REVIEW

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Modulation of anxiety behavior in gonadectomized animals

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Anxiety is a complex psychological state which happens after stressful life experiences. Many factors such as daily life events, neurotransmitter systems, and different brain areas could influence anxiety behavior in humans and animals. For example, opioids and androgens decrease anxiety behavior both in humans and animals. Furthermore, removing the testes (gonadectomy) causes higher levels of anxiety-like behaviors, in which the administration of testosterone and opioid antagonist can reverse some of these behaviors. We review the effects of morphine and androgens on the modulation of anxiety behavior in gonadectomized animals. We begin by highlighting the effects of opioid drugs and androgens on the modulation of anxiety behavior that have been implicated in anxiety behavior. We then discuss the functional consequences of gonadectomy on anxiety behavior. Finally, we consider how the opioids and androgens may contribute to adaptive responses associated with anxiety.

Key words: anxiety, morphine, testosterone, gonadectomy

INTRODUCTION

Anxiety is a complex psychological process that often occurs after stressful life experiences. In a number of cases, it is adaptive since it prepares the organism for future stressful encounters. Nevertheless, if prolonged or exaggerated over time, anxiety induces many abnormal and maladaptive thoughts and behaviors (Leuner and Shors, 2012). Anxiety disorders are the most common of all psychiatric disorders; though, the current human and animal investigation has yet to provide a clear understanding of the neural mechanisms underlying their etiology. Understanding the effect of hormones on the neurobiological systems which modulate anxiety behavior will increase our capacity to develop new drug targets to treat several mental illnesses in humans (McHenry et al., 2014). In fact, animal behavioral profiles are usually employed to evaluate new therapeutic agents to treat anxiety disorders and to evaluate the mechanism of action of anxiolytic drugs (Siepmann and Joraschky, 2007).

Several lines of research support the role of opioid receptors on the modulation of anxiety (Perrine et al., 2006; Erbs et al., 2012; Miladi-Gorji et al., 2012). For example, anxiety-like responses in mice are differentially affected by the activation of opioid receptors which the effects depend on the social status of the animals (Kudryavtseva et al., 2004). Several studies indicated that systemic administration of μ-opioid receptor agonists induces anxiolytic-like effect (Zarrindast et al., 2005; Solati et al., 2010; Eslimi et al., 2011), while the opioid receptor antagonists increase anxiety (Zarrindast et al., 2008; Rezayof et al., 2009; Zarrindast et al., 2010).

Moreover, many investigations demonstrate that some androgens possess anxiolytic-like activity both in humans and animal models (Frye and Edinger, 2004; Giltay et al., 2012; McDermott et al., 2012; Terburg et al., 2016). Testosterone is the main circulating androgen. It interacts with classic androgen receptors, thereby induce anxiolytic-like activity (Fernandez-Guasti and Martinez-Mota, 2005). Investigations from human

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and rodent studies have revealed that levels of testosterone are inversely correlated with levels of anxiety (Frye and Seliga, 2001; Khera, 2013; Khakpai, 2014; Dossat et al., 2017).

Removing the testes (gonadectomy), the main source of testosterone, causes higher levels of behavior indicative of anxiety in a variety of tasks, in male rats (Justel et al., 2012a). Testosterone deficiency syndrome, also recognized as late-onset hypogonadism, is a clinical and biochemical syndrome that can happen in men in relation to advancing age. The condition is characterized through deficient testicular production of testosterone. It may influence many organ systems and can result in substantial health consequences (Morales et al., 2015).

The appropriate use of testosterone replacement therapy advised the management of testosterone deficiency syndrome (Morales et al., 2015). Also, the relationship between testosterone levels and anxiety disorders in humans and animals is evident with hypogonadism (long-term) and gonadectomy (short- and long-term) in male humans and rodents, respectively. Numerous researches indicated that testosterone-replacement therapy for short- and long-term in hypogonadal men and gonadectomized male rodents critically alleviates anxiety (Fernandez-Guasti and Martinez-Mota, 2005; Zarrouf et al., 2009; McHenry et al., 2014).

Interestingly, opioids play a role in the effects of androgen in modulating anxiety behavior. So, investigations show the involvement of testosterone and opioid system in anxiogenic-like behaviors induced by gonadectomy in adult male rats for short-term (10 days) (Khakpai, 2014). Here, we review the effects of morphine and androgens on the modulation of anxiety behavior. We also consider how gonadectomy may induce anxiety behavior, as well as gonadectomy-treatment, may reverse responses associated with anxiety.

