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Food Restriction-Mediated Adrenal Influences on Antigen-Induced Bronchoconstriction and Airway Eosinophil Influx in the Guinea Pig

Key Words

Bronchoconstriction
Epinephrine
Cortisol
Stress
Guinea pig

Abstract

The aim of this study was to measure the effects of food restriction on antigen-induced bronchoconstriction and inflammatory cell influx in guinea pigs and to determine the role of plasma cortisol and catecholamine concentrations. Ovalbumin (OA; 0.3 mg/kg, i.v.) was administered to OA-sensitized, anesthetized guinea pigs which had been allowed free access to food or had been food restricted for 18 h prior to OA challenge. In addition to higher plasma levels of epinephrine (30% increase) and cortisol (33% increase), fasted guinea pigs had significantly lower (60% decreased) maximal bronchoconstrictor responses to OA than nonfasted, sensitized litter mates. Additionally, groups of fasted or fed animals were subdivided into two additional treatment groups: (1) saline-pretreated or (2) polyethylene glycol 400 (PEG)-pretreated (1 ml/kg, p.o., 1 h prior to antigen challenge). In saline-treated, fasted animals, bronchoconstrictor responses to antigen were significantly diminished (67% decreased) and epinephrine and cortisol levels were increased (64 and 34%, respectively) compared to the corresponding fed group. In both fasted and fed groups, the PEG-treated guinea pigs had higher plasma epinephrine and cortisol levels than animals which received saline, but no significant differences were detected within the PEG-treated group. Plasma norepinephrine concentrations were lower in all fasted groups. In a separate model in conscious guinea pigs, there were no differences in aerosol OA-induced bronchoconstriction and eosinophil influx between fasted and fed groups. However, compared to the saline pretreatment group, PEG administration reduced the antigen-induced bronchoconstriction and eosinophilia in both fed and fasted guinea pigs. We speculate that the reduced responsiveness to antigen in fasted versus fed animals may result from food-restriction-induced, stress-related release of epinephrine and cortisol from the adrenal glands, thereby suppressing mast cell degranulation or reducing responsiveness to spasmogenic and chemotactic mediators. In addition, the results suggest that oral dosing with 100% PEG may enhance this phenomenon.

Introduction

Enhanced airway reactivity or hyperresponsiveness is a feature of bronchial asthma. Adrenergic involvement in this phenomenon is suggested by the finding of decreased β -adrenoceptor function or number [1–3], nocturnal fluctuations in plasma epinephrine concentrations [2, 4, 5], increased airway sensitivity [6, 7] or bronchoconstriction [8–10] in asthmatics following administration of β -adrenoceptor-blocking drugs. Increases in cellular concentrations of cAMP mediate the relaxation of airway smooth muscle and inhibit chemotaxis, cytotoxicity and degranulation of inflammatory cells [11–13]. cAMP is formed from ATP by the catalytic action of adenylyl cyclase. The activity of this enzyme is stimulated by endogenous hormones and auto-coids, such as epinephrine and prostaglandin E₂, which act via cognate cell surface receptors [14].

Administration of antigen to sensitized animals results in acute bronchoconstriction mediated by mast-cell-derived mediators (histamine, leukotrienes, prostaglandins) acting directly on airway smooth muscle [15]. This acute response to antigen can be regulated by catecholamines in at least two ways. First, activation of β -adrenoceptors by circulating catecholamines suppresses mast cell degranulation [16]. Second, the responsiveness of airway smooth muscle to a number of mast-cell-derived bronchoconstrictors is reduced by the presence of endogenous catecholamines [17]. This fundamental tenet underscores the widespread use of β -adrenoceptor agonists for the treatment of airways disease and is the scientific basis for the recent investigation of selective phosphodiesterase inhibitors for the treatment of bronchial asthma [13, 18]. We have previously demonstrated an increased reactivity to antigen in guinea pigs which had been adrenalectomized or pretreated with β -adrenoceptor antagonists [17]. In addition, we have shown that the effects of phosphodiesterase 4 inhibition against antigen-induced bronchoconstriction may require adenylyl cyclase activation by endogenous catecholamines for optimal activity [19].

