Hormonal Influences on Lung Function and Response to Environmental Agents

Lessons from Animal Models of Respiratory Disease

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Numerous studies in humans and experimental animals have identified considerable sex differences in respiratory physiology and in the response of the lung to environmental agents. These differences appear to be mediated, at least in part, by sex hormones and their nuclear receptors. Moreover, animal models are increasingly used to study pathogenic mechanisms and test potential therapies for a variety of human lung diseases, many of which appear to be influenced by sex and sex hormones. In this article, data are summarized from studies of lung function and disease in which sex differences have been observed. Specific attention is paid to animal models of acute lung injury, nonallergic and allergic lung inflammation, and lung fibrosis. It is anticipated that continued investigation of the role of sex and sex hormones in animal models will provide valuable insight into the pathogenesis and potential treatments for a variety of acute and chronic human lung diseases.

Keywords: sex; sex hormones; respiratory mechanics; inflammation; airway

A considerable body of epidemiologic data suggests that the incidence and pathogenesis of a variety of lung diseases are influenced by sex. While genetic and environmental factors clearly have important roles, chronic obstructive pulmonary disease (COPD), asthma, lung fibrosis, lung cancer, and other respiratory ailments have been reported to be influenced in some way by sex (1–3). Delineating the underlying contribution of sex (and, by association, sex hormones) to these diseases is complicated by the inherent difficulties associated with identifying the roles of specific factors from epidemiologic data. Fortunately, from a mechanistic research perspective, the potential contribution of sex hormones to sex disparities in lung diseases also has been addressed in numerous animal studies.

In contrast to epidemiologic studies, animal models provide relatively controlled conditions under which to study the pathogenesis of lung diseases. Mice in particular have gained widespread use as a model to study lung diseases and associated functional alterations. This is due in part to their short breeding time, our evolving understanding of and ability to manipulate their immune system, and the development of sensitive and reliable methods to measure respiratory function in small animals. The ability to surgically and/or chemically alter sex hormone levels in mice and other experimental animals provides unique opportunities to tease out the potential involvement of sex hormones in observed sex differences. Furthermore, recent studies using estrogen receptor knockout mice have provided

valuable information on the role of these nuclear receptors in the lung. In the following sections, data are summarized from studies that highlight the influence of sex and sex hormones on basal lung function and on lung disease and associated lung dysfunction in mice, rats, and other species commonly used as models of human respiratory diseases. Some of the discussion of these studies has been previously published (4, 5).

SEX DIFFERENCES IN RESPIRATORY PHYSIOLOGY

The inclusion of lung function parameters in the assessment of experimental lung disease models provides important insight into physiologic outcomes that cannot always be predicted based solely on biochemical and morphological assessments. Furthermore, and perhaps most importantly, inclusion of lung function assessments more closely relates these models to the human conditions they are intended to mimic. Sex differences have been reported in certain baseline lung function parameters in many strains of mice, including C57BL/6, BALB/c, A/J, and others (6–8). For example, compared with values in females, male C57BL/6 mice have been reported to have higher tidal volume, minute ventilation, and peak inspiratory and expiratory flow rates (8).

As is the case with baseline lung function, reports of the comparative pulmonary response of male and female mice to challenge with a bronchoconstrictive agent (e.g., a cholinergic agonist) are limited. Airway hyperresponsiveness to cholinergic agents is a cardinal feature of asthma. Studies of sex differences in human airway responsiveness to cholinergic stimulation have reported greater sensitivity to inhaled methacholine in females than in males (9, 10). It has been suggested, however, that taking the relative differences in lung and airway sizes into account can at least partially explain this disparity (11, 12). Cholinergic airway responsiveness is markedly different in male and female mice. Although no differences in baseline respiratory mechanics were observed, males of the C57BL/6 and BALB/c strains were found to be more sensitive than females of these strains to inhaled methacholine (13). Specifically, greater increases in total respiratory system resistance, elastance, and other mechanical parameters were observed in males in response to methacholine aerosol (13). Subsequent studies revealed that this sex difference appears to be due to *in vivo* effects of androgens on vagus nerve-mediated reflex pathways and not to differences in innate responsiveness of airway smooth muscle (14). Airway responsiveness in castrated male mice to inhaled methacholine was equivalent to that in intact females, while females administered exogenous testosterone responded to inhaled methacholine in a fashion similar to that of intact males (14). Conversely, no difference between the sexes was observed in the contractile response to carbachol in isolated tracheal or bronchial rings (14). Similar to findings in mice, the responsiveness of the airways of male guinea pigs to methacholine has been reported to be greater than that observed in females (15).