The effect of opioid system on anxiety behavior

Opioid peptides play a role in many functions, including pain perception, respiration, homeothermy, nutrient intake, and the immune response. Moreover, studies have demonstrated the role of opioid receptors in regulating baseline anxiety states and related behaviors (Roeska et al., 2008; Solati 2011; McHugh et al., 2017; Wang et al., 2017). These functions are mediated by three major classes of G protein-coupled receptors, μ, δ and κ, whose activation inhibits adenylyl cyclase (Kahveci et al., 2006). It is well known that systemic injection of μ-opioid receptor agonists including morphine causes the anxiolytic-like effect (Zarrindast et al., 2005; Solati et al., 2010; Eslimi et al., 2011), probably by interacting with the GABAergic system (Le Merrer et al., 2006). In contrast, the opioid receptor antagonists enhance anxiety in various behavioral animal tests such as the elevated plus-maze (Zarrindast et al., 2008; 2010; Rezayof et al., 2009). It has been revealed that both intra-peritoneal (Shin et al., 2003), and intra-cerebral (Zarrindast et al., 2005) injections of morphine potently induced anxiolytic effects. Studies performed in rodents demonstrated that μ- and δ-opioid receptors are involved in the control of emotional responses, including anxiety and depressive-like behaviors (Erbs et al., 2012). Cat odor exposure produced a significant increase in the expression of pro-opiomelanocortin and μ-opioid receptor genes in the brain structures related to anxiety (amygdala) and motivation (mesolimbic area). Anxiety response produced *via* the odor of a predator is an innate behavioral response and evolutionarily highly conserved. There is a report showing that a cloth impregnated with cat odor placed on the cage of rats caused a robust anxiogenic-like action in rats. This is also coherent with the hypothesis that morphine enhances defensiveness in a situation related to the cat odor stimuli and also morphine eliminates ultrasonic vocalizations evoked by cat odor, which supports the assumption that the opioid system mediates behavioral responses associated with anxiety (Areda et al., 2005). Moreover, withdrawal from chronic opiates is related to an increase in anxiogenic-like behaviors, but the anxiety profile in the morphine-dependent animals is not clear (Buckman et al., 2009; Pooriamehr et al., 2017; Kim et al., 2018). Additionally, recent investigations have revealed that voluntary exercise can decrease anxiety levels in rodents. Miladi-Gorji and coworkers (2012) reported that voluntary exercise decreases the severity of the anxiogenic-like behaviors in both morphine-dependent and withdrawn rats. Therefore, voluntary exercise could be a potential natural method to ameliorate a number of the deleterious behavioral consequences of opiate abuse. In addition, anxiety has been described as key comorbidity in patients suffering from chronic pain. It has been reported that rats subjected to neuropathic pain models develop anxiety-like behavior which can be reversed through appropriate analgesic treatment such as morphine and gabapentin (Roeska et al., 2008). Many neurotransmitter systems including cannabinoid, acetylcholine, histamine, dopamine (Zarrindast et al., 2005; 2008; Rezayof et al., 2009) in different sites of the central nervous system (CNS) such as the hippocampus and amygdala have been proposed to be involved in the modulation of morphine functions on anxiety behavior (Solati et al., 2010; Kesmati et al., 2014).

Collectively, μ- and δ-opioid receptors are involved in the modulation of anxiety-like behaviors (Erbs et al., 2012). So that administration of opioid agonists induced

anxiolytic-like effect (Zarrindast et al., 2005; Solati et al., 2010; Eslimi et al., 2011), but the application of opioid antagonists induced anxiogenic-like response (Zarrindast et al., 2008; 2010; Rezayof et al., 2009). In the present review, the possible mechanism(s) between the opioid system and androgens in the modulation of anxiety-like behaviors have been investigated.

The effect of androgens on anxiety behavior

Many investigations demonstrate that some androgens possess anxiolytic-like activity both in humans and animal models (Frye and Edinger, 2004; Giltay et al., 2012; McDermott et al., 2012; Terburg et al., 2016). Testosterone is a main circulating androgen. Preliminary researches suggest that testosterone may have anxiety-decreasing and cognitive-increasing properties in animals and people (Frye and Seliga, 2001; Hermans et al., 2006; Miller et al., 2009). It interacts with classic androgen receptors, proposing that its anxiolytic-like activity could be mediated *via* this mechanism (Fernandez-Guasti and Martinez-Mota, 2005). Testosterone secretion is under the quick pulsatile control of gonadotropin-releasing hormone (GnRH) that in turn activates the production of luteinizing hormone (LH). The brain has receptors for testosterone and is capable of synthesizing and metabolizing testosterone too, for example, estradiol. It has been reported that low salivary testosterone levels are related to both depressive and anxiety disorders (Giltay et al., 2012).

