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Interaction of Adrenal and Gonadal Axes during Stress in Males

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ABSTRACT

In mammals, chronic exposure to different types of stressors, its strength and duration has long been recognized as a disruptive factor in the reproductive function. In males, stress has a suppressive effect on testosterone secretion, spermatogenesis and sexual behavior. Severe suppression of reproduction appears to be caused by hormones secreted when the hypothalamic-pituitary-adrenal (HPA) axis is activated during stress. Since Hans Selye suggested in 1946 that chronic stress increases activity in the HPA axis and at the same time suppresses activity in the hypothalamic-pituitary-gonadal (HGA) axis, there have been several studies demonstrating this antagonistic relationship. The centrally mediated inhibition of gonadotropin releasing hormone (GnRH) by the hormones of the HPA, such as corticotropin releasing hormone (CRH), β-endorphins, and glucocorticoids, as well as the gonadotropin inhibitory hormone (GnIH), the decrease in pituitary responsiveness to GnRH, and the direct effects of stress-hormones on testis leading to the reduction in testosterone secretion are discussed. The aim of this review is focused on the suppression of testicular axis caused by stress, as well as its reproductive consequences in fertility and sexual behavior in males.

INTRODUCTION

Living organisms maintain a dynamic equilibrium of biological variables inside limits of fluctuation, which is known as homeostasis, through adjustments in the internal environment controlled by biological systems. Homeostasis is constantly threatened by internal or external adverse forces called stressors[1], and the response to them is known as stress. Stress has been defined as a physiologic state of threatened homeostasis, which can be reestablished through complex physiological adaptive responses of organisms[1]. At the present time, stress is restricted to conditions where environmental demands exceed the regulatory capability of the organism to stressors, which are unpredictable and uncontrollable[2]. The process by which the organism responds to stressors and maintains stability is known as allostasis[3], which means, "reach stability through change" and does not pretend substitute the term homeostasis[4]. The adaptive response to stress depends on the stressor characteristics, which can be emotional or physical, acute or chronic; but also the strength of the stressor should be considered[5]. The stress response is mediated by the stress system, involving the central nervous system (CNS) and peripheral components[1,6]. The central components of the stress system include: 1) parvocellular neurons in the hypothalamic paraventricular nucleus (PVN), which releases corticotropin releasing hormone (CRH) and arginine vasopresin (AVP); 2) the locus coeruleus (LC) in the brain stem, and other mostly noradrenergic (NE) cell groups in the medulla and pons (LC/NE). The peripheral components include the adrenal gland of the hypothalamic-pituitary-adrenal (HPA) axis, and the efferent sympathetic-adrenomedullary system[1,6,7]. The stress response

facilitates the neural pathways that promote acute, time limited adaptive functions, such as arousal, alertness, vigilance, improved cognition and focused attention, and inhibits the neural pathways controlling acutely vegetative, non-adaptive functions, such as eating, growth, and reproduction. Coordination of the endocrine response to chronic stress by the CNS includes the participation of the HPA axis, which is an important regulator of homeostatic functions[5]. During the stress response glucocorticoid levels increase in blood plasma. Glucocorticoid synthesis and secretion from the adrenal cortex is stimulated by the adrenocorticotropic hormone (ACTH), which in turn, is released from the anterior lobe of the pituitary gland. ACTH secretion is regulated by CRH and to a minor extent by AVP[1,5,6]. Glucocorticoids have an important role in the basal activity regulation of the HPA axis, as well as in the ending of the stress response by acting on extra-hypothalamic centers, such as hippocampus, hypothalamus and the pituitary gland. The negative feedback of glucocorticoids on the secretion of CRH and ACTH limits the total duration of tissue exposure of the organism to glucocorticoids, thus minimizing the catabolic, lipogenic, anti-reproductive, and the immunosuppressive effects of these hormones. Negative feedback by glucocorticoids in the CNS is exerted via two types of receptors: the mineralocorticoid receptor (MR) or type I, expressed mainly in hippocampus, amygdala and prefrontal cortex, which responds to low concentrations of glucocorticoids; and the glucocorticoid receptor (GR) or type II, expressed mainly in hypothalamic PVN and hippocampus[8]. The negative feedback control of the CRH and ACTH secretion after stress or during the circadian and ultradian peaks is mediated by GR type II[1,6,7]. Negative feedback by glucocorticoids on the HPA-axis through GR activation on the PVN is exerted by a genomic fashion[9]. However, corticosteroids also regulate HPA-axis activity through non-genomic actions[10] in the hypothalamus, pituitary and hippocampus through membrane located receptors[11]. In general, the duration of stress response is short or limited; this allows that temporary catabolic, immunosupressors, anti-growth and anti-reproductive effects to be beneficial and without consequences for the organism. However, chronic activation of stress response can cause a significant number of disorders resulting from prolonged CRH and glucocorticoid secretion[6]. For example, the reproductive function is one of the most affected during chronic stress and, in males of different species alters all the reproductive aspects.

