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Stress and sex determination in fish: from brain to gonads

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ABSTRACT Fish present remarkable malleability regarding gonadal sex fate. This phenotypic plasticity enables an organism to adapt to changes in the environment by responding with different phenotypes. The gonad and the brain present this extraordinary plasticity. These organs are involved in response to environmental stressors to direct gonadal fate, inducing sex change or sex reversal in hermaphroditic and gonochoristic fish, respectively. The presence of such molecular and endocrine plasticity gives this group a large repertoire of possibilities against a continuously changing environment, resulting in the highest radiation of reproduction strategies described in vertebrates. In this review, we provide a broad and comparative view of tremendous radiation of sex determination mechanisms to direct gonadal fate. New results have established that the driving mechanism involves early response to the brain's environmental stressors plus high plasticity of gonadal differentiation and androgens as by-products of stress inactivation. In addition to the stress axis, two other major axes – the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-thyroid axis are well known for their participation in the regulation of reproduction – have been proposed to reinforce brain-gonadal interrelationships in the fate of the gonad.

KEY WORDS: *stress, CRH, cortisol, masculinization, androgen, sex reversal, sex change*

Introduction

An organism's ability to adjust to changes in the environment is a selected adaptive mechanism that serves not only to recover homeostasis, but also to provide an opportunity to modulate the fate of different organs during development. One of these adaptations to changes in environment is the ability of some animals and plants to direct gonadal fate (Capel, 2017; Fernandino *et al.*, 2013; Ortega-Recalde *et al.*, 2020), a mechanism known as environmental sex determination. Although this mechanism is a universal process in sexual organisms, a misconception is that it has been highly conserved during evolution due to the importance of sexual destiny; however, mechanisms of sex determination are remarkably diverse.

Mechanism of sex determination and differentiation in fish

In vertebrates, gonadal fate can be directed by genetic sex determination (GSD), environmental sex determination (ESD), or the interaction of both. In ESD, sex is established by a nongenetic signal experienced early in life, while GSD occurs when sex is established by genetic factors at conception. The phylogenetic distribution of these types of sex determination mechanisms indicates that conversions between types have occurred many times (Ashman *et al.*, 2014; Capel, 2017). Nevertheless, ESD is considered to be an ancestral state (Pokorná and Kratochvíl, 2009), and transitions from ESD to GSD occur at higher rates than the opposite direction (Bull, 1981), suggesting that genetic sex determination is more stable (Kitano and Peichel, 2012). All the GSD mechanisms, ranging from purely polygenic controls to early evolved sex chromosomes with heterogametic (XY) males or heterogametic (ZW) females, have been described in fish species (Devlin and Nagahama, 2002). In recent years, the declining costs of the methodology based on next-generation sequencing (NGS) have enabled much more information to be gathered regarding the genes and molecular pathways involved in GSD (Martínez *et al.*, 2014). Interestingly, the initial path established by genetic factors can often be overridden by environmental factors in fishes, making sex determination more complex.

Many environmental switches that trigger gonadal fate have been characterized. They can be grouped into biotic and abiotic parameters. Among biotic parameters, density and social interac-

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Abbreviations used in this paper: CRH, corticotrophin-releasing hormone; ESD, environmental sex determination; GSD, genetic sex determination.

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tions have been well characterized in Anguilliformes and hermaphroditic fish, respectively (Geffroy and Bardonnet, 2016; Gemmell *et al*., 2019; Pla *et al*., 2020). Abiotic parameters may be chemical or physical. Hypoxia and pH are chemical abiotic parameters reported to affect sex ratios (Breitburg *et al*., 2009; Cheung *et al*., 2014; Pollock *et al*., 2010; Reddon and Hurd, 2013; Römer and Beisenherz, 1996; Rubin, 1985; Shang *et al*., 2006; Thomas and Rahman, 2012; Yu and Wu, 2006), and among physical abiotic parameters, temperature, photoperiod and light wavelength have been well studied (e.g. Baroiller *et al*., 1996; Bezault *et al*., 2007; Brown *et al*., 2014; Corona-Herrera *et al*., 2018; Hattori *et al*., 2007; Hayasaka *et al*., 2019; Navarro-Martín *et al*., 2011; Pavlidis *et al*., 2000; Strüssmann *et al*., 2010; Uchida *et al*., 2004; Wang *et al*., 2017; Yamaguchi *et al*., 2010).