Testosterone plays a role in many behaviors associated with sexual and reproductive function as well as fear and anxiety behaviors (King et al., 2005; Carrier and Kabbaj 2012; McDermott et al., 2012). A wide body of evidence demonstrates an anxiolytic-like effect of testosterone (Justel et al., 2012a; Kim and Spear 2016; Liang et al., 2018). There is also considerable document for fear- and anxiety-reducing properties of testosterone across a number of species, such as rats, mice, ewes, and humans (van Honk et al., 2005; Lacreuse et al., 2010; Domonkos et al., 2017). Studies revealed that subcutaneous administration of testosterone increases anti-anxiety behavior in the elevated plus-maze, zero mazes, and Vogel task and also enhances motor behavior in the activity monitoring test in aged intact male C57/B6 mice (Frye et al., 2008). In animal models, the anxiolytic-like activity of androgens have been reported after different schedules; so, whereas some found immediate actions (Frye and Edinger, 2004), others reported decreased anxiety only after a long chronic administration (Fernandez-Guasti and Martinez-Mota, 2005). It has been demonstrated that the androgen receptors play a role in regulating anxiety-related behaviors, as well as corticosterone responses and neural stimulation following exposure to a mild stressor in rodents (Zuloaga et al., 2011).

In humans, gonadal hormones affect mood disorders such as anxiety and depression. Women are detected with anxiety disorders and depression more often than are men, and these disorders often coincide with a decrease in levels of estrogen during menopause (Arpels, 1996). Moreover, estrogen replacement therapy has been revealed to decline anxiety in postmenopausal women (Yazici et al., 2003). In men, alike but a less abrupt decrease in androgen levels with age is also often accompanied through symptoms of anxiety and depression (Kaminetsky 2005; Eskelinen et al., 2007). Androgen treatment of aging men, or of younger men with reduced testicular production of testosterone, improves some of these symptoms in both aging and younger men (Eskelinen et al., 2007; Amore et al., 2009; Seidman et al., 2009; Zuloaga et al., 2011). Furthermore, disorders of anxiety and fear dysregulation are highly prevalent. These disorders affect women nearly 2 times more than they affect men, occur predominately during a woman's reproductive years, and are particularly prevalent at times of hormonal flux. This suggests that gender differences and sex steroids play a main role in the regulation of anxiety and fear (Toufexis et al., 2006). Gender differences in the age-of-onset and prevalence of psychiatric disorders such as anxiety and depression indicate that sex hormones may modify symptoms of mental illness. Fear-potentiated startle is a translational measure of fear and anxiety as recent investigations have shown fear-potentiated startle in monkeys is reliably decreased by anxiolytics such as diazepam and morphine. Fear-potentiated startle is also changed in people with depression and anxiety (Toufexis et al., 2006; Morris et al., 2010). Correspondingly, boys and girls with low testosterone levels display greater indices of depression and anxiety than those with high testosterone (Edinger and Frye 2005; Zuloaga et al., 2011). Granger et al. (2003) reported that young boys and girls with lower salivary testosterone levels are more likely to experience higher levels of anxiety, depression and attention problems throughout the day compared to boys and girls of the same age with higher salivary testosterone levels. Androgen reduction related to aging is associated with negative mood and increased anxiety in men and women. These results suggest that androgens may have organizational and/or activational properties on mood and anxiety in people (Edinger and Frye, 2005; Domonkos et al., 2018). Although the clinical researches of testosterone therapy in women are more limited, some studies support anxiolytic roles for testosterone (Miller et al., 2009). In fact, women with a type of anxi-

ety disorder, such as generalized anxiety express lower levels of salivary testosterone, compared to emotionally healthy women (Giltay et al., 2012). Clinical documents suggest that testosterone has anxiolytic benefits, with the potential to promote improved mood in both women and men (McHenry et al., 2014).

At least two non-exclusive mechanisms may mediate the behavioral functions of steroid hormones. A classic genomic mechanism contains the coupling of the steroid hormone to intracellular receptors which are translocated to the nucleus and activate protein-synthesis. Furthermore, an alternative mechanism includes the activation of membrane receptors coupled to neurotransmitter receptor systems, as is the case of the $GABA_A$-benzodiazepine receptor complex. There is evidence showing that testosterone exerts its anxiolytic-like activity *via* its conversion to the reduced metabolites with the consequent activation of the $GABA_A$ receptor complex (Aikey et al., 2002; Fernandez-Guasti and Martinez-Mota, 2005). In addition, testosterone activates the hypothalamic-pituitary-adrenal axis, anxiety-related behavior, corticosterone responses, and sensorimotor gating in rodents (Zuloaga et al., 2011). Additionally, injection of either estrogens or androgens generally results in reduced indices of anxiety and depression-related behaviors in rodents (Frye et al., 2008). Studies suggest that anxiolytic functions of estrogens are largely mediated *via* activation of the estrogen receptor (Lund et al., 2005). Particularly, testosterone treatment declines, whereas estrogen treatment enhances, the release of stress hormones adrenocorticotropic hormone (ACTH) from the pituitary gland, and corticosterone from the adrenal cortex (Zuloaga et al., 2008; 2011). There is a wide body of evidence to propose that sexual experience may affect androgen secretion in many species, in turn, androgens may also affect anxiety. Sexual experience may change anxiety behavior and secretion of endogenous androgens. So, sexual experience is related to lower levels of anxiety-like response and higher levels of androgen secretion (Edinger and Frye, 2007b). Endogenous and exogenous testosterone affects some behavioral traits as revealed in human and animal studies. The effects of testosterone can be mediated through androgen or estrogen receptors, but also through rapid non-genomic effects. Endogenous testosterone levels have been revealed to be inversely related to anxiety and depression severity (Hodosy et al., 2012).

