

Sex differences in mouse models of asthma

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Abstract: Differences in disease susceptibility and prognosis between men and women are known to occur in the incidence and development of neurodegenerative, cardiovascular, and immunological disorders. In the lung there are also sex-based differences in the incidence, prevalence, and pathogenesis of lung cancer, cystic fibrosis, COPD, and asthma. In the general population, sex-based differences in asthma have been shown by epidemiologic studies, but unfortunately these studies are not consistent in their conclusions. This variability in human epidemiological studies justifies the need for more focused studies of the effects of specific hormones. Such specific mechanistic studies can most easily be performed in animal models, and since mouse models have the potential for separating specific genetic factors from environmental and exogenous factors, this species has become increasingly important in the design, analysis, and interpretation of asthma research. This review will document the male and female differences in airway function of naïve and sensitized mouse models, as well as the great variability in the functional measurements of airway tone. Until the situation is better understood, this variability between males and females should be kept in mind when designing, analyzing, and interpreting studies of smooth muscle responses in animal models and human subjects.

Key words: male, female, sex, airway responsiveness, methacholine challenge.

Résumé : On sait qu'il existe des différences dans la susceptibilité aux maladies et le pronostic entre les hommes et les femmes en ce qui a trait à l'incidence et à l'évolution des désordres neurodégénératifs, cardiovasculaires et immunologiques. Dans le poumon, on observe aussi des différences selon le sexe dans l'incidence, la prévalence et la pathogénèse du cancer du poumon, de la fibrose kystique, de la COPD et de l'asthme. Dans la population, les différences liées au sexe pour ce qui est de l'asthme ont été démontrées par des études épidémiologiques; malheureusement, les conclusions de ces études divergent. Cette variabilité dans les études épidémiologiques chez les humains justifie le besoin d'études ponctuelles plus précises des effets d'hormones spécifiques. De telles études peuvent facilement être faites sur des modèles animaux; comme les modèles de souris permettent de séparer les facteurs génétiques spécifiques des facteurs environnementaux et exogènes, cette espèce a pris une grande importance dans la conception, l'analyse et l'interprétation de la recherche sur l'asthme. La présente synthèse décrit les différences liées au sexe dans la fonction des voies aériennes de modèles de souris naïves et sensibilisées, de même que la grande variabilité dans les mesures fonctionnelles du tonus bronchique. Cette variabilité devrait être gardée à l'esprit au moment de la conception, de l'analyse et de l'interprétation des études des réponses des muscles lisses dans les modèles animaux et chez les humains jusqu'à ce qu'on comprenne mieux la situation.

Mots-clés : mâle, femelle, sexe, réactivité des voies aériennes, épreuve à la méthacholine.

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Introduction

Differences in disease susceptibility and prognosis between men and women are well known and have been well characterized in the literature. Sex differences are found in the incidence and development of neurodegenerative, cardiovascular, and immunological disorders (Johannesson et al.

2000; Jounieaux et al. 1996). In the general population, we also find sex-based differences in the incidence, prevalence, and pathogenesis of lung cancer, cystic fibrosis, and chronic obstructive pulmonary disease (COPD) (Caracta 2003). Additionally, male and female differences exist in asthma, a disease characterized by airway inflammation, hyperresponsiveness, and reversible obstruction (Cressy and DeBoisblanc 1998; National Asthma Education Program 1991).

In the general population, sex-based differences in asthma are of particular interest because disease incidence and prevalence are correlated with age and the onset of puberty (Crawford and Beedham 1976). There is substantial evidence that asthma afflicts more children than adults (Crawford and Beedham 1976; Fagan et al. 2001; Kjellman and Gustafsson 2000; Laprise and Boulet 1996). It is less well-appreciated, however, that, compared with girls, boys

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have a higher incidence of asthma during childhood, but a lower incidence after the onset of puberty (Crawford and Beedham 1976; Fagan et al. 2001; Kjellman and Gustafsson 2000; Laprise and Boulet 1996). As adults, women exhibit higher prevalence of airway hyperresponsiveness to cholinergic challenge than men, where a large proportion of these women were also hyperresponsive during childhood (Jansen et al. 1997; Roorda et al. 1994).

In women, the exacerbation of asthma symptoms has been associated with the menstrual cycle, and the syndrome of premenstrual or perimenstrual asthma has been well documented since the late 1800s (Agarwal and Shah 1997; Chhabra 2005; Salter, 1882; Tan 2001). Studies since the early 1940s have consistently shown that up to 40% of asthmatic women have reported pre-menstrual, circummenstrual, or perimenstrual aggravation of their asthma symptoms (Rees 1963). In the earliest reports documenting premenstrual asthma, it was hypothesized that a high level of estrogen may play a role in the etiology of the syndrome (Rees 1963; Salter, 1882). Endogenous allergens or "endocrine allergy" was thought to be one factor responsible for asthmatic symptoms (Zondek and Bromberg 1945).