In addition to having a high level of radiation in fundamental sex determination triggers (Capel, 2017), fish exhibit all types of reproductive strategies, including unisexuality, gonochorism, simultaneous (or synchronous) and sequential hermaphroditism (Devlin and Nagahama, 2002), demonstrating polyphyletic origin because they arose independently in different lineages during evolution (Ashman *et al*., 2014).

Stress brain response to traduce environmental sex fate

It was initially assumed that gonads sensed environmental change directly, responding with a change in sex fate. In reptiles, cultured gonads at female-promoting temperature (FPT) and the contralateral gonads at male-promoting temperature (MPT) of *Lepidochelys olivacea* showed female and male-pattern of Sox9, a gene involved in testis development (Moreno-Mendoza *et al*., 2001). Moreover, it was recently established that in the red-eared slider turtle *Trachemys scripta elegans*, the epigenetic regulator Kdm6b plays a causal role in testicular fate determination by demethylating trimethylated histone H3 lysine 27 (H3K27me3) in the Dmrt1 promoter in the gonad (Ge *et al*., 2018). Although many groups of reptiles and amphibians present interaction between environment and sex fate, fish are outstanding for the enormous radiation of environmental factors that can lead to shifts in the sex ratio of a population (Ashman *et al*., 2014). Their extraordinary diversity of reproductive strategies cannot be explained only by the high plasticity of gonad development. The fish brain is highly malleable, even in adulthood (Baroiller and D'Cotta, 2016; Chi *et al*., 2017), and maybe involved in the induction of gonadal sex reversion/change.

The environmental factors mentioned above that can change the sex bias can be considered as stressors for fish (Baroiller and D'Cotta, 2016; Fernandino *et al*., 2013; Geffroy and Douhard, 2019; Goikoetxea *et al*., 2017; Ospina-Alvarez and Piferrer, 2008). This reasoning, supported experimentally, led to establishing the central role of stress in this process (Fernandino *et al*., 2013; Goikoetxea *et al*., 2017). A decade ago, the crucial role of cortisol was elucidated. Cortisol is the main glucocorticoid participating in the stress response inducing testis development in "pejerrey", *Odontesthes bonariensis*, a fish with temperature-dependent sex determination (TSD) (Hattori *et al*., 2009). In this species, high temperature similar to that observed in summer, induces high cortisol synthesis, producing androgen as a by-product of cortisol inactivation, with the concomitant testis development (Fig. 1) (Fernandino *et al*., 2012; Hattori *et al*., 2009). Moreover, when larvae are reared at a temperature expected to induce an equal sex ratio, cortisol included in their diet was found to promote testis development (Hattori *et al*., 2009). This result was later corroborated in other gonochoristic fish, even in species with GSD (e.g. Medaka); however, the rearing temperature in GSD species needs to be higher than the range of temperature normally experienced by fish in the wild for these species (Hayashi *et al*., 2010; Yamaguchi *et al*., 2010).

Additionally, cortisol appears involved in the sex change of hermaphroditic fish (Chen *et al*., 2020; Frisch *et al*., 2007; Nozu and Nakamura, 2015; Olivotto and Geffrey, 2017; Solomon-Lane *et al*., 2013; reviewed by Gemmell *et al*., 2019; Goikoetxea *et al*., 2017; Liu *et al*., 2017; Ortega-Recalde *et al*., 2020). Hermaphroditic fish present a fascinating example of phenotypic plasticity by environmental cues. In this reproductive strategy, which exists only in fish, individuals begin life as one sex and can subsequently restructure and develop into the opposite sex via any of three strategies: protogynous (female to male), protandrous (male to female) and sequentially bidirectional sex change (sex change in either direction)