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Dijkstra and coworkers have reported that polymorphisms in the gene encoding estrogen receptor- α (ER α) are associated with airway hyperresponsiveness and lung function decline in humans, particularly in female subjects with asthma (16). Studies in rats and mice have generally revealed a suppressive effect of estrogens on cholinergic airway responsiveness. Estradiol treatment of ovariectomized rats was shown to decrease acetylcholine-induced airway reactivity, an effect that was associated with increased epithelial acetylcholinesterase activity (17). In mice, incubation with estrogen reduced cholinergic constriction in isolated tracheal and bronchial rings that were made "asthmatic" by passive sensitization with serum from humans with asthma (18). More recently, studies that used estrogen receptor knockout mice to investigate the involvement of estrogen in lung function and airway responsiveness have been reported. Compared with values in wild-type controls, a marked reduction in breathing frequency was found in naïve male and female ER α knockout (α ERKO) mice (8). As mentioned earlier, tidal volume was found to be significantly increased in male wild-type mice compared with that in female wild-type mice; however, this pattern was reversed in α ERKO mice (8). Similarly, whereas minute ventilation, peak inspiratory flow, and peak expiratory flow were higher in male than in female wild-type mice, this pattern was not seen in α ERKO mice (8). These data suggest that functional disruption of ER α leads to changes in a variety of respiratory parameters and that this nuclear receptor may be a critical regulator of breathing and respiratory rhythmogenesis in mice.

In terms of airway responsiveness, naïve female α ERKO mice were found to exhibit substantially enhanced airway responsiveness to inhaled methacholine compared with that observed in wild-type mice (8) (Figure 1). Moreover, expression of the M2 muscarinic receptor was markedly reduced in the lungs of α ERKO female mice relative to that in wild-type controls, and tracheas isolated from α ERKO female mice released more acetylcholine in response to electrical field stimulation than did tracheas from wild-type controls. In addition, the contractile response of α ERKO tracheas to electrical field stimulation was unaffected by the selective M2 muscarinic receptor agonist gallamine, indicating that M2 muscarinic receptors were also dysfunctional in these mice (8). Down-regulation of M2 muscarinic receptor expression and function leads to increased acetylcholine in the neuromuscular junction and results in enhanced bronchoconstriction following cholinergic agonist stimulation. These data cumulatively suggest significant roles for estrogen and particularly for ER α in modulating critical mechanisms involved in airway responsiveness in both rats and mice.

In addition to the data reported by Carey and colleagues (8), there are several examples in the literature of the influence of sex and sex hormones on the control of breathing in animals. Breathing frequency has been reported to be higher in male than in female rats (19, 20). ER α , ER β , and the androgen receptor are expressed in respiratory motor neurons of male and female rats (21) and intraventricular infusion of an ER α antisense vector was shown to decrease brain ER α protein levels and to affect ventilation in rats of both sexes (22). Mortola and Saiki found that conscious adult female rats have a greater hyperventilatory response than males to hypoxia; of note, this observation also was made in studies conducted with ovariectomized females and with prepubertal rats, suggesting that the difference was not mediated by ovarian hormones (23). Significantly increased tidal volume and minute expiratory ventilation, reduced arterial PCO₂, and enhanced ventilatory response to CO₂ inhalation were observed in male rats after combined administration of a synthetic potent progestin and