With the advent of hormone replacement therapy (HRT) and oral contraceptives (OCP), there have been several epidemiological studies specifically examining the effect of estrogen on asthma in women. The evidence, however, is contradictory, showing both beneficial and detrimental effects of these treatments with asthma (Barr and Camargo 2004; Haggerty et al. 2003). Estrogen replacement therapy (ERT) in women with natural or surgical menopause has been shown to increase the risk of asthma onset, but in other studies, ERT correlates with improvement in asthmatic symptoms (Myers and Sherman 1994; Troisi et al. 1995). Pulmonary function testing in post-menopausal women taking estrogen or combined estrogen and progesterone show higher forced expiratory volume in 1 s (FEV1) and less airway obstruction than women not on HRT (Carlson et al. 2001). With histamine challenge, post-menopausal women have decreased responsiveness with HRT than without (Hepburn et al. 2001; Lieberman et al. 1995a, 1995b).

Similarly perplexing are the reported effects of OCP use, which has been correlated with reduced wheezing in asthmatic women, but increased wheeze in non-asthmatic women (Salam et al. 2006). In other studies, however, oral contraceptives improve pulmonary function and decrease risk of asthma onset (Lam and Huang 1998; Matsuo et al. 1999).

Other sex steroids may also play a role in modulating asthma symptoms, but studies of their effects are as controversial as those with estrogen. Asthmatic women show significant worsening of peak expiratory flow compared with asthmatic women using hormone therapy without progesterone. (Lieberman et al. 1995a, 1995b) Further complications also arise from the fact that female sex hormones may not be the sole modulators of asthma: epidemiological evidence suggests a role for male sex hormones (Mulligan et al. 2006). In one study, men with hypogonadism were more likely to have asthma than men with normal testosterone levels, suggesting that androgens may be protective (Mulligan et al. 2006).

The epidemiological landscape for sex hormones in

asthma is thus as confusing as it is inconclusive. This variability in human epidemiological studies justifies the need for more focused studies of the effects of specific hormones. Such specific mechanistic studies can most easily be performed in animal models. In the remainder of this review, we will therefore focus on lung structure and function in animal models. Structural and functional differences between males and females will be highlighted as well as studies that examine the effects of both male and female sex hormones. Many different species have been studied, and we will show that, although there is not yet a consistent explanation for sex differences in airway constriction and inflammation, animal models have the potential to clarify the confusion in human studies.

Since mouse models have the potential for separating specific genetic factors from environmental and exogenous factors, this species has become increasingly important in the design, analysis, and interpretation of asthma research. Although the mouse has rapidly become the most popularly accepted experimental tool for probing pathologic mechanisms that might be operative in asthma, it has clear limitations. Mouse lungs are neither structurally nor anatomically identical to the human; they have 6 to 8 generations of airways leading to the terminal bronchioles, alveolar ducts, and alveoli. Humans have 20 to 30 generations of airways ending with terminal bronchioles that open into respiratory bronchioles, alveolar ducts, and alveoli. Additionally, although the inflammatory and immune response of mice is relatively well known, they require highly aberrant methods of allergen sensitization to produce asthma-like symptoms, and most importantly, without manipulation, do not intrinsically develop asthma.

Nevertheless, despite these limitations, the mouse model has several advantages. The short and rapid reproductive cycle and small size of mice have made them an economical choice among researchers. Furthermore, the mapping of the mouse genome with known genetic markers facilitates the identification of loci that can be linked to phenotypic traits. The mouse genome can be manipulated by targeting genes and creating transgenic mice. Additionally, examination of strain differences in response to airway challenges could reveal novel gene targets and biochemical pathways, one of the great strengths of the mouse model. Thus, overall, the mouse model will continue to serve as an important experimental tool for understanding the pathogenesis of asthma, but it is important to keep in mind its specific strengths and shortcomings.

