行政院國家科學委員會補助
大專學生參與專題研究計畫研究成果報告

計畫名稱：雌激素對活性氧族群引發呼吸道過度敏感的影響

執行計畫學生：張惟雅
學生計畫編號：NSC 98-2815-C-040-051-B
研究期間：98 年 07 月 01 日至 99 年 02 月 28 日止，計 8 個月
指導教授：阮婷

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執行單位：中山醫學大學醫學檢驗暨生物技術學系（所）

中華民國 99 年 05 月 12 日

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雌激素對活性氧族群引發呼吸道過度敏感的影響

Effect of Estrogen in Reactive Oxygen Species Induced Airway Hypersensitivity

執行計畫學生：張惟雅

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ABSTRACT

Enhanced airway reflex reactivity (EARR) and increased lung reactive oxygen species (ROS) are two features of asthma. A deterioration of asthma symptoms is associated with a declining estrogen level in females with unknown mechanisms. Sensitization of lung vagal C-fiber afferents (LVCFAs) has been implicated in the pathogenesis of asthma. We investigated the influence of estrogen on the LVCFA-mediated EARR induced by increased lung ROS in anesthetized female rats. Apneic response to intravenous capsaicin, a selective stimulant of LVCFAs, before and after spontaneous inhalation of aerosolized H_2O_2 (a major type of ROS; 0.05 % for 90 s) was measured as an index of airway reflex reactivity. We found that H_2O_2 inhalation induced a LVCFA-mediated EARR in intact rats and this EARR was more prominent during the metestrus/diestrus (low estrogen) phase than during the proestrus (high estrogen) phase. In ovariectomized rats, subcutaneous 17β-estradiol replacement, at a dose that mimics its level during the proestrus surge, suppressed the H_2O_2-induced EARR. The suppressive effect of 17β-estradiol was reversed by pretreatment with ICI 182,780, an estrogen receptor antagonist. These results suggest that endogenous and exogenous estrogen may alleviate ROS-induced EARR in female rats possibly via suppression of sensitization of LVCFAs.

INDRODUCTION

Asthma is characterized by increased reactive oxygen species (ROS) in the lungs and enhanced airway reflex reactivity (EARR). Increasing evidence suggests that estrogen influences airway reactivity during woman's reproductive years when levels of estrogen undergo cyclical variations. Indeed, epidemiologic data show an increase in asthmatic symptoms is associated with a low estrogen level in females. Lung vagal C-fiber afferents (LVCFAs) has been implicated in the development of EARR and the pathogenesis may involve increased ROS in the lungs. We thus aimed to test the hypothesis that estrogen may suppress the LVCFA-mediated EARR induced by increased lung ROS.

OBJECTIVES

(1) To study whether changes in estrogen levels during the estrous cycle may influence the EARR induced by increased lung H_2O_2 in female rats.

(2) To investigate whether the H_2O_2-induced EARR in ovariectomized rats can be prevented by 17β-estradiol replacement.

(3) To exam the role of LVCFAs in the H_2O_2-induced EARR in ovariectomized rats.

METHODS

Pulmonary ROS was increased by inhalation of 0.05 % aerosolized H_2O_2 for 90 s in anesthetized female Sprague-Dawley rats which breathed spontaneously. Respiratory flow, tidal volume, and arterial blood pressure were continuously monitored. Reflex apneic responses to intravenous administration of various doses of capsaicin before and 1 min after H_2O_2 inhalation were measured as the index of airway reflex reactivity. Apneic index was defined as the apneic duration divided by the baseline expiratory time. The estrous stage was assessed daily by vaginal lavage. Measurements were made when the rats were in either the proestrus (high estradiol levels) or metestrus/diestrus (low estradiol levels) stage. In the ovariectomized group, rats were ovariectomized 3 weeks ahead of experiments and were given single subcutaneous (s.c.) injections of estrogen (10 μg/kg in 0.2 ml of sesame oil) or its vehicle 4~8 hrs before measurement.

