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Steroid modulation of neurogenesis: Focus on radial glial cells in zebrafish

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ABSTRACT

Estrogens are known as steroid hormones affecting the brain in many different ways and a wealth of data now document effects on neurogenesis. Estrogens are provided by the periphery but can also be locally produced within the brain itself due to local aromatization of circulating androgens. Adult neurogenesis is described in all vertebrate species examined so far, but comparative investigations have brought to light differences between vertebrate groups. In teleost fishes, the neurogenic activity is spectacular and adult stem cells maintain their mitogenic activity in many proliferative areas within the brain. Fish are also quite unique because brain aromatase expression is limited to radial glia cells, the progenitor cells of adult fish brain. The zebrafish has emerged as an interesting vertebrate model to elucidate the cellular and molecular mechanisms of adult neurogenesis, and notably its modulation by steroids. The main objective of this review is to summarize data related to the functional link between estrogens production in the brain and neurogenesis in fish. First, we will demonstrate that the brain of zebrafish is an endogenous source of steroids and is directly targeted by local and/or peripheral steroids. Then, we will present data demonstrating the progenitor nature of radial glial cells in the brain of adult fish. Next, we will emphasize the role of estrogens in constitutive neurogenesis and its potential contribution to the regenerative neurogenesis. Finally, the negative impacts on neurogenesis of synthetic hormones used in contraceptive pills production and released in the aquatic environment will be discussed.

1. Introduction

Despite the early establishment of the basic architecture of neural circuits, the adult brains of all vertebrates studied so far retain the capacity of remodeling in order to adapt their neuronal networks to environmental demands or to damages [1,2]. For the past twenty years, the dogma according to which the number of neurons is defined at birth without new formation and replacement in adulthood has been challenged by a series of research highlighting the capacity of the adult brain to generate new cells. The pioneering work of Altman and Das in 1960s reported the production of new neurons in a very limited number of brain areas in rodents [3]. This new concept of adult neurogenesis, initially rejected, was reinforced two decades later by the work of Nottebohm who has demonstrated that neurons were generated in the forebrain of adult birds and incorporated in the vocal control center, allowing the annual learning of new song [4]. Since the 1990s, with the introduction of new methods for labeling dividing cells, the existence of proliferative activity in the adult brain of mammals was indeed evidenced in confined regions such as the subventricular zone of the lateral ventricles and the dentate gyrus of the hippocampus [5–7] and findings strongly suggest that adult neurogenesis also takes place in the hypothalamus [8–10]. With the development of the thymidine analog 5-bromo2′-deoxyuridine (BrdU) incorporation technique as a tool to label newborn neurons, it clearly appeared that adult neurogenesis is not limited to mammals and birds but is a feature

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conserved across vertebrate evolution. The data generated with that simple and fast technique showed unambiguously that adult neurogenesis occurs in reptiles [11], amphibians [12,13], fishes [14–17] and mammals notably in humans [18–20]. Currently, the adult neurogenesis concept is well accepted and defined as a complex and multistep process by which functional neurons are generated from resident neural stem/progenitor cells. In fact, neurogenesis encompasses the birth, the maturation and the migration of new neurons that integrate into existing neuronal networks [21,22]. Although this phenomenon is common, comparative investigations have brought to light major differences between vertebrate groups in terms of neurogenic niches in the brain [23]. While the generation of new neurons is obvious in two main regions in mammals, the neurogenic potential in adult teleost fish is spectacular in many proliferative areas. This continuous production of new neurons in adulthood is notably supported by the persistence and abundance of radial glial cells (RGCs) [17,24], known in mammals to serve as neural "stem" cells during embryonic neurogenesis [25,26]. Fish are also distinguished by their remarkable potential to regenerate their CNS from mechanical and chemical injuries by replacing damaged neurons such as shown in the cerebellum, the telencephalon and olfactory bulbs, the retina [27–32]. Indeed, a massive and transient increase in cell proliferation is observed in response to injuries applied to the brain and the spinal cord and newly generated neurons repopulate the wounded site allowing a complete regeneration of nervous tissue while the regenerative capacity of the adult mammalian brain is limited and the long-term survival of newborn cells is generally impaired [33]. The great neurogenic activity associated with the extraordinary repairing properties of the adult brain have made teleost fish valuable models to study and decipher mechanisms underlying adult neurogenesis in a constitutive or a regenerative context.

In mammals, a wealth of factors, notably neurotransmitters, growth factors and hormones, have been shown to modulate adult neurogenesis [34–38]. With respect to hormones, estradiol, is recognized as a major modulator of adult vertebrate neuronal plasticity [39,40] and neurogenesis under physiological conditions and many data also demonstrated its neuroprotective actions in damaged brains [35,41–47].

