Review

Environmental stress-induced testis differentiation: Androgen as a by-product of cortisol inactivation

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A B S T R A C T

This review deals with the gonadal masculinization induced by thermal stress in fish with focus on the action of 11β-hydroxysteroid dehydrogenase (11β-HSD) as this mechanism key transducer. High temperatures have been reported to produce male-skewed sex ratios in several species with TSD (temperature-dependent sex determination), and in some of them, this process was reported to be associated with high levels of cortisol, the hormone-related stress in vertebrates, during early gonad development. In addition, in pejerrey larvae reared at high-masculinizing temperatures, 11-ketotestosterone (11-KT), the main and most potent androgen in fish, was also detected at high levels. In testicular explants, cortisol induced the synthesis of 11-KT, suggesting that its synthesis could be under the control of the stress axis at the time of gonadal fate determination. 11β-HSD is one of the enzymes shared by the glucocorticoid and androgen pathways; this enzyme converts cortisol to cortisone and also participates in the finals steps of the synthesis of the 11-oxigenated androgens. Based on these data and literature information, here we propose that the masculinization induced by thermal stress can be considered as a consequence of cortisol inactivation and the concomitant synthesis of 11-KT and discussing this as a possible mechanism of masculinization induced by different types of environmental stressors.

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1. Forewords

The manifestation of sex in vertebrates is the result of two closely and interrelated processes: sex determination and subsequent gonadal differentiation. Sex determination refers to the proximate factors that determine gonadal fate whereas gonadal differentiation is defined as the process by which a morphologically undifferentiated gonad is then transformed into an ovary or a testis through a series of molecular, cellular, and histological benchmarks (Penman and Piferrer, 2008).

Two major types of sex determination systems have been described in gonochoristic species: Genotypic (GSD) and Environmental (ESD) Sex Determination. In GSD species, sex is determined at fertilization and can involve a single "master sex-determining gene" located in a sex chromosome, or several genes on different chromosomes. Within teleosts, XX-XY GSD systems can be found as in medaka (*Oryzias latipes*) (Matsuda et al., 2002; Nanda et al., 2002), rainbow trout (*Onchorynchus mykiss*) (Yano et al., 2012), fugu (*Takifugu rubripes*) (Kamiya et al., 2012) and the Patagonian pejerrey (*Odontesthes hatcheri*) (Hattori et al.,

2012), among others. Some species such as the tilapias *Oreochromis* spp. have the ZZ-ZW GSD system which is common in birds. However, the master sex determining gene is not conserved implying that the trigger has emerged more than once during teleost evolution (Volff et al., 2003). On the other hand, teleosts present a variety of ESD strategies and therefore are interesting models to study the effect of the environment on the gonadal fate (Devlin and Nagahama, 2002; Kobayashi et al., 2013; Penman and Piferrer, 2008; Strüssmann et al., 2004; Strüssmann and Nakamura, 2002). Gonadal sex in ESD species is environmentally determined at an early and critical period of life (Barske and Capel, 2008; Devlin and Nagahama, 2002; Penman and Piferrer, 2008; Strüssmann and Nakamura, 2002; Valenzuela et al., 2003) and environmental influences during this period can affect the process of genetic determination and gonadal fate even in species with genotypic sex determinations as the Nile tilapia (*Oreochromis niloticus*) (Baroiller et al., 1995).

In this review we focus on the influence of the environment on sex determination through possible stressing factors. Thus, we put particular emphasis in reviewing the direct or indirect evidences of stress associated with environmental factors and, propose a unifying model for the triggering of environmental-dependent testicular differentiation.

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2. Forms of environmental sex determination in fish

2.1. Social interactions

One of the social factors that might affect sex determination in some fish species is the population density. This factor seems to be crucial for sex determination in Anguilliformes (Beullens et al., 1997; Colombo and Grandi, 1996; Chiba et al., 2002; Davey and Jellyman, 2005; Degani and Kushnirov, 1992; Egusa, 1979; Holmgren, 1996; Krueger and Oliveira, 1999; Tzchori et al., 2004). For example, in feral American eels (*Anguilla rostrata*), a catadromous fish, it was reported that low population densities are correlated with a high percentage of females (Krueger and Oliveira, 1999). Furthermore, rearing captive Japanese eel (*Anguilla japonica*) at a high density induced a male-biased sex ratio (Davey and Jellyman, 2005) while the sex ratio in wild populations was either balanced or varied widely (Chu et al., 2006; Davey and Jellyman, 2005). Chronic stress and high cortisol plasma levels in eels reared under high densities possibly reflect the fact that Japanese eels are solitary and territorial (Chiba et al., 2002) (Table 1). Nevertheless it should be taken into account the interaction between growth rate and density because both have been implicated in sex determination (Davey and Jellyman, 2005).