Additionally to endogenous androgens' effect on anxiety behaviors, exogenous androgens may be used in part for their effects on mood. Men with low endogenous androgen levels due to aging or hypogonadism indicate more anxiety symptoms and declined mood than do their androgen-replete counterparts (Edinger and Frye, 2005; Meyers et al., 2010). Testosterone-replacement to such individuals can decrease some of the negative effects related to androgen decline (Edinger and Frye, 2005). Also, gonadectomy in adult rats for short-term (two weeks) declines open field activity in male rats and supplementation with testosterone propionate in gonadectomized rats recovers open field activity (Zhang et al., 2011; McDermott et al., 2012) (Table I). Testosterone is metabolized to neuroactive steroids through diverse pathways: in one pathway, it is converted to androstenedione and further reduced to androsterone; in other, it is converted to dihydrotestosterone which may be further reduced to 3α-androstanediol. This last pathway has been suggested to involve in the decreased anxiety produced *via* androgens in intact male rats and proposes that the anxiolytic-like action of androgens may require 5α-reduction (Edinger and Frye 2005; Fernandez-Guasti and Martinez-Mota, 2005). In support of this idea, Frye and Edinger (2004) indicated that the intrahippocampal injection of a 3α-hydroxysteroid-dehydrogenase inhibitor, indomethacin, to dihydrotestosterone-treated rats prevented the anxiolytic-like action induced by this steroid. Physiological levels of testosterone replacement in adult gonadectomized male, but not female rats, show protective properties against the development of anxiety-like behaviors in a model of chronic social isolation (Carrier and Kabbaj, 2012). Likewise, in intact aged male rodents with lower levels of testosterone, the application of testosterone decreases anxiety-like behaviors in the open field test and light-dark box test (Frye et al., 2008). These reports thus support the hypothesis that the activational effects of testosterone can decrease behavioral measures of anxiety in male rodents (McHenry et al., 2014; Domonkos et al., 2018).

Altogether, testosterone by interacting with classic androgen receptors induced anxiolytic-like effect (Fernandez-Guasti and Martinez-Mota, 2005). In animals (Frye and Edinger, 2004) and human studies, gonadal hormones affect anxiety behavior. Gender differences in the age-of-onset and prevalence of anxiety indicate that sex hormones may modify symptoms of anxiety (Edinger and Frye, 2005; Zuloaga et al., 2011). Androgen reduction related to aging is associated with the enhancement of anxiety in men and women. It seems that androgens may have organizational and/or activational effects on anxiety in people (Edinger and Frye, 2005). Testosterone-replacement to such persons can decline some of the negative effects associated with androgen decrease (Edinger and Frye, 2005). Similarly, gonadectomy in rats induced anxiogenic-like effect which testosterone supplementation in gonadectomized rats recovered this effect (Zhang et al., 2011; McDermott et al., 2012).

Table I . The table explains experimental design.

Gonadectomy	Post-operative period	Drug treatment	Species	Age	Effect on anxiety	Reference
↓ Testosterone	10 days	(Testosterone: 200, 300 and 450 mg/kg)	Rat	3 month	Gonadectomy → anxiogenic Testosterone treatment → anxiolytic Morphine treatment → anxiolytic Naloxone treatment → anxiogenic	(Khakpai, 2014)
↓ Testosterone	2 weeks	(Testosterone: 2 mg/kg)	Rat	3 month	Testosterone treatment → anxiolytic	(Zhang et al., 2011; Shin et al., 2003)
↓ Testosterone	10 days	(Testosterone: 25 mg/kg)	Rat	2-3 month	Testosterone treatment → anxiolytic	(Frye and Edinger, 2004)
↓ Testosterone	4 to 6 weeks	(Testosterone: 10 mg per rat)	Rat	2 month	Testosterone treatment → anxiolytic	(Edinger and Frye, 2004)
↓ Testosterone	4 to 6 weeks	(Testosterone: 1 mg/kg)	Rat	2 month	Testosterone treatment → anxiolytic	(Edinger and Frye, 2005)
↓ Testosterone	3 weeks	(Testosterone: 0.25, 0.50, 1.0 mg/rat)	Rat	3 month	Testosterone treatment → anxiolytic	(Fernandez-Guasti and Martinez-Mota, 2005)
↓ Testosterone	7 days	(Estradiol: 5 or 10 mg/rat)	Rat	2 month	Estradiol treatment → anxiolytic	(Walf and Frye, 2005a)
↓ Testosterone	4 weeks	(Morphine: 5 and 10 mg/kg)	Rat	3 month	↓ Opioid transmission in relation to LHRH release	(Almeida et al., 1988)
↓ Testosterone	2 to 4 weeks	(Testosterone: 1 mg/rat, naloxone: 1 mg/kg)	Rat	3 month	Gonadal factors → opiate activational and/or organizational effect on LH secretion	(Masotto and Negro-Vilar, 1988)
↓ Testosterone	13 days to 31 days	(Morphine: 1.5 mg/kg)	Rat	3 month	Tolerance → effect of morphine on LH secretion	(Cicero et al., 1982)
↓ Testosterone	10 days	(Oestradiol: 1.25 μg/kg, naloxone: 600 μg/kg)	Ferret	Adult	Interaction between steroids and opioids → regulation of LH secretion	(Lambert et al., 1990)
↓ Testosterone	4 weeks	(μ-receptor agonist (DAMGO): 1 nM, δ-receptor agonist (DSLET): 5 nM)	Rat	3-4 month	Gonadectomy → failed to affect μ- or δ-receptor agonists induced analgesia	(Kepler et al., 1991)

