ACUTE STRESS, HYPOTHALAMIC-HYPOPHYSEAL-GONADAL AXIS AND TESTICULAR FUNCTION – A REVIEW

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Abstract

The fact that the stress response affects testicular function in several animal species, has been established for several decades. The aim of the present review was to describe how the mechanisms involved in the acute stress response affect directly or indirectly the testicular function at different levels. Both neuroendocrine axes linked to the stress response, i.e., the sympathetic-adrenomedullar (SAM) axis and the hypothalamic-hypophyseal-adrenal (HHA) axis, affect the functioning of the hypothalamus-hypophyseal-gonadal (HHG) axis. Furthermore, both axes affect testicular function directly. Several reports support the concept that, in general terms, the HHA axis (as well as the hormones belonging to it) inhibits the HHG axis and also has direct effects on testicular function. The information regarding the effects of the SAM axis either on the HHG axis or as direct effects on testicular function are contradictory, and depends on whether the studies were performed *in vivo* or *in vitro*. Nevertheless, in general, stimulative effects are reported by the SAM axis. Both hormonal axes linked to the stress response exert direct effects on testicular function, on Leydig, Sertoli, myoid, and germ cells.

Key words: stress response, hypothalamus, hypophysis, testis

Over the last decades, it has been well established that the stress response affects reproduction, including testicular function, in several animal species (see reviews: Rivier and Rivest, 1991; Sapolsky et al., 2000; Hardy et al., 2005; Chichinadze and Chichinadze, 2008; Breen and Mellon, 2014). However, the mentioned reviews have only partially analysed the effects of the acute stress response on the hypothalamic-hypophyseal-gonadal (HPG) axis and testicular function (some made more emphasis on the hypothalamus-hypophyseal-adrenal axis and others rather on the sympathetic-adrenomedullar axis). None of these reviews offers an integrated global view of this process, nor its specific effects at different levels, especially on testicular cell types. Therefore, the aim of the present review is to describe how the mechanisms

involved in the acute stress response affect directly or indirectly the testicular function at different levels, using an integrative approach. Particular interest is dedicated to catecholamines mediated (adrenaline and noradrenaline) and cortisol mediated mechanisms.

Stress and the stress response

Stress is defined as a 'state of homeostatic imbalance' (Sapolsky, 2004), induced by stimuli, noxious or events known as 'stressors' (Möstl and Palme, 2002; Romero, 2004; De Kloet et al., 2005). The stress response is the physiological response that the organism generates when exposed to stressors and it is aimed at reestablishing homeostasis and returning to normal function (Moberg, 2000). Two main systems or axes are activated during the stress response. The axis to be activated earlier in response to a stressor includes the sympathetic portion of the autonomous nerve system, which stimulates the adrenal medulla to secrete catecholamines (adrenaline and noradrenaline) (Moberg, 2000; Sapolsky et al., 2000). This system is known as the sympathetic-adrenomedullar (SAM) axis. The second system to respond (slower over time) is the hypothalamic-hypophyseal-adrenal (HHA) axis (Sapolsky et al., 2000). When the HHA axis is activated in response to a stressor, the neurons in the hypothalamic paraventricular nucleus secrete a peptide known as corticotropin releasing factor (CRH). The CRH stimulates the adenohypophyseal secretion of adrenocorticotrope hormone (ACTH) which, once secreted into peripheral blood, stimulates the adrenal cortex into secreting glucocorticoids (GC) (cortisol or corticosterone, depending on the species) (Korte et al., 2005). In summary, the increase in plasmatic catecholamines and cortisol releases responses which enhance body energy availability, as glucose, lipids and amino acids in blood by stimulating glycogenolysis, gluconeogenesis, lipolysis and proteolysis (Sapolsky et al., 2000), facilitating adequate responses to the emergency generated by the stressor (Ewing et al., 1999).