Consequences of stress on the reproductive function

It is known that chronic exposure to stressors increases HPA axis activity and concomitantly reduces hypothalamic-pituitary-gonadal (HPG) axis activity[12]. Males under chronic stress exhibit suppression of testosterone secretion[13,14], spermatogenesis[15-17], fertility[18] and sexual behavior[14,19]. Since the system responsible for reproduction is directly linked to the stress system[20], the suppression of reproduction is caused by the HPA axis hormones secreted during stress, such as CRH, ACTH, β-endorphins, and glucocorticoids[21,22]. These hormones inhibit the reproductive axis at all levels. In the hypothalamus, GnRH release from neurons of the Medial Preoptic Area (MPOA) is suppressed by CRH, directly, through synaptic connections between CRH and GnRH neurons in the MPOA[23] or indirectly, through β-endorphins from arcuate Proopiomelanocortin (POMC) neurons[6,21].

Glucocorticoids and HPG axis during stress

Glucocorticoids (cortisol in humans, corticosterone in rats) are considered “stress hormones” because their concentration in plasma rises during stress response[5,24]. These hormones reduce the responsiveness of pituitary gonadotroph to GnRH, resulting in decreased LH secretion[25,26]. Glucocorticoids also have direct effects on gonads, decreasing the sensitivity of Leydig cells to LH, or decreasing testicular receptors to this hormone[27], with subsequent alteration in sex steroid output[28,29]. Besides, glucocorticoids induce resistance in target tissues of gonadal steroids by decreasing the concentration of sexual hormones receptors[5,30].

The suppression of gonadal function due to chronic activation of the HPA axis has been demonstrated in males of different species. Chronic stress by different types of stressors such as immobilization[13,15,31], electric foot shock[14,32], prolonged exercise[33], constant illumination[34], forced swimming in cold water[14,32], noise, fasting[35], surgery[36], crowding and social stress[37] in rats, hamsters[38] and humans of both genders, ballet dancers[39], prolonged physical exercise[40], military training[41] or sleep deprivation[42], causes an increase in plasma levels of ACTH and glucocorticoids, as well as a general inhibitory effect on the HPG axis, decreasing LH and testosterone.