Although the concept of sex change is different to that of sex differentiation, both processes share some molecular mechanisms (Gardner et al., 2003; Wu et al., 2010). In this context, protandrous and protogynous hermaphroditic fish are also interesting models to study the sex inversion mechanism. Protandrous fish first develop as males and at some point in their lifespan change to females. Protogynous fish first develop as females and then change to males (Devlin and Nagahama, 2002). In protogynous fish such as the labrids *Coris julis* and *Thalassoma pavo* (Lejeune, 1987; Wernerus and Tessari, 1991) and the pomacanthid angelfish (*Centropyge potteni*) (Lutnesky, 1994), sex change is favored by high density. In contrast, sex inversion in the protandrous species *Gonostoma bathyphylum* is initiated when a critical low density is reached in the population (Badcock, 1986). Thus, these and other fish species can use behavioral interactions between sexes, size relative to peers, sex ratio, visual or chemical stimuli (e.g., pheromones), as key factors for sex change (Devlin and Nagahama, 2002; Ospina-Álvarez and Piferrer, 2008).

Some others examples of social control of sex determination can be found in the paradise fish (*Macropodus opercularis*, Anabantidae) (Francis, 1983, 1984), the Midas cichlid *Amphilophus citrinellus* (Cichlidae) (Francis, 1990; Francis and Barlow, 1993; Oldfield et al., 2006), the Okinawa rubble gobiid fish (*Trimma okinawae*) (Kobayashi et al., 2013), the bluehead wrasse, *Thalassoma bifasciatum* (Perry and Grober, 2003) and, in the coral coral-dwelling fish (*Gobiodon erythrospilus*) (Hobbs et al., 2004). However there are differences between species. In the paradise fish for example, the response varies according to density; individual isolation favors maleness differentiation, whereas grouping individuals induces a

Table 1
Summary of the current knowledge of glucocorticoids and sexual steroids related to environmental factors that direct the gonadal fate. An upward pointing arrow indicates an increase in gene expression, hormone concentration or enzyme activity; whereas a downward pointing arrow indicates a decrease in these parameters. "n/q": not quantified.

Species	Treatment	Glucocorticoid	Stress-related gene	Sex steroids	Sex steroid-related enzyme or genes	Physiological response and suggested mechanism	Citation
Anguilla anguilla (European eel)	Low density	n/q	n/q	n/q	↑ cyp19a1a	Feminization at low population density	Tzchori et al. (2004)
Danio rerio (zebrafish)	High Temperature	n/q	n/q	n/q	↓ cyp19a1a ↓ aromatase	Female-to-male sex inversion. Apoptosis of PGCs	Abozaid et al. (2012), Uchida et al. (2002, 2004)
	Hypoxia	n/q	n/q	↑ T/E2 ratio	↑ cyp11a ↑ cyp17 ↑ 3β-hsd ↓ cyp19a1a	Male-based sex ratio in hypoxic condition. Induction of PGCs apoptosis.	Baroiller et al. (2009), Shang and Wu (2004), Shang et al. (2006), Yamamoto (1969)
Dicentrarchus labrax (European sea bass)	High Temperature	n/q	n/q	n/q	↓ cyp19a1a	Masculinization at MPT. Hipermethylation of cyp19a1a promoter	Blazquez et al. (2009) Blázquez et al. (1998), Koumoundouros et al. (2002), Navarro-Martin et al. (2011), Pavlidis et al. (2000)
Odontesthes bonariensis (pejerrey)	Warm Temperature and cortisol treatments	↑ cortisol	↑ hsd11b2 = cyp11b1	↑ T ↑ 11-KT	↑ hsd11b2 = cyp11b1 ↓ cyp19a1a ↑ fshr	Masculinization at MPT and cortisol treatment. Apoptosis of gonadal cells. Elevation of 11-KT as a by-product of cortisol inactivation	Blazquez and Somoza (2010), Fernandino et al. (2011), Hattori et al. (2009), Shinoda et al. (2010), Strüssmann et al. (2008)
Oreochromis niloticus (Nile tilapia)	High temperature	n/q	n/q	n/q	↓ cyp19a1a	Masculinization at high temperature.	Baroiller et al. (1995)
Oryzias latipes (medaka)	High Temperature and cortisol treatments	↑ Cortisol	n/q	n/q	↑ fshr	Female-to-male sex inversions. Inhibition of female-type proliferation of PGCs	Hattori et al. (2007), Hayashi et al. (2010), Selim et al. (2009)
Paralichthys olivaceus (Japanese flounder)	High Temperature and cortisol treatments	↑ Cortisol	n/q	n/q	↓ cyp19a1a ↑ cyp26b1	Masculinization at high temperature. Delay in meiotic initiation of germ cells.	Kitano et al. (1999), Yamaguchi and Kitano (2012), Yamaguchi et al. (2010)
Pseudocrenilabrus multicolor victoriae (Egyptian mouth-brooder)	Hypoxia	n/q	n/q	↑ T/E2 ratio	n/q	Male-based sex ratio in hypoxic condition.	Friesen et al. (2012)

female differentiation (Francis, 1983). Nonetheless, selection for dominance increased the male proportion, suggesting a social more than density control of sex determination in this specie (Francis, 1984). In the Okinawa rubble goby, that present bi-directional sex change, a single isolated female changes sex whereas sex change was not observed in isolated males (Kobayashi et al., 2013). Both in the Midas cichlid as in the Okinawa rubble gobiid fish, relative body size is the main factor and also larger individuals suppress growth of smaller subordinates through behavioral interactions (Francis and Barlow, 1993; Manabe et al., 2008). Thus, in the Okinawa rubble gobidd fish when two females are grouped together, the larger one differentiates as a male, protogyny and, if two males are grouped together, the smaller one differentiate as a female, protandry (Manabe et al., 2007, 2008; Sunobe and Nakazono, 1993).

