

ASTHMA PHYSIOLOGY AND BIOMAKERS

Age and Sex Influences on Airway Hyperresponsiveness

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Objective. The precise relationship between age and gender and their influence on airway reactivity has not been clearly defined. Previous studies of age and gender influences on airway reactivity have been confounded by environmental influences such as cigarette smoking. The objective of this study was to examine the effect of age and gender on airway reactivity in C57BL/6 mice housed under controlled conditions, independent of confounding environmental factors. **Methods.** Mice were separated into four experimental groups based on age and gender: males at 6 and 12 weeks of age and females at 6 and 12 weeks of age. Airway reactivity to inhaled methacholine was examined in each group. **Results.** Significant differences in methacholine-induced airway reactivity were observed between the sexes at both age groups. At 6 weeks of age, the males demonstrated a significantly increased airway reactivity to methacholine as compared to females ($p < .001$). Paradoxically, at 12 weeks of age, the males demonstrated a significantly lower response to methacholine as compared to females ($p < .001$). Hence the relationship between age and airway reactivity is markedly different in males as compared to females. Examination of intragender differences revealed that in female mice, airway reactivity increased significantly with age ($p < .001$). In contrast, males demonstrated a significant reduction in airway reactivity with age ($p < .001$). **Conclusion.** These findings demonstrate important differences in airway reactivity related to age and gender that are observed independent of any environmental influences. Furthermore, these findings highlight the importance of careful age and sex matching in studies of airway reactivity.

Keywords age, airway reactivity, asthma, C57BL/6, mice, Penh, sex

INTRODUCTION

Exposure of the airways to methacholine chloride (MCh) causes the smooth muscles that line the airway to contract, resulting in airway narrowing and elevated airway resistance (1). The extent to which the airways respond to MCh provocation is known as airway hyperresponsiveness (AHR). The level of AHR exhibited is used as a predictor of general airway health and is frequently employed to assess and monitor the severity of airway inflammatory diseases, such as asthma. In normal healthy individuals AHR has been shown to vary with age and sex. However, the precise relationship between these parameters and AHR has not been directly examined.

Studies of airway response in adults have demonstrated significantly greater AHR in females as compared to males (2–4). In contrast, other investigators have shown that measurements of airway response in children reveal greater AHR in males as compared to females (5–7). All of these studies have been confounded by environmental influences such as cigarette smoking, making it difficult to determine whether the findings in adults reflect environmental changes or intrinsic sex-related differences. Moreover, sex differences in environmental exposures and responses to environmental variables have made it difficult to directly explore these disparities in the human population.

We have sought to redress this problem by directly examining the effects of age and sex on AHR in laboratory mice under controlled environmental conditions. This study

specifically examines age and sex influences on AHR independent of extrinsic environmental factors.

METHODS

Mice

Six- and 12-week-old male and female C57BL/6 mice were obtained from Walter and Eliza Hall Institute (Melbourne, Australia). All mice were maintained in a standard animal care facility (Department of Agriculture, University of Melbourne, Australia) and all procedures were approved by the Royal Children's Hospital Animal Experimentation Ethics Committee.

Measurement of Airway Responsiveness

Methacholine chloride-induced AHR was assessed in unrestrained mice by barometric, whole-body plethysmography (Buxco Electronics, Troy, New York, USA) as described by Hamelmann et al. (8). Airway response is expressed as an enhanced pause (Penh), a dimensionless value representing a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of inspiration. There is some debate concerning the use of Penh although the technique remains widely used in studies of AHR in mice (9–11). In brief, the Bio-system was first calibrated with a rapid injection of 1 ml of air into the mouse chambers. Mice were then placed in individual chambers and allowed to settle for approximately 10 to 15 min, after which a baseline Penh reading was taken. Following the baseline reading the mice were exposed to increasing concentrations of aerosolized MCh (1.5, 3, 6, 12, 25, 50, and 100 mg/ml in phosphate-buffered saline [PBS]) for a period of 2 min. After each MCh exposure Penh values were recorded at 20-s intervals over 5 min. A median value over the 5-min period was

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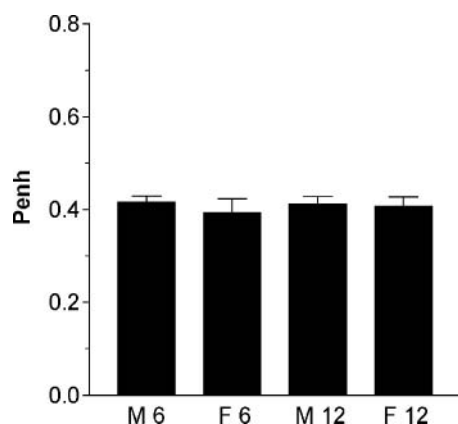


FIGURE 1.—Baseline Penh in C57 black 6 mice under normal breathing conditions. Mice were grouped according to age and sex: Males at 6 weeks (M 6), females at 6 weeks (F 6), males at 12 weeks (M 12), females at 12 weeks (F 12). No significant differences were observed in baseline Penh values between any of the groups.

determined to give a single Penh value for each consecutive dose.