The testosterone decrease (0.25 ng/ml, 90%) during chronic stress in rats has been long attributed to glucocorticoids, since the increased levels of these hormones during stress (up to 550 ng/ml, 200%) is associated with testicular involution and a significant drop in testosterone secretion, thus diminishing sexual stimulation and fertility[43,44]. Glucocorticoids are capable of directly inhibit testosterone synthesis in Leydig cells, both in vitro[28,45] and in vivo[46], then this effect is mediated through glucocorticoid receptors expressed in Leydig cells[47]. Glucocorticoid receptors are also expressed in primary spermatocytes, peritubular myoid cells[48], suggesting that these hormones can directly affect spermatogenesis in the testis. The underlying mechanisms of steroidogenic decrease in Leydig cells by glucocorticoids during stress include the inhibition of the activity of 17 αHydroxylase and 17-20 lyase, as well as inhibition in the transcription of genes transcription encoding testosterone biosynthetic enzymes such as 3β-hydroxisteroid dehydrogenase (3βHSD) and cholesterol side-chain cleavage cytochrome P450 (P450scc)[28,49], and reduction of steroidogenic enzymes content, including P450scc, or 20-22 lyase, which catalyzes conversion of cholesterol to pregnenolone; 3β-hydroxysteroid dehydrogenase, which catalyzes the biosynthesis of progesterone from pregnenolone; and 17α-hydroxylase/C17-20 lyase, wich catalyzes the conversion of hydroxyprogesterone into androgens[28,50,51]. The inhibition of steroidogenesis in Leydig cells mediated by glucocorticoids during stress, occurs when the activity of the enzyme 11β-hydroxysteroid dehydrogenase 1 (11βHSD1), which dehydrogenates and inactivates intracellular corticosteroid, is low[43,52,53]. Glucocorticoids can also inhibit testosterone biosynthesis in Leydig cells through a non-genomic mechanism by reducing the production of cytoplasmic

cAMP, which is part of the LH signaling transduction pathway that regulates steroidogenic activity in those cells[54]. In addition to the inhibition of testosterone biosynthesis in stressful conditions, the high levels of the glucocorticoid corticosterone during stress can also initiate apoptosis in rat Leydig cells[43]. The molecular mechanisms of corticosterone-induced apoptotic death in isolated Leydig cells involve FasL/Fas system and caspase-3. Apoptotic signaling pathways include increased expression of Fas receptor, Fas ligand (FasL), and the members of the Bcl2 family: Bid, Bak, Bad, Bax proteins, the proteases caspase-8 and caspase-9 markers, as well as tumor protein p53 (inducer of apoptosis), all of them proapoptotic, along with a simultaneous decrease in the B-cell lymphoma 2 (Bcl-2), which is an antiapoptotic protein. Both intrinsic and extrinsic apoptotic pathways activate proteolytic machinery causing apoptosis within hours[55-57] and these apoptotic signaling pathways activate during acute and chronic stress in testes[58]. The cleavage of procaspase-3 is responsible for the proteolysis of a large number of protein substrates and cell disassembly during the execution phase of apoptosis. Corticosterone causes loss of mitochondrial membrane potential ($\Delta\Psi$) leading to the leak of cytochrome C and other proteins from the mitochondria, causing activation of caspases[59,60]. The respiratory chain becomes uncoupled from ATP synthesis giving rise to reactive oxygen species (ROS) generation and further potentiating the death process[61]. Together, all of these events are implicated in the process of corticosterone-induced Leydig cell apoptosis[62,43] resulting in testosterone decline.

Oxidative stress in the testis is one of the major factors inducing apoptosis not only in Leydig[63], but also in germ cells, as has been demonstrated in numerous studies. In vitro[64] or *in vivo*[65] heat stress, chemicals[66], sexual hormones such as estradiol[67], or testosterone[68], ischemia[69], as well as corticosterone administration[62,70] cause oxidative stress inducing apoptosis in those cells, being spermatids and spermatocytes the most susceptible to chronic stress. At the same time, lipid peroxidation and levels of hydrogen peroxide increase, while superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase activities decrease, indicating that physiological processes of oxidative stress have a direct linkage to the extent of germ cell apoptosis in the seminiferous epithelium[71,72]. Although the molecular mechanisms of apoptosis induction by oxidative stress in testis have not been completely identified, some of the pathways involved have been revealed from in vitro studies by the exposure of germ cells to H_2O_2[73]. Induction of germ cell apoptosis is directly associated with a significant increase in lipid peroxidation and a concomitant decrease in SOD and catalase activity.

The suppression of testosterone due to steroidogenic inhibition and apoptosis of Leydig[46] and testicular germ cells[74] by glucocorticoids during stress has been proposed to be the main factor for the impairment of spermatogenesis, fertility and male sexual behavior. These are very important issues concerning reproductive problems observed in males of several species of vertebrates, in which infertility and/or diminished sexual motivation are stress-induced difficulties of reproductive success.