Interestingly, Perry and Grober (Perry and Grober, 2003) found that the process of behavioral sex change in the blue head wrasse is related to the social environment and modulated at the brain level. These authors suggest that the brain is sensitive to changes in cortisol levels which result from changes in the social environment (Perry and Grober, 2003), implying that sexual plasticity involves both, the gonads and the brain (Blazquez and Somoza, 2010). These results also suggest that stressing factors have to be taken into account in the relationship between social interaction and sex differentiation in different species.

2.2. Hypoxia and pH

The effect of low dissolved oxygen concentrations (hypoxia) on sex differentiation has also been studied; e.g., sex ratio of zebrafish (Danio rerio) reared under hypoxia was male-biased in comparison to those at normoxic conditions (Shang and Wu, 2004; Shang et al., 2006). It was recently proposed that the mechanism involved in hypoxia leading to the male-biased sex ratio is by modulating the expression of steroidogenic genes that produced an alteration of the Testosterone/17β-estradiol (T/E_2) balance (Yu et al., 2012). Earlier information available on the relation between hypoxia and reproductive disorders in fish supports this hypothesis (Breitburg et al., 2009; Pollock et al., 2010; Thomas and Rahman, 2012; Wu et al., 2003). In the African cichlid Egyptian mouth-brooder, Pseudocrenilabrus multicolor victoriae, for example, it was reported that fish exposed to hypoxic conditions exhibited higher levels of T than normally-oxygenated fish (Friesen et al., 2012); although the E_2 levels were not affected, the sex hormone balance favored T.

Baroiller and co-authors had previously hypothesized that, as in mammals, hypoxia can induce apoptosis of the primordial germ cells (PGCs) in zebrafish (Baroiller et al., 2009), and it is known that PGCs depletion is related to testicular differentiation (Fernandino et al., 2011; Hattori et al., 2009; Strüssmann et al., 2008; Tanaka et al., 2008; Uchida et al., 2002; Villamizar et al., 2012). In this species, which is an undifferentiated gonochorist, all fish first develop ovaries regardless of their genotypic sex. The genotypic males eventually undergo an apoptotic process leading to the loss of oocytes in the transition from an ovary to a testis (Uchida et al., 2002, 2004). Finally, hypoxia is likely perceived by the animals as an stressor, as other environmental factors, particularly at the earlier stages of development. Thus, a cortisol rise following hypoxic conditions was observed in rainbow trout at early stages of development (Fuzzen et al., 2011), suggesting that all these physiological steps are interrelated and active during this period.

The influence of pH on sexual differentiation has been studied in several species of Poeciliids, e.g. the swordtail (Xiphophorus helleri) and the blackbelly limia (Poecilia melanogaster), and in Chiclids (Apistogramma spp. and Pelvicachromis pulcher). In these species, acidic and alkaline conditions induced male- and female-biased sex ratios, respectively (Oldfield et al., 2006; Römer and Beisenherz, 1996; Rubin, 1985). In contrast, another Poeciliid (Poecilia sphenops) showed the opposite gender-bias effects in combination with rearing temperature (Barón et al., 2002).

2.3. Temperature

Among the effectors of ESD, water temperature is the most studied factor in teleosts (Temperature-dependent sex determination or TSD) (Baroiller et al., 2009; Kobayashi et al., 2013; Ospina-Álvarez and Piferrer, 2008; Sandra and Norma, 2010). In the Atherinopsidae family for instance, several species with TSD have been reported (Strüssmann et al., 2010; Strüssmann and Nakamura, 2002), including the pejerrey Odontesthes bonariensis (Strüssmann et al., 1997). In this species, female monosex populations can be obtained when larvae are maintained during the critical period of sex determination (between hatching and morphological gonadal differentiation) at low temperatures (13–19 °C) and mixed-sex populations are produced at intermediate temperature (24–25 °C). Conversely, all-male populations can be obtained at high temperatures (29 °C) (Strüssmann et al., 1997). The link between masculinizing temperatures and sex differentiation was elucidated in pejerrey (Fernandino et al., 2012; Hattori et al., 2009). Others atherinopsid fish in which temperature can affect in different degrees the sex determination process are Menidia menidia (Conover and Fleisher, 1986; Duffy et al., 2010), Menidia peninsulae (Middaugh and Hemmer, 1987), Odontesthes argentinensis (Strüssmann et al., 1996), and O. hatcheri (Strüssmann et al., 1996).