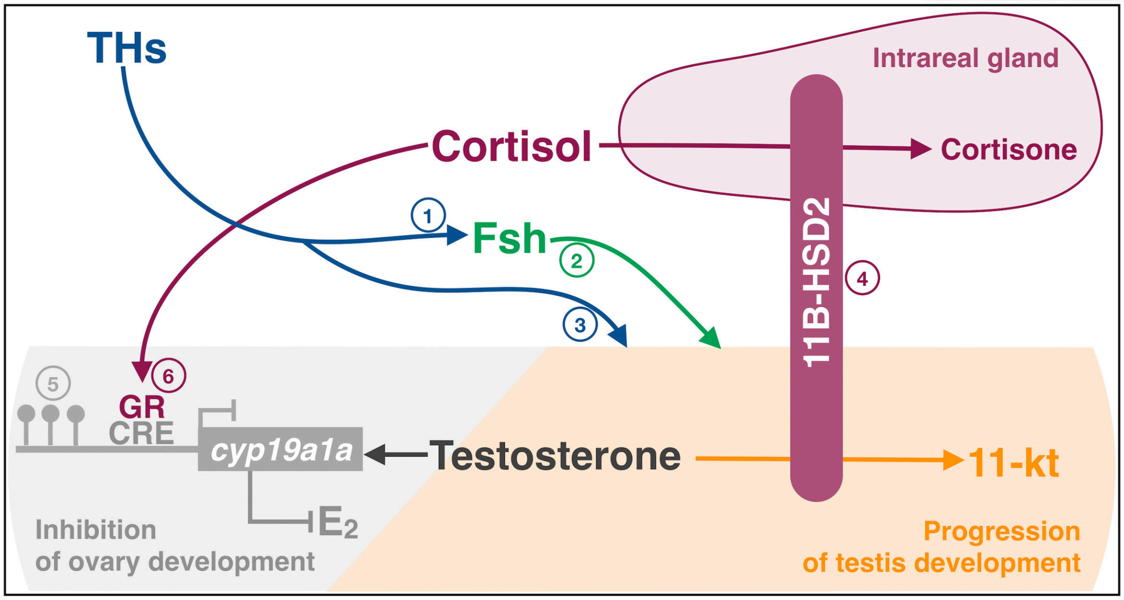

Fig. 1. Hypothetical model of male bias. *The final inductors of the different hypothalamic-pituitary pathways, thyroid (THs, blue) and gonadal hormones (FSH, green), as well as cortisol (dark red) promote the development of the testis in fish sex change. Numbers in the circles are the references for each step: 1) Morais* et al.*, 2013. 2) Kobayashi* et al.*, 2010. 3) Sharma and Patiño, 2013; Sharma* et al.*, 2016. 4) Chen* et al.*, 2020; Fernandino* et al.*, 2012; Goikoetxea* et al.*, 2017; Hattori* et al.*, 2009; Todd* et al.*, 2019. 5) Navarro-Martín* et al.*, 2011. 6) Yamaguchi* et al.*, 2010.*

(Devlin and Nagahama, 2002). Thus, sequential hermaphroditism is a clear example of the fish gonadal plasticity in response to environmental stimuli, resulting in changes in reproductive behavior and external morphology (Gemmell *et al.*, 2019).

Perry and Grober (2003) studied the tropical coral reef bluehead wrasse *Thalassoma bifasciatum*, a sequential hermaphrodite that displays social control of sex change, initially suggesting that brain activity is a consequence of cortisol levels modulated by environmental stimuli. They hypothesized that dominant males in social groups of protogynous species are responsible for inhibiting socially induced sex change of subordinate females by displaying aggressive behavior. Removal or loss of the dominant male triggers the sex change of the alpha female due to the high cortisol level. However, this hypothesis suggests that the high level of cortisol is necessary to the sex change, but not sufficient, and that another mechanism must be involved in the process. Moreover, cortisol administration promoted sex change in three-spot wrasse, *Halichoeres trimaculatus* (Nozu and Nakamura, 2015). Recently, it has been reported that cortisol administration induces the female-to-male sex change in the protogynous orange-spotted grouper, *Epinephelus coioides* (Chen *et al.*, 2020), corroborating the importance of this glucocorticoid in the induction of testicular development. However, the key regulators of cortisol levels are still elusive.

