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Sex Steroidal Hormones and Respiratory Control

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Abstract

There is a growing public awareness that sex hormones can have an impact on a variety of physiological processes. Yet, despite almost a century of research, we still do not have a clear picture as to the effects of sex hormones on the regulation of breathing. Considerable data has accumulated showing that estrogen, progesterone and testosterone can influence respiratory function in animals and humans. Several disorders of breathing such as obstructive sleep apnea (OSA) and sudden infant death syndrome (SIDS) show clear sex differences in their prevalence, lending weight to the importance of sex hormones in respiratory control. This review focuses on questions such as: How early do sex hormones influence breathing? Which is the most effective? Where do sex hormones exert their effects? What mechanisms are involved? Are there age-associated changes? A clearer understanding of how sex hormones influence the control of breathing could enable sex- and age-specific therapeutic interventions for diseases of the respiratory control system.

1. Introduction

There is a growing awareness that sex hormones play a major role in virtually all physiological processes, and the research literature reflects this with an increasing number of studies including female subjects. Public awareness of the potential impact of sex hormones on cardiovascular function, sleep and breathing has been elevated by the findings of large, population based studies including the Nurses Health Study (Grodstein et al., 2000), the Womens Health Initiative (Rossouw et al., 2007), the Wisconsin Sleep Cohort Study (Young et al., 2003) and the Sleep Heart Health Study (Shahar et al., 2003).

For almost a century it has been known that sex hormones influence breathing. Studies by Hasselbach reported that women have decreased alveolar PCO₂ and lowered arterial PCO₂ during pregnancy (Hasselbach, 1912; Hasselbach and Gammeltoft, 1915). Later studies described cyclic fluctuations in ventilation during the normal menstrual cycle that ceased with menopause (Griffith et al., 1929). In the ensuing years, considerable data have accumulated showing that throughout life, estrogen, progesterone and testosterone can influence respiratory function in animals and humans (for reviews, see Dempsey et al., 1986; Tatsumi et al., 1995; Behan et al., 2003). Additionally, several disorders of breathing such as obstructive sleep apnea (OSA), sudden infant death syndrome (SIDS) and Rett syndrome also show clear sex differences in their prevalence, lending weight to the importance of sex hormones in respiratory control (Kapsimalis and Kryger, 2002a,b; Chahrour and Zoghbi, 2007; Hauck, 2001).

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This review focuses on sex hormones and their receptors in the respiratory control system. Contemporary questions revolve around issues such as: How early do sex hormones exert their effect on breathing? Which of the sex hormones, estrogen, progesterone or testosterone is the most potent, and in which sex? Where do sex hormones exert their effects in the nervous system and by what mechanisms?; and finally, Does aging alter the impact of sex hormones on the control of breathing? Ideally, a clearer understanding of where, when and how sex hormones influence the control of breathing in males and females will lead to sex- and age-specific therapeutic interventions for diseases of the respiratory control system.

2. Sex hormones in the central nervous system

Sex steroid hormones include androgens (e.g., testosterone), estrogens (of which 17 β -estradiol is the most potent in mammals), and progesterone, all of which are derived from cholesterol (Stoffel-Wagner, 2001). Steroid hormones are synthesized primarily in the gonads, the adrenal gland and the placenta. Neurosteroids are synthesized *de novo* in the central and peripheral nervous system. Steroidogenic enzymes are present in both neurons and glia, and many of these enzymes are region specific (Mellon and Griffin, 2002). Neurosteroids can be synthesized from cholesterol or steroid hormone precursors (e.g., progesterone, pregnenolone, allopregnanolone, 5 α -dihydrosterone, estradiol, dehydroepiandrosterone), or from circulating sex steroid hormones such as testosterone and progesterone, all of which readily cross the blood-brain barrier.