Hypothalamic-hypophyseal-gonadal axis

It is well known that testicular function is controlled by the hypothalamic-hypophyseal-gonadal axis (HHG) (Knobil and Neill, 2006). The hypothalamus secretes the gonadotropin releasing hormone (GnRH), which stimulates pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Figure 1) (Hall et al., 1988; Knobil and Neill, 2006). Both gonadotropins are secreted into the peripheral blood and reach the testis, where they provoke several responses. Stimulation of steroidogenesis by testicular Leydig cells is the main function of LH in males (Purvis and Hansson, 1978; Feldman and Nelson, 2007), whereas FSH stimulates spermatogenesis, via Sertoli cell function stimulation (Knobil and Neill, 2006; Feldman and Nelson, 2007). Testosterone, estrogens and inhibin as well as other hormones, are secreted from the testis into the bloodstream and once they reach the hypothalamus and pituitary gland exert negative feedback on the release of GnRH, LH and FSH (Amann, 1993; Feldman and Nelson, 2007; Bilezikjian et al., 2012). Activin, also testes secreted, acts at two different levels, first on the hypothalamus stimulating the release of GnRH, and second on the pituitary gland stimulating FSH secretion by the

gonadotrope cells (Carroll et al., 1991; MacConell et al., 1999; Feldman and Nelson, 2007; Bilezikjian et al., 2012).

Figure 1. Model of the effect of stress hormones (CRH: corticotropin releasing factor, ACTH: adrenocorticotrophic hormone, Gcs: glucocorticoids, and catecholamines) on hypothalamic-hypophyseal-gonadal axis and testicular cells

Stress, hypothalamic-hypophyseal-gonadal axis and testosterone secretion

Several authors, studying different animal species and stressors, reported that plasma testosterone concentrations decrease after applying the stressor: surgical stress in rats (Gray et al., 1978); electroejaculation-associated stress in rams (Damián and Ungerfeld, 2011) and bulls (Welsh and Johnson, 1981); movement restriction stress in rams (Stellflug et al., 2006); immobilization stress in rats (Stojkov et al., 2012); stress linked to capture and immobilization in monkeys (Sapolsky et al., 1985); capture-induced stress in birds (Deviche et al., 2010). The drop in testosterone secretion due to stress can be provoked directly or indirectly at different levels of the HHG, hypothalamic, pituitary and gonadal level, which are discussed below and separately in each level.

Effects of stress at the hypothalamic level

Gambacciani et al. (1986) performed *in vitro* studies on male rats hypothalamus and reported that CRH administration inhibited GnRH secretion. Nikolarakis et al. (1986) noticed that the inhibitory effect of CRH on GnRH secretion in hypothalamic sections (*in vitro*) is dose-dependent. Furthermore, synaptic contact between axonic endings of CRH-reactive neurons and dendrites of neurons reactive to GnRH has been reported (MacLusky et al., 1988), suggesting that the inhibitory effect of CRH on GnRH secretion can occur directly on GnRH-releasing neurons. Glucocorticoid

receptors (GR) were identified in hypothalamic neurons that secrete GnRH (Ahima and Harlan, 1992). Dubey and Plant (1985) reported that in rhesus monkeys, GC inhibit GnRH secretion. The expression of GnRH mRNA and the secretion of GnRH on the GT1 cell line (which both expresses GnRH mRNA and secretes GnRH) can be inhibited by GC (Attardi et al., 1997). Similar results were reported by Gore et al. (2006), who found that treating adult male rats with corticosterone decreases GnRH mRNA levels.

Moreover, several studies reported that catecholamines stimulate GnRH secretion (Kamberi, 1974; McCann and Moss, 1975; Kalra, 1977; Ojeda et al., 1979; Barraclough and Wise, 1982; Parvizi and Ellendorff, 1982; Clarke and Pompolo, 2005). According to Kamberi (1974), this effect of catecholamines stimulating GnRH secretion “seems to be linked to the α -adrenergic receptors of the hypothalamus”. Histological studies demonstrated two sites of action and regulation of catecholamines on the release of GnRH. One site of action has been shown by colocalization of GnRH and norepinephrine neurons (Kalra and Kalra, 1983); furthermore, GnRH neurons are in contact with catecholamine axons (with norepinephrine probably stimulating and dopamine probably inhibiting GnRH activity) (Hoffman et al., 1982). These results demonstrate a direct action of catecholamines on GnRH neurons. The second site of action is the tuberoinfundibular dopamine system, whereby dopamine inhibits prolactin secretion, and thus facilitates gonadotrophin secretion by acting either at the pituitary level, GnRH cell, or both (Hoffman et al., 1982). In addition, epinephrine innervations were visible in the periventricular area of the hypothalamus, paraventricular nucleus and supraoptic nucleus (Kalra and Kalra, 1983). However, Negro-Vilar et al. (1979) observed no evidence of stimulation of GnRH release by epinephrine in studies *in vitro* from the median eminence of the hypothalamus of normal male rats.