In men, stressful situations can lead to erectile dysfunction as well as decrease in sexual motivation (libido) and fertility. Although stress is not the only factor in the etiology of infertility, it is considered an additional risk factor for idiopathic infertility[75]. Also, emotional stress related to work or the depressive reaction to infertility or its therapy, causes decreased semen quality, with low sperm concentration[76]. Psychological stress also has a negative effect on semen volume, sperm motility[77], concentration[78], and morphology, increasing sperm abnormalities, in healthy[79] and subfertile (reduced fertility with prolonged time of unwanted non-conception) men[80]. Severe psychological stress brought on by the death of a relative or spouse consistently lowers sperm count[81], in some cases leading to oligospermy or azoospermia[82,83]. This potential reduction in fertility is most likely caused by the stress-induced decline of testosterone[82] but also it is possible that stress hormones can cause direct effects on the seminiferous epithelium, impairing spermatogenesis[79]. Considering that spermatogenesis is a testosterone-dependent process, which is suppressed during chronic stress, it can be expected to be significantly impaired in stressful conditions, as it has been reported in a wide number of studies. Chronic stress in rats causes a reduction in seminiferous epithelial area, with degenerative signs such as vacuoles in the basal epithelium, picnosis, and exfoliation of degenerative germinal cells in the tubule. Also, the mean number of type A spermatogonia, spermatocytes and spermatids in the seminiferous tubules decreases[44,84]. Beside these effects in seminiferous epithelium, chronic stress in male rats decreases quality in epididymal sperm, reducing sperm viability, motility, and concentration[85], as well as a decrease in the number of offspring[44].

GnIH/RFRP hormone and HPG axis during stress

A recently discovered peptide hormone is also implicated in the inhibition of the HPG axis, both in physiological conditions, as well as during stress. Research in the field of reproduction regulation involves the participation of this recently identified peptide. This peptide acts directly on the pituitary gland inhibiting gonadotropin release in quail, so it was named gonadotropin-inhibitory hormone (GnIH)[86]. GnIH homologues have been identified in other vertebrates including mammals, called RFamide related peptides (RFRPs)[87]. This peptide was discovered in the hypothalamus[88] and is expressed in RFRP neurons within the dorsomedial nucleus (DMH) in rats, with fibers extending both to the median eminence and to the preoptic area, making putative contact with GnRH neurons within the hypothalamus[86]. GnIH receptors are expressed on GnRH-I and -II neurons in birds[89]. As in birds, mammalian GnIH/RFRP inhibits pituitary synthesis of the gonadotropin subunits α and β[90] as well as gonadotropin release in several mammalian species, such as rats, sheep[91], hamsters[92], and humans[87]. Furthermore, GnIH/RFRP and its receptor have been demonstrated both in avian and mammalian gonads. This means that RFRP can act directly at multiple levels: within the brain, on the pituitary, and in the gonads[89]. This also indicates that GnIH and its related peptides are important modulators of reproductive function at the level of GnRH neurons, the gonadotroph and the gonads. GnIH/RFRP is also involved

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in the negative regulation of the HPG axis during stress, as both acute and chronic immobilization stress leads to an up-regulation of GnIH/RFRP expression in the dorsomedial hypothalamus (DMH) of adult male rats[93]. These current findings provide a novel mechanism by which HPA axis activation can influence reproductive function. The mechanism by which the HPA axis inhibits the HPG axis involves adrenal glucocorticoids, then, these steroid hormones released during the stress response bind to GR in hypothalamic GnIH/RFRP neurons, increasing transcription of the GnIH/RFRP gene, as well as its expression in the DMH[94]. GnIH/RFRP released from this hypothalamic area inhibits GnRH release through a direct postsynaptic mechanism, causing hyper polarization in GnRH neurons[86], and decreases pituitary sensitivity to GnRH[94], thus contributing to hypothalamic suppression of reproductive function. The latest evidence in the study of mechanisms regulating spermatogenesis in testes is the finding that GnIH/RFRP and its receptor are present both in testis and accessory reproductive organs of birds such as quail[89] and mammals, such as the hamster[95]. This hormone is located in the testicular interstitial cells and germ cells, such as spermatocytes and elongated spermatids; its receptors have been identified in spermatocytes, and round and elongated spermatids[89,95] **(Figure 1).**