Sex steroid hormones and neurosteroids can affect gene expression by binding to classical nuclear receptors, binding to steroid receptors located on cell membranes, acting on ion-gated membrane receptors, or modulating neurotransmitter receptor function. For example, metabolites of progesterone such as allopregnanolone, can bind to GABA_A receptors and potentiate GABA inhibition (Smith et al., 2007). Neurosteroids may act at other neurotransmitter receptors such as NMDA, and they are also involved in myelination and neurite outgrowth (Mellon and Griffin, 2002). Neurosteroid synthesis is high during the perinatal period and also during exposure to physiological stressors such as hypoxia (Mellon and Vaudry, 2001; Nguyen et al., 2004). In perinatal rats, respiratory rhythm is modulated by neurosteroids acting at the GABA_A receptor (Ren and Greer, 2006). Specifically, the efficacy of GABA_A receptor-mediated modulation of respiratory membrane potential and rhythmogenesis is enhanced by allopregnanolone and depressed by dehydroepiandrosterone sulphate. Thus, the balance of neurosteroids in the pre-Bötzinger complex could modulate rhythmogenesis by altering the efficacy of specific inhibitory inputs to this region. In adult rats, respiratory plasticity in response to intermittent hypoxia is significantly reduced following gonadectomy in male rats. This effect is reversed by supplementing with testosterone, but only a form that can be converted to estradiol by aromatase in the brain (Zabka et al., 2006). Endogenous concentrations of neurosteroids and steroidogenic enzymes can be modulated by the ovarian cycle, stress, hormone manipulations and aging. Additionally, neurosteroids have been implicated in a variety of behavioral and neurological disorders including the regulation fear and anxiety, seizure disorders, memory, neurodegeneration and postpartum depression (Mellon and Griffin, 2002).

Estrogen, progesterone and testosterone are present in both males and females from birth, although circulating levels differ greatly. For example, in neonatal rats testosterone levels are higher in males than in females, whereas progesterone levels are approximately equal (Döhler and Wuttke, 1974; Weisz and Ward, 1980). Serum estrogen levels are almost undetectable in immature rats and are similar in males and females, although sex differences in hormone levels have been measured in the cortex and hypothalamus, but not in the brainstem, of neonatal rats (Rhoda et al., 1984; Amateau et al., 2004).

Male testosterone levels peak at ~2–6 months in rats and ~18–40 years in humans (Ghanadian et al., 1975; Smith et al., 1992; Bhasin et al., 2006). In regularly cycling adult women, estradiol levels increase during the follicular phase and are at their lowest at the end of the luteal phase (Hotchkiss and Knobil, 1994). Progesterone levels peak in the luteal phase and are low in the follicular phase. In female rats, estradiol levels increase progressively during diestrus. Early in estrus when estradiol levels are low, progesterone levels peak (Freeman, 1994). In male rats, progesterone levels are much lower than in females at any stage of the estrus cycle, whereas estradiol levels approximate the lowest levels measured in female rats (Gonzalez-Arenas et al., 2004).

With increasing age there is a decline in circulating levels of testosterone, estradiol and progesterone in males (Ghanadian et al., 1975; Ferrini and Barrett-Connor, 1998; Smith et al., 1992). In female rats, the regular 4–5 day estrous cycle slows, and animals eventually become acyclic. Estradiol levels are relatively unchanged, although progesterone levels can fluctuate (Chakraborty and Gore, 2004). In contrast, women and some higher primates show a sharp decline in levels of estradiol and progesterone at menopause (Chakraborty and Gore, 2004).

2.1. Mechanisms by which sex hormones exert their effect

Many of the actions of sex steroid hormones are mediated by specific intracellular receptors, members of the classical nuclear receptor superfamily. Estrogen exerts its effects by means of two mechanisms: genomic or non-genomic. In the former mechanism, estrogen binds to its receptor (ER α , 66 kDa; ER β , 54 kDa; encoded by different genes) and translocates to the nucleus with a subsequent regulation of target genes (Nilsson et al., 2001). In the latter mechanism, estrogen acts at the plasma membrane, resulting in activation of signal transduction pathways, accompanied by calcium influx (Vasudevan and Pfaff, 2007). Latencies differ considerably between these two mechanisms: minutes to hours for the former, seconds to minutes for the latter (Brailoiu et al., 2007), and either mechanism may be engaged within a single neuron (Vasudevan and Pfaff, 2007).

Considerable attention has focused recently on GPR30, an orphan G-protein coupled membrane receptor that binds estrogen with high affinity followed by adenylyl cyclase activation (Thomas et al., 2006). In the rat central nervous system GPR30 immunoreactivity has been reported in several respiratory-related structures including the nucleus of the solitary tract, the hypoglossal nucleus and the nucleus ambiguus in both male and female rats (Brailoiu et al., 2007). Whether the G-protein coupled ER is associated with the plasma membrane or whether classical ERs are temporarily tethered to the membrane remains controversial (Vasudevan and Pfaff, 2007).

Two progesterone receptor isoforms have also been described in rat, PR-B (120 kDa) and the N-terminally truncated PR-A (80 kDa), both of which are derived from a single gene (Kastner et al., 1990). Similar to estrogen, progesterone exerts its effect by classical genomic mechanisms (Auger, 2004). Non-genomic effects of progesterone metabolites include allosteric modulation of the GABA $_A$ receptor (Smith et al., 2007).