RESULTS

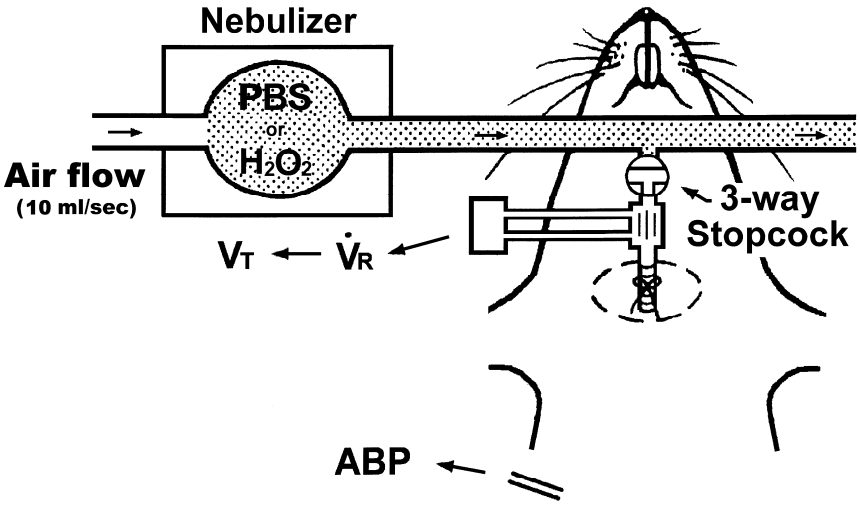

Figure 1. Schematic illustration showing the experimental setup. Animals breathed room air during control period. To produce airway sensory sensitization by H_2O_2, the 3-way stopcock was turned so that animals inhaled aerosolized H_2O_2 or PBS spontaneously via a sidearm from the lumen of the outlet tubing of the nebulizer. $\dot{V}_R$, respiratory flow; V_T, tidal volume; ABP, arterial blood pressure.

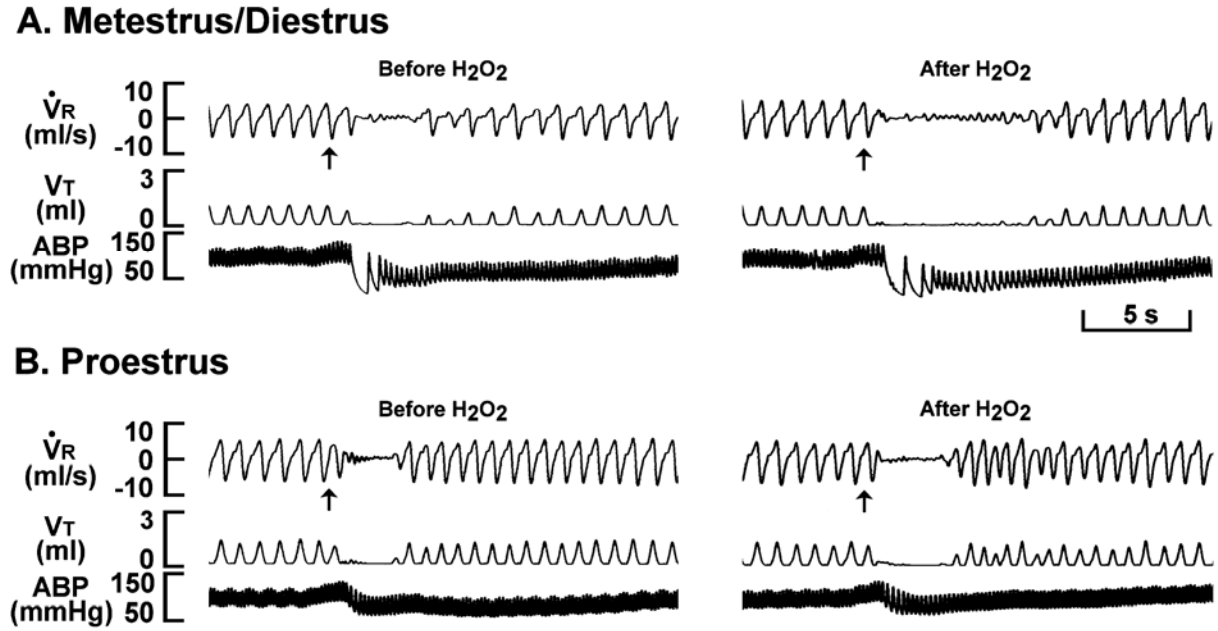

Figure 2. Reflex apneic responses to intravenous injections of capsaicin after airway sensory sensitization by H_2O_2 measured from a rat in metestrus/diestrus stage (A) and another rat in proestrus stage (B). Capsaicin (0.75 μg/kg) is a selective chemical stimulant for LVCFAs. The onset of stimulant challenge is indicated by arrows. Between the two capsaicin injections, 40 min were allowed to elapse. $\dot{V}_R$, respiratory flow; V_T, tidal volume; ABP, arterial blood pressure. Note that H_2O_2 sensitization induced an EARR, which was more prominent during the metestrus/diestrus (low estrogen) stage than during the proestrus (high estrogen) stage.