Anatomy and physiology

In the rat model, significant structural differences exist between males and females at the site of the lung's gas-exchange region. Females tend to have smaller alveoli and greater total surface area for gas-exchange than males (Massaro et al. 1995), perhaps evolutionarily developed to optimize for the increased oxygen demands during pregnancy and lactation (Wilborn 1968). Additionally, there is evidence that estrogen maintains the total number of alveoli in the lung (Massaro and Massaro 2004; Massaro et al. 1995), as depletion of sex hormones via bilateral ovariectomy resulted in loss of alveoli and alveolar surface

area with no change in lung volume (Massaro and Massaro 2004; Massaro et al. 1995). The number of alveoli and the alveolar surface area were not significantly different after estrogen treatment following ovariectomy, indicating that estrogen may possibly help to regenerate alveolar septa (Massaro et al. 1996).

There are few comprehensive studies examining the differences in lung function between males and females. In mice, sex-based differences in dead space volume, vital capacity, static compliance, and inspiratory reserve capacity (defined as the volume inspired from the functional residual capacity (FRC) to a total lung capacity (TLC) defined at 25 cmH₂O airway pressure), are seen in certain strains of mice, but not in other age-matched strains (Schulz et al. 2002). The evidence suggests that genetic background independent of sexual identity may interact with female responsiveness. Males show consistently higher total lung capacity than females in 7 strains of mice (Reinhard et al. 2002), but in one study (Schulz et al. 2002), dead space volume was significantly higher in females than in males of the C57BL/6 mouse strain. Females of this strain may therefore have larger conducting airways than do their male counterparts. The data are controversial, however, because no male–female differences were found in the same strain of mice in another study by Reinhard and colleagues (2002). Despite these observations, Reinhard and colleagues nevertheless concluded that females have a smaller lung, but relatively larger conducting airways, than do their male counterparts (Reinhard et al. 2002).

It is unclear if there are differences in the basal respiratory mechanics between male and female mice. Baseline measurements are often inconsistent and quite variable even when age-matched mice of the same strain are measured by using similar, if not identical, measurement techniques. This variability is potentially problematic in the comparison of studies from different authors, as well as experiments within the same study.

Airway hyperresponsiveness is considered to be an important outcome of inflammation and airway remodeling in asthma (Boulet et al. 1998). Nevertheless, there are few comprehensive studies of male–female differences where the primary endpoint is the innate responsiveness of the airways after challenge with a bronchoconstrictive agent. One commonly used model of innate hyperresponsiveness is the murine model, which has been used to study the interaction between environment and genetics (Bolego et al. 1997; Duguet et al. 2000; Levitt and Mitzner 1988; Martin et al. 1988; Rivera-Sanchez et al. 2004; Shore et al. 2003). Bronchial reactivity is known to be influenced by genetic background, as several studies have shown that bronchial hyperresponsiveness differs significantly among inbred mouse strains (Card et al. 2006; Duguet et al. 2000; Levitt and Mitzner 1988). Molecular techniques that compare gene–gene interactions in ovalbumin-induced allergic airway inflammation of different strains of mice have been useful in isolating potentially important genes in asthma (Walker et al. 2006). Likewise, this method could potentially isolate genes which regulate sex-based lung function differences. Nevertheless, very few studies compare males and females, and in most studies, experiments are commonly restricted to just one sex and not the other.

In the few studies that have compared responses of both sexes, airway responsiveness to cholinergic challenge seems to differ between the sexes, although there is great strain dependence in the responses. In A/J mice, known to be a hyperresponsive strain, no sex difference was found in airway resistance changes after bronchoconstrictive challenge (Levitt and Mitzner 1988). On the other hand, measurement of respiratory mechanics and airway responsiveness in mechanically ventilated, naïve C57BL/6 mice showed that males are innately more responsive than females to aerosolized methacholine (Card et al. 2006).

The published literature thus shows a pressing need for comparative analysis between males and females in the architecture and function of the lung in commonly used animal models. Without this information on basic anatomy and physiology, the analysis and comparison of research among different groups using different models will remain exceedingly difficult. In an effort to begin to address this concern, we have compiled 2 tables from the published literature using the mouse model in which data on males and females were presented separately.

Three different methods of assessing airway function were used by these different investigators: the respiratory system resistance, R_{rs} , measured across the lung and thorax; the lung resistance, R_L , measured across the whole lung; and the Newtonian resistance, R_n , a measurement made at very high frequencies that is purported to better reflect airway resistance (Tomioka et al. 2002). Although the R_{rs} includes the mechanics of the chest wall, in the mouse both the elastance and tissue resistance are trivially small (Sly et al. 2003; Tankersley et al. 1997), so the R_{rs} should not be too different from the lung resistance. The lung resistance, however, does include the lung tissue resistance, so the magnitude of the lung resistance may not equal to that of the airways (which is what we really are interested in). In the literature we reviewed, the R_{rs} and lung resistance have been measured by end-inflation occlusion and forced oscillation techniques, as well as those developed by Mead and Whittenberger 1953 and Amdur and Mead 1958. Measurement of lung resistance has also been made in the isolated perfused lung with the technique by Mead and Whittenberger 1953. The measure of R_n is obtained by a mathematical model partitioning the airway resistance from the tissue resistance of the entire respiratory system. These different methods of estimating airway resistance often result in quite different quantitative magnitudes even at baseline; nevertheless, they should be essentially equivalent in their abilities to assess relative changes with most interventions.