Extreme temperatures can affect gonadal fate in many other species (Penman and Piferrer, 2008; Sarre et al., 2004). For example, in medaka, a differentiated gonochoristic fish with a well characterized GSD (Matsuda et al., 2002; Nanda et al., 2002), phenotypic males can be produced from genotypic females when the eggs are exposed to warm temperatures during embryonic development (Hattori et al., 2007). This female-to-male sex inversion can be produced by an inhibition of PGCs proliferation (Selim et al., 2009) and high cortisol levels (Hayashi et al., 2010). Temperature can also affect gonadal fate in zebrafish (Abozaid et al., 2012) and genotypic females exposed to high temperatures differentiated as males (Uchida et al., 2002, 2004) (Table 1). Similar results have been obtained in Japanese (Paralichthys olivaceus) and Southern flounders (Paralichthys lethostigma) (Kitano et al., 1999; Luckenbach et al., 2003; Yamaguchi and Kitano, 2012; Yamaguchi et al., 2010), Nile tilapia (Abucay et al., 1999; Baroiller et al., 1995, 1996, 2009; Tessema et al., 2006; Wessels and Hörstgen-Schwark, 2007) and European sea bass (Dicentrarchus labrax L) (Blazquez et al., 2009; Blázquez et al., 1998; Koumoundouros et al., 2002; Navarro-Martin et al., 2011; Pavlidis et al., 2000).

3. Sexual steroids and the canalization of the gonad

Steroidogenesis is the biochemical process by which cholesterol is converted into steroid hormones, including sex steroids, glucocorticoids and mineralocorticoids by a battery of oxidative enzymes in several steroidogenic organs like the gonads, the interrenal gland, and the brain (Blazquez and Somoza, 2010; Wendelaar Bonga, 1997). Sex steroids are related to sex differentiation in different vertebrate taxa (Morohashi et al., 2013; Nakamura, 2010).

Generally, estrogens and androgens have been associated with female and male traits, respectively, as well as with gametogenesis and the development of secondary sexual characteristics. Although steroidogenesis consists of a series of interdependent metabolic pathways, the relationship between androgens and estrogens is particularly close; estrogen synthesis is totally dependent on the supply of an androgen substrate, T (Fig. 1). This is because T is at

the metabolic crossroad of two enzymes, aromatase (P450arom) and 11β-hydroxylase P450 (P45011β). These two enzymes produce E_2, the main bioactive estrogen in fish, and 11β-hydroxytestosterone (11β-OHT) respectively. The latter is subsequently converted into 11-ketotestosterone (11KT, the principal bioactive androgen in fish) by the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). This crossroad points to the need for a coordinated regulation of the respective enzymes' activities to catalyze the synthesis of androgens and/or estrogens (Yaron, 1995).

In the gonads of developing embryos/larvae, steroid-producing cells express the steroidogenic enzymes related genes and differentially synthesize their respective sex steroids that can be detected even before the morphological differentiation of the gonads (Devlin and Nagahama, 2002; Matsuda, 2003; Nakamura, 2010; Yamamoto, 1969). In this context, two hypotheses have been put forth with respect to the influence of steroids on the gonadal differentiation in teleosts. The first one considers that the development of a sexually undifferentiated gonad into an ovary or a testis depends on the differential activity of gonadal aromatase (P450arom) or P45011β/11β-HSD2, respectively. Thus ovarian differentiation begins by the action of estrogens while testicular differentiation by the action of 11-oxygenated androgens (Baroiller et al., 2009; Guiguen et al., 2010; Nakamura, 2010). The alternative hypothesis proposes that the expression and activity of gonadal aromatase induces ovarian differentiation, meanwhile its down-regulation and/or inhibition of its activity is related to testicular differentiation, and androgens do not participate in early testicular differentiation being a consequence of this process (Guiguen et al., 2010). Beyond these two assumptions, it is well known that the sex of the fish is labile to hormone treatments at a critical time early in life, suggesting their importance in these processes. After this "labile period", stronger and/or longer treatments may be necessary to change the direction of gonadal development once it has been set in motion (Baroiller et al., 1999; Piferrer, 2001).

4. Glucocorticoids and sex determination

In teleost fish the production of glucocorticoids by the interrenal gland is primarily regulated by the adrenocorticotropic hormone (ACTH), whose release is under control of the hypothalamic peptide corticotrophin releasing hormone (CRH) (Mommsen et al., 1999; Wendelaar Bonga, 1997). The main glucocorticoid in teleost fish is cortisol which has an important role on modulation of the adaptive intermediary metabolism (Vijayan et al., 1994), ionic regulation (Sakamoto and McCormick, 2006), and immune function (Salas-Leiton et al., 1858). The glucocorticoids mechanism of action involves their passage through the plasma membrane, and subsequent binding to cytoplasmic receptors. The hormone-receptor complex is then transported to the nucleus, where it acts as a ligand-dependent transcription factor on the transactivation or repression of glucocorticoid responsive genes, by binding to glucocorticoid response elements in the regulatory DNA sequences (Bury and Sturm, 2007). In teleosts, two paralog genes that code glucocorticoid receptors have been described (Bury and Sturm, 2007; Greenwood et al., 2003), with the only exception of zebrafish that presents one variant (Schaaf et al., 2009).