The effect of gonadectomy on anxiety behavior

Previous evidence indicates that sexual behavior induces an anxiolytic-like effect decreasing the impact of several types of stressors. Second, androgens have been described to have anxiolytic effects in other situations including emotional stress. For example, male rats administered testosterone are less disrupted during punished drinking testing in the Vogel paradigm (Bing et al., 1998) and display declined signs of anxiety in the elevated plus-maze (Aikey et al., 2002), open field test (Edinger and Frye 2004), defensive burying test (Fernandez-Guasti and Martinez-Mota, 2003), and defensive freezing (Edinger and Frye, 2005) relative to vehicle-treated rats. Additionally, enhancement of endogenous androgen release *via* sexual stimuli also enhances exploratory behavior in the open arms of the elevated plus-maze in male mice (Aikey et al., 2002). As mentioned previously, gonadectomy in adult male rats during short-term (10 days) as well as long-term (70 days) induces higher levels of anxiety behavior (Svensson et al., 2000; Justel et al., 2012a; Khakpai 2014), which testosterone administration can reverse some of the effects of gonadectomy (Frye and Edinger, 2004; Justel et al., 2012a; Khakpai, 2014). Toufexis et al. (2005) reported that castration of male rats produced a more consistent light-enhanced startle (anxiogenic response), similar in magnitude to that observed in female rats. Replacement of testosterone, at high physiological doses, significantly attenuated light-enhanced startle in castrated males and further reduced it in intact male rats. This shows that circulating testosterone acts to decrease the response of male rats to the anxiogenic stimulus of bright light (Toufexis et al., 2005).

In the open field test, animals treated with anxiolytics display an enhanced tendency to explore the central location of the field (Prut and Belzung, 2003; Yan et al., 2015). So, it was expected that exogenous testosterone treatment would increase activity in the central area of the open field. Conversely, gonadectomy in adult male rats for short-term reduce activity in the central area of the open field (Justel et al., 2012a). The effects of gonadectomy and hormone replacement for short-term in adult rats on various measures of anxiety have also

been studied in the elevated plus-maze (Walf and Frye, 2005b), avoidance (Frye et al., 2004; Edinger and Frye, 2007a) and acoustic startle response paradigms (Turvin et al., 2007), consequently introducing the further probability that anxiety in the gonadectomy and/or hormone-replaced subjects could affect NOR testing and outcome (Aubele et al., 2008).

Researches demonstrated that testosterone-replacement can alleviate anxiety behavior related to gonadectomy from 4 to 6 weeks following surgery. As mentioned previously, testosterone's anti-anxiety effects may be modulated in part by its metabolites. Testosterone can be aromatized to estrogen, which has been revealed to decline anxiety behavior in people and animals. However, testosterone is also metabolized to dihydrotestosterone. Administration of dihydrotestosterone, a nonaromatizable metabolite, can decrease the anxiety behavior of gonadectomized rats similarly to testosterone administration (Edinger and Frye, 2004; Frye and Edinger, 2004). Dihydrotestosterone can metabolize to 3α-androstanediol and systemic injection of 3α-androstanediol can also decrease anxiety behavior of intact or gonadectomized male or female rats (Edinger and Frye, 2005). Studies indicated that blocking dihydrotestosterone metabolism to 3α-androstanediol with indomethacin, a 3α-hydroxysteroid dehydrogenase inhibitor also increases anxiety behavior in the open field, elevated plus maze, and defensive freezing tasks of intact or dihydrotestosterone replaced male rats (Frye and Edinger, 2004). These findings propose that testosterone's anti-anxiety properties may be due in part to the actions of its 5α-reduced metabolites, independent of its aromatization to estrogen.