Androgen receptors which are also members of the nuclear receptor superfamily, are found throughout the brain in males and females (Simerly et al., 1990). Increasing evidence suggests that androgens can also exert rapid, non-genomic effects via membrane receptors (Michels and Hoppe, 2008).

Changes in circulating levels of sex hormones across the estrus cycle, or with increasing age, have frequently been correlated with neuroanatomical or physiological measures. However, care must be taken when making such associations. Classical steroid receptor levels can vary considerably within the space of a few hours: PRs can be upregulated by estradiol and

downregulated by progesterone, and estrogen receptors are negatively regulated by their ligand (Chakraborty and Gore, 2004). Unique combinations of coactivators and corepressors in a transcriptional complex can selectively control the expression of genes in different neuronal populations. Additionally, *in vitro* studies have shown that steroid hormone receptors can be activated by non-steroidal mechanisms such as phosphorylation, or by the neurotransmitter dopamine (Auger, 2004).

In studies that compare respiratory control measures in males and females, some knowledge of circulating sex hormone levels is extremely valuable. Additionally, in studies where animals are gonadectomized and supplemented with sex hormones, the timing and duration of hormone administration may be critical for correct interpretation of the data. For example, there is a broad range of testosterone levels in male rats at all ages. Gonadectomy may not reduce testosterone levels significantly, and it is difficult to achieve physiological levels of hormone with supplementation (see discussion in Zabka et al., 2006). Additionally, delivery of testosterone may affect endogenous levels of other hormones. For example, when testosterone levels were suppressed by leuprolide acetate in healthy men, estradiol, follicle stimulating hormone and luteinizing hormone levels were also suppressed (Mateika et al., 2004). In female rats the relationship between estrogen and progesterone varies considerably across the five-day estrus cycle, and mimicking this relationship by ovariectomy and hormone supplementation is particularly challenging. Many factors influence serum estradiol concentrations including the age at ovariectomy, post ovariectomy duration, type and duration of estrogen replacement therapy, as well as species, strain and age (Chakraborty and Gore, 2004).

Possible mechanisms whereby sex hormones can modulate breathing include direct or indirect effects on gene expression in respiratory neurons. Anatomically distinct populations of respiratory neurons have been identified by marker genes such as peptides, receptors and calcium binding proteins (Alheid et al., 2002). One example of this is the expression of neurokinin-1 receptors in respiratory rhythm-generating neurons in the pre-Bötzinger complex (Stornetta et al., 2003). Changes in neurokinin-1 receptor expression in the uterus across the estrus cycle and during pregnancy, suggests that circulating sex hormone levels could alter gene expression in select groups of respiratory neurons and thereby alter breathing (Candenas et al., 2001). Recently it has been hypothesized that groups of respiratory neurons express unique combinations of transcription factors during development which ultimately determine their role in brainstem respiratory networks (Gray, 2008). For example, selective lesions of Phox2b- expressing neurons in the retrotrapezius nucleus result in alterations in the apneic threshold (Takakura et al., 2008). Were transcription factors such as Phox2b shown to be influenced by sex hormones or neurosteroids, this might help to explain how elevated steroid hormone levels during development in response to stress could alter respiratory responses in adulthood (Genest et al., 2004).

3. Breathing in males and females

There are a number of reports of differences in ventilatory measures between men and women at different ages (White et al., 1983; Saaresranta and Polo, 2002; Jensen et al., 2005a). Similarly, several studies have reported variations in ventilatory measures across the menstrual cycle, and in pregnancy (Driver et al., 2005; Jensen et al., 2005b; da Silva et al., 2006). Nonetheless, despite many studies in both animals and humans, no clear picture has emerged as to how sex and age impact normal breathing as well as responses to respiratory challenges such as hypoxia and hypercapnea.

3.1. Sex differences and age-associated changes in ventilation

The data are conflicting as to whether there are age-associated changes in ventilatory responses to hypoxia or hypercapnia in males and females. Some studies in humans reported a blunting of the hypoxic ventilatory response and the hypercapnic ventilatory response with age (Kronenberg and Drage, 1973; Brischetto et al., 1984), whereas others found little or no difference (Patrick and Howard, 1972; Rubin et al., 1982; Ahmed et al., 1991). These human studies included relatively small groups of subjects, and comparisons were made more difficult by sex differences in anthropometric, lung function and baseline ventilatory indices, and how the data were normalized (Van Klaveren and Demedts, 1998). Additionally, some studies did not control for the menstrual cycle stage of the female subjects or the use of oral contraceptives and hormone replacement therapy (Sebert et al., 1990). The data from animal studies are sparse. Schlenker and Goldman (1985) reported an age-associated decrease in the hypoxic ventilatory response in male rats, whereas female rats had an age-associated increase in the hypoxic ventilatory response. As with the human studies, not all age groups were represented for each sex in these animal studies, and sex hormone levels were not measured.