Statistical Analysis

Data are expressed as mean values $\pm$ SEM. One-way analysis of variance was used to test for significance in AHR measurements taken at baseline and in comparisons between groups in bronchoalveolar (BAL) cell count. Two-way analysis of variance was used to test for the influence of age and sex upon airway MCh dose-response curves. In addition, significant differences in airway response at individual MCh doses were assessed using a Bonferroni post-test.

RESULTS

Baseline measurements of Penh taken prior to MCh nebulization showed no significant differences between the four

TABLE 1.—Methacholine-induced airway responsiveness in male and female mice at 6 weeks and 12 weeks of age.

		Methacholine concentration (mg/ml)						
		0	3.125	6.25	12.5	25	50	100
Females	6 weeks	0.39	0.39	0.44	1.09	2.03	3.06 ^{*,#}	3.26 ^{*,#}
	12 weeks	0.41	0.41	0.56	1.57	2.81	4.57	5.63 [#]
Males	6 weeks	0.42	0.47	0.50	2.10	3.77	5.71	5.97 [*]
	12 weeks	0.41	0.48	0.65	1.07	2.11	3.60	3.12

* $p < .05$ for comparisons between 6- and 12-week-old mice of the same sex.

$p < .05$ for comparisons between males and females of the same age.

groups (Figure 1). Bronchoalveolar lavage (BAL) cell counts were equivalent in all groups (data not shown).

Both age and sex were shown to significantly influence MCh-induced AHR in mice. The MCh dose-response curve for 6-week-old males was significantly increased as compared to that for 6-week-old females ($p < .001$) (Figure 2A). Comparison of airway response at individual doses of MCh revealed significantly greater airway response in males as compared to females at doses of 50 mg/ml ($p < .05$) and 100 mg/ml ($p < .05$) (Table 1). Conversely at 12 weeks of age, the MCh dose-response curve for the females was significantly greater than that for males ($p < .001$) (Figure 2B). Twelve-week-old females demonstrated significantly greater airway response to MCh at a dose of 100 mg/ml as compared to 12-week-old males ($p < .001$) (Table 1).

Comparison of MCh-induced airway responses in 6-week-old and 12-week-old female mice revealed a significant increase in AHR with age (difference in response curves $p < .001$) (Figure 3A). Penh was significantly higher in 12-week-old females as compared to 6-week-old females at MCh concentrations 50 mg/ml ($p < .05$) and 100 mg/ml ($p < .001$) (Table 1). Comparison of MCh-induced AHR in males at 6 and 12 weeks of age also showed significant differences. However, in contrast to the female mice, MCh dose-response curves decreased significantly with age ($p < .001$).

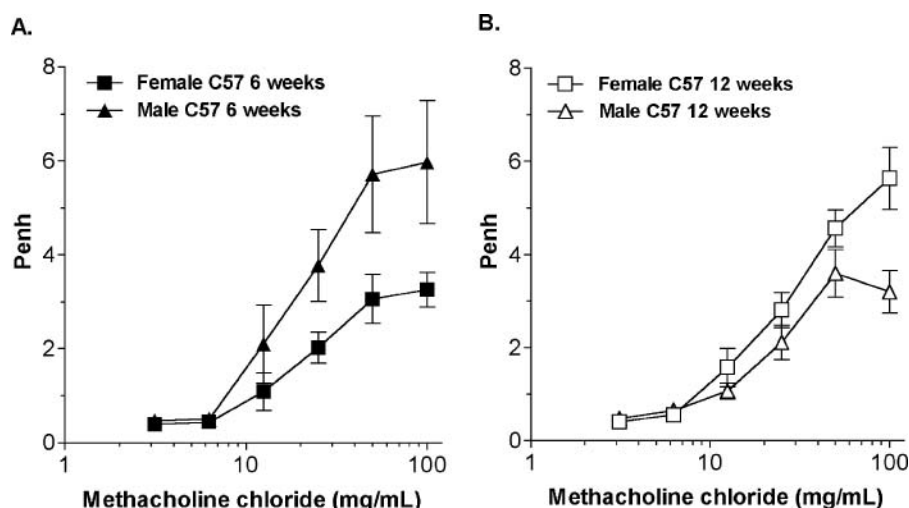


FIGURE 2.—Influence of sex on MCh-induced airway responses at (A) 6 weeks and (B) 12 weeks of age. (A) Six-week-old male mice (▲) demonstrated significantly increased MCh-induced airway reactivity as compared to 6-week-old female mice (■); $p < .001$. (B) Twelve-week-old male mice (△) demonstrated significantly reduced MCh-induced airway reactivity as compared to 12-week-old female mice (□); $p < .001$.

.001) (Figure 3B) and Penh was significantly lower in 12-week-old males as compared to 6-week-old males at MCh dose 100 mg/ml ($p < .05$) (Table 1).