Besides the well-known effects of stress on reproduction (Aluru and Vijayan, 2009; Consten et al., 2002; Milla et al., 2009; Mommsen et al., 1999; Schreck, 2010; Wendelaar Bonga, 1997), recently, the effects of cortisol on gonadal differentiation have begun to receive attention. In this respect, and as previously described, each of the already mentioned environmental factors that trigger sex determination can be considered as potential stressors and, in most cases they can be correlated to a male-biased sex ratio. Unfortunately, cortisol and/or androgens plasma levels during the sex determination and gonadal differentiation period were examined only in very few species (Table 1). In the pejerrey, a TSD specie, the role of cortisol in gonadal differentiation was examined under different temperatures. The results revealed high
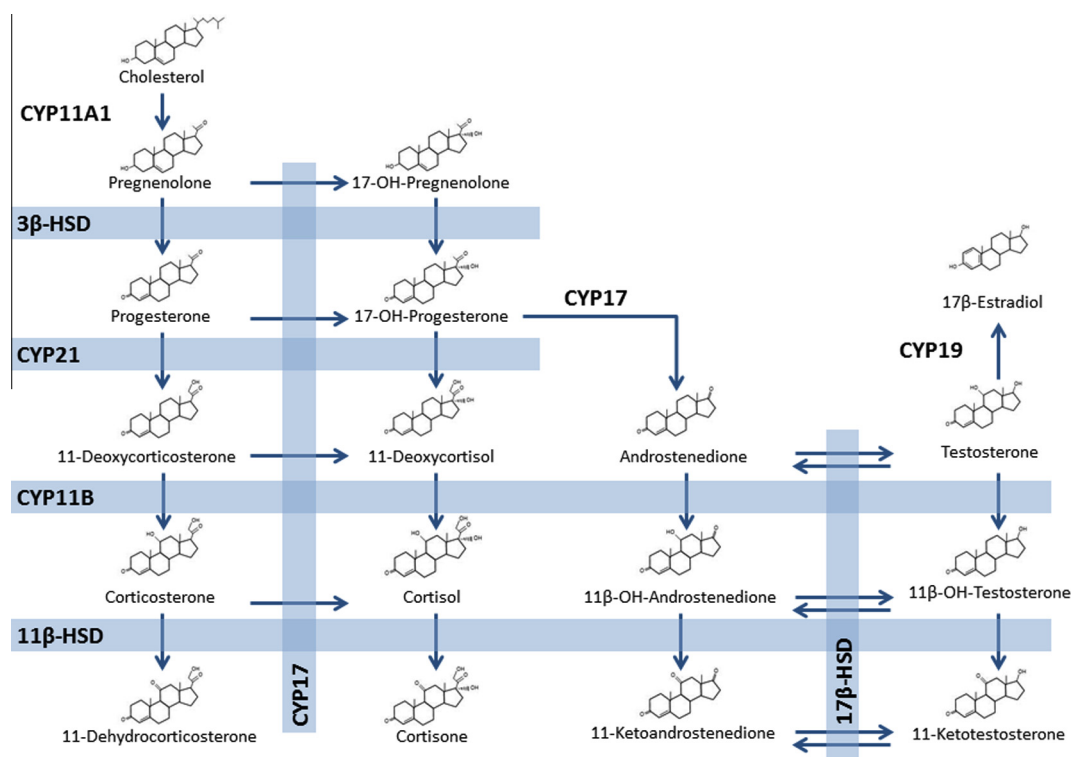

Fig. 1. Simplified pathways of biosynthesis of sex steroids and glucocorticoids in fish. The steroidogenic enzymes are indicated by arrows and are divided into two classes, the hydroxysteroid dehydrogenases (3β-HSD; 11β-HSD, 17β-HSD), and the cytochrome P proteins (CYP followed by the specification of the family, subfamily and, where necessary, polypeptide). The 11β-HSD, as others enzymes, is shared by the glucocorticoid and androgen pathways (light blue rectangles).

cortisol levels in larvae reared at warm, male-inducing temperatures, that at lower temperature (Hattori et al., 2009). In the same species, cortisol and the cortisol agonist dexamethasone induced male-biased sex-ratios in larvae reared at an intermediate temperature that is generally associated with balanced sex ratios. Interestingly high levels of T and 11-KT were quantified in these groups with respect to their respective controls (Hattori et al., 2009).

The role of glucocorticoids on the gonadal fate at masculinizing temperatures was also evidenced in other teleost species as Japanese flounder and medaka (Hayashi et al., 2010; Yamaguchi et al., 2010), suggesting a common mechanism among these species and probably in other fish groups. However, the direct effect(s) of cortisol responsible for triggering the male pathway is(are) still not clearly elucidated. Three possibilities are under consideration: (i) cortisol inhibits aromatase expression, (ii) cortisol causes reduction in the number of PGCs and, (iii) cortisol affects 11KT synthesis.