Recently a study of sex differences in ventilation that included male and female rats at three different ages, arterial blood gas analysis, and measurement of circulating sex hormone levels was undertaken (Wenninger et al., 2007). One of the key findings of this study is that the impact of aging on ventilatory control is non-linear. For example, breathing frequency decreased with age in both males and females, but in males the decrease was step-wise at each age studied (3, 12, >20 months of age), whereas in females the decrease occurred only between middle-age and old. Similar non-linear effects of aging were observed in the hypoxic and hypercapnic ventilatory responses. As male and female rats have different responses at a given age, it is not surprising that data from a number of prior studies appear to conflict with each other. Repeating this rat study with more animals at a wider range of ages might highlight critical periods in ventilatory control in males by comparison with females.

Studies in anesthetized rats have shown sex differences in a measurement of respiratory plasticity in response to episodic hypoxia known as long term facilitation. Hypoglossal and phrenic long term facilitation was greater in anesthetized young male rats than in age-matched female rats in estrus or diestrus, but by middle-age, long term facilitation was greater in female than in male rats (Zabka et al., 2001a,b). Long term facilitation has been measured in awake humans, but, in contrast to rats, the magnitude of the sustained increase in minute ventilation following intermittent hypoxia was similar in men and women (Wadhwa et al., 2008). These data point to the possibility of species differences in respiratory plasticity, but also highlight the difficulties of comparing studies in awake and anesthetized preparations.

3.2 Does aging alter the impact of sex hormones on the control of breathing?

Although the respiratory system is capable of maintaining adequate ventilation and gas exchange throughout life, shortcomings are revealed when the system is challenged (e.g., hypoxia, hypercapnia, exercise, disease). Some of the effects of aging are associated with structural changes in the airway as well as in muscle and lung function (Janssens et al., 1999). Other aging effects are likely related to alterations in carotid chemoreceptors (Conde et al., 2006), respiratory neuronal circuits (McGinnis and Yu, 1995; Behan and Brownfield, 1999; Seebart et al., 2007), and cellular and molecular events that accompany the aging process. Some of these ventilatory changes are equally present in males and females, but others seem to be more pronounced in one sex. As the levels of most sex hormones decline with age, it is reasonable to hypothesize that age-related changes in respiratory control are linked to changes in specific sex hormone or sex hormone receptor levels (Behan et al., 2002).

In males, there is an overall decline in levels of testosterone, estrogen and progesterone with age (Smith et al., 1992; Ghanadian et al., 1975; Ferrini and Barrett-Connor, 1998; Bhasin et al., 2006). Some effects of sex hormones on respiratory control in male rats are mediated by the conversion of testosterone to estradiol by aromatase in the brain (Zabka et al., 2006). However, it is not clear whether there is an age-associated decline in aromatase levels that might contribute to the loss of respiratory plasticity associated with increasing age (Zabka et al., 2005; Ishunina et al., 2005). Furthermore, testosterone has been shown to regulate aromatase activity in some brain regions in adult rats (Roselli et al., 1998). Whether this regulation is maintained in old age with reduced testosterone levels remains to be determined. In women there is a precipitous decline in levels of estrogen and progesterone at menopause, but testosterone levels decrease gradually (Burger, 2002). Despite no major loss of sex hormones with increasing age in female rats, there are age-associated changes in respiratory function (Schlenker and Goldman, 1985; Zabka et al., 2001b, 2003).

Few studies have systematically addressed age-associated changes in sex hormone receptors in the brain. Nonetheless, there is evidence from studies in rodents that the effects of steroid hormones in the aging brain may differ from those in the young brain due to changes in steroid hormone receptors (Chakraborty and Gore, 2004; McGinnis and Yu, 1995). Furthermore, it is likely that there are also age-associated changes in signaling molecules downstream of sex hormone receptors. Ultimately, the capacity to respond to sex hormones in an aged neuron may be fundamentally different from that of a young neuron.

