number of MSH sequences, ACTH is consistently present in all vertebrate species.

Receptors for ACTH and MSH, termed MC receptors, are G protein-coupled receptors (GPCRs) with seven transmembrane domains (Cone, 2000, 2006). The presence of five subtypes, MC1R–MC5R, has been shown in mammals and birds (Cone, 2000; Takeuchi et al., 2003). In addition, fish such as goldfish (Kobayashi et al., 2011a; Cerdá-Reverter et al., 2003a,b) and zebrafish (Logan et al., 2003) have been shown to possess the five subtypes, whereas MC5R is further subdivided into two molecular types in zebrafish. In the case of the medaka, stickleback, and puffer fish, the repertoire of MC receptors comprises four subtypes, excluding MC3R (Klovins et al., 2004; Logan et al., 2003; Selz et al., 2007), which might have been deleted during the course of evolution. Among the five MC receptor subtypes, MC2R interacts with ACTH, but not with other MC peptides such as α-MSH (Cone, 2000, 2006). The other MC receptors can bind MSHs in addition to

* Corresponding author. Address: School of Marine Biosciences, Kitasato University, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0373, Japan. Fax: +81 42 778 5010.
E-mail address: akiyoshi@kitasato-u.ac.jp (A. Takahashi).
[1] Present address: Graduate School of Integrated Arts and Sciences, Hiroshima University, Higashi-hiroshima, Hiroshima 739-8521, Japan.

0016-6480/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2013.03.005

ACTH. Therefore, MC2R is a specific receptor for ACTH. Recently, however, it was reported that the elephant shark MC2R recognizes both ACTH and α-MSH (Reinick et al., 2012).

Fish are divided into jawless (Agnatha) and jawed (Gnathostomata) types (Nelson, 2006). Jawless fish comprise Myxini, containing hagfish, and Cephalaspidomorphi, containing lampreys; jawed fish include lobe-finned (Sarcopterygii), cartilaginous (Chondrichthyes), and ray-finned fish (Actinopterygii). In fish, the association of MC systems with the pituitary–interrenal axis has been investigated in lampreys (Takahashi and Kawauchi, 2006a), cartilaginous fish (Anderson, 2012), and ray-finned fish (Wendellar Bonga, 1997). We demonstrated for the first time the presence of the pituitary–interrenal axis in jawless fish (lampreys) by detecting ACTH and MC receptors, and by determining the steroidogenic activity of ACTH. In ray-finned fish (flounders), we showed the association of MC2R and MC5R with the activities of ACTH and α-MSH. This review focuses on recent advances relating to the pituitary–interrenal axis on these two fish groups.

2. The pituitary–interrenal axis of jawless fish with reference to the sea lamprey

2.1. Structure and distribution of ACTH precursor

Sea lamprey, *Petromyzon marinus* (subfamily Petromyzontinae) possesses two different types of POMC, called proopiocortin (POC) and proopiomelanotropin (POM). As their names suggest, POC is a precursor for ACTH and β-END, and POM for MSHs and a different β-END (Heinig et al., 1995; Takahashi et al., 1995b). Thus, the lamprey differs from jawed fish in which a common POMC contains ACTH, MSH, and β-END. Both POC and POM are also present in the pouched lamprey *Geotria australis* (subfamily Geotriinae) and the short-headed lamprey *Mordacia mordax* (subfamily Mordaciinae) in the Southern Hemisphere as in sea lamprey in the Northern Hemisphere (Takahashi et al., 2006b).

Poc and *Pom*, the genes for POC and POM, are expressed in the sea lamprey pituitary in the pars distalis (PD) and pars intermedia (PI), respectively (Takahashi et al., 1995b). Immunocytochemical studies also showed ACTH-producing cells and MSH-producing cells in the PD and PI, respectively (Nozaki et al., 1995). Southern hemisphere lampreys show a similar distribution (Takahashi et al., 2006b). The distribution pattern of these cells appears comparable to those in jawed fish. The difference between lampreys and jawed fish is that two different tissue-specific genes are expressed in lampreys; whereas common genes are found in the two lobes in jawed fish. Although the expression of the gene types is different, the role of the PD as the source of ACTH is the same in lampreys and jawed fish. These findings suggest that POC and POM have diverged from a common ancestor in concert with the functional differentiation of the pituitary gland prior to the separation of the three lamprey groups.