In the brain, androgen receptors are expressed through both neurons and glial cells and are predominantly found in the hippocampus, amygdala, thalamus, hypothalamus, and cerebral cortex (Moghadami et al., 2016). The hippocampus is a putative site of action for androgens' anti-anxiety properties. The hippocampus modulates the anxiety process (Bannerman et al., 2002). Androgens can have actions in the hippocampus. In the rat hippocampus, the androgen receptor is mainly concentrated in the CA1 pyramidal cells. It is reasonable to assume the presence of an association between androgen receptors and cognitive activities (Moghadami et al., 2016). Castration decreases neuronal firing, enhances vulnerability to cell death, and decreases synapse density in the hippocampus, effects which can be reversed by androgen-replacement (Hajszan et al., 2004). The enzymes necessary for testosterone's metabolism, 5α-reductase, and 3α-hydroxysteroid dehydrogenase, are also located within the hippocampus (Rhodes and Frye, 2004). As such, testosterone and dihydrotestosterone are readily metabolized to 3α-androstanediol in the hippocampus (Edinger and Frye, 2004). These data propose that testosterone's 5α-reduced metabolites may have actions in the hippocampus to modulate the anxiety process (Frye and Edinger, 2004; Edinger and Frye, 2005).

Overall, sexual behavior caused an anxiolytic-like effect. Gonadectomy for short-term and long-term induced higher levels of anxiety behavior (Svensson et al., 2000; Justel et al., 2012a; Khakpai 2014), which administration of testosterone can reverse some effects of gonadectomy (Frye and Edinger, 2004; Justel et al., 2012a; Khakpai 2014), showing that circulating testosterone decreased the response of males to the anxiogenic stimulus (Toufexis et al., 2005). Testosterone's anti-anxiety effects produced *via* 5α-reduced metabolites (Edinger and Frye, 2004; Frye and Edinger, 2004).

The effects of opioid antagonist and testosterone on the modulation of anxiety behavior

Opioids are known to play a role in mediating the effects of androgen. Nonetheless, opioids have many adverse effects, including opioid-induced androgen deficiency (Chrastil et al., 2014). The effect of the opioid system on the modulation of testosterone levels is suggested to be mediated *via* effects on both the hypothalamus and the testes. Opiates are proposed to affect the release of GnRH from the hypothalamus. In the CNS, endogenous opioids inhibit pulsatile GnRH release, partly mediating the stress response within the central nervous-pituitary-gonadal axis (Bottcher et al., 2017). This, in turn, causes a decrease in the release of LH from the anterior pituitary gland, which is necessary for the activation of Leydig cells to produce testosterone. In addition, opiates were also shown to increase the sensitivity of the hypothalamus to the negative feedback effects of testosterone causing a marked suppression in LH release (Lambert et al., 1990; Hofford et al., 2010; Ruka et al., 2016). Opioidergic transmission reduced, in relation to LHRH release, after long term castration. Opioid receptor activity (evaluated *via* responsiveness to an opioid receptor agonist) of female rats is maintained, while that of male rats is lost, after long term gonadectomy (Almeida et al., 1988). Studies indicated that naloxone can stimulate LH release when rats gonadectomized for a few weeks, were injected with either oestradiol benzoate or testosterone propionate. Masotto and Negro-Vilar (1988) reported that male rats indicated no variation in any parameter of pulsatile LH secretion in response to naloxone 8 weeks after castration whereas a small enhance in mean LH level and in LH pulse amplitude was observed 1 to 2 weeks after gonadectomy. In gonadally intact ewes the opioid antag-

onist, WIN 44,441-3, increased LH pulse frequency and amplitude at selected times during the estrous cycle, however, it had no influence in subjects ovariectomized for 4 months or more. Ewes retained an ability to indicate a small enhance in LH pulse frequency when given an opioid antagonist 1 week after ovariectomy (Whisnant and Goodman, 1988). In other researches, however, LH responses to opioid manipulation have been observed following short- and long-term castration. For example, Cicero and coworkers (1982) found alike LH response to naloxone in male rats tested 3 and 31 days after castration. In female rats, plasma LH levels enhanced in response to naloxone 24 h, 4 days and 8 days after ovariectomy (Babu et al., 1988). Also, intraventricular injection of the opioid receptor agonist, β-endorphin to female rats which had been ovariectomized for 3 weeks, produced significant reductions in LH pulse frequency and amplitude as compared to the LH output observed during a comparable saline injection. Female rabbits displayed dramatic rises in LH pulse amplitudes and mean LH levels when given an intravenous injection of naloxone 2 weeks after gonadectomy. Although LH responses to naloxone are variable after short-term gonadectomy in the species as mentioned, gonadally intact subjects or animals given sex steroids after short-term gonadectomy generally show an enhance in LH secretion in response to opioid receptor antagonists (Lambert et al., 1990). Otherwise, modulation of the sensitivity of the hypothalamus-pituitary axis *via* opiates was also proposed to result from a declined sensitivity of the pituitary to GnRH. Additionally to their effects on the hypothalamus, the opioid system was revealed to inhibit gonadal function through specific opioid receptors within the testes. This was confirmed to be mediated by suppression of testicular steroidogenesis, which results in reductions in plasma testosterone levels (Hofford et al., 2010).