Stress in laboratory animals, initiated by a number of approaches including fasting, hypoxia, anesthesia or morphine administration, has been shown to induce substantial stimulation of the pituitary-adrenal axis, resulting in altered circulating levels of catecholamines and cortisol [20–25]. In addition, acute, not chronic, stress in humans results in a reduction in airway responsiveness to antigen [26, 27]. The purpose of the present study was threefold: (1) to determine the effect of food restriction on the airway sensitivity to intravenously administered antigen in anesthetized guinea pigs; (2) to determine the effect of food restriction on air-

way reactivity and airway eosinophilia to inhaled antigen in conscious guinea pigs, and (3) to investigate whether any observed changes in responsiveness are due to increases in catecholamines or corticosteroids in animal models of the early- and late-phase response to antigen.

Materials and Methods

All animal protocols in this manuscript were approved by the Animal Care and Use Committee at SmithKline Beecham Pharmaceuticals, King of Prussia, Pa., USA.

Sensitization Procedure

Male Hartley guinea pigs (200–250 g; Hazelton Research Animals, Denver, Pa., USA) were sensitized by intramuscular injections of 0.35 ml of a 5% (w/v) ovalbumin (OA)/saline solution into each thigh (0.7 ml total) on days 1 and 4. The guinea pigs were ready for use after day 25.

Food Restriction and Vehicle Administration Procedure

The animals, housed in groups of 5 and accustomed to ad libitum access to Purina™ guinea pig chow, were fasted by removal of the 'j-type' feeders from their metal cages at approximately 2 p.m. the day before being brought into the laboratory for testing.

Measurement of Bronchoconstriction in Anesthetized Guinea Pigs

Male Hartley guinea pigs (500–600 g), actively sensitized to OA, were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and the jugular vein, carotid artery and trachea were cannulated (PE tubing 50, 60 and 260, respectively) for drug administration, blood pressure monitoring and ventilation, respectively. Bilateral vagotomies were performed to minimize neural reflex influences. The animals were paralyzed with pancuronium bromide (0.1 mg/kg i.v.) and ventilated at 45 breaths/min with a Harvard Rodent Respirator (model 683, Harvard Apparatus, South Natick, Mass., USA). Airway pressure changes were measured via a side arm of the tracheal cannula with a Druck PDCR 10/21, 70-mbar transducer (Druck Incorporated, New Raifield, Conn., USA). The ventilatory stroke volume was set to produce a side arm pressure of 8 cm of H₂O (approximately 5 cm³ room air). Blood pressure was measured with a Statham P23XL Physical Pressure Transducer (Viggo-Spectramed, Oxnard, Calif., USA). Pressures were recorded on a Grass Model 7D Polygraph (Grass Instrument Co., Quincy, Mass., USA). The animals were kept on a heated surgical table throughout the experiment to maintain body temperature. OA-induced bronchoconstriction was elicited by administration of 0.1 mg/kg OA. At the plateau of the initial response to OA, a subsequent dose of 0.2 mg/kg OA (0.3 mg/kg, cumulative) was added. At the peak of the antigen-induced response, 1 cm³/kg of a saturated KCl solution was administered. The responses to OA were measured as changes in airway insufflation pressure (Δ cm H₂O) and expressed as percentage of the maximal bronchoconstriction induced by KCl.

Catecholamine and Cortisol Measurement

One milliliter of arterial blood was drawn into a syringe containing 0.1 cm³ heparin solution (300 units/ml saline) 10 min after initial ventilation. The blood was replaced with 1 ml of isotonic saline. The blood samples were centrifuged at 10,000 rpm (12,000 g) for 10 min,

and the plasma was immediately frozen for catecholamine and cortisol analysis.

Fractional catecholamine analyses were performed by alumina extraction and HPLC electrochemical detection by SmithKline Bioscience Clinical Laboratories (Van Nuys, Calif., USA). Cortisol concentration was measured by radioimmunoassay by SmithKline Beecham Bioscience Clinical Laboratories, Collegeville, Pa., USA.

Conscious Animal Body Plethysmography and Bronchoalveolar Lavage

The characteristics of early- and late-phase responses to antigen challenge in this conscious guinea pig model have been reported previously [17, 19]. Conscious guinea pigs (500–600 g) were placed into a double-flow body plethysmograph (Penn-Century, Philadelphia, Pa., USA), consisting of a nasal (head) chamber and a thoracic (body) chamber. Each chamber was equipped with a pneumotachograph consisting of 6 fine (325) mesh screens stacked in series. A bias air flow of 250 ml/min was passed into the nasal chamber to provide continuous fresh air, and a bias air flow of 125 ml/min was passed into the thoracic chamber to maintain a comfortable ambient temperature in the plethysmograph. A seal was made between the 2 chambers by placing a magnetic collar containing a slab of black rubber sandwiches between 2 pieces of thin latex around each animal's neck. The magnet-containing collar secured the guinea pig such that essentially no air flowed between chambers. Each chamber was connected to a Buxco noninvasive respiratory analyzer (Buxco Electronics, Sharon, Conn., USA) via a Validyne Differential pressure transducer (± 2 cm) to determine measurement of tidal volume, minute volume, tidal air flow, respiratory rate, resistance and specific airway conductance (sGaw).