In this regard, it has been postulated that environmental disturbance regulates changes in the neuropeptide arginine vasotocin (Avt) mRNA in the preoptic area of the hypothalamus (Fig. 2) (Godwin *et al.*, 2000). It has been suggested that this neuropeptide participates in social behavioral sex change. It is the teleost homolog of mammalian vasopressin (Urano and Ando, 2011). As mentioned above, it was observed in the bluehead wrasse that *avt* expression is down-regulated in neurons involved in glucocorticoid suppression – the parvocellular neurons of the hypothalamus –, contributing to the social behavior observed during sex change. Additionally, this modulation of Avt would release a different ratio with corticotrophin-releasing hormone (Crh) (Perry and Grober, 2003), which ultimately regulates the cortisol increase necessary to sex and behavioral change. However, it was recently proposed that low levels of Avt maintains high cortisol levels during the early stages of sex change (Liu *et al.*, 2017). Likewise, the neuropeptide norepinephrine (NE), which regulates CRH neurons in mammals (Fig. 2) (Itoi *et al.*, 1999) and stimulates the blood cortisol level in rainbow trout *Oncorhynchus mykiss* (Overli *et al.*, 1999), was increased in the hypothalamic preoptic area of saddleback wrasses *Thalassoma duperrey*, and bluebanded gobies *Lythrypnus dalli*, early in sex change (Larson *et al.*, 2003; Solomon-Lane *et al.*, 2013). In addition to cortisol regulation inducers, other neuropeptides, such as dopamine (DA) and serotonin (5-HT), have been suggested to inhibit sex change (Kramer *et al.*, 1993; Larson *et al.*, 2003; Perreault *et al.*, 2003; Semsar *et al.*, 2004) (see review by Gemmel *et al.*, 2019). Although hypotheses have been proposed about cerebral regulation of the hypothalamus-pituitary-interrenal (HPI) axis in sex change, further evidence is still needed to establish the general mechanism in synchronous hermaphrodites.

Recently, the brain has been corroborated as a key factor linking external environmental stimuli with internal physiological responses during sex reversal of gonochoristic fish, establishing the brain's importance in changing gonadal fate (Castañeda Cortés *et al.*, 2019). It is well known that in vertebrates, the hypothalamic-pituitary-adrenal/interrenal (HPA/I) axis transduces environmental changes to physiological responses (Kovács, 2013). The paraventricular nucleus of the hypothalamus (PVN) synthesizes Crh when there is an environmental stressor (Wamsteeker Cusulin *et al.*, 2013), promoting the release of the adrenocorticotropic hormone (Acth) from the pituitary by binding to two Crh receptors, finally promoting synthesis and release of cortisol from the adrenal/interrenal gland (Figs. 1, 2) (Füzesi *et al.*, 2016). An important point in elucidating the brain's participation in environmentally-induced sex reversal was the establishment of the temporal overlap between the induction of stress response and the gonadal sex determination period. Castañeda-Cortés *et al.,* (2019) observed an increase in the expression of corticosteroid-related hormone type b (*crhb*) during this period in medaka embryos incubated at high temperature (Fig. 2), a well-characterized environmental stressor for this species (Hattori *et al.*, 2007). Moreover, *crhb* was the only CRH-like gene that responded to an environmental stressor at early stages of development, because its paralog *crha* and the three Crh-like urocortins were not over-expressed. Another interesting point in this study was that the transcriptional response to environmental changes was no sexually dimorphic, showing that the environmental response is not genotypically sex-dependent. To corroborate the participation of the HPI axis in environmentally-induced sex reversal, biallelic mutants of both *crh* receptors were generated, in which a decrease of cortisol synthesis was observed, with levels similar to those in embryos incubated at unstressed temperature, with the concomitant complete lack of sex reversal (Castañeda Cortés *et al.*, 2019). Further studies should be conducted in other fish species, primarily hermaphroditic fish, to corroborate the participation of Crh as the transducer of environmental stimuli to change gonadal fate.

Having postulated Crh as the transducer of environmental changes in the reversion of gonadal sex, it is important to establish the mechanisms of direct regulation of this neuropeptide during environmental sex change. Above, we have outlined the knowledge of the potential role of Avt, Ne, Da and 5-HT in the up-regulation and down-regulation of Crh in hermaphrodite fish during the induction of sex change. However, confirmatory experiments will be necessary to corroborate this endocrine regulation in the brain. Transcriptional regulation of *crhb* has recently been established in the medaka fish (Uchimura *et al.*, 2019). In this gonochorist, two heat shock proteins, hsp70.1 and hsp30, up-regulate the expression of *crhb* in the hypothalamus of embryos incubated at high temperature (Fig. 2). Furthermore, heat shock proteins act as chaperones of steroid hormone receptors, such as glucocorticoid, androgen, and estrogen receptors (Pratt and Toft, 1997), all well known to be involved in gonadal differentiation, hence the role of heat shock proteins in sex reversal induced by environmental changes should be also evaluated downstream of Crh. Together, endocrine and transcriptional Crh regulations would provide an idea of the complexity of control in response to stress under environmental change, suggesting that Crh is a common regulator in environmental sexual determination in fish.