Figure 1. Sensitivity to methacholine-induced airway responsiveness in mice is exaggerated in the absence of ER α . The lungs of naïve female α ERKO mice (open bars) were found to respond more robustly than the lungs of naïve female wild-type mice (solid bars) to methacholine aerosol. Specifically, lower provocative concentrations of methacholine aerosol were required in α ERKO mice to cause a 200% increase (PC₂₀₀) over baseline values in total respiratory resistance (R), Newtonian resistance (Rn), and tissue resistance (G), and to cause a 50% increase (PC₅₀) over baseline values in total elastance (E) and tissue elastance (H). **P* < 0.05 versus corresponding wild-type group. Reprinted by permission from Reference 8.

estradiol for 5 days (24). In addition, in mice of the OF1 strain, males were found to be less resistant than females to a normobaric hypoxia, while treatment of castrated males or ovariectomized females with estradiol increased hypoxic survival (25).

In total, the data described in this section highlight the significant influence that sex and sex hormones can have on respiratory function and on airway responsiveness in mice, rats, and guinea pigs. A summary of the reported effects is presented in Table 1. Perhaps not surprisingly, in some instances the effects of male sex hormones appear to be responsible for the observed sex differences while in others it is female sex hormones that have been implicated.

SEX DIFFERENCES IN MODELS OF LUNG INJURY AND DISEASE

Numerous studies have examined the effect of sex and sex hormones in a variety of models of lung injury and disease. These include models of nonallergic and allergic inflammation, acute lung injury, chronic obstructive pulmonary disease, and lung fibrosis. The reported effects are discussed in the following subsections and a summary is presented in Table 2.

Nonallergic Lung Inflammation and Acute Lung Injury

Bacterial lipopolysaccharide (LPS), a ubiquitous airborne contaminant and recognized risk factor for asthma (26), causes airway inflammation and hyperresponsiveness in humans and animals. Despite this knowledge, surprisingly little has been reported with regard to the potential influence of sex or sex hormones on the pulmonary response to LPS in humans or experimental animals. A study by Kline and colleagues revealed that individuals who were sensitive to inhaled LPS (defined as those displaying a > 20% decline in FEV₁ after inhalation of no more than 6.5 μ g of LPS) were more likely to be female, while hypo-responsive individuals were more often male (27). Many studies investigating LPS-induced lung inflammation in experimental animals use only one sex or do not consider potential differences between the sexes if both are used, thus making comparisons among various studies difficult. To our knowledge the first study to report a sex difference in airway inflammatory parameters after LPS exposure in experimental animals was

TABLE 1. SUMMARY OF SEX DIFFERENCES IN RESPIRATORY PHYSIOLOGY

Reported Endpoint	Animal Model	Sex Difference	Hormone Effect	Reference
Total lung capacity	Mouse	M > F	NR	6
Airway size	Mouse	F > M	NR	6
Specific lung volume	Mouse	F > M	NR	7
Tidal volume	Mouse	M > F	ER α -mediated decrease	8
	Rat	Only M were studied	Estrogen- and progestin-mediated increase	24
Minute ventilation	Mouse	M > F	ER α -mediated decrease	8
Peak inspiratory flow	Mouse	M > F	ER α -mediated decrease	8
Peak expiratory flow	Mouse	M > F	ER α -mediated decrease	8
Breathing frequency	Mouse	M = F	NR	8, 13
	Rat	M > F	NR	19, 20
Airway responsiveness	Mouse	M > F	Androgen-mediated increase	13, 14
		α ERKO F > wild-type F	ER α -mediated	8
		M = F	NR	14
		<i>In vitro</i> study	Estrogen-mediated decrease	18
	Rat	Only ovariectomized F were studied	Estrogen-mediated decrease	17
	Guinea Pig	M > F	NR	15

Definition of abbreviations: ER = estrogen receptor; ERKO = ER knockout; F = female; M = male; NR = not reported.

that of Tesfaigzi and coworkers (28). In this study the sex difference in airway effects after intranasal LPS administration was limited to higher bronchoalveolar lavage (BAL) fluid IL-6 content in males than in females. No other lung inflammatory parameters differed between the sexes, although males also displayed a more severe hypothermia than did females (28).