Table 1 shows baseline resistance values in male and female mice of several different strains. The different columns show the age of the mice, the kind of anesthesia and paralytic, the method and type of resistance measured, and the mean values of resistance. This table shows that there is no consistent difference in the baseline resistance between males and females in these strains. The sex differences are small and lie within the variability of the measurement. Table 2 shows the airway responsiveness to cholinergic challenges given by aerosol in males and females in 2 strains of mice. Responsiveness assessed with either R_{rs} or R_n showed a decreased responsiveness (increased PC₂₀₀, concentration required to produce a 200% increase in resistance) in female

Table 1. Baseline measurements of respiratory system resistance in different strains of male and female mice.

Strain	Age, weeks	Anesthesia, paralytic	Method	Baseline Rrs, cmH ₂ O·mL ⁻¹ ·s ⁻¹		Reference
				Males	Females	
129SV/ImJ	12–14	Ketamine, xylazine, isoflurane – pancuronium	Mead and Whittenberger 1953, at 130 br/min	1.03±0.26	0.88±0.18	Reinhard et al. 2002
A/J	12–14	Ketamine, xylazine, isoflurane – pancuronium	Mead and Whittenberger 1953, at 130 br/min	0.83±0.18	0.97±0.25	Reinhard et al. 2002
BALB/cByJ	12–14	Ketamine, xylazine, isoflurane – pancuronium	Mead and Whittenberger 1953	0.84±0.20	0.78±0.15	Reinhard et al. 2002
C3H/HeJ	12–14	Ketamine, xylazine, isoflurane – pancuronium	Mead and Whittenberger 1953, at 130 br/min	1.15±0.41	0.80±0.20	Reinhard et al. 2002
C3HeB/FeJ	10	Dead after i.p. overdose of ketamine and xylazine	Amdur and Mead 1958, at 130 br/min	0.60±0.24	0.63±0.06	Schulz et al. 2002
C57BL/6J	12–14	Ketamine, xylazine, isoflurane, pancuronium	Mead and Whittenberger 1953, at 130 br/min	0.74±0.09	0.77±0.14	Reinhard et al. 2002
C57BL/6	10	Dead after i.p. overdose of ketamine and xylazine	Amdur and Mead 1958, at 130 br/min	0.66±0.14	0.58±0.12	Schulz et al. 2002
C57BL/6	8–10	Urethane – pancuronium	Forced oscillation at 2.5 Hz	0.60*	0.68*	Card et al. 2006
C57BL/6	8–10	Urethane – pancuronium	Forced oscillation at 2.5 Hz	0.90*	0.60*	Card et al. 2006
FVB/J	12–14	Isoflurane, ketamine, xylazine – pancuronium	Mead and Whittenberger 1953, at 130 br/min	0.82±0.24	0.81±0.15	Reinhard et al. 2002
JF1	10	Dead after i.p. overdose of ketamine and xylazine	Amdur and Mead 1958, at 130 br/min	0.78±0.14	0.94±0.21	Schulz et al. 2002
WR/J	12–14	Isoflurane, ketamine, xylazine – pancuronium	Mead and Whittenberger 1953, at 130 br/min	0.75±0.23	0.79±0.25	Reinhard et al. 2002

Note: The respiratory system resistance (Rrs) includes the chest wall; br/min, breaths per minute.

*There are no standard errors for the Card et al. 2006 data, since these mean values could be obtained only from the published graph.

mice in both strains studied. Table 3 shows the baseline resistance and airway responsiveness to cholinergic challenges given intravenously in male mice of 4 strains. There are no published studies comparing sexes with IV challenges.

These data are presented to emphasize the wide variability among the different methods to assess airway responsiveness in mice. Baseline resistance values vary over a 6-fold range among or even within different studies. Table 1 highlights the wide variation in methods, baseline values, and responsiveness commonly observed in the published literature. Reasons for this variability are not always clear, but until there is some rational explanation, comparison among the many different labs will remain difficult.