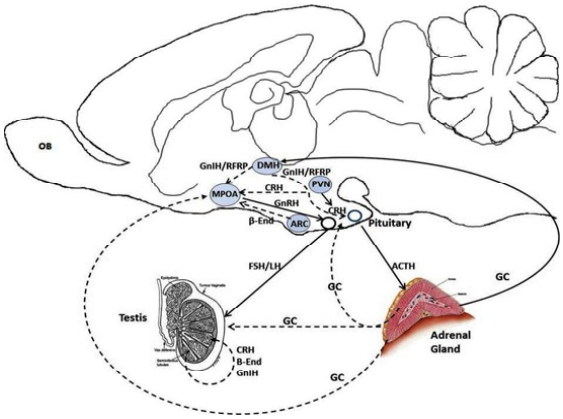

Figure 1. Neuroendocrine pathways involved in the suppression of HPG axis by HPA axis.

During stress CRH neurons in PVN activate and stimulate the secretion of ACTH in the anterior lobe of the pituitary. This hormone stimulates synthesis and secretion of glucocorticoids in the adrenal cortex. HPG axis is inhibited at all levels by HPA axis. GnRH release from MPOA is inhibited by CRH, β-endorphins, and GnIH/RFRP directly in the hypothalamus. At the same time, gonadotropin release is also inhibited by GnIH/RFRP. Adrenal glucocorticoids (GC) reduce the responsiveness of gonadotrophs to GnRH, reduce steroidogenesis in testis, and inhibit GnRH release directly and indirectly through GnIH/RFRP. In the testis, CRH acts as a direct and indirect (β-endorphin mediated) anti-reproductive peptide, where both peptides function as negative regulators of gonadotropin actions. Solid lines=stimulation. Dashed lines=inhibition.

Opioids and the HPG axis during stress

Suppression of serum testosterone levels caused by chronic stress can be attenuated by the opioid antagonist naltrexone (NTX), which suggests that the effects of stress on testosterone levels could be partially mediated by endogenous opioids, mainly β-endorphins, besides from CRF and glucocorticoids[32]. The inhibitory effects of both peptides could be exerted directly in the testis, inhibiting androgen synthesis, and by decreasing GnRH release from the MPOA. Both CRH and β-endorphins can exert effects on the HPG axis by inhibiting GnRH release from the hypothalamus[96], inhibiting LH release from the pituitary[97], and inhibiting testosterone synthesis directly in Leydig cells[98,99], thus decreasing testosterone in plasma. All these effects caused by CRH, opioids and stress can be reverted or prevented by the administration of the opioid antagonists naloxone or NTX, increasing GnRH[96], as well as LH and testosterone[98].

In addition to its known antireproductive functions in the hypothalamus, CRH acts as a direct and indirect (β-endorphin mediated) anti-reproductive peptide in the testis, where both peptides function as negative regulators of gonadotropin actions[100,101]. CRH secretion in Leydig cells is stimulated by serotonin through 5-hidroxitriptamine type 2 (5HT2) receptors[102], and it has been proposed that, during stress, serotonin is released and stimulates CRH secretion in Leydig cells, causing inhibition of LH-induced cAMP production and steroidogenesis in cultured Leydig cells[100]. Testosterone synthesis can be suppressed not only by glucocorticoids[54], but also by endogenous opioid peptides, which are capable of inhibiting the enzymes involved in steroidogenesis[99]. The inhibition of testicular steroidogenesis by local opioids, specifically β-endorphin, can be prevented by the infusion of opioid antagonists such as naloxone and NTX methobromide (a peripheral opioid antagonist), suggesting that these effects are mediated, in part by the opioid μ-receptor subtype[98]. When rats are treated with NTX the decrease in plasma testosterone due to stress is attenuated[28]. NTX and NTX methobromide can stimulate an increase in testicular testosterone, as well as in pituitary LH in male rats[99], and rhesus monkeys[103]. The stimulatory effect of NTX in males could be due to the stimulation of LH release from the pituitary, as well as to stimulation of testicular testosterone secretion by increasing the activity of the enzymes involved in testosterone biosynthesis[98]. All this evidence supports the idea that the inhibitory effects of stress on testicular steroidogenesis are mediated, at least to some extent, by opioids. However, more studies are necessary to demonstrate opioid release in brain structures, pituitary, and testis in the male rat during stress **(Figure 1).**