Alternative axes for environmental sex determination

Although the stress axis seems to have a key role in sex reversal and sex change induced by environmental factors, the participation of two major axes, the hypothalamic-pituitary-gonadal (HPG) and the hypothalamic-pituitary-thyroid (HPT) (Castañeda Cortés *et al.*, 2014), has been proposed as reinforcing the brain and gonadal interrelationships in the fate of the gonad.

Miranda *et al.,* (2003) first hypothesized that the brain directs gonadal differentiation through GnRH regulation. Following this statement and taking into account the extensive information available on the role of HPG in adult reproduction (Acevedo-Rodriguez *et al.*, 2018) and the observation that adult reproductive regulators are recurrent during early gonadal development, has been suggested the participation of HPG in sex reversal and sex change. The main regulator of reproduction in vertebrates is the gonadotropin-related hormone (GnRH) in the hypothalamus, which modulates the release of both gonadotropins – follicle-stimulating hormone beta (FSHb) and luteinizing hormone beta (LHb) – from the pituitary. These gonadotropins regulate the synthesis of steroid sex hormones and the reproductive cycle of the gonad (Figs. 1, 2) (Young *et al.*, 2005). The participation of GnRH in gonadal differentiation was initially postulated during the sex reversal of the "pejerrey" (Miranda, 2003). During the gonadal sex determination period characterized in this species (Strüssmann *et al.*, 1997), three groups of neurons were identified: the terminal nerve ganglion (TNG), the midbrain tegmentum (MT), and the preoptic area (POA). However, only neurons in the POA increased during the gonadal sex determination period at high temperature, which presented male bias. In tilapia, GnRH-III immunoreactive neurons in the TNG only increased in males, and androgen induction of sex reversal produced an increase in the number of GnRH-III positive neurons in the TNG of females (Kuramochi *et al.*, 2011). Reinforcing the participation of GnRH in male gonadal development, in the protandrous cinnamon clownfish *Amphiprion melanopus*, drastic down-regulation was observed for all three of *gnrh* variants – GnRH-I, GnRH-III and GnRH-II – during the sex change (Kim *et al.*, 2012), prior to female development. Moreover, in the protandrous dusky anemonefish *Amphiprion melanopus*, it was observed that the number of GnRH-positive neurons in the POA, though not TNG, differed between sexual phases, with males displaying higher numbers than females (Elofsson *et al.*, 1997).

It has been postulated that other players in the GnRH-regulating HPG axis participate in the gonadal fate induced by environmental changes. The neuropeptide Kisspeptin, a member of the Arg-Phe (RF)-amide peptide family, regulates sexual maturation of reproduction by stimulating GnRH release from the POA of the hypothalamus (Mechaly *et al.*, 2013; Roa *et al.*, 2011). In "pejerrrey", a dimorphic expression of *kiss2* was observed at a temperature that induced an all-male bias (Fig. 2) (Tovar Bohórquez *et al.*, 2017). This up-regulation in the brain during testis development is consistent with the GnRH up-regulation in the POA previously reported in this species (Miranda, 2003). Similar results were observed in the protogynous orange spotted grouper (Shi *et al.*, 2010). The expression of *kiss2* and *gnrh1* was up-regulated during the induced sex change. Moreover, the administration of synthesized Kiss2 increased *gnrh1* transcription in the hypothalamus and *fshb* levels in the pituitary (Shi *et al.*, 2010). However, similar administration of Kiss in the protandrous dusky anemonefish induced the maturation of spermatocytes and oocytes in males and females, but did not induce sex reversal (Kim *et al.*, 2014).