Effects of specific hormones

Female sex hormones

In rodents, the estrous cycle occurs every 4–5 days and is characterized by 4 stages: proestrus, estrus, metestrus, and diestrus (Champlin et al. 1973). Rodents do not menstruate at the end of the cycle, as do humans (Grummer 2006). Similar to humans, the estrous cycle in the rodent is under regulation by the hypothalamus, which releases gonadotropin-releasing hormone (GnRH) to stimulate follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Brenner and West 1975). During proestrus, ovarian follicles develop under the influence of FSH and LH (Demeestere et al. 2005). Under this hormonal influence, theca cells produce androgens from cholesterol, which are then converted to 17 β -estradiol by the granulosa cells. Estrogen levels peak during proestrus, right before the LH surge, whereas progesterone

is the highest right after the LH peak (Brenner and West 1975). During the estrus phase, which follows proestrus, the rodent is sexually receptive and ovulates 3–5 h after the onset of the dark cycle. The estrous cycle is greatly influenced by the light–dark cycle, and rodents can be maintained in a chronic state of anovulation during long periods of constant light (Singh 2005). After estrus, the corpora lutea forms and the mature egg moves from the ovary through the oviduct to the uterus in the metestrus phase. Follicles begin to undergo rapid development and the previous egg atrophies during diestrus in preparation for the next cycle. Estrogen is maintained at low concentrations but begins to increase during diestrus (Brenner and West 1975).

The sex hormones exert their primary effects on the reproductive organs, but they also exert significant second-order effects on non-reproductive organs. Estradiol has been shown to have a dose-dependent effect on the smooth muscle constriction of aortic rings explanted from ovariectomized Sprague–Dawley rats (Bolego et al. 1997). Sex hormones have also been shown to modulate the immune system via the alteration of such immune cells as B and T cells, natural killer cells, and macrophages (Ahmed and Talal 1990).

Studies have shown that estradiol has antiinflammatory properties, (Balzano et al. 2001; Bodel et al. 1972; Sunday et al. 2006) and can attenuate carbachol-induced smooth muscle constriction in sensitized tracheal rings from C57BL/6 male mice (Dimitropoulou et al. 2005). More importantly, the timing of estradiol inhibition during the sensitization of the immune system is important in priming the airways to yield a less responsive phenotype. Estradiol treat-

Table 2. Airway responsiveness to cholinergic challenges given by aerosol in 2 strains of male and female mice.

Strain	Age, weeks	Anesthesia, paralytic	Method	<i>R</i>	PC ₂₀₀ , mg/mL		Reference
					Males	Females	
BALB/c	8–10	Urethane – pancuronium	Forced oscillation at 2.5 Hz	<i>Rrs</i>	4.9±0.67	12±1.3	Card et al. 2006
BALB/c	8–10	Urethane – pancuronium	Forced oscillation at 20 Hz	<i>Rn</i>	6.4±1.1	16±2.4	Card et al. 2006
C57BL/6	8–10	Urethane – pancuronium	Forced oscillation at 2.5 Hz	<i>Rrs</i>	5.8±0.83	14±1.3	Card et al. 2006
C57BL/6	8–10	Urethane – pancuronium	Forced oscillation at 2.5 Hz	<i>Rrs</i>	6.3±0.6	13±1.9	Card et al. 2006
C57BL/6	8–10	Urethane – pancuronium	Forced oscillation at 20 Hz	<i>Rn</i>	7.5±0.8	27±4.0	Card et al. 2006
C57BL/6	8–10	Urethane – pancuronium	Forced oscillation at 20 Hz	<i>Rn</i>	5.6±0.62	9.3±1.3	Card et al. 2006
C57BL/6	12–16	Urethane – pancuronium	Forced oscillation at 2.5 Hz	<i>Rrs</i>	—	11±1.6	Carey et al. 2007
C57BL/6 + vehicle	12–16	Urethane – pancuronium	Forced oscillation at 2.5 Hz	<i>Rrs</i>	—	24±4.6	Carey et al. 2007
C57BL/6 sham + placebo	12–16	Urethane – pancuronium	Forced oscillation at 2.5 Hz	<i>Rrs</i>	—	59±4.1	Carey et al. 2007
C57BL/6	12–16	Urethane – pancuronium	Forced oscillation at 20 Hz	<i>Rn</i>	—	7.0±1.5	Carey et al. 2007
C57BL/6 + vehicle	12–16	Urethane – pancuronium	Forced oscillation at 20 Hz	<i>Rn</i>	—	18±2.5	Carey et al. 2007
C57BL/6 sham + placebo	12–16	Urethane – pancuronium	Forced oscillation at 20 Hz	<i>Rn</i>	—	45±6.1	Carey et al. 2007

Note: Measurements were made of the respiratory system resistance (*Rrs*) and the Newtonian resistance (*Rn*). The PC₂₀₀ (concentration required to produce a 200% increase in resistance) is significantly higher in females of both strains with either measurement.

ment during sensitization of the immune system in female BALB/c mice does not change airway inflammation, whereas ovariectomy before ovalbumin (OVA) challenge significantly inhibits lung eosinophilia and depresses the development of lung inflammation (Riffo-Vasquez et al. 2007).