DISCUSSION

In this study, age and sex were shown to significantly influence MCh-induced airway responses in C57BL/6 mice. At 6 weeks of age, male mice demonstrated significantly elevated AHR as compared to the females. In contrast, at 12 weeks of age, this relationship was reversed, with the females demonstrating a significantly greater AHR than males. Examination of age-related changes in AHR within each sex revealed opposing trends in males as compared to females. In males, AHR declined with age; conversely in females, AHR increased with age. Thus both age and sex significantly influenced MCh-induced AHR. These findings in mice confirm the observational findings in the human population previously published by Paoletti et al. (5). There is a reversed relationship between AHR in males and females in childhood as compared to adulthood.

In previous studies it was suggested that age and sex effects upon AHR reflected environmental influences on lung function. In this present study, age and sex differences in MCh-induced AHR were observed despite all mice being housed in the same controlled environment. This indicates that the age- and gender-dependent changes in MCh-induced AHR occurred independently of any environmental influences. In addition, as baseline Penh was not significantly different between the various groups studied, these age and sex effects did not relate to anatomical differences between the groups. These findings suggest that the age- and sex-related changes in AHR reflect intrinsic differences between males and females, possibly related to differences in sex hormones, with male hormones being protective against elevated AHR.

The influence of sex hormones on lung function has attracted significant attention recently, with particular interest directed to their potential role in disease states such as asthma (12). Several studies have shown close correlations

between AHR and hormonal variations occurring during the menstrual cycle (12, 13). In addition, estrogen and progesterone correlate closely with key factors influencing bronchoconstriction (12, 14–16). Specifically, in healthy females progesterone levels directly correlate with expression of β_2 -adrenoceptors, the key receptors responsible for relaxation of airway smooth muscle (17). In contrast, estrogen levels correlate with prostaglandin $F_{2\alpha}$ formation, a molecule with bronchoconstrictive and proinflammatory actions (18). Further studies are required to establish the role of sex hormones in influencing AHR and the potential impact this may have on the incidence of airways disease in the general population. This is relevant because the age- and sex-related changes in AHR in mice mimic the incidence of allergic airways disease across the human life span. The incidence of asthma is significantly higher in young boys than in girls and adult males, and declines steadily post adolescence to middle age (14). Conversely, the incidence of asthma in females increases significantly with age (14). Similarly, hospital admissions for asthma are more common in adult females as compared to young females, whereas more admissions were seen for males only in the prepubertal period (19). Longitudinal studies conducted on asthmatic children followed through to adulthood (age of 30) have demonstrated deterioration of lung function (forced expiratory volume in one second [FEV_1]) from childhood to adulthood in females but improvement in males (20).

In summary, this study confirms and defines the complex interactions between age, sex, and AHR to MCh. These age and sex differences are likely to reflect intrinsic physiological differences related to sex hormones, rather than extrinsic environmental influences. This study also emphasises the importance of appropriate age- and sex-matched controls in studies of AHR. Further investigation to identify the mechanisms leading to these sex differences in AHR are required. Improved understanding of the influence of sex hormones on lung function may provide important insights leading to improved treatment of airway inflammatory disorders such as asthma.

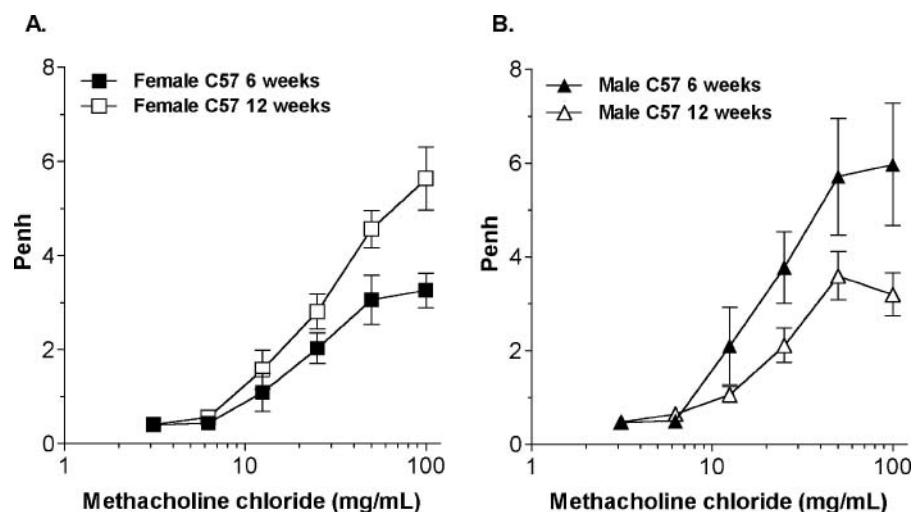


FIGURE 3.—Influence of age on MCh-induced airway responses in (A) females and (B) males. (A) Female mice at 12 weeks (□) demonstrated significantly increased MCh-induced airway reactivity as compared to 6-week-old female mice (■); $p < .001$. (B) Twelve-week-old male mice (△) demonstrated significantly reduced MCh-induced airway reactivity as compared to 6-week-old male mice (▲); $p < .001$.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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