2.2. Transcription of Poc and Pom

The 5′-flanking regions of *Poc* and *Pom* could reveal their functional differences. Several kinds of transcriptional elements are present in the 5′-flanking region of *Poc* and *Pom* (Takahashi et al., 2005b). Although a Pit-1-binding site, an E box, STAT, and RAIE are observed in the 5′-flanking regions of both genes, there was virtually no similarity in the distribution of these elements. Moreover, while *Poc* contains a TATA box near the transcription initiation site, which is conserved in the zebrafish, *Xenopus laevis*, human, rat, and bovine *Pomc*, *Pom* does not, while some TATA boxes are present in distal region. It is conceivable that deletion, duplication, and insertion of regulatory elements after gene duplication resulted in the specific expression of *Poc* and *Pom* in the PD and PI, respectively, which coincided with mutations in the coding regions so that the two distinct precursors diverged to separately encode ACTH and the two MSHs.

Ptx1 and Tpit are important transcription factors for *Pomc* (Lamolet et al., 2001; Lamonerie et al., 1996). There are several putative regulatory elements, including binding sites for Ptx1 and Tpit, along with a TATA box close to the transcription initiation site in the promoter region of *Poc* (Takahashi et al., 2005b). In contrast, the promoter region of *Pom* has binding sites for neither Ptx1, nor Tpit, nor a proximal TATA box. On the basis of these characteristics, we expected the promoter activity of *Poc* to be greater than that of *Pom* in AtT-20/D16v cells, derived from mouse pituitary epithelial-like tumor. However, we found the opposite results (Takahashi et al., 2005c). Specifically, reporter-plasmid constructs for *Poc* exhibited virtually no promoter activity, whereas those for *Pom* exhibited substantial activity. It is suggested that transcription of the sea lamprey *Poc* is not initiated by machinery in AtT-20/D16v cells – in other words, it may lack some essential element(s) for expression in AtT-20/D16v cells, or that transcription factor(s) in AtT-20/D16v cells are insufficient to drive the transcription of *Poc*. Thus, the transcription activities of *Poc* and *Pom* are apparently different from each other. It is plausible that some elements in the promoter region of the ancestral gene for *Poc*, which are essential for transcription in mammalian cell lines, have been deleted in a process of functional differentiation after the gene duplication that generated *Poc* and *Pom*.

2.3. Production of ACTH in the pituitary

In the sea lamprey pituitary, ACTH is located in the middle part of POC (Takahashi et al., 1995b). We originally isolated an ACTH peptide consisting of 60 residues ($ACTH_{1-60}$) (Takahashi et al., 1995a). Later, it was found that some of the $ACTH_{1-60}$ is further processed to $ACTH_{1-59}$, and additional modification occurs on $ACTH_{1-59}$ and $ACTH_{1-60}$ at Ser^{35} (Takahashi et al., 2006c). The modification is thought to be phosphorylation, as is the case in humans and rats (Bennett et al., 1981, 1983; Mains and Eipper, 1983).

2.4. Steroidogenesis

In sea lamprey, the *in vitro* corticotropic activity of ACTH isolated from sea lamprey pituitary was examined using pronephric and mesonephric tissues (Takahashi et al., 1995a). The results showed that ACTH converted 11-deoxycortisol to at least two steroid products, one of which was confirmed as 11-deoxycorticosterone. Thus, it was demonstrated that a functional pituitary–adrenal (interrenal) axis is present in the lamprey.

2.5. Melanocortin receptors

As reported by Haitina et al. (2007), two MC receptors have been cloned from the river lamprey. The lamprey receptors, designated MCa receptor and MCb receptor, correspond to the MC1R and MC4R of jawed fish, respectively. Expression and pharmacological characterization showed that the lamprey MCa receptor could bind to and was activated by both lamprey and human MSH peptides. When expressed in the HEK-293 EBNA cell line, the affinity of the lamprey MCa receptor for the lamprey peptides including $ACTH_{1-31}$, MSH-A, and MSH-B was not greater than that for the human peptides such as ACTH and α-MSH. Among the lamprey peptides, MSH-B had the highest affinity, followed by $ACTH_{1-31}$; MSH-A had the lowest affinity.

The lamprey MCa receptor has the ability to bind all the MC ligands including ACTH and α-MSH examined (Haitina et al., 2007). Thus, the binding properties of the lamprey MCa receptor are sim-