In vivo studies in female Wistar rats show that estradiol decreases airway responsiveness to cholinergic challenge (Degano et al. 2001). Similar to the effect of estradiol on the vascular smooth muscle, estradiol attenuates airway smooth muscle constriction in a dose-dependent manner in female Wistar rats (Degano et al. 2003). Somewhat surprisingly, low doses of estradiol compared with high doses have been found to cause greater relaxation in explanted tracheal smooth muscle (Degano et al. 2001, 2003). This relaxation was correlated with an increase in acetylcholinesterase activity at the site of the airway epithelium (Degano et al. 2003; Degano et al. 2001). Thus, in vivo studies do not yet necessarily help clarify the role of estradiol. In another study, the maximal response to methacholine challenge, but not PC₂₀₀, was significantly reduced in ovariectomized female BALB/c mice (Riffo-Vasquez et al. 2007). In these same mice, however, OVA challenge before ovariectomy failed to elicit the same attenuation of airway responsiveness (Riffo-Vasquez et al. 2007).

Estrogen receptors

Estrogen acts on the alpha and beta estrogen receptors (ER α and ER β), which are differentially distributed throughout the body in the non-reproductive organs. ER α is preferentially distributed in the uterus, ovary, kidney, and heart; ER β is distinctly expressed in the gastrointestinal tract, hematopoietic and central nervous systems, and lung (Couse et al. 1997; Kuiper et al. 1997). In the lung, there is a 3:1 ratio of ER β to ER α (Couse et al. 1997). Although both receptors share similar mechanisms of action, evidence suggests that the receptors may regulate distinct pathways (Paech et al. 1997). Novel estrogen receptors have also been discovered to exist on the plasma membrane in a variety of cells (Le

Mellay et al. 1997; Levin 1999). Because estrogen has been found to activate membrane adenylate cyclase via phospholipase C β , thereby mobilizing calcium from the endoplasmic reticulum (Le Mellay et al. 1997; Levin 1999), cell surface estrogen receptors are thought to modulate the receptor-mediated cholinergic pathway.

ER α is the receptor most likely involved in modulating airway hyperresponsiveness. In human studies, airway responsiveness to inhaled histamine was found to be associated with 2 estrogen receptor polymorphisms in the ER α gene (*ESR1*) (Dijkstra et al. 2006). Additionally, these single nucleotide polymorphisms (SNPs) were found to be correlated with the longitudinal decline in FEV1 particularly in female subjects, although no particular SNPs were associated with asthma (Dijkstra et al. 2006).

The evidence in animal models indicates that ER α may be responsible for the attenuation of airway hyperresponsiveness. ER α knock out (α ERKO) mice on C57BL/6 background are more hyperresponsive and have greater maximal response to aerosolized methacholine challenge than either ER β knock out (β ERKO) mice on C57BL/6 background or wild type C57BL/6 mice (Carey et al. 2007). In addition in the same study, α ERKO mice treated with estradiol show increased hyperresponsiveness, although curiously, ovariectomized α ERKO mice show the most attenuation of airway responsiveness (Carey et al. 2007). These data suggest that ER α , in combination with estrogen, may play a role in regulating airway constriction.

Progesterone

Human studies have found that progesterone treatment may alter the manifestation of asthma symptoms. Three case studies in one report demonstrate that intramuscular injections of progesterone eliminated a striking decrease in premenstrual peak expiratory flow rate to the extent that patients' daily doses of corticosteroids could be successfully reduced (Beynon et al. 1988). Studies in isolated guinea pig trachea supported these clinical observations, showing that progesterone inhibits histamine- or carbachol-induced con-

Table 3. Airway responsiveness to cholinergic challenges given intravenously to 2 strains of male mice.