Effects of stress at the pituitary gland level

In several works (*in vivo* or *in vitro*) it has been observed that the major hormones of the HHA axis (CRH, ACTH and GC) affect the release of LH. Rivier and Vale (1984) showed that intracerebroventricular CRH administration in rats decreases plasma LH. Then, Rivier et al. (1986) reported that intracerebroventricular administration of CRH antagonist blocked stress-induced decreases in plasma LH concentrations. In addition, intravenous administration of CRH to rhesus monkeys decreased both plasma LH and FSH, and this effect was independent of GC secretion (Olster and Ferin, 1987; Gindoff and Ferin, 1987; Xiao et al., 1989). The LH secretion in response to GnRH administration was inhibited by previous administration of GC in both bulls (Chantaprateep and Thibier, 1978) and male rats (Rosen et al., 1988), as well as by a previous dosage of ACTH in rams (Fuquay and Moberg, 1980, 1983; Matteri et al., 1984). Rosen et al. (1991) demonstrated that a GC treatment inhibited the GnRH-induced expression of LH mRNAs. Furthermore, GR were also identified in pituitary gonadotroph cells of rat (Kononen et al., 1992, 1993) and mouse (Breen et al., 2012). Briski (1995) reported GR mediates an inhibitory effect on LH release in intact male rats. This was confirmed by Breen and Karsch (2004) and Breen et al. (2008), who found that the GC receptor is implicated in the direct pituitary effect of

GC. Li and Wagner (1983), performing an *in vitro* study with bovine pituitary cells, observed that GC decreased GnRH-induced LH release. Similar results were observed by Kamel and Kubajak (1987), who reported that GC inhibit LH secretion in rat primary pituitary cell cultures. In another *in vitro* study, in ram pituitary cultured cells, CRH and ACTH inhibited GnRH-induced LH release (Smart, 1994). Recently, Breen et al. (2012) demonstrated that glucocorticoids suppress GnRH induction of LH gene expression, acting directly upon the anterior pituitary gonadotrope cells at genomic levels. Based on all this information we conclude that the main hormones of the HHA axis (CRH, ACTH and GC) had negative effects on the release of LH and can act directly at the pituitary level.

Unlike the clear effects of GC on the LH release at pituitary level, the effects of GC on the release of FSH may be contrasting. For example, CRH and GC administration inhibited GnRH-induced FSH release in cultured pig pituitary cells (Li, 1993). However, subsequent studies reported a positive effect of GC on the release of FSH in rat pituitary cultures (Kilen et al., 1996; Bohnsack et al., 2000; Leal et al., 2003). Furthermore, GC induce transcription of FSH in the pituitary of male rats (McAndrews et al., 1994; Breen and Mellon, 2014).

Recently, Phogat and Parvizi (2010) reported that an ACTH treatment provoked an increase in LH release in male pigs. However, a previous study carried out in sows reported a decrement in LH release after an ACTH treatment (Brandt et al., 2007). Interestingly, gender differences might explain differences in LH release in response to an ACTH treatment, as Van Lier et al. (1999) had reported for sheep. Although these works (performed in pigs and sheep) demonstrated ACTH influenced LH release, it was not possible to establish whether ACTH effects were exerted at the hypothalamic or at the pituitary level, since both were *in vivo* studies which did not use a challenge with GnRH. In this regard, Tilbrook et al. (1999) showed that a treatment with ACTH inhibited the LH response to GnRH in both gonadectomized rams and ewes, with no differences between sexes. However, they also observed that isolation/restraint stress decreased LH release to GnRH challenge in gonadectomized rams but not in gonadectomized ewes, suggesting also that this effect is independent of sex steroids. Based on this information, we suggest that the sex effect in the pituitary response to GnRH differs according to the type of stressor.