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Stress, testosterone and sexual behavior

Sexual behavior in males is possibly the most vulnerable aspect of male reproduction. Studies in animal models indicate that chronic stress attenuates both motivational (appetitive behavior that brings the male into contact with a female, which would elicit relatively stereotypic behaviors) and consummatory (motor patterns during copulation) components of male sexual behavior[104], thus diminishing the reproductive success. In male rats, stress causes increase in mount, intromission and ejaculation latencies, as well as in the number of mounts. On the contrary, the number of ejaculation decreases[14,105].

This impairment of sexual behavior in male rats caused by physical stress is associated with increased plasma corticosterone and concomitant decrease in plasma testosterone[14,32]. Nevertheless, the impairment of sexual behavior can hardly be attributed to a direct effect of corticosterone, since administration of different doses (0.5-4 mg) to sexually experienced male rats during 4 or 8 consecutive days does not replicate the effects of stress as none of the copulatory parameters is modified. Plasma testosterone is not modified by corticosterone administration either[106]. These findings might be explained by the relatively low doses administered, since larger doses of corticosterone (5-7.5 mg/100 g BW) are needed in order to induce apoptosis in testis, for example[62,73]. Another factor that must be considered to explain the lack of corticosterone effect of on sexual behavior is the sexual experience of males, since this factor modifies the extent to which stressful situations alter testis functionality and sexual behavior, probably through a stress-protective effect of sexual activity and mating in male rats, decreasing the endocrine and neuroendocrine response to stress.

Stress induces severe alterations on sexual parameters, such as mount and ejaculation latencies, as well as ejaculatory frequency in naive males compared to sexually experienced males[32]. However, other components of the stress response have been involved in the disruption of sexual behavior due to chronic stress. The endogenous opioid system is activated in response to a number of stressors, both physical and psychological, such as immobilization, cold-water immersion, and electric foot shocks[107]; and plasma β-endorphins increase with these stressors[108]. These stressors also cause an increase in POMC synthesis rate, as well as β-endorphin release from the arcuate nucleus in the hypothalamus[109]. In men, social or psychological chronic stress causes erectile dysfunction, increases plasma levels of β-endorphins, and decreases libido. Treatment with NTX improves these symptoms and increases penile blood flow and the number of sexual acts in a dose-dependent manner[110]. In healthy sexually active men, NTX increases the number and intensity of orgasms; this suggests that endogenous opioids modulate orgasmic response and the perceived intensity of sexual arousal and orgasm in men[111]. The inhibition of sexual behavior caused by stress can be blocked by NTX administration before exposure to stressors. These findings indicate that endogenous opioids could be involved in the effects of stress on sexual behavior[32]. Endogenous opioid peptides are commonly accepted as having an inhibitory role in male sexual behavior[112]. These inhibitory effects are apparently mediated by μ-opioid receptors, since the administration of a selective μ-receptor agonist (morphiceptin) into the MPOA of rats delays the initiation of male copulatory behavior[113,114], and opioid receptor antagonists, such as naloxone and NTX, prevent these effects[115,116]. The hypothalamic PVN is another area where endogenous opioids and/or opioid μ-receptor agonists can also inhibit male sexual behavior by preventing dopamine receptor agonists and oxytocin induced penile erection[117], and must be considered to explain the effects of stress and naltrexone on stressed males. Endogenous opioid peptides exert a dual effect on sexual behavior: They facilitate the anticipatory phase of sexual behavior by improving sexual arousal and motivation, acting in the ventral tegmental area to increase the activity of the mesolimbic dopaminergic system[112,114]. On the contrary, they inhibit the consummatory phase of sexual behavior, impairing sexual performance, by acting in the MPOA[115]. Therefore, it is possible that some of the alterations in sexual parameters observed in stressed males might be due to the action of endogenous opioids in the MPOA and PVN[118]. Most hypothalamic endogenous opioids are located in POMC neurons situated in the arcuate nucleus, which project to the MPOA, a brain structure of central importance for the display of sexual behavior in males[104]. This is one of the central structures that expresses μ-receptors[119] and is commonly recognized as a brain area in which opioid peptides act to inhibit male sexual behavior[120], mainly through opioid receptors of the μ type[113]. Therefore, prevention of the inhibitory effects of stress on sexual behavior by NTX administration suggests that the effects of stressors are mediated, partially, by endogenous opioids, mainly β-endorphins, probably acting on μ-opioid receptors, since NTX is an antagonist of μ-opioid receptors[108].