All data were recorded on a Grass model 7D polygraph (Grass Instruments) and digitized by a Boxco Data Logger (Buxco Electronics). Readings were taken continuously and averaged over 15 s. After a 10 min stabilization period, the animals were exposed to aerosols of 0.5% solutions of OA generated by a Pulmosonic nebulizer (DeVilbiss Inc., Somerset, Pa., USA) and delivered for 5 s at a rate of 250 ml/min via a nose cone built into the plethysmograph. In all animals, the results were calculated as percent change in sGaw from baseline reading taken just prior to the initial antigen challenge and reported as peak change (mean $\pm$ s.e.) every 30 s for at least 10 min after OA challenge.

Twenty-four hours after OA challenge, the guinea pigs were sacrificed by cervical dislocation and exsanguinated by severing the axillary arteries. The lungs were lavaged with 50 ml of Dulbecco's phosphate-buffered saline (5 aliquots of 10 ml), which was aspirated after gentle chest massage. The bronchoalveolar lavage (BAL) fluid was centrifuged at 2,200 rpm (1,100 *g*) for 10 min, the supernatant was aspirated and the pellet was resuspended in 5 ml 0.25% NaCl to lyse residual erythrocytes. This dispersion was centrifuged at 2,200 rpm (1,100 *g*) for 10 min, the supernatant was aspirated and the pellet was resuspended with 5 ml 0.9% NaCl. Total cell counts were done by hemocytometry using trypan blue stain. Slides were prepared on a Shandon Cytospin 2 (Pittsburgh, Pa., USA) at 300 rpm for 5 min, fixed and stained. Differential counts on at least 200 cells were done using standard morphologic criteria to classify cells as eosinophils, neutrophils or mononuclear cells, and the results were expressed as percentages of total cells counted.

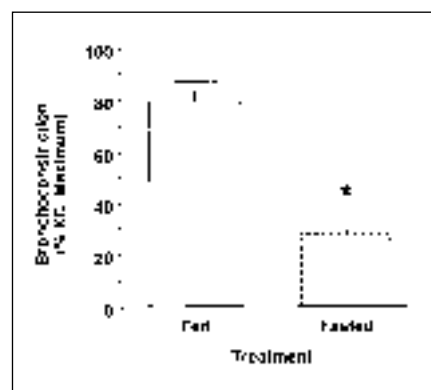


Fig. 1. Effect of fasting for 18 h on OA (0.3 mg/kg, i.v.)-induced bronchoconstriction in anesthetized, paralyzed and ventilated guinea pigs. The results are measured as airway insufflation pressure responses to OA and expressed as percentage of KCl-induced maximal bronchoconstriction; *n* = 10–11 for each treatment group. **p* < 0.05, Student's unpaired *t* test, two-tailed, when compared to fed group.

Statistical Analysis

Bronchoconstriction, catecholamine concentration and cortisol concentration results were compared between two treatment groups by Student's unpaired *t* test and among multiple treatment groups with analysis of variance (ANOVA) and Fisher's protected least square Differences (PLSD) test. Statistical significance was determined at a minimum 95% level of confidence. Statistical analysis for bronchoconstriction and inflammatory cell influx was performed using Statview Version 4.5 software (Abacus Concepts Inc., Berkeley, Calif., USA) on a Macintosh computer. Statistical analysis of the catecholamine concentrations was performed on a SAS main-frame statistical analysis system.

Drugs

OA (chicken egg, grade V) and polyethylene glycol (PEG) 400 were purchased from Sigma Chemical Co. (St. Louis Mo., USA).