The effects of catecholamines at the pituitary level are contradictory and depend on whether the studies were performed *in vivo* or *in vitro*. *In vivo* catecholamines treatment induced the release of LH during acute stress in male rats, and this effect was mediated through activation of alpha 2-adrenergic receptors (Martín et al., 1995). Moreover, in studies *in vitro* it has been reported that catecholamines increase the LH and FSH release only when administered intraventricularly or when anterior pituitary halves have been co-incubated in the presence of ventral hypothalamic fragments (Kamberi et al., 1970; Kamberi, 1974). However, when catecholamines were infused directly into the anterior pituitary via a cannulated hypophyseal portal vessel or when anterior pituitary halves were incubated without any hypothalamic fragment, no effect was observed (Kamberi et al., 1970; 1971; Kamberi, 1974; Barraclough and Wise, 1982). These experiments show that catecholamines increase the LH and FSH release by acting at the hypothalamic level rather than at the pitui-

tary level. Moreover, Li (1989) reported that GnRH-induced secretion of LH decreased when cultured pituitary cells were exposed to either adrenaline or noradrenaline.

Effects of stress at the gonadal (testicular) level

Effects on Leydig cells

Epithelial, endocrine Leydig cells are the main source of androgens in males, and secrete discrete amounts of estrogens and oxytocin, as well as a number of paracrine factors regulating Sertoli cell activity. It is well known that both GnRH and LH levels control serum testosterone secretion (Knobil and Neill, 2006). However, the stress response can affect testosterone secretion directly at the testicular level as well.

Several studies have reported that Leydig cells express GR (Evain et al., 1976; Stalker et al., 1991; Ge et al., 1997; Hu et al., 2008), which could mediate the stress-induced inhibition of testosterone secretion. Bambino and Hsueh (1981) reported both *in vitro* and *in vivo* studies of a direct inhibitory effect of GC on testicular steroidogenesis mediated through a decrement in testicular LH receptor content. Charpenet et al. (1982) found that movement restriction-induced stress in rats provoked both a decrease in gonadotropin sensitivity of Leydig cells and in testosterone secretion, with no change in serum LH levels. Moreover, Orr and Mann (1990) reported that the decrease in testosterone levels due to immobilization-induced stress in rats is LH independent, since it does not affect LH levels nor the binding capacity of LH receptors in Leydig cells. Two years later, these same authors reported that during immobilization-induced stress in rats, the increase of plasma GC acting via GR on Leydig cells suppresses the testicular response to gonadotropins, and consequently provokes a decrease in testosterone secretion (Orr and Mann, 1992). However, hydrocortisone (a GC) stimulated testosterone production in porcine Leydig cells (Li, 1991). Orr et al. (1994) observed that acute immobilization stress affected testicular steroidogenesis (inhibiting the activities of both 17 α -hydroxylase and 17,20-lyase or P450c17), and this effect was independent of both circulating levels of LH and the binding characteristics of LH/hCG receptors. Similar results were reported by Srivastava et al. (1993) and Marić et al (1996), who observed *in vivo* that acute immobilization stress in rats decreased testosterone levels without detectable changes in plasmatic LH levels, and *in vitro* reduced activity of 3 β -hydroxysteroid dehydrogenase (3 β -HSD) on Leydig cells, suggesting direct effects on steroidogenesis at testicular level. Hales and Payne (1989) found that GC diminish the synthesis of the enzyme P450 (essential for testosterone synthesis) induced by cAMP in cultured mouse Leydig cells. Further works demonstrated that GC decreased the testosterone production in Leydig cells mediated through reduced cAMP activity and steroidogenetic enzymes (11 β -HSD, 3 β - and 17 β -HSD) (Gao et al., 1996, 1997; Sankar et al., 2000 a, b; Dong et al., 2004). In addition to genomic effects, Dong et al. (2004) suggested possible non-genomic effects of GC on the decrease in testosterone secretion, since they observed a rapid decrement in cAMP and testosterone levels in Leydig cells incubated with GC. These possible non-genomic effects of GC could be implied in relation to a membrane form of the GR, by the activation of protein kinases and phosphorylation events, by action on ion channels, by induction of phospholipid turnover or

