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Review

Aromatase, estrogen receptors and brain development in fish and amphibians ☆

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ABSTRACT

Estrogens affect brain development of vertebrates, not only by impacting activity and morphology of existing circuits, but also by modulating embryonic and adult neurogenesis. The issue is complex as estrogens can not only originate from peripheral tissues, but also be locally produced within the brain itself due to local aromatization of androgens. In this respect, teleost fishes are quite unique because aromatase is expressed exclusively in radial glial cells, which represent pluripotent cells in the brain of all vertebrates. Expression of aromatase in the brain of fish is also strongly stimulated by estrogens and some androgens. This creates a very intriguing positive auto-regulatory loop leading to dramatic aromatase expression in sexually mature fish with elevated levels of circulating steroids. Looking at the effects of estrogens or anti-estrogens in the brain of adult zebrafish showed that estrogens inhibit rather than stimulate cell proliferation and newborn cell migration. The functional meaning of these observations is still unclear, but these data suggest that the brain of fish is experiencing constant remodeling under the influence of circulating steroids and brain-derived neurosteroids, possibly permitting a diversification of sexual strategies, notably hermaphroditism. Recent data in frogs indicate that aromatase expression is limited to neurons and do not concern radial glial cells. Thus, until now, there is no other example of vertebrates in which radial progenitors express aromatase. This raises the question of when and why these new features were gained and what are their adaptive benefits. This article is part of a Special Issue entitled: Nuclear receptors in animal development.

1. Introduction

In 1943, Edward A. Doisy (1893–1986) received the Nobel prize for his discovery of the chemical nature of vitamin K, but Doisy and his colleagues should also be remembered as the discoverers of estriol, estrone and estradiol [1]. Estradiol is a cholesterol derivative mostly known for its actions on the estrus cycle and the maintenance of female sexual characters. Estradiol was originally considered as a female hormone [1], until it was found, unexpectedly at the time, that E2 was present in the urine of the stallion [2]. This finding sets the stage for the discovery that estrogens in fact derive from androgens thanks to the action of cytochrome P450 aromatase, the only enzyme capable of aromatizing the A ring of C19 androgens to convert them into C18 estrogens. With the exception of suidae, in which there are 5 aromatase genes (also named *cyp19a1*), most vertebrates have a single *cyp19a1* gene whose tissue specific expression is driven by multiple aromatase promoters [3]. Apart from the gonads, aromatase is expressed in a large variety of tissues such as the bones, the skin, the adrenals, the adipose tissue, the fetal liver, the placenta and some breast cancers. In addition, aromatase is also well expressed in the central nervous system of all vertebrates where it is supposed to play complex and still poorly understood roles [4–6].

Since the seminal work of Alfred Jost on the hormonal control of sex differentiation in the mammalian fetus [7] and the development of the aromatization hypothesis [8], our views on aromatase and estrogen functions in the brain are largely influenced by the mammalian literature. In rodents, there is considerable evidence that the masculinization effects of testosterone on the organization of male-specific circuits are caused by aromatization and are in fact mediated by estradiol. The molecular and cellular mechanisms underlying those estrogenic effects have been thoroughly studied and involve complex age-, sex- and region-specific actions on cell proliferation, apoptosis and differentiation [9–11]. This provides evidence that, at least in this case, estrogens can modulate neurogenesis to cause irreversible sexual dimorphism of some brain structures. In addition, over the last ten years, a number of

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studies reported potential effects of estrogens and xeno-estrogens, notably bis-phenol A, in either embryonic neurogenesis and/or in the adult neurogenesis in the hippocampus [12–16]. There has been already several excellent reviews dealing with the putative functions of aromatase in mammals or birds and the role of estrogens in modulating brain functions [11,17–22]. On the other hand, a number of reports have shown that upon mechanical or chemical lesions, there is an up regulation of aromatase expression in either reactive astrocytes surrounding the lesions or in radial glial cells (RGCs) facing the lesions as shown in birds, suggesting that aromatase expression in cells of the astroglial lineage would be part of the mechanisms supporting brain repairs after lesion [11,21,23–28]. Thus aromatase can, under certain circumstances, be expressed in cells of the astrocyte lineage, notably under situation of brain repair, while it is admitted in mammals and birds that aromatase is expressed primarily in neurons [29,30].

In contrast to estradiol, testosterone is usually perceived as a male hormone, but this is not the case in all vertebrates. In fish for example, a wealth of data have demonstrated that testosterone is present in the blood of both males and females [31,32] and that fish have some particular non aromatizable androgens, such as 11-keto-testosterone that is found only in males and triggers male secondary sexual characters and sexual behavior [33]. Fish are also unique in that they exhibit an amazing sexual plasticity. They are the only vertebrates capable of total sex change, either naturally or upon hormonal treatment. Thus, the above-mentioned aromatization hypothesis probably does not apply to teleost fishes, but it is possible that this sexual plasticity is linked to some of the remarkable characteristics of aromatase expression and functions in the brain of teleost fish that will be reviewed in this article.

Teleost fishes offer an alternative model in which there is a clear neuroanatomical link between estrogen production in the brain and neurogenesis. Indeed, in teleost fishes, radial glial cells strongly express aromatase and the questions are to understand when and why this feature emerged during evolution. Whether this particular situation is found only in fishes or is also observed in basal tetrapods is unclear. Recently released information on frogs will also be examined in this review for evolutive and comparative purposes.

