Steroids 103 (2015) 123–144

Contents lists available at ScienceDirect

Steroids

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

Steroids in teleost fishes: A functional point of view

Janina Tokarz [a], Gabriele Möller [a], Martin Hrabě de Angelis [a,b,c], Jerzy Adamski [a,b,c,*]

[a] Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Experimental Genetics, Genome Analysis Center, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany
[b] Lehrstuhl für Experimentelle Genetik, Technische Universität München, 85350 Freising-Weihenstephan, Germany
[c] Member of German Center for Diabetes Research (DZD), Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

ARTICLE INFO

Article history:
Received 7 April 2015
Received in revised form 11 June 2015
Accepted 15 June 2015
Available online 20 June 2015

Keywords:
Steroidogenesis
Nuclear receptor
Endocrine disruption

ABSTRACT

Steroid hormones are involved in the regulation of a variety of processes like embryonic development, sex differentiation, metabolism, immune responses, circadian rhythms, stress response, and reproduction in vertebrates. Teleost fishes and humans show a remarkable conservation in many developmental and physiological aspects, including the endocrine system in general and the steroid hormone related processes in particular. This review provides an overview of the current knowledge about steroid hormone biosynthesis and the steroid hormone receptors in teleost fishes and compares the findings to the human system. The impact of the duplicated genome in teleost fishes on steroid hormone biosynthesis and perception is addressed. Additionally, important processes in fish physiology regulated by steroid hormones, which are most dissimilar to humans, are described. We also give a short overview on the influence of anthropogenic endocrine disrupting compounds on steroid hormone signaling and the resulting adverse physiological effects for teleost fishes. By this approach, we show that the steroidogenesis, hormone receptors, and function of the steroid hormones are reasonably well understood when summarizing the available data of all teleost species analyzed to date. However, on the level of a single species or a certain fish-specific aspect of physiology, further research is needed.

1. Introduction

Bony fishes (osteichthyes) are a very successful class of vertebrates with over 25,000 living species [1]. The first osteichthyes emerged about 450 million years ago and since then, a tremendous diversity of species has evolved [2]. Osteichthyes are subdivided into lobe-finned fishes (sarcopterygii) and ray-finned fishes (actinopterygii) [2]; among the latter the teleostei are the most representative [3] and best studied group [1]. Teleost fishes have adapted to diverse ecological habitats ranging from fresh water over seawater to environmental extremes (e.g., emerging onto land) [1].

Teleost fishes are of high interest for humans in two large areas, namely as part of the diet and as model organisms for research purposes. For humans, fishes have been and are still an important nutritional resource: on the one hand, the evolution of hominids and the early brain development was dependent on fish-rich food, and on the other hand, humans are still reliant on essential nutrients provided in high concentrations in fishes [4,5]. However, due to overfishing and other environmental factors like pollution or ocean acidification, wild stocks of fishes were dramatically decreased [4,6]. To respond to declining wild populations and increased demand for seafood, aquaculture has grown and is still growing [4,7]. The number of species cultured for human nutrition, however, is relatively small [1,7].

The second aspect, where fishes in general and teleost fishes in particular are of importance for humans, is their usage in research as model organisms. The basal processes underlying embryogenesis and organogenesis are strikingly conserved between teleost fishes and tetrapods [8–11]. The understanding of vertebrate development has advanced considerably by studying model organisms, among these are also teleost fishes [12,13]. The most popular fish model species are also increasingly used to analyze human diseases like genetic disorders [14], brain disorders [15,16], or toxicological [17] and immunological [18] aspects, among others [9,19]. Teleost fishes share not only developmental aspects with their mammalian counterparts, but also the endocrine system including hormones, receptors, and signaling cascades displays a striking homology [9,20]. Compared to mammalian model organisms like mouse and rat, the widely used teleost fish species like zebrafish, medaka, fathead minnow, or three-spined stickleback have several

* Corresponding author at: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Experimental Genetics, Genome Analysis Center, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany.
E-mail address: adamski@helmholtz-muenchen.de (J. Adamski).

http://dx.doi.org/10.1016/j.steroids.2015.06.011
0039-128X/

practical advantages [10,12,21]. Their small size allows for large fish stocks in relatively small facilities. The fishes have a high fecundity, fertilize externally, their embryos are often optically transparent thus enabling microscopic observations, the embryonic development occurs rapidly, and both adults as well as embryos are amenable to genetic modifications like microinjection, chemical mutagenesis, and transgenesis [10,19,22–25]. Furthermore, most endocrine hormones and receptors are prenatally active in mammals, which impair the investigation of their developmental role in mammals. Here, teleost fishes represent an ideal model for the analysis of prenatal hormone action [20].

However, when working with teleost fishes, one has to consider that the lineage of teleostei underwent whole genome duplication about 350 million years ago, a process that did not occur in terrestrial vertebrates and is as such termed the teleost specific whole genome duplication [26–28]. This offers a unique opportunity to study evolutionary processes in teleost fishes [20]. It is considered that genome duplications are crucial for the generation of complexity and for the provision of raw material for adaptation and innovation [28]. After a whole genome duplication event, the duplicated gene copies can have different fates. Non-functionalization by silencing mutations is the most likely outcome, but it is also common that the duplicated genes are preserved by subfunctionalization (i.e., division of gene function on both copies), neofunctionalization (i.e., gaining a novel function), and parallel existence with diverging regulation and expression [28–30]. All of these processes have been observed in teleost fishes [28].