acting by second messengers such as cAMP and intracellular calcium (Dong et al., 2004; Hardy et al., 2005). Moreover, GC provoke a decrease in the expression of both the LH receptor and the enzyme 11β -HSD mRNAs in rat cultured Leydig cells, in a dose-dependent manner (Rengarajan and Balasubramanian, 2007). Recently, Stojkov et al. (2012) observed that immobilization-induced stress in rats affected the steroidogenic machinery in Leydig cells. They showed that immobilization-induced stress incremented serum corticosterone concentrations and decreased serum testosterone concentrations. Furthermore, the testosterone production in culture medium with Leydig cells isolated from immobilized rats was lower as compared to their controls. The authors observed that the expression of *Scarb1* (gene for scavenger receptor class B, member 1), *Cyp11a1* (gene for cholesterol side-chain cleavage enzyme or P450scc), *Cyp17a1* (gene for enzyme P450c17), *17βHSD3* (gene for 17β -hydroysteroid dehydrogenase type 3) and the cAMP production were inhibited by the acute stress of immobilization. In addition, they showed that the stress induced an increase in the level of transcript for *11βHSD2* (unidirectional oxidase that inactivates glucocorticoids in Leydig cells), as a protective mechanism against GC. Moreover, Stojkov et al. (2012) confirmed previous studies (Srivastava et al., 1993; Orr et al., 1994; Marić et al., 1996), which observed that acute immobilization stress affected testicular steroidogenesis, independently of circulating LH levels. Recently, Ing et al. (2014) reported that GC administration in horses decreased serum testosterone concentration, and caused the down-regulated expression of the enzymes involved in the steroidogenesis (*Cyp19a1*: aromatase; *SQLE*: squalene epoxidase, which has a role in cholesterol biosynthesis; *INSL3*: Insulin-like 3, which is associated with testosterone synthesis; *DHCR24*: 24-dehydrocholesterol reductase; and *GSTA3*: glutathione S-transferase A3, which produce the immediate precursor of testosterone), as well as glucocorticoid receptor alpha (*NR3C1*) and luteinizing hormone receptor (*LHCGR*), genes localized in the Leydig cells. Based on all this information, we conclude that there is a direct action of GC on the decrease of testosterone secretion at the testicular level, mainly by acting on the steroidogenic machinery of Leydig cells, but also by a decrease in the expression of LH receptor in Leydig cells and by other possible non-genomic effects.

Interestingly, LH appears not to be determinant on the decrease in the concentration of testosterone during the acute stress response, since the concentration of testosterone decreases without any decrease in LH levels (Charpenet et al., 1982; Orr and Mann, 1990; Srivastava et al., 1993; Orr et al., 1994; Marić et al., 1996; Stojkov et al., 2012). Collu et al. (1979) reported that acute stress of exercise, cold and immobilization did not change the levels of LH in male rats. In this sense, it is possible that the GC provoke the decrease in serum testosterone concentrations through direct effects at the Leydig cells level, and that such decrement may be independent of LH levels in blood. This effect may be partly due to the increase in GC during the stress response, which causes a decrease in sensitivity (receptors) of Leydig cells to LH (Orr and Mann, 1992; Rengarajan and Balasubramanian, 2007; Ing et al., 2014). Moreover, the fact that LH is not a decisive factor on the decrease in the concentration of testosterone during acute stress response, can also be explained by the direct effect of GC on testicular steroidogenesis, highlighting that this effect is independent

of circulating levels of LH (Srivastava et al. 1993; Orr et al., 1994; Marić et al., 1996; Stojkov et al., 2012).

The stress effect of diminishing testosterone production also implies Leydig cells apoptosis. Gao et al. (2003) noticed that high levels of GC induce apoptosis of Leydig cells but no change in serum LH concentrations. Hardy et al. (2005) showed preliminary results indicating that both acute and chronic movement restriction stress induce greater apoptosis of Leydig cells.

The increment in serum catecholamine concentrations due to a stress response can affect Leydig cells. However, information regarding the effects of catecholamines on steroidogenic activity is scarce and contradictory, and depends on whether the studies were performed *in vitro* or *in vivo*. For instance, some studies performed *in vivo* reported that adrenalin treatments reduce testosterone production and plasma concentrations in both men (Levin et al., 1967) and in adult rats (Götz et al., 1983) while others, performed *in vitro*, observed that catecholamines stimulate cAMP-induced testosterone production in mouse Leydig cells (Cooke et al., 1982). Moger et al. (1982) and Moger and Murphy (1983) reported similar results working with Leydig cells primary cultures. Furthermore, adrenaline and noradrenaline stimulated testosterone synthesis in cultured fetal mouse Leydig cells (Breuiller et al., 1988) and in adult cultured Leydig cells of the Siberian hamster (*Phodopus sungorus*) (Mayerhofer et al., 1993). These results were later confirmed by Stojkov et al. (2012), who observed that adrenaline increased cAMP and testosterone production by Leydig cells.