Animal studies also support links between anabolic-androgenic steroids and opioids. At the physiologic level, testosterone increases the response to opioids. At the pharmacologic level, testosterone self-administration intracerebroventricular causes autonomic depression alike to opioid overdose, which is inhibited by the opioid antagonist naltrexone. In other studies, anabolic-androgenic steroids increase morphine-induced hypothermia (Celerier et al., 2003), even as they decrease the analgesic response of morphine (Philipova et al., 2003), and weaken tolerance to morphine's antinociceptive effect (Celerier et al., 2003). This is consistent with anabolic-androgenic steroids-induced opioid receptor binding in the brain (Cooper and Wood, 2014). The effect of castration may be nociceptive because it enhanced morphine analgesia on the hot-plate test (Ali et al., 1995). Alike to results in female rats, intracerebroventricular morphine infusions in castrated male rats induce analgesia on the tail-flick and jump tests which are decreased in efficacy but not potency (Kepler et al., 1989). This effect may be CNS area-dependent because morphine potency after infusion into the ventrolateral periaqueductal gray in castrated male rats is slightly enhanced. Generalizations cannot be made from morphine to other μ-receptor agonists because gonadectomy in adult male rats during 4 weeks failed to consistently affect analgesia induced by intracerebroventricular infusions of μ-receptor-selective agonist D-Ala2-MePhe4-Gly-ol5-enkephalin (DAMGO). Gonadectomy in adult male rats for 4 weeks was similarly without action on the δ-receptor analgesia of [D-Ser2, Leu5]enkephalin-Thr6 (DSLET) (Kepler et al., 1991). In male mice, morphine analgesia after castration is enhanced on the hot-plate test and against abdominal writhing produced by acetic acid but declined on the tail-flick test (Ali et al., 1995). Testosterone reversed the decreased morphine sensitivity of the castrated rat (Kest et al., 2000). Results of nociceptive testing procedures examining the activational roles of gonadal hormones on opioid antinociception are somewhat variable. In male rats, gonadectomy for short- and long-term enhanced, declined or failed to change μ-opioid antinociception. Also, in females, gonadectomy during short- and long-term enhanced, declined or did not change opioid antinociception. The variability across investigations that have manipulated gonadal hormones in adult rats might be due to the wide array of methodologic differences across investigations. Almost every investigation has used different gonadectomy test intervals (short- and long-term), hormone replacement regimens, opioid injection procedures, and nociceptive testing procedures. Investigations suggest that both testosterone in adult male rats and estradiol in adult female rats contribute to the sex difference in morphine antinociception (Craft et al., 2004). Sex differences in the antinociceptive effects of opioids have been revealed in both non-human primates and rodents, with males being usually more sensitive than females (Terner et al., 2002; Loyd et al., 2008; Bai et al., 2015). There is abundant evidence showing that pharmacokinetic factors cannot fully account for these differences, as opioids are more potent in males following central injection, and systemic injection of morphine causes comparable brain and plasma levels in males and females (Kepler et al., 1991; Kest et al., 1999; Terner et al., 2002). There is also evidence proposing that pharmacodynamic factors do not play a key role, as sex differences have not been indicated in opioid binding affinity and receptor density (Terner et al., 2002).

In mammals, opioids control food intake and energy balance, and gonadal androgens interact with the

opioid system neurochemically and behaviorally (Mateo et al., 1992). So, pretreatment with the long-acting opioid blocker naltrexone inhibited the physiologic and behavioral symptoms of testosterone injection and blocked the reinforcing effects of testosterone self-administration (Peters and Wood, 2005).

Investigations show the involvement of testosterone and opioidergic system in anxiogenic-like behaviors induced by gonadectomy during the short- and long-term. Several studies have reported an anxiolytic function for morphine and μ-opioid receptor agonists when injected peripherally, whereas μ-opioid receptor antagonists tend to be anxiogenic (Le Merrer et al., 2006; Zarrindast MR 2008). As mentioned above, the endogenous opioid system could influence testosterone levels *via* effects on the hypothalamic-pituitary-gonadal axis and the testes (Hofford et al., 2010). Thus, the possible mechanism(s) between testosterone and opioid system in anxiety behavior control seem possible. Administration of opioids in males causes opioid produced androgen deficiency, i.e. a significant reduction in plasma testosterone levels. This effect is reported in humans as well as in experimental animals, for example, rodents (Khakpai, 2014). This opioid effect is dramatic, a single administration can cause a robust decrease in testosterone levels comparable to castration. Moreover, tolerance does not develop to this opioid mediated effect, consequently, this decrease lasts for the entire duration of opioid administration (Aloisi et al., 2005).