4. Key questions

4.1. How early do sex hormones exert their effect?

Although circulating levels of maternal hormones increase dramatically during gestation, the fetus has limited exposure to sex steroid hormones (Doan et al., 2004; Soliz and Joseph, 2005). Nonetheless, gestational blockade of these maternal sex hormones has long-lasting effects on respiratory control (Doan et al., 2004). The respiratory control system undergoes a prolonged period of postnatal maturation that is influenced by a number of environmental factors (Carroll, 2003; Soliz and Joseph, 2005; Bavis and Mitchell, 2008). Postnatal critical periods during which exposure to hypoxia or hyperoxia can have lasting effects on chemoreflex sensitivity in later life have been described (Bavis et al., 2004; Reeves and Gozal, 2005). Perinatal exposure to steroid hormones may also have a long-lasting impact on the respiratory control system. For example, neonatal stress associated with removal of rat pups from the mother for 3 hours per day from day 3 to 12, results in elevated cortisol levels (Francis et al., 1999). In adulthood, these rats have an elevated hypoxic ventilatory response (Genest et al., 2004; Kinkead et al., 2005a,b). However, female rats seem to be less susceptible to the effects of neonatal maternal stress by comparison with males who show a 25% increase in the hypoxic ventilatory response (Genest et al., 2004). Implanting young pups with cortisol-releasing pellets also results in augmentation of the hypoxic ventilatory response in a sex-specific manner (Fournier et al., 2007). Sexual dimorphism has also been described in the chemoreflex of animals living at high altitude, perhaps resulting from early exposure to hypoxia (Joseph et al., 2000). Whether sex differences in the response to early hormone exposure are due to differences in sex hormone receptor expression in peripheral chemosensors such as the carotid body, or in central respiratory control regions is not known. Sex differences in the expression of sex hormone receptors in some brainstem respiratory nuclei have been reported in male and female weanling rats (postnatal days 21–25; Schlenker and Hanson, 2006), although it is not clear whether these differences persist to adulthood (Behan and Thomas, 2005). Ren and Greer (2006) proposed that physiological stressors during the perinatal period (including hypoxia, asphyxia, parturition, ethanol exposure and infection) increase neurosteroid synthesis, which in turn regulates GABA_A receptor-mediated modulation of respiratory frequency (Ren and Greer, 2006). Neurosteroid synthesis could be regulated in a sex-specific manner. Additionally,

gonadal hormones could control the expression of specific GABA_A receptor subunits in males and females, as has been shown in the rat hypothalamus (Clark et al., 1998).

Male rats are exposed to high levels of testosterone (and its metabolite, estradiol) at day 18 of gestation, and within 4 hours of birth there is a further surge in testosterone levels (Weisz and Ward, 1980; Rhoda et al., 1984). These testosterone surges result in behavioral masculinization and defeminization of the brain. Testosterone exerts its effect on the brain primarily by conversion to estradiol by aromatase. The developing female brain, in contrast, is exposed to significantly lower levels of circulating testosterone and estradiol (Wilson and Davies, 2007). Differential exposure of male and female brains to sex hormones may contribute to the development of sex differences in central and peripheral components of the respiratory control system. Additionally, testosterone exposure in the first week of life can significantly alter aromatase activity, and permanently alter the conversion of testosterone to estradiol (Roselli et al., 1998). Carotid body hypertrophy, together with enhanced excitatory dopaminergic drive has been reported following prenatal blockade of estradiol synthesis (Soliz and Joseph, 2005). Thus, abnormal exposure to sex hormones during development may have a permanent impact on the capacity of the respiratory control system to respond to hypoxic insults. Moreover, the impact of steroid hormones on the developing respiratory control system may be sex-specific.

4.2. Which sex hormone is the most effective in the regulation of breathing?

Progesterone—For many years, hyperventilation in human pregnancy has been associated with elevated progesterone levels. In the luteal phase of the menstrual cycle when the level of progesterone is high, hyperventilation and decreased $P_{ET}CO_2$ have been reported (Slatkowska et al., 2006). Upper airway resistance during sleep is also lower in the luteal compared to the follicular phase (Driver et al., 2005). In a study of healthy men, administration of the synthetic progestin, medroxyprogesterone increased minute ventilation as well as the hypoxic and hypercapnic ventilatory responses (Skatrud et al., 1978). Nonetheless, the potential clinical benefits of progesterone analogues to induce hyperventilation and treat sleep apnea syndrome are mixed, with some studies reporting positive outcomes (Strohl et al., 1981; Collop, 1994), and other studies reporting no improvement in symptoms (Cook et al., 1989).