Strain	Age, weeks	Anesthesia	Method	<i>R</i>	Baseline <i>R</i> , cmH ₂ O·mL ⁻¹ ·s ⁻¹	PC ₂₀₀ , µg/kg	Reference
A/J	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 1.5 Hz	<i>R</i> _{rs}	1.7±0.1	20±2.9	Duguet et al. 2000
A/J	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 5.5 Hz	<i>R</i> _{rs}	0.8±0.2	14±2.6	Duguet et al. 2000
A/J	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 20 Hz	<i>R</i> _{rs}	0.5±0.1	10.1±1.31	Duguet et al. 2000
A/J	6–8	Halothane – none	End-inflation occlusion	<i>R</i> _{rs}	1.8	12.5	Ewart et al. 1995
A/J	6–8	Halothane – none	End-inflation occlusion	<i>R</i> _{rs}	1.3	—	Ewart et al. 1995
A/J	N/A	Pentobarbital – pancuronium	Mead and Whittenberger 1953	<i>R</i> _{rs}	0.7	11	Shinagawa and Kojima 2003
A/J	6–8	Pentobarbital – none	Forced oscillation at 20 Hz	<i>R</i> _n	0.24±0.02	0.03±0.01 (PC ₃₀₀)	Wagers et al. 2007
A/J	N/A	Dead after pentobarbital overdose	Isolated lung with Mead and Whittenberger 1953, at 90 br/min	<i>R</i> _L	0.30	—	Held and Uhlig 2000
BALB/c	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 1.5 Hz	<i>R</i> _{rs}	1.7±0.1	20.9±6.55	Duguet et al. 2000
BALB/c	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 5.5 Hz	<i>R</i> _{rs}	0.8±0.1	13.4±3.08	Duguet et al. 2000
BALB/c	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 20 Hz	<i>R</i> _{rs}	0.5±0.1	12.1±2.69	Duguet et al. 2000
BALB/c	8	Xylazine, pentobarbital – none	Forced oscillation at 2.5 Hz	<i>R</i> _{rs}	0.8	3	Shore et al. 2006
BALB/c	N/A	Pentobarbital – pancuronium	Mead and Whittenberger 1953	<i>R</i> _{rs}	1.3	15	Shinagawa and Kojima 2003
BALB/c	6–8	Pentobarbital – none	Forced oscillation at 20 Hz	<i>R</i> _n	0.34±0.03	0.1±0.03 (PC ₃₀₀)	Wagers et al. 2007
BALB/c	N/A	Dead after pentobarbital overdose	Isolated lung with Mead and Whittenberger 1953, at 90 br/min	<i>R</i> _L	0.29	—	Held and Uhlig 2000
C3H/HeJ	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 1.5 Hz	<i>R</i> _{rs}	1.7±0.2	28.5±6.48	Duguet et al. 2000
C3H/HeJ	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 5.5 Hz	<i>R</i> _{rs}	0.8±0.1	42.18±6.29	Duguet et al. 2000
C3H/HeJ	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 20 Hz	<i>R</i> _{rs}	0.5±0.03	25.04±4.66	Duguet et al. 2000
C3H/HeJ	6–8	Halothane – none	End-inflation occlusion	<i>R</i> _{rs}	1	48	Ewart et al. 1995
C3H/HeJ	N/A	Pentobarbital – pancuronium	Mead and Whittenberger 1953	<i>R</i> _{rs}	1.4	160	Shinagawa and Kojima 2003
C57BL/6	N/A	Pentobarbital – pancuronium	Mead and Whittenberger 1953	<i>R</i> _{rs}	1.6	110	Shinagawa and Kojima 2003
C57BL/6J	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 1.5 Hz	<i>R</i> _{rs}	1.7±0.1	37.7±9.02	Duguet et al. 2000
C57BL/6J	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 5.5 Hz	<i>R</i> _{rs}	0.9±0.1	45.7±6.75	Duguet et al. 2000
C57BL/6J	5–6	Xylazine, pentobarbital – doxacurium	Forced oscillation at 20 Hz	<i>R</i> _{rs}	0.5±0.1	28.3±3.64	Duguet et al. 2000
C57BL/6	4–12	Pentobarbital – none	Amdur and Mead 1958, at 150 br/min	<i>R</i> _L	0.63	133	Martin et al. 1988
C57BL/6	N/A	Dead after pentobarbital overdose	Isolated lung with Mead and Whittenberger 1953, at 90 br/min	<i>R</i> _L	0.30	—	Held and Uhlig 2000

Note: Measurements were made of the respiratory system resistance (*R*_{rs}), the lung resistance (*R*_L), and the Newtonian resistance (*R*_n). PC₂₀₀, concentration required to produce a 200% increase in resistance; N/A, not available; br/min, breaths per minute. Measurements without estimates of error indicate that the values were only available by reading from the published graphs. The values reported here for the Shinagawa and Kojima 2003 data have been changed to micrograms per kilogram from the milligrams per kilogram reported in their paper, since we think this must have been a typographical error in their publication.