Estradiol may also play significant roles in teleost neurogenesis as the brain of fish is well known for harboring a high expression of aromatase, the only enzyme that catalyzes the final step of estrogen biosynthesis. Aromatase is expressed in the brain of all vertebrates, but in teleost fish, the enzymatic activity is much higher than in mammal and bird. In addition, the three estrogen receptors are described in many brain areas of teleost fish [48–50].

In the last few years and for the above-mentioned reasons, the zebrafish has emerged as an interesting vertebrate model to elucidate the cellular and molecular mechanisms of adult neurogenesis, and notably its modulation by steroids, in normal and in reparative conditions. The main scope of this review is to summarize recently released information on the functional link between estrogens production in the brain and neurogenesis in fish with a particular focus on the zebrafish model. First, we will document the capacity of adult fish brain to produce steroids. We will present data demonstrating that RGCs could be an endogenous source of steroids and are directly targeted by local and/or peripheral steroids. We will next emphasize the role of estrogens in constitutive neurogenesis and its potential contribution to the regenerative neurogenesis. Finally, we will provide findings that point out the deleterious impacts on neurogenesis of synthetic hormones used in contraceptive pills production and released in the aquatic environment.

2. The brain of adult zebrafish: a source of neurosteroids?

2.1. De novo neurosteroids synthesis

While neurosteroids synthesis is widely documented in mammals, only few studies focused on *de novo* steroid synthesis in the brain of teleost fish [51–55] Such a feature raises the question of the origin, local and/or peripheral, of C19 androgens available for brain aromatization. Our laboratory recently demonstrated that the brain of adult zebrafish was able to *de novo* synthesize a wide variety of radiolabeled neurosteroids from [^{3}H]-pregnenolone. Among these locally-produced steroids, there are notably dehydroepiandrosterone (DHEA), androgens (i.e., testosterone), estrogens (i.e., estrone and 17β estradiol), progesterone and derivatives [51,55]. Such results clearly evidence that 17β-hsd, 3α and 3β-hsd, cyp17, 5α-reductase and cyp19a1b (AroB) are expressed and biologically active in the brain of adult zebrafish. As no specific well-characterized antibodies are available, apart from AroB, and in order to determine the sites of production of neurosteroids in the zebrafish brain, *in situ* hybridization was performed for the main steroidogenic enzymes leading to estrogen synthesis (*cyp11a1* -P450$_{SCC}$-, *3β-hsd*,*cyp17* and *cyp19a1b*). These experiments show that these steroidogenic enzymes are widely expressed in the whole brain, notably in the telencephalon, the preoptic area, the hypothalamus, the mesencephalon and the cerebellum. Moreover, they exhibit an overall similar pattern suggesting a potential co-expression, at least in some regions such as the hypothalamus. Thus, by performing *cyp11a1*, *3β-hsd* and *cyp17* ISH followed by AroB immunohistochemistry, some steroidogenic enzymes transcripts were detected in AroB-radial glial cells, raising the question of the steroidogenic capacity of RGCs in zebrafish. In addition, the distribution of these main steroidogenic enzymes (apart from AroB) also strongly argues in favor of a neuronal expression. This is notably reinforced by the fact that 3βHSD-like immunoreactivity was observed in neurons throughout the adult zebrafish brain [52]. Last but not least, steroidogenic enzymes expression in microglia and oligodendrocytes is not excluded, but it would require further investigations. Together, all these results show that the brain of adult zebrafish is a true steroidogenic organ, RGCs being a source of neurosteroids [51,55,56], and raise the question of the targets of such steroids in the brain as well as their functions.

2.2. Aromatase and radial glial cells

Cytochrome P450 aromatase (aromatase), the rate-limiting enzyme that transform C19 androgens into estrogen, is described in the gonads and the brain of all vertebrates species studied so far, with a broader distribution in mammals [57]. Strikingly, when compared to other vertebrates and especially to mammals, the brain of adult fish exhibits an exceptionally high aromatase activity in anterior regions such as olfactory bulbs, telencephalon, preoptic area and hypothalamus [48,58,59]. The *cyp19a1* genes, which encode aromatase, are highly conserved throughout vertebrate lineages but their expressions are driven by different regulatory mechanisms. In the mammalian genome a single *cyp19a1* gene has been characterized (except in the pig) and its expression is driven by the use of distinct tissue-specific promoters and alternative splicing [60,61]. As a result of teleost specific whole genome duplication [62], two *cyp19a1* genes, *cyp19a1a* and *cyp19a1b,* have been identified in most fish, except in the Japanese and European eel [63,64]. As evidenced in a growing number of teleost species, including the zebrafish, those two genes encode different enzyme isoforms, aromatase A (AroA, produced by the *cyp19a1a* gene), which is mostly described in the gonads and aromatase B (AroB, the product of the *cyp19a1b* gene), which is strongly expressed in

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