Two recent studies have more thoroughly investigated the influence of sex and sex hormones on LPS-induced airway inflammation in mice. Speyer and colleagues administered LPS by intratracheal administration to male, female, and ovariectomized female C57BL/6 mice and assessed lung inflammatory endpoints 6 hours later (29). Male mice were found to have greater albumin and neutrophil content, and higher myeloperoxidase activity in BAL fluid, than did female mice. Female mice that had been ovariectomized responded to LPS in a male-like fashion (i.e., elevated BAL fluid albumin, neutrophils, and myeloperoxidase activity) unless they were administered a single dose of exogenous estradiol, in which case they responded in a fashion similar to that of intact females (29). These data suggest a protective effect of female sex hormones on LPS-induced airway inflammation in mice. A similar sex difference was reported by Card and coworkers in that airway inflammatory and lung functional responses of male C57BL/6 mice to airway administration of LPS were more severe than those of females (13). However, in this study ovariectomy did not alter the response of female mice, while castration was found to prevent the exaggerated LPS-induced inflammatory and functional effects that were observed in male mice. Moreover, administration of 5- α -dihydrotestosterone to female mice resulted in a significant augmentation of their inflammatory responses to LPS such that they resembled and often surpassed those of intact males (13). The data from these two studies collectively suggest that androgens promote and estrogens inhibit inflammatory responses to LPS in the mouse airway (13, 29).

There are several other examples of models of lung inflammation and acute lung injury that appear to be influenced by sex hormones. A recent study by Mikerov and coworkers reported that male mice were more susceptible than female mice to lung infection with *Klebsiella pneumoniae* but that this pattern was reversed in mice that had been exposed to ozone before establishment of the infection (30). Tissue damage associated with carrageenan-induced pleurisy in rats was reported to be attenuated by estrogen; this effect was blocked by coadministration of the ER antagonists ICI 182780 or tamoxifen, suggestive of a receptor-mediated effect (31). In a study of granulomatous lung inflammation induced in female

rats by heat-killed bacilli Calmette-Guérin (BCG), ovariectomy increased granuloma formation, lung-to-body weight ratio, and inflammatory cells in BAL fluid, while administration of exogenous estradiol to ovariectomized rats prevented these effects (32). Two studies examining chemical-induced Clara cell toxicity in mice have reported sex differences believed to be due to differences in metabolism. Van Winkle and colleagues observed that the airways of female mice were more susceptible than those of male mice to injury after a single intraperitoneal dose of naphthalene, a recognized environmental contaminant and component of cigarette smoke (33). The airways of female mice in this study were found to generate more naphthalene dihydrodiol, a potential toxic metabolite, than were the airways of male mice, suggesting that sex-specific metabolism might underlie the observed sex difference in toxicity. Enhanced metabolism in female mice also was proposed as underlying the increased sensitivity of females compared with males to bronchiolar cytotoxicity observed 24 hours after intraperitoneal administration of the prevalent water contaminant 1,1-dichloroethylene (34).

A potential sex disparity in the resistance and susceptibility to shock-induced lung injury has been investigated in several animal studies. In general, male sex hormones have been found to be detrimental and female sex hormones to be protective in these studies. Estrous or proestrous rats are more resistant to shock-induced intestinal and lung injury than rats in other phases of the estrous cycle, and it is believed that the resistance to intestinal injury underlies the resistance to secondary lung injury (35). Furthermore, castration of male rats decreases susceptibility to both lung and intestinal injury after trauma-hemorrhagic shock (36). Yu and colleagues showed that the protective effects of estrogen on lung injury in male rats after trauma-hemorrhage were ER β -mediated, possibly *via* ER β -induced down-regulation of inducible nitric oxide synthase (37). Other data suggest that decreased neutrophil priming and activation in female rats compared with male rats may be an underlying mechanism for decreased cellular injury and tissue damage in females after trauma hemorrhage (38).