Regarding the HPG axis, it has been postulated that gonadotropins play an important role in gonadal sex differentiation in fish. It has been hypothesized that in many fish, gonadotropin participates in gonadal development, with *fshb* being expressed earlier than *lh* before the gonadal sex differentiation period (Chen and Ge, 2012; Gao *et al.*, 2019; García Ayala, 2003; Guzmán *et al.*, 2009; Moles *et al.*, 2007; Molés *et al.*, 2011; Pandolfi *et al.*, 2006), but with the exception of "pejerrey", in which *lh* was observed first (Miranda *et al.*, 2001). To study the participation of gonadotropin, especially Fsh, mutant Fsh receptors were generated in medaka fish, showing induction of some female-to-male reversal (Murozumi *et al.*, 2014). Nevertheless, zebrafish

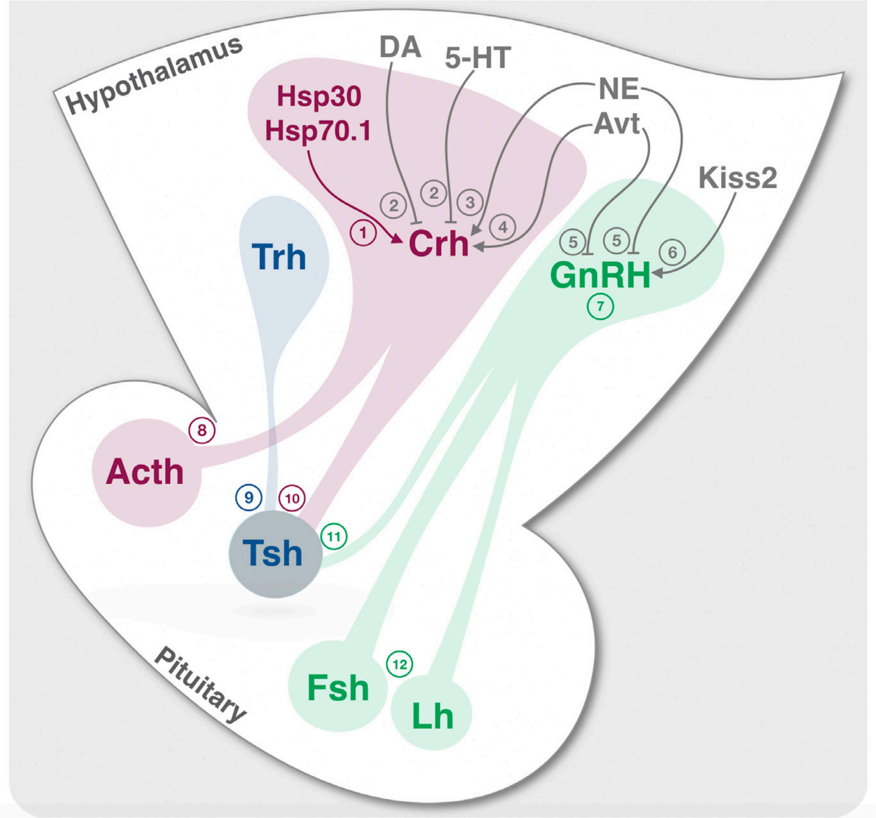

Fig. 2. Schematic representation of the role of the brain in sex change in fish. *The hypothetical interaction between the hypothalamic-pituitary-thyroid gland (Trh-Tsh, in blue), adrenal/interrenal (Hsp30/70.1-Crh-Acth, in dark red), and gonadal (GnRH-Fsh-Lh, in green) axes, with the positive or negative input of different neuropeptides (in gray), guide the sex change to male in fish. Numbers in the circles are the references for each step: 1) Uchimura* et al., *2019. 2) Gemmel* et al., *2019. 3) Larson* et al., *2003; Overli* et al., *1999; Solomon-Lane* et al., *2013. 4) Liu* et al., *2017; Perry and Grober, 2003. 5) Goikoetxea* et al., *2017. 6) Shi* et al., *2010; Tovar Bohórquez* et al., *2017. 7) Elofsson* et al., *1997; Kim* et al., *2012; Miranda* et al., *2003. 8) Castañeda Cortés* et al., *2019. 9) Chatterjee* et al., *2001; Han* et al., *2004; Larsen* et al., *1998. 10) Castañeda Cortés* et al., *2014; Larsen* et al., *1998; Peter, 2011. 11) Chiba* et al., *2004; Roy* et al., *2000. 12) Chen and Ge, 2012; Gao* et al., *2019; García Ayala, 2003; Guzmán* et al., *2009; Miranda* et al., *2001; Moles* et al., *2007; 2011; Pandolfi* et al., *2006; Zhang, Zhu,* et al., *2015.*