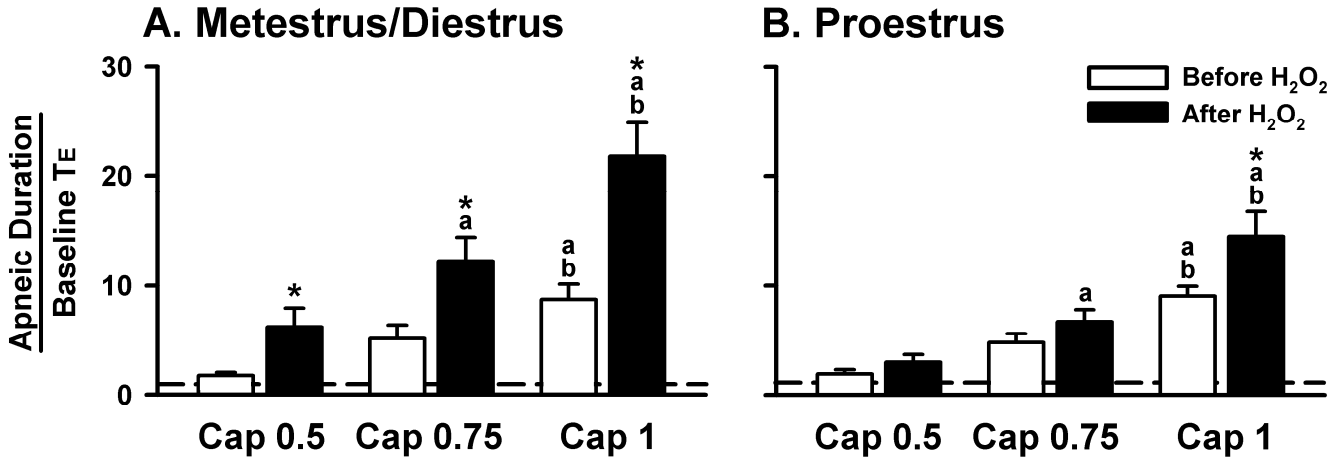

Figure 3. Difference in the airway reflex reactivity to intravenous capsaicin after airway sensory sensitization by H_2O_2 between the metestrus/diestrus (A) and in the proestrus study group (B). Apneic Index was defined as the apneic duration divided by the baseline expiratory time to serve as an index of airway reflex reactivity. T_E, expiratory time; *, significantly different from response before H_2O_2 sensitization; a, significantly different from response to 0.5 μg/kg of capsaicin; b, significantly different from response to 0.75 μg/kg of capsaicin. Data in each group are mean ± SE from 8 rats. Note that H_2O_2 sensitization induced an EARR, which was more prominent during the metestrus/diestrus (low estrogen) stage than during the proestrus (high estrogen) stage.

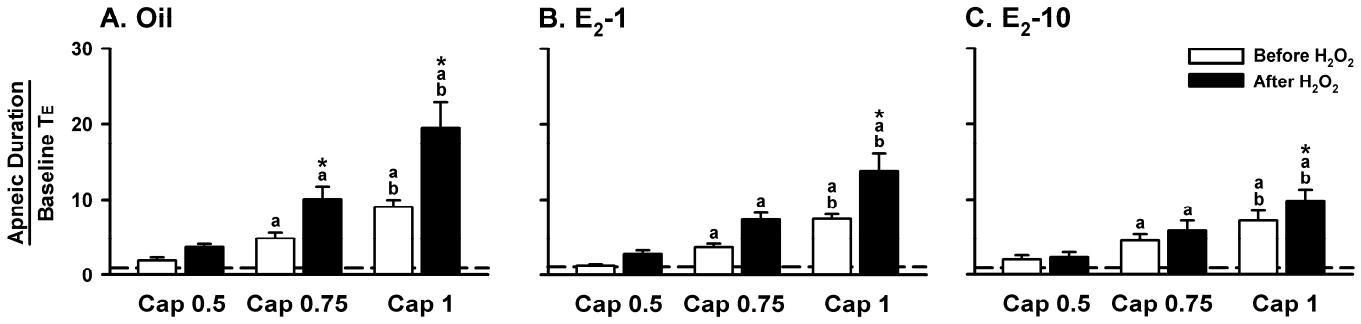

Figure 4. Difference in the airway reflex reactivity to intravenous capsaicin after airway sensory sensitization by H_2O_2 in ovariectomized rats with or without 17β-estradiol replacement. Animals in panels A, B and C were subcutaneously pretreated with sesame oil (Oil, the vehicle), 1 μg/kg 17β-estradiol (E_2-1), and 10 μg/kg 17β-estradiol (E_2-10), respectively. Apneic Index was defined as the apneic duration divided by the baseline expiratory time to serve as an index of airway reflex reactivity. T_E, expiratory time; *, significantly different from response before H_2O_2 sensitization; a, significantly different from response to 0.5 μg/kg of capsaicin; b, significantly different from response to 0.75 μg/kg of capsaicin. Data in each group are mean ± SE from 8 rats. Note that the H_2O_2-induced EARR in ovariectomized rats can be alleviated by 17β-estradiol replacement.