Allergic Lung Inflammation

There is considerable evidence to suggest that there is a role for sex and sex hormones in the pathogenesis of asthma (3, 4, 39). Asthma prevalence in the general population is higher in women than in men, although several studies indicate distinctive changes in asthma prevalence and severity with age. Male children have asthma more frequently than do female children,

TABLE 2. SUMMARY OF SEX DIFFERENCES IN MODELS OF LUNG INJURY AND DISEASE

Disease Model	Species	Sex Difference	Noteworthy Observations	Reference
Nonallergic lung inflammation and acute lung injury				
LPS-induced inflammation	Mouse	M > F	More severe hypothermia and BAL fluid IL-6 content in M	28
		M > F	Hypothermia, inflammation, and airway responsiveness all greater in M; androgens shown to be exacerbatory	13
		M > F	BAL fluid inflammatory parameters greater in M; estrogens shown to be protective	29
<i>Klebsiella pneumoniae</i> infection	Mouse	M > F	Prior ozone exposure reversed the observed sex difference	30
Carrageenan-induced pleurisy	Rat	Only F were studied	Tissue damage in ovariectomized F attenuated by estrogen	31
Granulomatus lung inflammation	Rat	Only F were studied	Tissue damage and BAL inflammatory parameters in ovariectomized F attenuated by estrogen	32
Chemical-induced injury	Mouse	F > M	Sensitivity of F to naphthalene-induced injury associated with levels of toxic metabolite generated	33
		F > M	Sensitivity of F to 1,1-dichloroethylene-induced injury associated with enhanced metabolism	34
Shock-induced injury	Rat	Only F were studied	Estrous or proestrous F resistant to injury	35
		M > F	Estrous or proestrous F resistant to injury	36
		Only M were studied	Estrogen shown to be protective via ER- β	37
		M > F	Less neutrophil priming and activation in F	38
Allergic lung inflammation				
Ovalbumin-induced inflammation	Mouse	F > M	Higher serum IgE in F	40, 41, 42, 43
		F > M	Greater airway inflammatory cell and cytokine content in F	43, 47
		F > M	Bronchial-bronchiolar inflammation increased in castrated M	44
		M > F	No sex difference in inflammation but airway hyperresponsiveness present in M; suppressive effect of estrogen demonstrated	45
		Only F were studied	No difference in inflammation but airway hyperresponsiveness present in α ERKO F	8
		Only F were studied	Inflammation and airway responsiveness decreased by ovariectomy when conducted before but not after allergic sensitization	46
	Rat	Only F were studied	Inflammation decreased by ovariectomy or ER antagonist	49
Chronic obstructive pulmonary disease				
Cigarette smoke exposure	Mouse	F > M	Emphysematous-like changes occurred earlier in F	57
Lung fibrosis				
Bleomycin-induced fibrosis	Mouse	M > F	Histologic damage greater in M	62
		M > F	No difference in fibrosis, but static compliance decreased in M; androgens shown to be exacerbatory	63
	Rat	F > M	Higher mortality and more severe fibrosis in F; estrogens shown to be exacerbatory	61

Definition of abbreviations: BAL = bronchoalveolar lavage; ER = estrogen receptor; ERKO = ER knockout; F = female; M = male.

but a reversal of this incidence pattern occurs around the time of puberty, leading to a female predominance during middle age. The difference between the sexes is not apparent later in life (around the fifth or sixth decade), and some reports suggest that there is an increase once again in male prevalence (3, 4, 39). These data collectively suggest a role for female sex hormones in promoting the asthmatic phenotype.

Consistent with observations in adult humans, numerous but not all studies in mice have reported an increased susceptibility to various components of allergic airway disease in females compared with males. Corteling and Trifilieff observed increased serum IgE and decreased sensitivity to the therapeutic effects of budesonide in allergic female mice compared with allergic male mice (40), while Seymour and colleagues reported significantly higher total and allergen-specific IgG1 and IgE in the serum of allergic females compared with allergic males (41). Cui and coworkers also observed higher allergen-specific IgE in the serum of allergic female mice (42). Increased airway inflammatory cell and cytokine content and serum IgE levels were noted in allergic female mice compared with allergic male mice in a study by Okuyama and colleagues (43). In a study by Hayashi and coworkers, less severe bronchial-bronchiolar inflammation was observed in allergic male mice compared with allergic female mice; this reduced level of inflammation was not evident when males were castrated, suggesting a protective role