Collectively, the opioid system could modulate testosterone levels by affecting the release of GnRH from the hypothalamus. Endogenous opioids inhibited pulsatile GnRH release (Bottcher et al., 2017) which caused a decrease in the release of LH (Lambert et al., 1990; Hofford et al., 2010; Ruka et al., 2016). Opioid transmission decreased, in correlation with LHRH release, after long-term castration (Almeida et al., 1988). Naloxone stimulated LH to release following short- and long-term castration in adult male rats (Cicero et al., 1982). Interaction between testosterone and opioidergic system may modulate anxiogenic-like responses induced by gonadectomy during the short- and long-term. (Le Merrer et al., 2006; Zarrindast, 2008).

Treatment of anxiety disorders with androgens alone or in a combination with different anxiolytics

Many investigations have demonstrated that anxiety-like behaviors are influenced *via* peripheral and central factors including hormones and neurotransmitters in the diverse regions of CNS. Many types of research have revealed the anxiolytic effect of androgens in various methods. The most cited paper exploring the effects of testosterone on anxiety behavior in animals and humans has presented in numerous experiments that testosterone either endogenous or exogenous reduced anxiety (Frye and Seliga, 2001; Aikey et al., 2002; Khera, 2013; Khakpai, 2014; Dossat et al., 2017). Furthermore, a similar experiment indicated that this anxiolytic response of testosterone is dose-dependent and very probable mediated *via* 5-alpha reductase which reduces testosterone to dihydrotestosterone. Some experiments on gonadectomized rats indicated that dihydrotestosterone 3-alpha metabolites can be the mediators of testosterone anxiolytic effects (Edinger and Frye, 2005). Furthermore, blockade of the dihydrotestosterone transformation to 3-alpha androstanediol *via* a 3-alpha hydroxysteroid dehydrogenase inhibited or prevented the anxiolysis (Frye and Edinger, 2004; Celec et al., 2015).

The hypothalamic-pituitary-gonadal axis is regulated through a complex series of outside effects as well. Opioids are one of a number of such effects. Studies propose that opioids, both endogenous and exogenous, can couple to opioid receptors principally in the hypothalamus, but potentially also in the pituitary and the testis, to regulate gonadal function. Opioids have been presented to decline the release of GnRH or restrict its normal pulsatility at the level of the hypothalamus, resulting in a reduced release of LH and FSH from the pituitary and a second fall in the gonadal steroid production, that is, hypogonadism. Direct influences of opioids on the testis, including reduced secretion of testosterone and testicular interstitial fluid, have also been revealed. Opioid receptors have also been distributed in ovarian tissue cultures and opioids have been revealed to directly suppress ovarian steroid production in vitro. Opioids have also been revealed to change the adrenal production of dehydroepiandrosterone, the main precursor of both testosterone in men and estradiol production in women (Katz and Mazer, 2009). Therefore, opioids by influencing hypothalamic-pituitary-gonadal activity as well as GnRH and LH secretion interact with androgens to modulation of anxiety behavior.

CONCLUSION

Anxiety can be produced by various endocrine, autoimmune, metabolic and toxic disorders as well as the adverse effects of medication (Kessler et al., 2005). Several studies have reported anxiolytic function for morphine and androgens. One the other hand, withdrawal of morphine (Buckman et al., 2009; Poori-

amehr et al., 2017; Kim et al., 2018) and gonadectomy for short- and long-term (Svensson et al., 2000; Justel et al., 2012a; Khakpai 2014) cause to anxiogenic behavior. Interestingly, the opioid system was revealed to play a role in gonadal hormone regulation (Hofford et al., 2011). The effects of opioids on testosterone levels have several implications for the short and long term health of patients requiring pain management and for drug addicts. Opioid treatment decreases plasma testosterone levels in males (Aloisi et al., 2009; Hofford et al., 2011). This effect induces *via* modulation of the hypothalamic-pituitary-gonadal axis activity (Hofford et al., 2011; Khakpai, 2014). Researches indicated the effect of opioids on the modulation of anxiety-response induced by androgen. Also, opioids by modulation of plasma testosterone levels could modulate anxiety behavior in gonadectomized animals (Khakpai, 2014). There are reports showing that injection of the opioid antagonist, naloxone, produces a rise in testosterone concentrations and so, administration of naloxone in low doses is capable of modifying testosterone concentrations in plasma (Gartner, 2001; Khakpai, 2014). Therefore, naloxone may have an effect on the modulation of anxiety behavior in gonadectomized animals (Khakpai, 2014). Moreover, future studies are needed to fully understand the nature and causes of the possible mechanisms between opioid and androgens on the modulation of anxiety behavior in gonadectomized animals, as this might simultaneously target the cortical/cognitive as well as subcortical/reflexive characteristics of anxiety while avoiding the apparent side-effects of chronic hormone administration or opiate abuse.

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