Few studies in animals have investigated the effects of progesterone on ventilation. Bayliss and Millhorn (1992) showed that in both male and female cats, a progesterone receptor agonist, R5020, increased peak integrated phrenic nerve activity and respiratory frequency. In female cats and guinea pigs, estrogen enhanced the effects of progesterone on ventilation, possibly by upregulating progesterone receptor expression (Bayliss and Millhorn, 1992; Hosenpud et al., 1983). Similarly, in male rats when a synthetic progestin and estradiol were given simultaneously, hyperventilation and decreased $P_{ET}CO_2$ were observed together with an enhanced response to CO_2 (Tatsumi et al., 1991). Taken together these data suggest that progesterone can alter ventilatory responses in both males and females, especially in combination with estrogen. Thus far, the mechanism whereby progesterone exerts its stimulatory effect on breathing, and its site(s) of action are still unknown. In many brain and spinal cord nuclei, progesterone receptors (PR) are upregulated by estrogen pre-exposure (MacLusky and McEwen, 1980; Monks et al., 2001). However, Bayliss and Millhorn (1992) failed to find progesterone receptor expression in the brainstem of cats that hyperventilated following progesterone administration. Based on lesioning studies, they argued that the respiratory effects of progesterone are mediated by PR-containing cells in the hypothalamus, regulated by estrogen. Although PR expression is minimal in brainstem respiratory nuclei, progesterone could act by a non-genomic mechanism in those nuclei. Progesterone could also act by altering the release of neuromodulators such as serotonin in brainstem respiratory nuclei,

as has been shown in the hypothalamus (Farmer et al., 1996). Finally, progesterone and its metabolites can bind to GABA_A receptors and modulate their function (Smith et al., 2007).

Estrogen—The impact of estrogen on ventilation has not been as well characterized as that of progesterone. Nonetheless, estradiol has been implicated in the control of breathing as decreased levels in menopause are linked to the respiratory disorder, obstructive sleep apnea (OSA) (Young et al., 2003; Shahar et al., 2003). Although a decrease in progesterone levels with menopause may also contribute to increased upper airway resistance, it is likely that estrogen plays an important role. In two pilot studies of sleep-disordered breathing in postmenopausal women, estrogen monotherapy was associated with a significant reduction in the apnea/hypopnea index (Keefe et al., 1999; Manber et al., 2003). Combined estrogen and progesterone also had a therapeutic effect, but only in one of the studies (Keefe et al., 1999). However, in a large study of sleep-disordered breathing in postmenopausal women taking estrogen or combined estrogen and progesterone therapy, there was a stronger inverse association of indices of hypoxemia with combined use of estrogen with progesterone than with use of estrogen alone (Shahar et al., 2003). This finding indicates a synergistic effect of estrogen and progesterone. Women with obstructive sleep apnea hypopnea syndrome have lower levels of estradiol and progesterone than control (Netzer et al., 2003). However, in a study of young healthy women in which estrogen and progesterone levels were reduced pharmacologically to postmenopause levels for one month, no clinically significant sleep-disordered breathing was detected, suggesting that the clinical impact of reduced sex hormone levels may not become apparent for months to years (D'Ambrosio et al., 2006).

Testosterone—As OSA affects middle-aged men far more than middle-aged women (Young et al., 1993), it is not surprising that testosterone has been implicated in the control of breathing. There are reports of low serum testosterone levels in men with OSA, independent of age (Grunstein et al., 1989; Kirbas et al., 2007). Although many clinical reports that suggest testosterone supplementation in hypogonadal men is associated with induction or worsening of OSA symptoms (Saareanta and Polo, 2002; Liu et al., 2003), overall it appears that OSA is an uncommon adverse event following testosterone administration (Hanafy, 2007). Nonetheless, elevated testosterone levels in normal men and women may have a measurable impact on respiratory parameters. Women with elevated androgen levels due to polycystic ovarian syndrome have a higher apnea/hypopnea index than healthy controls (Fogel et al., 2001). In healthy women, testosterone administration increased baseline ventilation during wakefulness, and altered the apneic threshold and increased ventilatory sensitivity to CO₂ during sleep (Zhou et al., 2003; Ahuja et al., 2008).

As with most reports of the effects of sex hormones in humans, it is difficult to gain a clear picture of the role of testosterone in breathing. Animal studies provide some suggestions as to the mechanisms whereby testosterone can alter respiratory parameters. Only a few studies have probed the effects of testosterone on ventilation in animals. Acute testosterone administration in neutered male cats decreased phrenic nerve output, and increased the hypoxic ventilatory response and hypercapnic ventilatory response (Tatsumi et al., 1994). Previous studies in our laboratory showed that both age and gonadectomy diminished hypoxia-induced long term facilitation of hypoglossal and phrenic motor output in male rats (Behan et al., 2003; Zabka et al., 2005). Recently we found that phrenic and hypoglossal long term facilitation could be restored by testosterone replacement in gonadectomized male rats, an effect that requires the conversion of testosterone to estrogen by aromatase (Zabka et al., 2006). Thus, the impact of testosterone on the control of breathing in animals and humans may ultimately be mediated by estrogen, the availability of which is controlled by aromatase. In healthy young men and women, testosterone administration and its conversion to estradiol in the brain could upregulate progesterone receptors and thereby augment minute ventilation and the hypoxic and hypercapnic ventilatory responses. However, in hypogonadal men and women with polycystic

ovarian disease, aromatase levels may be altered, perhaps contributing to the adverse respiratory events reported following testosterone administration. Similarly, aromatase levels may change with age in a site-specific manner (Simpson et al., 2002).