of androgens (44). Conversely, a recent study by Matsubara and colleagues revealed that allergic airway inflammation in male and female mice after repeated aerosol allergen exposure did not differ, but that airway hyperresponsiveness was present in males and not in females (45). Experiments conducted by these authors with mice that were ovariectomized, treated with estradiol, or treated with the ER antagonist ICI 182780 indicated that this difference was due to suppressive effects of estrogen. Consistent with the notion of a suppressive effect of estrogen on allergic airway responsiveness, Carey and colleagues reported that airway inflammatory indices were comparable in allergic α ERKO and wild-type female mice, but that significant airway hyperresponsiveness developed in the former but not in the latter (8).

A recent report indicated that while ovariectomy before sensitization to an experimental allergen decreased allergic airway inflammation and responsiveness in female mice, no such reduction in allergic outcomes was observed when ovariectomy was performed at the end of the sensitization protocol (46). These data highlight the different influences that sex hormones may have on the sensitization and effector phases of an allergic response. Melgert and colleagues reported increased allergic airway inflammation in female mice and suggested that this was due in part to the lower number of naturally occurring regulatory T cells that were observed in

female mice compared with male mice (47). This is an important observation given that regulatory T cells are thought to play an important role in controlling T helper type 2-biased responses and that impaired function of this cell type has been associated with allergic diseases (48).

There are other data that suggest that female sex hormones promote allergic lung inflammation in animal models. In female rats, ovariectomy or treatment with the ER antagonist tamoxifen was found to decrease allergic airway inflammation, while estrogen replacement in ovariectomized rats returned inflammation to levels observed in intact females (49). In female mice, administration of progesterone was shown to accentuate allergic airway disease (50). It has been demonstrated that inhalation of environmental tobacco smoke enhances the pulmonary allergic response in mice (51, 52). Related to this, female mice exposed to environmental tobacco smoke before allergen sensitization and exposure have been shown to possess more IgE-positive cells in their lungs than their male counterparts (41), while progesterone and environmental tobacco smoke were found to synergistically exacerbate allergic airway disease in ovariectomized female mice (53).

Chronic Obstructive Pulmonary Disease

Female smokers appear to be more susceptible than male smokers to developing COPD (2), although there also is a preponderance of females among the small percentage of patients with COPD who are nonsmokers (54). Ben-Zaken Cohen and coworkers (55) recently reviewed some of the evidence in support of a role for sex-dependent metabolism of components of cigarette smoke as underlying the increased susceptibility of females to COPD. Estrogens have a recognized capacity to up-regulate cytochrome P450 expression and activity while not affecting conjugation (Phase 2) enzymes, thereby potentially leading to an imbalance in the formation and removal of toxic intermediates of cigarette smoke components (2, 55).

An animal model that displays all of the morphologic and functional aspects of COPD has not yet been established, although cigarette smoke-induced lung injury has been suggested to be the closest (56). In this regard, it is interesting to note that exposure of mice to cigarette smoke for up to 22 weeks was found to result in emphysematous-like changes that occurred earlier in females than in males (57). The underlying mechanism(s) for this difference is not presently clear but might be related to sex differences in pulmonary metabolism of chemicals present in tobacco smoke, similar to what has been proposed in humans (55). The study in mice that was conducted by Van Winkle and colleagues supports this possibility (33). These authors reported an increased susceptibility of female mouse airways to injury after a single intraperitoneal dose of naphthalene, a polycyclic aromatic hydrocarbon present in cigarette smoke. Notably, more of the potentially toxic metabolite naphthalene dihydrodiol was produced in female airways than in male airways (33). A study by Forkert and colleagues (34) demonstrated that the airways of female mice also were more sensitive than those of male mice to the toxic effects of an intraperitoneal administration of another compound that is metabolized by cytochrome P450—namely, 1,1-dichloroethylene. Thus, although limited, the available data support the notion of enhanced metabolism of pulmonary toxicants as contributing to the susceptibility of female airways to chemical-induced injury and emphysematous-like changes that are characteristic of COPD.