The contrasting effects of catecholamines on testosterone secretion according to *in vivo* and *in vitro* studies can be explained in part by catecholamine effects on testicular blood flow. For instance, catecholamines stimulate testicular vasoconstriction (Waites et al., 1975; Davis, 1992; Rauchenwald et al., 1995; Chiochio et al., 1999). In studies *in vivo* the smaller testicular blood flow provoked by catecholamines could thus diminish circulating LH levels and inhibit testosterone production. It would be interesting to conduct further studies in order to determine which other factors could be affecting the inhibitory effects of catecholamines on testosterone production *in vivo*.

Although most studies found that the stress response decreases testosterone concentration in blood mainly by way of increased GC concentrations, some studies report that during the initial stages of the acute stress response a transient increase in testosterone levels can occur, from minutes to an hour after the stressor was activated (Frankel and Ryan, 1981; Rivier and Rivest, 1991; Wingfield and Sapolsky, 2003; Chichinadze and Chichinadze, 2008). This transitory increase in testosterone concentration could be associated to the rapid response of the sympathetic system (SAM axis) during the stress response, and the consequent action of catecholamines, which – as mentioned above – stimulate testosterone secretion (Chichinadze and Chichinadze, 2008). After this transitory increase of testosterone concentration during the acute stress response, the testosterone decreases possibly due to the inhibitory action of GC on steroidogenesis, which increases its concentrations in blood later than do catecholamines (see review: Sapolsky et al., 2000). Besides SAM axis activation and release of catecholamines, the testicle receives direct innervation by sympa-

thetic branches that increase the testosterone concentration (Chiocchio et al., 1999). Moreover, there are neural pathways from brain to the testis, independently of the pituitary (Lee et al., 2002; Selvage et al., 2004), which could contribute to the rapid increase of testosterone secretion during the initial stages of the stress response.

Interestingly, the hormone CRH can be isolated from testicular tissues, and it is produced by Leydig cells (Thompson et al., 1987; Audhya et al., 1989). There is contradictory evidence regarding the effect of CRH on testosterone production by Leydig cells. Some *in vitro* studies observed that CRH inhibits Leydig cells testosterone production (Ulisse et al., 1989; Fabbri et al., 1990; Duafu et al., 1993). However, subsequent reports demonstrated positive effects of CRH on steroidogenesis in Leydig cells. Huang et al. (1995) observed that CRH stimulates intracellular cAMP and testosterone production in primary mouse Leydig cell cultures. Similarly, Heinrich et al. (1998) showed that CRH acts directly stimulating the testosterone production in primary mouse Leydig cell cultures, in a time- and dose-dependent way.

Effects on Sertoli cells

Sertoli cells are diploid epithelial cells which regulate the microenvironment within the seminiferous tubules. Sertoli cells are of paramount importance for local regulation of spermatogenesis, both qualitatively and quantitatively. Sertoli cells secrete inhibin, steroid hormones and great quantities of paracrine factors. The cortisol had no significant effect on transferrin secretion in Sertoli cells of the calf testis (Jenkis and Ellison, 1989). It has been reported that Sertoli cells have GR (Levy et al., 1989; Lim et al., 1996; Konrad et al., 1998; Denolet et al., 2006). The GR content of Sertoli cells is greater in immature animals as compared to adults (Levy et al., 1989). In addition, this same article reports that GC treatments decrease GR mRNA levels and increase androgen binding protein (ABP) mRNA levels in Sertoli cells (Levy et al., 1989). Lim et al. (1996) confirmed these results in cultured Sertoli cells, with GC effects on ABP mRNA abundance regulated at the transcriptional level. This Sertoli cell response to stress with greater ABP synthesis might be a compensatory mechanism to diminished testosterone production by Leydig cells linked to stress. It was also reported that GC induce up-regulation of stanniocalcin 1 in cultured Sertoli cells (Li and Wong, 2008). Recently, Hazra et al. (2014) observed that Sertoli cell GR knockout in mice had lower serum concentrations of FSH and LH and testicular FSH and LH receptor mRNA levels, reduced Sertoli cell number and displayed atypical germ cells, as well as down-regulated testicular Cyp11a1 and up-regulated 17 β HSD3 expression. Based on these results, the authors suggest that the GR in Sertoli cells are important for normal testicular function, for maintaining the number of Sertoli cells and for the production of circulating gonadotropin hormones and testicular steroidogenesis.