Results

OA-Induced Bronchoconstriction in Anesthetized Guinea Pigs

In anesthetized, OA-sensitized guinea pigs, the administration of OA (0.3 mg/kg, i.v.) increased pulmonary inflation pressure from the initial inflation pressure setting of 8 cm H₂O to 69.5 $\pm$ 6.6 cm H₂O, which represented 79.1 $\pm$ 8.2% of the KCl-induced maximum bronchoconstrictor response (fig. 1; *n* = 10). In recent studies, we have shown that the administration of 0.1 mg/kg OA produces a threshold airway pressure response, and the subsequent addition of 0.2 mg/kg (0.3 mg/kg total) produces a near-maximal response [17, 19]. In animals which had been fasting

Table 1. Effects of different treatments on epinephrine, norepinephrine and cortisol plasma concentrations in anesthetized guinea pigs

Oral pretreatment	n	Fed or fasted	Epinephrine, pg/ml	Norepinephrine, pg/ml	Cortisol, ug/dl
None	10	Fed	1,273±163	491±87	52.6±3.5
	11	Fasted	1,655±120 ^a	351±38	69.9±3.1 ^a
Saline	5	Fed	1,064±173	592±140	54.6±7.1
	6	Fasted	1,749±88 ^a	323±60 ^a	73.8±4.1 ^a
PEG	6	Fed	2,173±509 ^b	630±83	77.0±7.6 ^b
	6	Fasted	2,335±345	299±64 ^a	85.8±10.9

Results are given as mean ± SEM. ^ap<0.05 compared to corresponding fed group; ^bp<0.05 compared to corresponding saline-pretreated group; Student's unpaired t test

for 18 h, bronchoconstrictor responses to OA (0.3 mg/kg, i.v.) were significantly decreased (60% reduction) to 27.2±8.2 cm H₂O, which represented 31.0±10.3% of the KCl-induced maximum (fig. 1, p<0.05, Student's unpaired t test, n = 10–11, each treatment). Maximal responses to KCl in fasted animals (82.7±3.8 cm H₂O) were not different from responses in corresponding fed animals (84.5±1.8 cm H₂O). In addition, there were significant differences in the plasma levels of epinephrine (fed = 1,273±163 pg/ml; fasted = 1,655±120 pg/ml) and cortisol (fed = 52.6±3.5 µg/dl; fasted 69.9±3.1 µg/dl) (table 1, p<0.05, Student's unpaired t test, n = 10–11, each treatment). These differences represented a 30% increase in epinephrine and a 33% increase in cortisol in guinea pigs which had been fasted compared to their nonfasted, sensitized litter mates. Plasma norepinephrine concentrations were not significantly different between fasted (491±87 pg/ml) and fed groups (351±38 pg/ml; table 1; p = 0.14, Student's unpaired t test, n = 10–11, each treatment).

A separate set of guinea pigs was subjected to the fasting/feeding protocol followed by an oral dose of normal saline or PEG (1 ml/kg) 1 h prior to antigen challenge. As in the initial study, in the saline-treated animals the bronchoconstrictor responses to OA in fasted animals (30.8±12.6% of the KCl-induced maximal response) were significantly less (67% reduced) than in corresponding saline-treated animals which had been allowed continuous access to food over the 18 h prior to antigen challenge (92.6±3.5% of the maximal bronchoconstrictor response; fig. 2, p<0.05, Student's unpaired t test, n = 5–6, each treatment). In addition, there were significant differences in the plasma levels of epinephrine (fed = 1,064±173 pg/ml; fasted = 1,749±88 pg/ml) and cortisol (fed = 54.6±7.1 µg/dl; fasted = 73.8±4.1 µg/dl) (table 1, p<0.05, Student's unpaired t test, n = 5–6, each treatment). These differences represent-

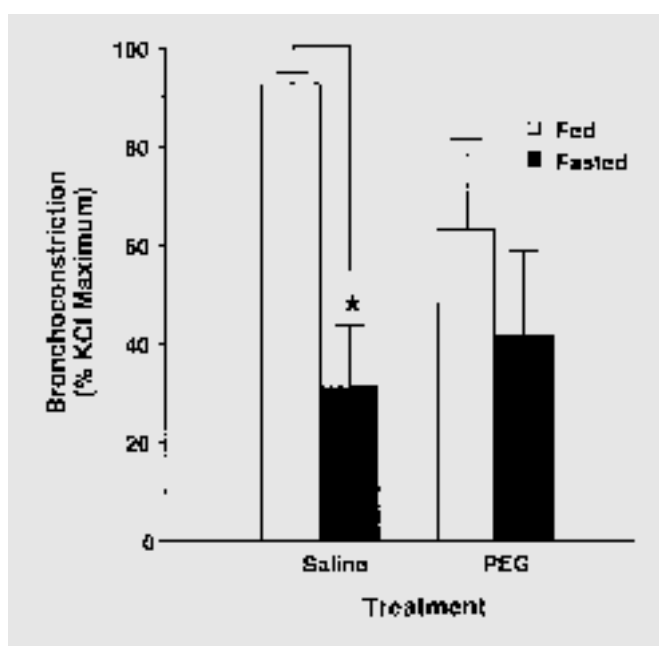


Fig. 2. Effects of different treatments: saline (1 ml/kg) or PEG 400 (1 ml/kg), administered 1 h prior to the antigen challenge, on OA (0.3 mg/kg, i.v.)-induced bronchoconstriction in anesthetized, paralyzed and ventilated guinea pigs. The results are measured as airway insufflation pressure responses to OA and expressed as percentage of KCl-induced maximal bronchoconstriction; n = 5–6 for each treatment group. *p<0.05 ANOVA followed by Fisher's PLSD.