Lung Fibrosis

Sex differences in the risk and prevalence of idiopathic lung fibrosis in humans have been reported, with males more

susceptible than females (58, 59). Sex-related differences also have been observed in animal models of lung fibrosis. Administration of bleomycin to mice, rats, and other experimental animals results in pulmonary fibrosis and has been extensively used as a model to study mechanisms and potential treatments for idiopathic lung fibrosis (60). Female rats administered intratracheal bleomycin exhibited a higher mortality rate and more severe fibrosis than males, as evidenced by higher levels of lung collagen deposition and fibrogenic cytokine expression (61). Ovariectomy was found to diminish fibrosis, whereas estradiol replacement restored the fibrotic response to that of intact females. Estradiol also was demonstrated to have a direct fibrogenic effect on fibroblasts isolated from fibrotic lungs, as indicated by increased expression of procollagen (α_1) I and TGF- β 1 mRNA after incubation with estradiol (61).

In contrast to observations in rats, bleomycin administration to mice has been reported to result in a more severe fibrotic response in males than in females (62). Increased histologic evidence of fibrosis was reported in male versus female mice after bleomycin administration via a subcutaneous osmotic minipump, an outcome that was suggested to be due to differential expression and/or activity of bleomycin hydrolase in the lungs of males and females (62). Conversely, a more recent study reported that male and female C57BL/6 mice did not differ in terms of their lung fibrotic responses, but that the bleomycin-induced decrease in static compliance was significantly greater in males than in females (63). This adverse effect on lung function was found to be due to male sex hormones, as castrated males exhibited a female-like response to bleomycin while female mice given exogenous 5- α -dihydrotestosterone exhibited a male-like response (Figure 2). Ovariectomy of females and disruption of estrogen receptor signaling in α ERKO and β ERKO mice were without effect (63).

Figure 2. Male sex hormones promote lung function decline in a model of lung fibrosis. Bleomycin-treated male mice demonstrated a decline in static compliance (indicative of increased lung stiffening) after bleomycin administration, whereas female mice did not. Castration of male mice resulted in decreased basal static compliance and a lack of a further decrease due to bleomycin (i.e., a female-like response). Conversely, administration of exogenous 5- α -dihydrotestosterone (DHT) to castrated males or intact females was associated with a decline in static compliance after bleomycin (i.e., a male-like response). * $P < 0.05$ versus corresponding saline (Sal) group. Reprinted by permission from Reference 63.

Figure 3. A simplified depiction of the types of studies and data summarized in this article that demonstrate an influence of sex hormones on lung function and disease in animal models.

Other studies support the notion of an enhanced susceptibility to lung and airway fibrosis in male mice. Markova and colleagues (64) reported that naïve, 16-week-old adult male C57BL/6 mice had approximately 25% more lung hydroxyproline, a measure of collagen content, than was observed in age-matched females. This increased level of lung collagen was not present in male mice deficient in the androgen receptor (ArTfm mice), suggesting a contribution of the androgen receptor pathway to the observed sex difference in lung collagen levels (64). Moreover, Lekgabe and colleagues (65) demonstrated an interesting synergism between the hormones relaxin and estrogen in the lung. They reported that airway fibrosis is under the influence of both relaxin and estrogen and that estrogen can partially protect the lung from airway fibrosis in the absence of relaxin (65).

CONCLUSIONS

It is clear from the data that have been summarized here that considerable sex differences exist in respiratory physiology and disease. An overview of the data discussed in this article is presented in Figure 3. It also is apparent that the underlying mechanism(s) for the sex differences that have been reported to date are not likely due to one sex hormone, sex hormone receptor, or intracellular signaling pathway. Rather, a complex interplay among these factors appears to underlie the differences in lung function and in the enhanced susceptibility (or lack thereof) of one sex over the other in a number of models of lung disease. Recognition of potential sex differences is important to the design and interpretation of experimental studies and to the comparison of the results of such studies with those from others. It is anticipated that continued investigation of these differences in animal models will provide important information related to our understanding of a variety of lung diseases and, ultimately, identify improved treatment options for affected patients.

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