2. Estrogen receptors, aromatase and radial glia in the brain of fish

Teleost fish (Teleostei) represent one of three infraclasses of Actinopterygians, corresponding to the so-called ray-finned fishes. This is a highly diversified group that arose 280–250 millions years ago and comprises around 26,000 species in about 40 orders and that include most of the living fishes, including the economically-relevant species that are the topic of intense research. A major characteristic of these animals is that, compared to tetrapods, they experienced an additional whole genome duplication that took place early in the emergence of the group. As a result of this duplication, known as the 3R [34], teleost fish have potentially twice as much genes than other vertebrates (2:4:8 rule). Although many of these duplicated genes have been lost during evolution, some of them are conserved and developed new or exaggerated functions.

2.1. Estrogen receptors in teleost fishes

The first estrogen receptor cloned in fish was that of the rainbow trout [35,36], soon after the cloning of the human ERα (esr1) [37]. Compared with the mammalian ERα, this rainbow trout (rt ERα) of 65 kDa, named ERα short, exhibited at the N-terminus a deletion of 45 amino acid residues corresponding to the A domain. However, subsequently a longer ERα form of 71 kDa was retrieved from an ovarian library [38,39]. By S1 nuclease protection assays, it was shown that these two isoforms derived from two classes of mRNA generated by an alternative usage of two promoters. Consequently, these mRNA species differ in their 5′-untranslated region and the presence of an ATG in exon 2a permits adding 45 residues at the N-terminus of rtERα-long. Analysis of the transcriptional activities of these isoforms in a yeast cell system demonstrated that, in contrast with rtERα-long, rtERα-short exhibits a ligand-independent transactivation capacity representing 15–25% of the full-length receptor activity. Structural analysis of the AF1 function showed that as it is the case of the mammalian ERα, in the absence of ligand, the A domain of the rtERα-long interacts with the C-terminal region in the absence of ligand, causing inhibition of the AF1 activity located in the B domain [38,39]. Studies in rainbow trout showed that the full-length ERα is expressed in liver, brain, pituitary, and ovary, whereas expression of the ERα short is restricted to the liver, demonstrating a tissue-specific expression of these two ERα isoforms [40].

Following these pioneer studies and the discovery of two estrogen receptors in mammals [41], it rapidly appeared that teleosts have not only one ERα, but also two ERβ resulting from an ancient duplication [42,43]. Zebrafish, probably the best-documented fish, has three ER, two ERβ, ERβ1 (*esr2b*), and ERβ2 (*esr2a*) and one ERα (esr1). These three estrogen receptors bind estradiol with a high affinity within the 0.5–0.7 nM range, similar to what has been observed in mammals [43]. They also have transactivation capacity on reporter genes bearing estrogen responsive element, with ERβ2 exhibiting a little more efficiency than the other two receptors, starting at 10^{-12}–10^{-11} M. All three ERs are expressed in a wide variety of tissues including the brain [43].

One of the characteristics of ERα, at least in the liver, is its spectacular up regulation by its own ligand. This phenomenon has been first deciphered at the molecular level in the rainbow trout and is the basis for the strong induction of vitellogenin expression in the liver upon estrogenic modulation [35]. Such an up regulation of ERα is also observed in the brain, but the induction is much lower than what is observed in the liver [44].

2.2. Estrogen receptors in the brain of teleosts

Estrogen receptor expression has been studied in detail in a limited number of species, in particular the rainbow trout [45,46], the zebrafish [43,47,48], the European sea bass [49,50] and the medaka [51,52]. As already documented in mammals, ERα is well expressed in the classical neuroendocrine regions of the brain such as the preoptic area and the mediobasal and caudal hypothalamus, whereas ERβs have a wider distribution in particular along the brain ventricles of the telencephalon and diencephalon. In the preoptic area and the mediobasal hypothalamus, it is very likely that the three receptors are expressed in the same cells (F. Adrio et al., unpublished). Antibodies against the fish estrogen receptors have been developed only in rainbow trout and they are limited to ERα. Although these antibodies permitted to obtain excellent stainings in line with the distribution of the mRNAs, they were rapidly exhausted due to low titer and a necessary purification enrichment step [45,46]. Due to the lack of good antibodies, the identity of cells bearing estrogen receptor expression is not entirely clear. However, there are evidences that ERα is expressed primarily in neurons, in particular in dopaminergic neurons of the preoptic region [53], GABA neurons [54], GnRH 3 neurons [52] and kisspeptin neurons [52,55,56]. Because expression of *cyp19a1b*, encoding aromatase B in fish, is so strongly regulated by E2 through an ER dependent mechanism (see below), it would be expected to observe ER in the RGC. In fact, by increasing the sensitivity of the in situ hybridization techniques, through either amplification of the signal or using radioactive probes with longer exposure, it appears that ERβ is likely expressed along the brain ventricles, notably in the telencephalon, preoptic area and mediobasal hypothalamus, but to much lower levels than in neurons of the preoptic area and hypothalamus (Fig. 1).

2.3. Radial glia progenitors express aromatase in teleost fishes

A striking feature of the brain of teleost fish is the fact that brain aromatase is massively expressed in radial progenitors of adult animals.

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