GnIH/RFRP and male sexual behavior

In addition to opioids, GnIH/RFRP should also be considered among the mechanisms by which sexual behavior is impaired by stress. Although evidence is scarce, there are studies showing that intracerebroventricular (ICV) GnIH/RFRP administration in adult rats suppresses sexual behavior, with increased mount, intromission and ejaculation latencies with a concomitant reduction in ejaculation frequency[92]. In birds, central GnIH/RFRP administration reduces the frequency of copulation solicitation display, which is a GnRH-dependent behavior in male[120] and female[121] sparrows. However, more research regarding the effects of RFRP in this important aspect of reproductive physiology of mammal males and females is needed. Also, the precise physiological mechanisms of GnIH/RFRP action in the brain and the differences between genders and possibly species will be the focus of future studies.

CONCLUSIONS

The consequences of stress on reproductive function have been observed at all levels of the HPG axis and are mediated

mainly by glucocorticoids, which are known as stress hormones¨ for that reason. These adrenal steroids induce the expression of GnIH/RFRP, which also plays a relevant role in the inhibition of the HPG axis during chronic stress, decreasing GnRH release in the hypothalamus, pituitary LH secretion by reducing its responsiveness to GnRH, and inhibiting testicular steroidogenesis directly, and indirectly by inducing apoptosis of Leydig cells. Likewise, glucocorticoids decrease spermatogenesis by causing apoptosis directly on testicular germ cells, or indirectly through the decrease of testosterone secretion due to apoptosis in Leydig cells. Apoptosis in germ cells, Leydig cells and spermatozoa is caused by oxidative stress. As a result of the actions of the HPA axis on the HPG axis function during stress, the impairment of sexual behavior, as well as infertility have been observed in males of different species of vertebrates, thus decreasing reproductive success. Therefore, the involvement of GnIH/RFRP, CRH, β-endorphins and glucocorticoids in the HPA-HPG interaction should be relevant to most vertebrates. The recent findings about GnIH/RFRP during stress are crucial for understanding the mechanisms underlying one of the most common suppressors of HPG activity.

As previously demonstrated, HPA axis activation is implicated in a number of causes of infertility and reproductive dysfunction related to stress, and can significantly influence the effectiveness of assisted reproductive procedures in humans. Moreover, the assessment of oxidative stress in testes and semen of captive animals will help to understand and propose antioxidant treatments for the improvement of captive breeding programs, as well as assisted reproduction in humans. In addition, chronic stress is of primary concern in captive breeding programs as well as in agricultural breeding programs. Finally, the discovery of GnIH in the avian brain and its role in the neuroendocrine control of reproduction opens new approaches to the study of reproductive neuroendocrinology across species. Now it is known that GnRH is not the only hypothalamic neurohormone controlling reproduction in vertebrates. Future research will elucidate the interactions of GnRH, GnIH, kisspeptin, and other neuropeptides related with the reproductive function in vertebrates.

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