traction via a pathway bypassing gene upregulation and possibly inducing bronchodilation of the airways (Perusquia et al. 1997). In contrast to this direct effect, progesterone is known to produce a pro-inflammatory response in C57BL/6 mice and is responsible for shifting the Th1 response to Th2 while blocking the Th1 response (Miyaura and Iwata 2002). The hormone has also been shown to increase eosinophilic airway inflammation in OVA-sensitized BALB/c mice while increasing bronchial hyperresponsiveness (Hellings et al. 2003; Perusquia et al. 1997). Very recently it was shown that serum progesterone levels are directly linked to increased Th-2 cytokines in allergic mice (Mitchell and Gershwin 2007). This study was able to isolate the effects of progesterone by studying ovariectomized mice given exogenous progesterone, but unfortunately, there were no functional measurements made of the airway responsiveness, so it is unclear how to translate this result to asthma. Thus, the role of progesterone in humans and animal models remains unclear.

Male sex hormones

The male reproductive hormones are under control by the hypothalamus, which influences the anterior pituitary to release FSH and LH through the release of GnRH. LH stimulates the Leydig cells to produce testosterone, which is then converted to dihydrotestosterone (DHT) in target tissues by 5 α -reductase. The onset of puberty is initiated by the pulsatile release of GnRH by the hypothalamus, initiating the production of testosterone. Although the effects of the female sex hormones on asthma have been the focus of greater study, the role of male sex hormones in asthma has not yet been thoroughly explored.

Both male and female fetuses express androgen receptors (AR) in non-reproductive tissues such as the intestine, kidney, thymus, lung, and trachea (Wilson and McPhaul 1996). Significantly more AR-B than AR-A is expressed in the lung; however, total AR expression is lower in the adult than in the fetus (Wilson and McPhaul 1996). Androgens are responsible for the maturation of the human lung in utero (Hume et al. 1996), where DHT has been shown to increase the lung terminal buds of the embryonic lung and play a role in the early lung-branching morphogenesis (Levesque et al. 2000). DHT has also been found to prolong the period of late-gestation cell proliferation, thus delaying lung maturation (Adamson and King 1984a, 1984b; Nielsen et al. 1990). This may explain why males have larger lung architecture than females (Adamson and King 1984a, 1984b; Nielsen et al. 1990).

In the cardiovascular literature, sex differences in the regulation of vascular smooth muscle and vascular tone are well-known (Thompson and Khalil 2003). Testosterone has been shown to dilate canine resistance arteries via an NO-dependent pathway in the epithelium (Chou et al. 1996). Evidence also suggests that testosterone-induced vasorelaxation in the mesenteric arterial bed of male Wistar rats is mediated by potassium channels (Tep-areenan et al. 2002).

Although there have been few reports of male sex hormones and their effects on airway hyperresponsiveness in vivo, in vitro studies demonstrate that testosterone causes a relaxation of the airway smooth muscle via a direct pathway not involved with upregulation of genes (Kouloumenta et al.

2006). Explanted rabbit tracheal muscle precontracted with carbachol shows a dose-dependent relaxation with testosterone, but not when the tissue is denuded of the epithelium or treated with an NO synthetase inhibitor (Kouloumenta et al. 2006). In one in vivo study, however, castration reduced the sensitivity of male C57BL/6 mice to aerosolized methacholine challenge, and instillation of DHT in C57BL/6 females caused increased airway responsiveness (Card et al. 2006). This same study showed that ovariectomized C57BL/6 females do not differ from sham-operated females in their response to lipopolysaccharide (LPS), whereas castrated C57BL/6 males show more inflammation compared with females. This evidence suggests that male sex hormones may promote a more robust inflammatory response in the mouse lung (Card et al. 2006).

Conclusion

In conclusion, the role of male and female sex hormones in airway smooth muscle responsiveness and asthma is not well understood in either humans or other animal models. Perhaps the biggest conundrum is the fact that women have a higher incidence of asthma than men during their reproductive years when estrogen levels are highest, yet the direct effect of estrogen on airway smooth muscle is generally to reduce responsiveness. This review has documented the male and female differences in lung function of naïve and sensitized animal models, as well as the great variability in the functional measurements of airway tone. Data from animal studies show dual roles of both female and male sex steroids in attenuating or exacerbating airway constriction. Additionally, we find no conclusive mechanistic explanation of the sex-based differences in airway constriction and inflammation. Until the situation is better understood, this variability between males and females should be considered when designing, analyzing, and interpreting studies of smooth muscle responses in animal models and human subjects.

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