Due to the marked conservation in many developmental and physiological aspects between fishes and mammals, fishes were long considered as simply being "aquatic mammals", which is not true [31]. For instance, teleost fishes have developed a large variety of reproductive strategies for adaptation to differing aquatic environments [32] and show an enormous plasticity concerning sexual determination processes [33,34], which is in contrast to mammals. Therefore, especially the endocrinology of teleost fishes compared to mammals in general and humans in particular has to be different in certain aspects. In this review, we will focus on the endocrinology and specifically on the steroid hormones of teleost fishes (the list of covered species and their taxonomy can be found in Appendix A, Supplementary data, Table S1). We will give an overview of steroidogenesis and steroid hormone receptors in teleost fishes and compare the obtained knowledge to the human system. Further, we will review the implications of the duplicated genome on the steroid biosynthesis and the steroid hormone receptors. The steroid hormone related processes, which are most dissimilar to the human system, will be illustrated as well as the effects occurring upon disruption of endocrine signaling. This review will highlight conserved and dissimilar aspects of steroid hormones in teleost fishes compared to humans and will point out that the research on these model organisms is beneficial for the well-being of the human population.

2. Synthesis of steroid hormones in teleost fishes compared to human steroidogenesis

In general, steroid biosynthesis in teleost fishes is controlled by the hypothalamus–pituitary–interrenal and the hypothalamus–pituitary–gonadal axis [9,35,36]. Steroidogenesis occurs primarily in different peripheral tissues like the gonads, the interrenal gland, and the brain [37–41]. The interrenal gland comprises specialized cells which are embedded in the head kidney of teleost fishes and is functionally homologous to the adrenal gland in mammals [42]. All classes of steroid hormones are synthesized *de novo* from the common precursor cholesterol [43,44]. Its availability for the cytochrome p450 side chain cleavage enzyme (Cyp11a1), which removes the side chain of cholesterol resulting in pregnenolone, is controlled by the steroidogenic acute regulatory protein (StAR) [45,46]. StAR transfers cholesterol across the barrier of the outer and inner mitochondrial membrane and is as such the rate limiting step of steroidogenesis [47]. Downstream of the synthesis pathway, several enzymes modify the steroid nucleus including side chain cleavage, Δ5/Δ4-isomerization, hydrogenation, and aromatization. Other enzymes add and modify functional groups by hydroxylation, reduction, or oxidation [48]. The postulated pathway of steroidogenesis in teleost fishes is outlined in Fig. 1. To date, all of the denoted genes are identified in a large number of different teleost species (see Table 1) and annotated in even more species. Most of those genes are cloned and their expression has been analyzed; however, the extent of characterization is strongly dependent on the gene, on the species, and on the focus of the respective study (Table 1 and references therein). For example, the cytochrome p450 enzymes cholesterol side chain cleavage (*cyp11a1*), 17α-hydroxylase/lyase (*cyp17a*), and aromatase (*cyp19a1*) are the best characterized genes in the pathway, because they constitute three important bottlenecks in the steroidogenesis. Cyp11a1 is the only enzyme that converts cholesterol to pregnenolone and is therefore the only entrance into the whole process of steroidogenesis. Cyp17a is the next bottleneck in the pathway, because it is the only enzyme responsible for the conversion of C21 steroids to C19 steroids. This enzyme can use a variety of substrates, but the two most important products (17α-hydroxyprogesterone and androstenedione) cannot be synthesized by other enzymes. Cyp19a1 is responsible for the formation of C18 steroids and is thus the most important enzyme in regard of hormonal control of sexual development in teleost fishes [49–51]. In contrast to the aforementioned important genes which have been deeply characterized or have been at least annotated in almost all teleost fish species analyzed to date, there are other genes in the pathway, which are only characterized in a few selected species. Among these genes are 17β-hydroxysteroid dehydrogenases type 3 and type 1 (*hsd17b3* and *hsd17b1*, respectively), and 21-hydroxylase (*cyp21a1*). Hsd17b3 is an essential enzyme for the synthesis of 11-ketotestosterone, the active androgen in fish [52], and has been characterized only in zebrafish and medaka up to now [52–54]. Due to sequence homology, the gene has been annotated in a number of further teleost species (Table 1). Hsd17b1 converts inactive estrone (E1) to active, receptor-binding estradiol (E2), and was identified and partially characterized in a few model fish species like Nile tilapia [55], Japanese eel [56], zebrafish [53,57], and Atlantic cod [58]. Similar to *hsd17b3*, *hsd17b1* is also annotated based on sequence similarity in many further teleost fish species (Table 1). The steroid 21-hydroxylase (*cyp21a1*) is by far the least characterized gene in the steroidogenic pathway of teleost fishes. This enzyme is supposed to be involved in the biosynthesis of 11-deoxycorticosterone and cortisol, where the latter is a deeply investigated stress hormone in teleost fishes [59]. Therefore, it is surprising that the mRNA was only detected in five fish species and that no functional evidence for this enzyme is shown and published to date (Table 1).

While all the genes associated with steroidogenesis in teleost fishes are known and the respective mRNAs were detected in various species (Table 1), the verification of the postulated pathway with respect to the function, i.e., the enzymatic level, is lagging behind. When summarizing the published evidence for all enzymes of the steroidogenic pathway over all teleost species, about 70% of the postulated reactions have been directly proven (Fig. 1, Table 1). However, when a single species is considered, the maximum coverage is only approximately 20–40% of the steroidogenic pathway, depending on the species. The maximum individual coverage is observed in well characterized model organisms like Nile tilapia, Japanese eel, rainbow trout, and medaka.