4.3. Where in the respiratory control system do sex hormones exert their effect?

The major structures involved in the neural control of breathing include the carotid body chemoreceptors, the nucleus of the solitary tract, the ventral respiratory column, the hypoglossal nucleus in the brainstem, the phrenic motor nucleus in the spinal cord, and neuromodulatory inputs to these regions (Feldman and McCrimmon, 2003). Amongst these, candidates for sex hormone modulation of breathing might include structures that contain sex hormone receptors and show sexual dimorphism in their expression. As more studies are designed to uncover the site(s) of action of circulating sex hormones, it is likely that most neuronal structures involved in the control of breathing will be implicated.

Carotid body—The carotid body is the primary peripheral chemosensory organ, the activity of which regulates overall respiratory drive. Changes in carotid body structure are associated with long-lasting changes in cardiorespiratory regulation. (Carroll, 2003). Only a few studies have focused on the impact of sex hormones on the carotid body. Hannhart et al., (1989) reported that the carotid sinus nerve activity increased following chronic elevation of progesterone levels in cats, and suggested that the carotid body was sensitive to circulating sex hormones. However, additional studies suggested that this effect might be potentiated centrally by estrogen (Hannhart et al., 1990; Tatsumi et al., 1994). Both estrogen receptor (ER β) and progesterone receptor (PR) immunoreactivity have been described in the carotid body of P2 male rats, although no information is available for adult animals or for androgen receptor expression in the carotid body (Soliz and Joseph, 2005). If sex hormones influence carotid body chemosensitivity in a sex-specific manner, tyrosine hydroxylase (and dopamine synthesis) may mediate the effect, as gonadectomy at one week of age increased tyrosine hydroxylase activity in the carotid body of female but not male rats (Joseph et al., 2002). Similarly, elevated cortisol levels in the first week also affected tyrosine hydroxylase mRNA levels in the carotid bodies of male but not female rats at 8–10 weeks of age, possibly contributing to a sex-specific augmented hypoxic ventilatory response (Kinkead et al., 2005b). Clearly, additional studies are needed to determine whether there are fundamental sex differences in the development of carotid body chemosensitivity that contribute to the sexual dimorphism of respiratory-related diseases.

Brainstem—Estrogen receptors have been localized throughout the brainstem and spinal cord including the nucleus tractus solitarius, ventral respiratory column, and in respiratory motoneurons in the hypoglossal and phrenic nuclei (Shughrue et al., 1997; Simerly, 1990; Behan and Thomas, 2005). In weanling rats (postnatal days 21–25) in which weight and hormone levels are comparable in males and females, there are sex differences in ER α and ER β immunoreactivity in the nucleus of the solitary tract and the hypoglossal nucleus (Schlenker and Hanson, 2006). In male and female mice, the distribution of ER α and ER β immunoreactive neurons throughout the brainstem was similar, although there were differences in the intensity of staining related to sex and endocrine status (VanderHorst et al., 2005). No sex differences in ER α and ER β immunoreactivity were detected in identified motoneurons the hypoglossal nucleus in adult rats (Behan and Thomas, 2005). Similarly, no sex differences were detected in androgen receptor immunoreactivity in hypoglossal or phrenic motoneurons (Behan and Thomas, 2005). Nonetheless, neuronal activity in hypoglossal and phrenic motor neurons can be influenced by circulating sex hormones. In male rats, long term facilitation of hypoglossal and phrenic motor output in response to intermittent hypoxia is reduced following gonadectomy (Zabka et al., 2005), and can be restored by testosterone replacement (Zabka et al., 2006). In female rats, there are clear differences in long term facilitation of hypoglossal

and phrenic motor output in estrus vs. diestrus (Zabka et al., 2001b). Clearly, a rigorous quantitative analysis of sex hormone receptor protein expression in brainstem respiratory nuclei and respiratory motoneurons in males and females is needed. Correlating these data with measurements of sex hormone levels could provide a clearer picture of whether a relationship exists between receptor expression and hormone levels in respiratory motor nuclei.