The first possibility was studied in pejerrey and Japanese flounder where was demonstrated that during cortisol-induced masculinization, cyp19a1a (gonadal aromatase) expression was downregulated (Hattori et al., 2009; Yamaguchi et al., 2010). Also, in the Japanese flounder, cortisol directly suppressed cyp19a1a transcription in vitro by binding to glucocorticoid responsive element in its promoter region (Yamaguchi et al., 2010). Nevertheless, it has to be taken into account that high temperature can also induce masculinization via epigenetic regulation of cyp19a1a. For instance, in the European sea bass hyper-methylation of cyp19a1a promoter down-regulated aromatase expression and then inhibited the female pathway (Navarro-Martin et al., 2011). Thus, in both species, the final result is the down-regulation of cyp19a1a expression, although under the control of different mechanisms.

Cortisol can produce depletion in the number of PGCs/GCs (primordial germ cells/germ cells) or a delay in the beginning of PGC proliferation. This would result in a low relation between PGCs and somatic cells (SC) in the gonad, which seems to be critical to direct the masculinization pathway as shown in medaka (Tanaka

et al., 2008). It is also well documented that apoptosis is involved in testicular differentiation in pejerrey and zebrafish (Fernandino et al., 2011; Hattori et al., 2009; Strüssmann et al., 2008; Uchida et al., 2002; Villamizar et al., 2012). In pejerrey, treatment with cortisol or high temperatures induced a male-biased sex ratio and an increased incidence of apoptosis in the gonadal primordia (Fernandino et al., 2011; Hattori et al., 2009; Strüssmann et al., 2008). Also, the induction of PGC apoptosis during gonadal differentiation produced masculinization in medaka (Nakamura et al., 2012; Saito et al., 2007). Another way to depress PGC/SC ratio during gonadal differentiation is to delay the beginning of PGC proliferation. The proliferation of PGCs in females has been well studied in medaka (Saito and Tanaka, 2009); in this species PGCs in the ovary start the proliferation of PGC around stage 37, close to the time of hatching, whereas in putative testes their proliferation starts only 20–30 days after hatching (Saito and Tanaka, 2009). The high temperature treatment produces a delay in the proliferation of the XX gonads (Selim et al., 2009), increasing cortisol levels (Hayashi et al., 2010) and inducing masculinization.

In pejerrey masculinized by cortisol treatment, both, the inhibition of aromatase and the induction of gonadal apoptosis were detected during the gonadal differentiation period (Fig. 2, Table 1) (Hattori et al., 2009; Strüssmann et al., 2008). Other characteristic of larvae reared at masculinizing temperatures, which were observed during the critical period of sex determination and therefore before the two previously mentioned event, consisted in high cortisol, T, and 11-KT levels (Hattori et al., 2009). These observations and the fact that both androgen have been shown to have a masculinizing effect in several teleosts (Devlin and Nagahama, 2002), hinted at a third possibility and that is that the early increase in these three steroids is the actual link between the stress axis and high-temperature-induced male differentiation. In this scenario, the inhibition of aromatase expression and induction of apoptosis could be the consequence of the increase in these androgens, perhaps to reinsure the masculinization triggered through cortisol.
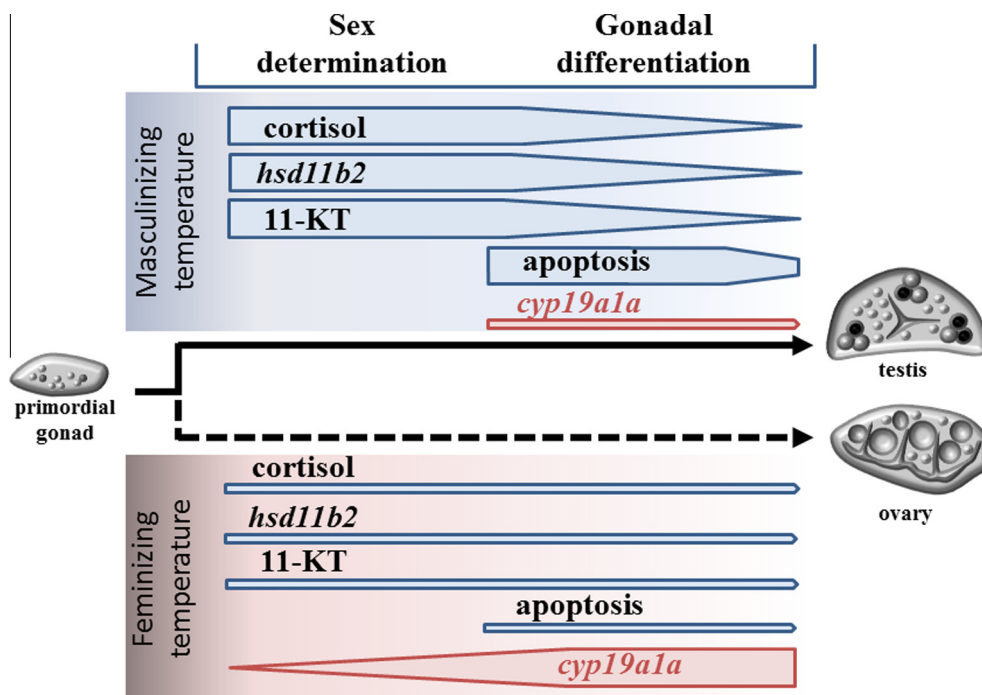

Fig. 2. Schematic representation of the temporal appearance and relative magnitude of whole-body cortisol and 11-KT levels, gonadal apoptosis, hsd11b2 and cyp19a1a mRNA expression in pejerrey larvae reared at masculinizing and feminizing temperatures. The results obtained in this species highlight a clear temporal hierarchy of events.