knockouts of *fshb* or *lhb* only showed an ovarian and testicular delay in development, but not sex reversal. However, the double mutant of the two gonadotrophins led to all males, and a delay in testicular development (Zhang, Zhu, *et al.*, 2015). Interestingly, the lack of the receptors *fshr* or *lhr* presented a different phenotypic pattern than their ligands, in which only *fshr* induced female-to-male reversal (Zhang, Lau, *et al.*, 2015). Additionally, the expression of gonadotropins has been studied in protogynous, protandrous and bidirectional hermaphroditic fish. For example, in the protogynous honeycomb grouper *Epinephelus merra*, *fsh* was strongly associated with testis development (Fig. 1). Moreover, Fsh treatments induced female-to-male sex change (Kobayashi *et al.*, 2010). Nevertheless, the patterns of gonadotropins across sex change are inconsistent even among closely related species (see review by Gemmell *et al.*, 2019), and further studies are needed to establish a consistent pattern of gonadotropins during sex change. Trudeau (2018) provided an explanation for these inconsistent results obtained for neuropeptide and gonadotropin knockouts. In his review, Trudeau hypothesized differences in the hypothalamus-hypophysial anatomy of fish, which present direct innervation of gonadotrophs because they have lost the average eminence present in mammals, with the possibility of independent regulation by > 20 neurohormones. Consequently, the elimination of any of these neurohormones could have minimal impact, because of the co-expressed stimulators of functionally intact gonadotropins. For this reason, genetic compensation in response to the loss of protein function can maintain sufficient gonadal development to sustain reproduction (Trudeau, 2018). Considering the late ontogenetic expression of GnRH during the period of gonadal sex determination, the present results establish GnRH as a consequence, rather than the cause, of testicle development.

The input process in the brain has been most intensively studied with regard to ESD is the stress axis, though there has recently been more interest in the thyroid axis (HPT; Figs. 1,2), due to its participation in adult reproductive health (Tovo-Neto *et al.*, 2018). Thyroid hormone (THs) is mainly involved in the regulation of metabolism, development, growth and reproduction. Its participation in gonadal development has been mostly studied in mammal species, while studies in fish remain limited (reviewed in Castañeda Cortés *et al.*, 2014). For example, in zebrafish, the endocrine manipulation of THs inducing hyperthyroidism during embryonic and larval stages through exposure to thyroxine (T4) caused male-biased populations (Sharma and Patiño, 2013, Sharma *et al.*, 2016). Conversely, the induction of hypothyroidism using goitrogens (substances that disrupt the production of THs) produced a female-biased population (Mukhi and Patino, 2006; Sharma *et al.*, 2016). Based on these results, induced hyperthyroidism during early gonadal development stages caused testis development, but induced hypothyroidism only delayed testis development. However, additional experiments and species should be studied to improve knowledge on the participation of THs in gonadal development. Additionally, in adult zebrafish, it was observed that THs can influence the proliferation and function of Sertoli cells regulating Fsh activity (Morais *et al.*, 2013). The crossover of THs regulated by GnRH has also been studied in fish (Fig. 2), although it has shown contradictory results between species. Some studies showed that GnRH-III injection increased T4 plasma levels (Chiba *et al.*, 2004; Roy *et al.*, 2000), while in others, no T4 changes were observed (MacKenzie *et al.*, 1987). Additional studies during early gonadal development stages will be necessary to clarify the effect of the crossover of HPT and HPG axes on gonadal fate.

Similarly, it has been suggested that the crossover between HPI and HPT axes in the pituitary may participate in stress-induced sex determination (Fig. 2) (Castañeda Cortés *et al.*, 2014). Environmental changes stimulate the hypothalamic tripeptide thyrotropin-releasing hormone (TRH), which stimulates the anterior pituitary to synthesize and secrete the thyroid-stimulating hormone (TSH). Nevertheless, in fish, a contradictory pattern of Tsh induced by Trh makes it difficult to understand THs homeostasis regulation and establishes that Trh is not the main Tsh-releasing factor. For example, in the bighead carp *Aristichthys nobilis*, and Japanese eel *Anguilla japonica*, *in vitro* Trh induction was shown to increase hypophyseal *tshb* expression (Chatterjee *et al.*, 2001; Han *et al.*, 2004), while in Coho salmon *Oncorhynchus kisutch*, a similar approach did not induce Tsh release (Larsen *et al.*, 1998). Interestingly, in the Coho salmon, Crh caused a significant concentration-dependent increase in TSH secretion (Larsen *et al.*, 1998), suggesting a remarkable crossover between HPI and HPT axes. Although this crossover between these major endocrine axes has been well established in tetrapods (Castañeda Cortés *et al.*, 2014; De Groef *et al.*, 2006; Okada *et al.*, 2009), and the importance of THs in stress response has been well documented in fish (Castañeda Cortés *et al.*, 2014; Peter, 2011), we are far from elucidating the role of THs in stress-induced sex reversal/change.