In gonadectomized, estrogen-primed female rats, progesterone receptor mRNA was detected in the nucleus tractus solitarius and the ventrolateral medulla (Kastrup et al., 1999). In contrast, our observations and those of Kastrup et al. (1999) suggest that progesterone receptor immunoreactivity in the rat brainstem is sparse with only a few labeled neurons in the nucleus of the solitary tract, and none in respiratory motoneurons. There are no data on progesterone receptor expression in phrenic motoneurons, although estrogen-inducible progesterone receptor expression has been reported in the lumbar spinal cord (Monks et al., 2001). In light of the fact that progesterone has such a marked impact on breathing, especially in females, the absence of robust receptor expression in respiratory brain regions is puzzling and raises the possibility that progesterone or its metabolites may be acting indirectly to alter respiratory function. GABA_A receptors have a steroid-specific site by which progesterone can enhance receptor binding and alter Cl⁻ influx. GABA_A receptor subunit composition is also affected by hormonal state or manipulation of estrogen and progesterone levels (Smith et al., 2007). By altering GABA_A receptor subunit composition in respiratory brain regions, circulating hormones could orchestrate a dynamic balance between excitation and inhibition across the life cycle in both males and females.

Serotonergic neurons—Brainstem serotonergic neurons have been implicated in the control of breathing, and could be a potential site for sexual dimorphism in respiratory control. Serotonergic neurons have been linked to SIDS, which is reported twice as frequently in male as in female infants (Hauck, 2001; Kinney et al., 2001). Following lesions of serotonergic neurons in newborn piglets, the ventilatory response to CO₂ was diminished, and in mice this effect was male-specific (Penatti et al., 2006; Hodges et al., 2005). The ventilatory response to hypercapnia was also reduced in male, but not female serotonin transporter knockout mice (E. Nattie, personal communication). Thus, sex differences in the serotonergic system could contribute to the differential respiratory responses of males and females. Although the number of serotonergic neurons is similar in the caudal raphe region of male and female rats (Penatti et al., 2006; Barker et al., 2007), female rats have more serotonin and greater serotonin-2A receptor immunoreactivity in respiratory motor nuclei (Behan et al., 2003; Seebart et al., 2007). Serotonin levels in respiratory motor nuclei fluctuate across the estrus cycle in female rats, suggesting that circulating levels of estrogen and progesterone directly influence serotonergic modulation of respiratory motoneurons (Behan et al., 2003). To determine whether there was a direct relationship between serum sex hormone levels and serotonin in the caudal raphe region where serotonergic neuronal cell bodies are localized, we ovariectomized six groups of middle-aged female rats (12 mo; n = 6/group) and supplemented with estrogen and progesterone, the timing of which mimicked different stages of the estrus cycle. Estrogen and progesterone levels were measured with radiimmunoassay and serotonin levels by ELISA. There was a statistically significant relationship between serum levels of estrogen and serotonin in the caudal raphe region ($R^2 = 0.73$; $p = 0.03$), and a weaker relationship between serum levels of progesterone and serotonin ($R^2 = 0.41$; $p = 0.17$). In preliminary studies we have also identified ER α immunoreactivity in serotonergic neurons in the caudal raphe in both male and female rats. Taken together, these data strongly suggest that the caudal raphe may be an important site of action of sex steroid hormones on respiratory control, as serotonin neurons in this area innervate both hypoglossal and phrenic motoneurons (Manaker et al., 1992).

5. Conclusions

From a body of work that spans almost a century, much progress has been made in our understanding of the role of sex hormones in the control of breathing. It is also clear that many questions remain, and a need for continued research to address the complex mechanisms that underlie the role of sex hormones in breathing in males and females throughout life. Future studies should focus on understanding the impact of combinations of sex hormones at different ages in males and females, dissecting the contributions of individual hormones at the cellular and molecular level, and identifying their site of action in the respiratory control system. In order to make accurate comparisons between different animal studies, and between animal and human studies, estrogen, progesterone, and testosterone levels should be measured in both males and females. If sex hormones are introduced, the duration and timing of their administration needs to be reported. Terms such as “young”, “middle-aged” and “old” need to be defined carefully as the range of ages within each of these terms varies enormously in both animal and human studies. With increased longevity in humans and prolonged exposure to both endogenous and exogenous sex hormones, the impact of sex steroids on breathing becomes ever more apparent. A greater understanding of how sex hormones can modulate the respiratory control system may make it possible to customize and target hormone therapies for respiratory disorders that preferentially affect males and females at all ages.

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