5. The glucocorticoid and androgen pathways cross talk

Although the essential role of estrogens on ovary differentiation is widely accepted, the participation of androgens in testicular differentiation is still controversial. Some authors consider them consequences of the testicular differentiation other than the inductors of masculinization (Guiguen, 2000; Guiguen et al., 2010; Ijiri et al., 2008; Vizziano et al., 2008); however, the exposure to androgens during early stages of fish development, does lead to masculinization, suggesting that they have a key role in the sex differentiation process (Devlin and Nagahama, 2002). Furthermore, either 11-oxygenated androgens or the expression of enzymes involved in their synthesis, have been found during the critical period of sex determination in different species (Hattori et al., 2009; Miura et al., 2008).

The synthesis of 11-oxygenated androgens has a direct relation to cortisol, because their synthesis and inactivation are performed by the same steroidogenic machinery. These enzymes are the 11β-hydroxylase (CYP11B) and the 11-βhydroxysteroid dehydrogenase (11β-HSD; Fig. 1). The first one catalyzes the 11-hydroxylation of steroids such as the androgens T and androstenedione (Δ4) to 11-hydroxy-testosterone (11-OH-T) and 11-hydroxy-androstenedione (11-OH-Δ4) respectively (Borg, 1994; Lokman et al., 2002). In fish, 11-OH-Δ4 has been considered as the principal steroid produced by the gonads whereas 11-KT is the predominant plasma androgen (Borg, 1994; Cavaco et al., 1997; Tesone and Charreau, 1980). Nonetheless, the expression of the gene that codifies for CYP11B showed an increase in males only at later stages of morphological gonad differentiation (Blasco et al., 2010; Raghuveer et al., 2011; Socorro et al., 2007). These observations suggest that larvae during the sex differentiation present accumulation of 11-OH-Δ4 that then, under the action of a stimulus, e.g. an environmental factors, this hormone would be converted into 11-ketoandrostenedione (11-KΔ4) by the 11β-HSD and finally to 11-KT by the 17β-hydroxysteroid dehydrogenase (17β-HSD). Based on this information, strong candidate enzyme for regulation of environmental sex determination is the 11β-HSD.

As already stated, 11β-HSD plays a key role in the synthesis of 11-oxygenated androgens and it is also known to be involved in the metabolism of glucocorticoids (Fig. 1) (Bury and Sturm, 2007; Oppermann et al., 1997). In mammals, two types of 11β-HSD are reported: the 11β-HSD1 type, which acts as an 11-oxo-reductase NADP(H)-dependent enzyme in the conversion of cortisone to cortisol, and the 11β-HSD2 type which acts as an oxidase NAD+-dependent enzyme that converts the active ligand cortisol to cortisone, an inactive form unable to bind to glucocorticoid receptors (Albiston et al., 1994). In fish, only one 11β-HSD has been described and it is considered to be the homologue of the mammalian 11β-HSD2 (Baker, 2004) acting on the conversion of cortisol to cortisone in the glucocorticoid metabolism and in the 11-oxygenate androgen synthesis pathway (Fig. 1) (Borg, 1994; Lokman et al., 2002). In rainbow trout, high levels of the gene codifying for 11β-HSD (hsd11b2) were reported in Leydig cells, suggesting that one of the roles of the 11β-HSD in the gonads is to catalyze 11-KT production and to protect the testicular tissue from circulating cortisol (Kusakabe et al., 2003). So, 11β-HSD seems to be important for the final step of the major fish androgen biosynthesis, the 11-KT, although the relationship with the glucocorticoid metabolism has not been fully elucidated.