Catecholamines also exert direct effects on Sertoli cells. It has been reported that Sertoli cells have adrenergic receptors (Heindel et al., 1981; Kierszenbaum et al., 1985; Troispoux et al., 1998). Verhoeven et al. (1979) observed that adrenergic neurotransmitters (e.g.: adrenaline and noradrenaline) stimulate the aromatization of testosterone by Sertoli cells in culture. Moreover, Troispoux et al. (1998) found that the stimulation of β -adrenergic receptors increases the production of transferrin

by Sertoli cells, in a process that involves cAMP and plasminogen activator. Sertoli cells in $\alpha 1b$ -adrenergic receptor knockout mice had a high degree of vacuolization in their cytoplasm, as well as a higher expression of inhibin (Mhaouty-Kodja et al., 2007). These results indicate that $\alpha 1b$ -AR has important direct effects on Sertoli cells and indirectly affects the relationship with other types of testicular cells. Furthermore, treatment with clenbuterol ($\beta 2$ -adrenergic agonist) affected the metabolism of pig Sertoli cells, which showed large lipid deposits, autophagic vacuoles and cytolysosomes (Blanco et al., 2002).

Effects on germ cells

The germ cells proliferate and differentiate from diploid spermatogonia into spermatozoa. The stress of capture in buffalo caused degeneration of germ cells, cytoplasmic vacuolation of primary spermatocytes and lower mean relative frequencies of late primary spermatocytes, and both round and early and elongated late spermatids (McCool and Entwistle, 1989). Immobilization stress led to high incidence of germ cells degeneration in rats (Lee et al., 1993).

The GR were identified in spermatocytes of rats (Schultz et al., 1993). These authors suggest that GC could affect directly the spermatogenesis. The percentage of apoptotic germ cells was greater 24 hours after immobilization stress in rats (Yazawa et al., 1999). One year later, the same team of investigators observed that exogenous GC induced apoptosis of testicular germ cells (Yazawa et al., 2000), and suggested that GR could mediate this process.

As was mentioned before, adrenergic receptors are very important in testicular function. Spermatogenesis of knock-out mice for the $\alpha 1b$ adrenergic receptor is altered, with damaged germ cells, apoptosis of spermatocytes and flawed communication between Leydig and Sertoli cells (Mhaouty-Kodja et al., 2007). The same authors suggested that "catecholamines act directly on maturing spermatocytes to maintain spermatogenesis" (Mhaouty-Kodja et al., 2007). Treatment with clenbuterol ($\beta 2$ -adrenergic agonist), added to the diet (1 ppm) in pigs and for a minimum time of two weeks provoked degeneration of germ cells, which were observed with vacuolized cytoplasm and irregular morphology (Blanco et al., 2002). It should be kept in mind that, since Sertoli cells are essential to normal germ cell development, the effects on germ cells which have been reported could be explained either as direct effects on germ cells, or as indirect effects mediated by Sertoli cells.

Effects on peritubular myoid cells

Peritubular myoid cells are found in the peritubular interstitial tissue, surrounding the testicular seminiferous tubules. Myoid cells contain abundant actin, myosin, desmin/vimentin and alpha-actinin. Myoid cells have been shown to be contractile, involved in the transport of spermatozoa and testicular fluid in the tubule lumen. Myoid cells contain androgen receptors, are involved in retinol processing, secrete a number of substances including extracellular matrix components (fibronectin, type I and IV collagens, proteoglycans) and growth factors (PModS, TGF beta, IGF-I, activin-A) which affect Sertoli cell activity (Maekawa et al., 1996). Schultz et al. (1993) identified GR in the peritubular myoid cells, suggesting a direct effect of GC

on these cells. Weber et al. (2000) reported that administration of GC on peritubular cells decreased the proliferation and fibronectin secretion. Miyake et al. (1986) reported the existence of adrenergic 2α and β receptors in the myoid cells of human seminiferous tubules. It was observed that tubular ischaemic atrophy provoked by adrenalin affected the peritubular myoid cells, which increased their secretion of extracellular matrix components and produced tubular fibrosis (Santamaria et al., 1995 a). The authors suggest that "this alteration may involve direct effects on the peritubular cells or the changes may be secondary to germ cell and/or Sertoli cell lesions". They also observed that intra-scrotal injections of adrenalin in rats increased both the proliferation indexes and the number of peritubular myoid cells between the third and the eighth weeks of treatment (Santamaria et al., 1995 b). The authors hypothesized that the proliferation of the peritubular cells occurs in order to increase their secretion of extracellular matrix components leading to enlargement of the lamina propria of the seminiferous tubule.