Regulation of sex steroid hormones during environmental sex reversal/change

Sex steroid hormones – estrogen and androgens – have been shown to be strongly involved in ovary and testis development, because when they are administered exogenously during the gonadal sex determination period, they induce female- or male-biased populations, respectively (Devlin and Nagahama, 2002). Nevertheless, not all fish need sex steroids to develop functional gonads. For example, in medaka fish, oral administration of androgens during the gonadal sex determination period induces the development of neo-males (Yamamoto, 1953), corroborating the presence of the molecular machinery. However, the *scl* mutant in medaka, lacking P450c17 (the gene that encodes a steroidogenic enzyme required for both estrogen and androgen production), develops normal testis and only the dimorphic sex character is absent in males (Sato *et al.,* 2008). Additionally, treatment with an androgen antagonist showed intersex gonad (testis-ova) in genotypic males (Kang *et al.*, 2006). On the other hand, *scl* genotypic female mutants showed intersex (ovo-testis) phenotypes (Sato *et al.*, 2008) and lack of gonadal aromatase (*cyp19a1a*), which is an enzyme involved in estrogen synthesis. Moreover, *scl* mutants promote partial female-to-male sex reversal in the same species (Nakamoto *et al.*, 2018). These disparities between experiments in the same species may be due to the target level of the axis or the steroidogenic pathway interrupted, in which the up-regulation of alternative compensatory mechanisms or pathways may be present.

When faced with an environmental change, the HPI axis is activated to synthesize cortisol, inducing female-to-male change by acting on the sex steroid hormone balance (Fernandino *et al.*, 2013; Goikoetxea *et al.*, 2017). As mentioned above, an increase in glucocorticoid levels induced high androgen synthesis by up-regulation of the *hsd11b2* gene, which encodes the enzyme needed to inactivate cortisol and synthesize 11-KT (Fernandino *et al.*, 2012; Todd *et al.*, 2019). To reinforce the masculinization pathway, the increase in cortisol level also inhibits the synthesis of estradiol, suppressing the synthesis of this sex steroid by binding through its receptor (GR, glucocorticoid receptor) to cAMP-responsive element (CRE) and methylating the *cyp19a1a* promoter (Fig. 1) (Chen *et al.*, 2020; Navarro-Martín *et al.*, 2011; Yamaguchi *et al.*, 2010), directing the development of the testis. In protogynous hermaphrodite fish, a mechanistic model between these axes during sex change has also been proposed (Goikoetxea *et al.*, 2017). The promotion of sex change behavior in females after the loss of a dominant male decreases GnRH and Lh by elevation of Avt and Ne levels (Fig. 2), inducing follicle apoptosis in the ovary while driving up serum cortisol to ultimately stimulate 11-KT synthesis and suppress estradiol synthesis (Fig. 1). As mentioned above, in gonochoristic fish, the participation of GnRH in gonadal development could be a consequence, rather than the cause; however, the role of this neuropeptide and the whole HPG axis in sex reversal induced by environmental stress is far from being elucidated.

In conclusion, the central role of the stress axis in the environmental response is a common mechanism, which is conserved in sex reversal and sex change in gonochoristic and hermaphrodite fish, respectively. Consequently, the main difference between these reproductive strategies of gonadal development could be the higher plasticity in the gonad of hermaphroditic fish. In gonochoristic fish, once the gonad has been differentiated, it acts as a suppressor mechanism to prevent transdifferentiation in adults (Nishimura and Tanaka, 2016), an essential property of hermaphrodite species. This key differential characteristic of the gonad between gonochoristic and hermaphroditic fish and similarities in brain response would establish the evolutionary conservation of a common response to an environmental change. At this point, most of the results obtained in the study of environmental sex determination are simple associations, wherefore further, more conclusive experiments using new methodologies are needed to distinguish cause from effect.

Disclosure summary

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