Recently, our group proposed the 11β-HSD as the key enzyme in the warm temperature-induced masculinization in pejerrey (Fernandino et al., 2012). In that work we observed that hsd11b2 mRNAs increased in presumable Leydig cells during the sex determination period at a male-producing temperature. These data suggested that the enzymatic machinery necessary for the local production of 11-oxygenated steroids was already active in the undifferentiated gonads during this critical period (Fig. 3). As the effects of steroid are mediated through specific intracellular
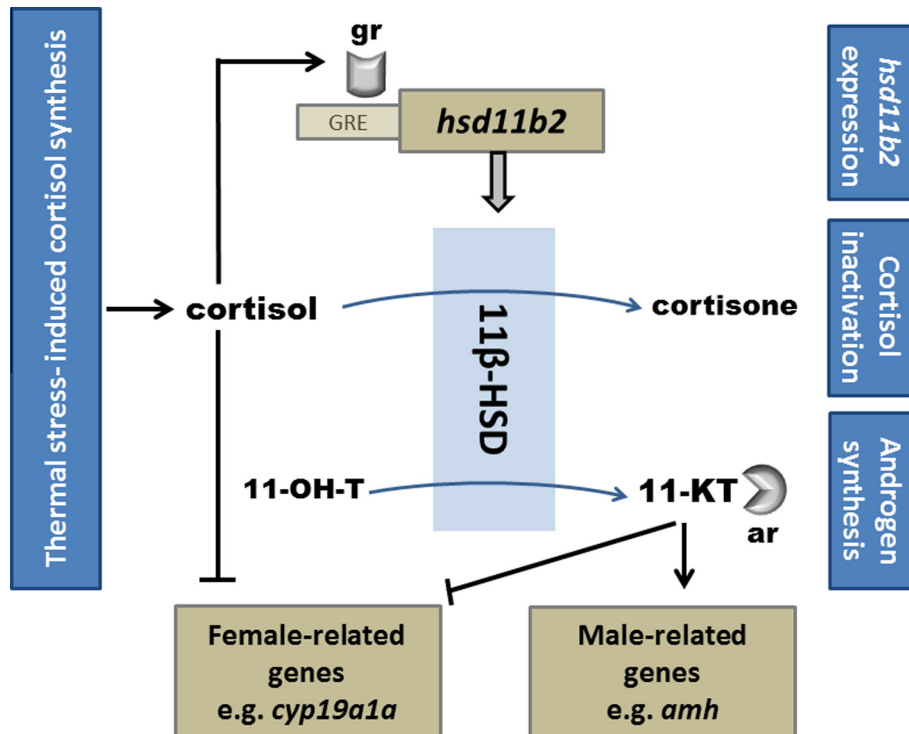

Fig. 3. Proposed mechanism of the temperature-induced masculinization in pejerrey. Larvae exposed to warm temperatures present high levels of cortisol increasing the hsd11b2 expression during the sex determination period. The high levels of 11-KT are due by the inactivation of cortisol by 11β-HSD, which is shared by both pathways (light blue rectangles). The expression of the gr1 and the ar1 also increase during the critical period. Arrows indicate direct or indirect induction; whereas inhibition is indicated by a bar at the end of the lines.

receptors (Alsop and Vijayan, 2008; Beato and Klug, 2000), the expression of the respective receptors was also measured. The results showed that the glucocorticoid receptor 1 (gr1) and androgen receptor 1 (ar1) were highly expressed during the same period compared to larvae at female-producing temperatures, suggesting that the gonad also show responsiveness to the ligands. In conclusion, that study showed that glucocorticoid and androgen signaling are triggered and active during the sex determination period in pejerrey.

It is interesting that cortisol can induce the 11-KT production and spermatogenesis in testes explants of Japanese eel (Ozaki et al., 2006). In pejerrey, treatment of testes with cortisol also up-regulated the synthesis of 11-KT in vitro (Fernandino et al., 2012). Moreover, cortisol was able to enhance 11-KT production in testis explants at low doses, whereas higher dosages suppressed this effect, presenting a non-monotonic dose-response curve (Vandenberg et al., 2012). A first explanation for the effects at higher doses is that cortisol acts on testicular steroidogenesis by competing for the enzymes involved in 11-KT synthesis, as suggested for common carp Cyprinus carpio L. (Consten et al., 2002) while at low (optimal?) doses the exposition to cortisol produces an up-regulation of the 11-KT synthesis (Fig. 3). These results suggest that a certain level of cortisol is necessary to induce 11-KT synthesis and that this response would be species-specific.

6. Conclusion and perspectives

The data presented in this review on the environmental factors that induce masculinization in fish suggest three possible mechanism of action: (i) cortisol inhibits aromatase expression, (ii) cortisol leads to depletion of PGCs and, (iii) there is a cross talk between the glucocorticoid and androgen pathways, with androgens as the final inducers of masculinization. At least in pejerrey these three hypotheses are not necessary mutually exclusive and all of them reach the same end, which is the masculinization of the gonad, but with temporal hierarchy.

In this respect, pejerrey larvae have the capacity to recover after a stressor stimulus that induced cortisol release by inactivating it to cortisone. One of the enzymes that modulate this process is 11β-HSD, which is shared by the glucocorticoid and androgen pathways Thus, the cross talk among stress and masculinization through the key enzyme 11β-HSD could be the transducer between cortisol and androgen synthesis in temperature-induced masculinization. Inhibition of aromatase expression and depletion of PGCs could be mechanisms to reinsure the masculinization process. Thus, the temporal hierarchy observed in pejerrey provides a novel perspective on the mechanism of environmental-induced masculinization. This mechanism need to be analyzed for other forms of environmentally induced masculinization.

Furthermore, the association between glucocorticoids and testicular differentiation could be important from the viewpoint of endocrine disruption. Glucocorticoid and glucocorticoid-like activity was found in different effluents and surface water samples (Chang et al., 2007; Fan et al., 2011; Herrero et al., 2012; Stavreva et al., 2012). Moreover taking into account that anti-stress compounds targeting glucocorticoid receptor are among the most frequently prescribed drugs worldwide (Lu et al., 2006), it might seem advisable to consider their effects on gonadal sex differentiation and masculinization in fish and perhaps other aquatic vertebrates as new form of endocrine disruption.

Disclosure summary

The authors have nothing to disclose.

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