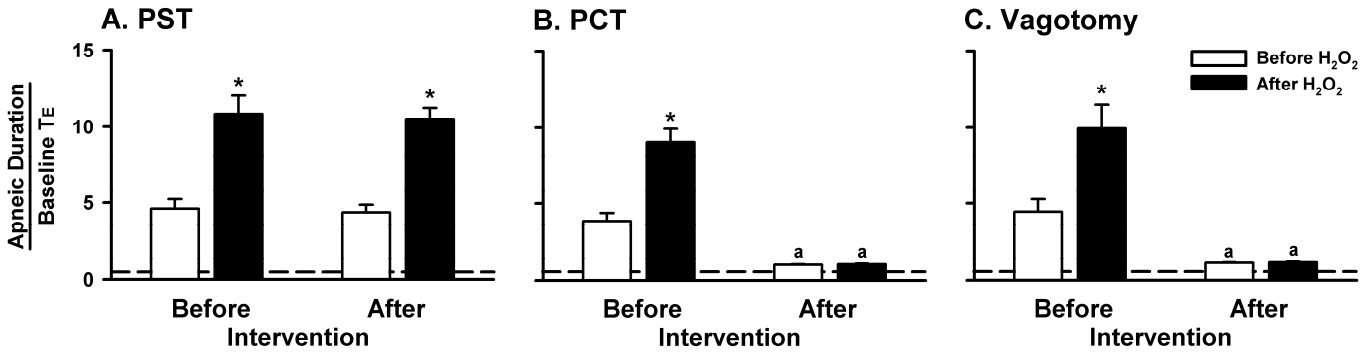

Figure 5. Effects of various experimental interventions of vagus nerves on the H_2O_2-induced EARR to intravenous capsaicin (0.75 μg/kg) in ovariectomized rats. PST, perivagal sham nerve treatment; PCT, perivagal capsaicin treatment; T_E, expiratory time; *, significantly different from the response before nerve treatment; a, significantly different from response before H_2O_2. Data in each group are mean ± SE from 8 rats. PCT is a technique that selectively blocks the neural conduction of LVCFAs. Note that the reflex apneic responses to capsaicin were abolished by either PCT or vagotomy, suggesting the H_2O_2-induced EARR is a consequence of sensitization of LVCFAs.

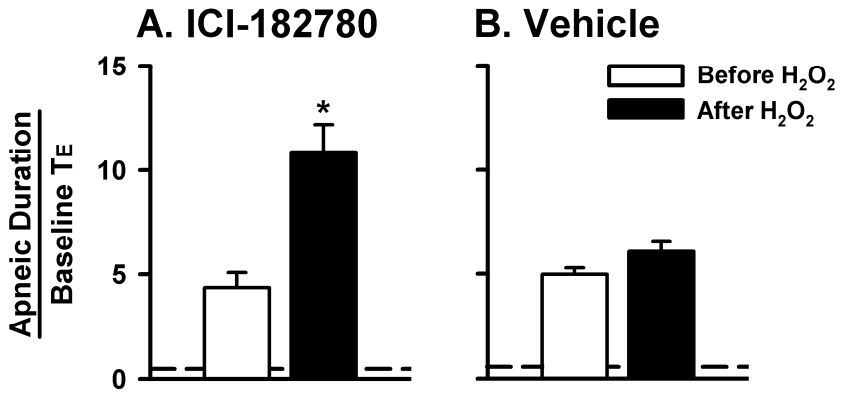

Figure 6. Effect of pretreatment with ICI-182780 (A) or its vehicle (B) on the H_2O_2-EARR to capsaicin in ovariectomized rats with 17β-estradiol replacement (10 μg/kg, s.c). ICI-182780 is a non-selective estrogen receptors antagonist. T_E, expiratory time; *, significantly different from the response before H_2O_2 sensitization. Data in each group are mean ± SE from 8 rats. Note that the suppressive effect of 17β-estradiol replacement on the H_2O_2-EARR was reversed by pretreatment with ICI-182780.

CONCLUSIONS

Estrogen may alleviate ROS-induced EARR in female rats possibly via suppression of sensitization of LVCFAs and this effect is mediated through estrogen receptors.