Effects on macrophages

Macrophages have roles in almost every aspect of an organism's biology; from development, homeostasis and repair, to immune responses to pathogens. Although they are well known for their phagocytic activity, they also secrete different cytokines (e.g. IL-1, IL-6, TNF α). In addition, at testicular level, supporting evidence shows close functional associations between the Leydig cell and macrophages. For instance, there is a close physical and developmental relationship between the Leydig cell population and the testicular macrophages (Hedger, 2002) by directly associating form junctional complexes (Miller et al. 1983; Hutson, 1990) and developing specialized interdigitations between the two cell types implying the potential for a direct exchange of information and materials (Miller et al., 1983). The macrophages have diverse origins, transcriptional complexity and lability, they are capable of phenotype switching in response to stress (Wynn et al., 2013). However, to our knowledge, there is little information on the direct effects of stress hormones on immune function at the testicular level, and their impact on the functionality in the different cell types in the testis. In general, the stress response (mediated mainly by GC) inhibits inflammatory reactions (either cytokines or other mediators) (see reviews: Homo-Delarche et al., 1991; Sapolsky et al., 2000). GC are associated with Th1 cytokines [such IL-1, IL-2, IL-6, IFN γ and tumor necrosis factor α (TNF α)] downregulation, and upregulation of Th2 cytokines (IL-4, IL-5, IL-10 and IL-13) (review: Webster Marketon and Glaser, 2008). Given that the GR also were identified in testicular macrophages of rats (Schultz et al., 1993) it is possible to speculate that GC may cause changes in the expression of cytokines produced by these macrophages, and that these cytokines may provoke direct changes in different cells at the testicular level. In this sense, the link between testicular macrophages and stress is indirectly based on the effects of certain cytokines at testicular level and its association with other cell types such as Leydig cells.

It is possible that cytokines or others products of macrophages could be involved in the local regulation of steroid synthesis in the testis. In the mature testis, macrophages influence steroidogenesis in Leydig cells, secreting 25 hydroxycholesterol,

which is used in testosterone synthesis (Lukyanenko et al., 2000; Nes et al., 2000). More specifically in relation to cytokines, testicular macrophages are able to produce IL-1 (Miller et al., 1983; Niemi et al., 1986; Cudicini et al., 1997; Jonsson et al., 2001). In addition, it has been observed that IL-1 inhibits (Lin et al., 1991; Hales, 1992; Mauduit et al., 1992) or stimulates the testosterone secretion by Leydig cells (Verhoeven et al., 1988; Warren et al., 1990; Svechnikov et al., 2001). Other cytokine, as IL-6, induced prolonged suppression of testosterone levels in healthy men; there were, however, no apparent changes in gonadotropin levels (Tsigos et al., 1999). These authors suggest that "IL-6 might induce persistent testicular resistance to LH action or suppression of Leydig cell steroidogenesis or both, with potential adverse effects on male reproductive function" (Tsigos et al., 1999). Since the GC produce a decrease in these proinflammatory cytokines (IL-1 and IL-6) and both appear to have different effects on testosterone production, it shows the complex of this whole relationship between stress hormones, testosterone production and testicular macrophages. Moreover, it has been reported in other tissues that macrophages have alpha and beta-adrenergic receptor and that catecholamines may affect its functions (Abrass et al., 1985; Spengler et al., 1990), and even it was reported that macrophages themselves can release catecholamines (Brown et al., 2003); however, there are very few studies on this topic at testicular level. Therefore, further studies are needed to know the complex role of stress hormones, macrophages and its mechanism of action between them and with other cell types at testicular levels.

Conclusions

In this review we have shown evidence that both axes involved in the acute stress response (SAM and HHA) affect the HHG axis activity and also have direct effects on the testis, via several hormones. Scientific literature backs up the concept that, in general terms, the HHA axis (and its hormones) inhibits both the HHG axis and testicular function (as was schematized in the model shown in Figure 1). Nevertheless, information regarding the effects of the SAM axis either on the HHG axis or directly on testicular function are contradictory, albeit in general, the SAM axis has stimulatory effects (as was schematized in the model shown in Figure 1). Both SAM and HHA axis exert direct effects on testicular function, on Leydig, Sertoli, myoid, as well as on germ cells.

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