ed a 64% increase in epinephrine and a 35% increase in cortisol in guinea pigs which had been fasted compared to their nonfasted, sensitized litter mates. Plasma norepinephrine concentrations were significantly lower (45% reduced) in fasted (323±60 pg/ml) versus fed (592±140 pg/ml) animals (table 1, p<0.05, Student's unpaired t test, n = 5–6, each treatment).

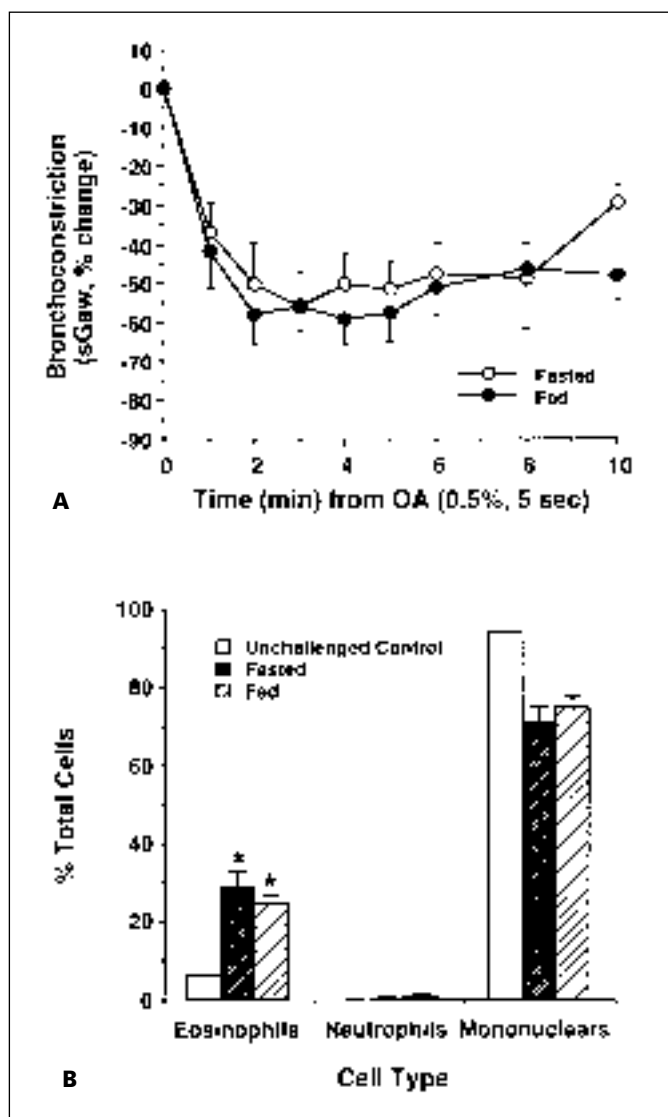


Fig. 3. Effect of fasting for 18 h on inhaled, nebulized OA (0.5% for 5 s)-induced reductions in sGaw (**A**) and airway eosinophil influx, as measured by BAL 24 h after OA challenge (**B**) in conscious guinea pigs. Bronchoconstriction results are expressed as percent decrease in sGaw from baseline measured immediately prior to OA challenge; eosinophils is expressed as percentage of total inflammatory cells recovered in the BAL fluid; $n = 10-11$. *Significantly different from BAL of guinea pigs unchallenged with OA; $p < 0.05$, ANOVA followed by Fisher's PLSD.

In the experiments employing PEG-treated animals, OA-induced bronchoconstrictor responses were not different between fed ($62.8 \pm 18.6\%$ of maximum) and fasted animals ($41.2 \pm 17.4\%$) (fig. 2, $p > 0.05$, Student's unpaired t test, $n = 6$, each treatment). Similarly, the plasma levels of epinephrine (fed = $2,173 \pm 509$ pg/ml; fasted = $2,335 \pm 345$

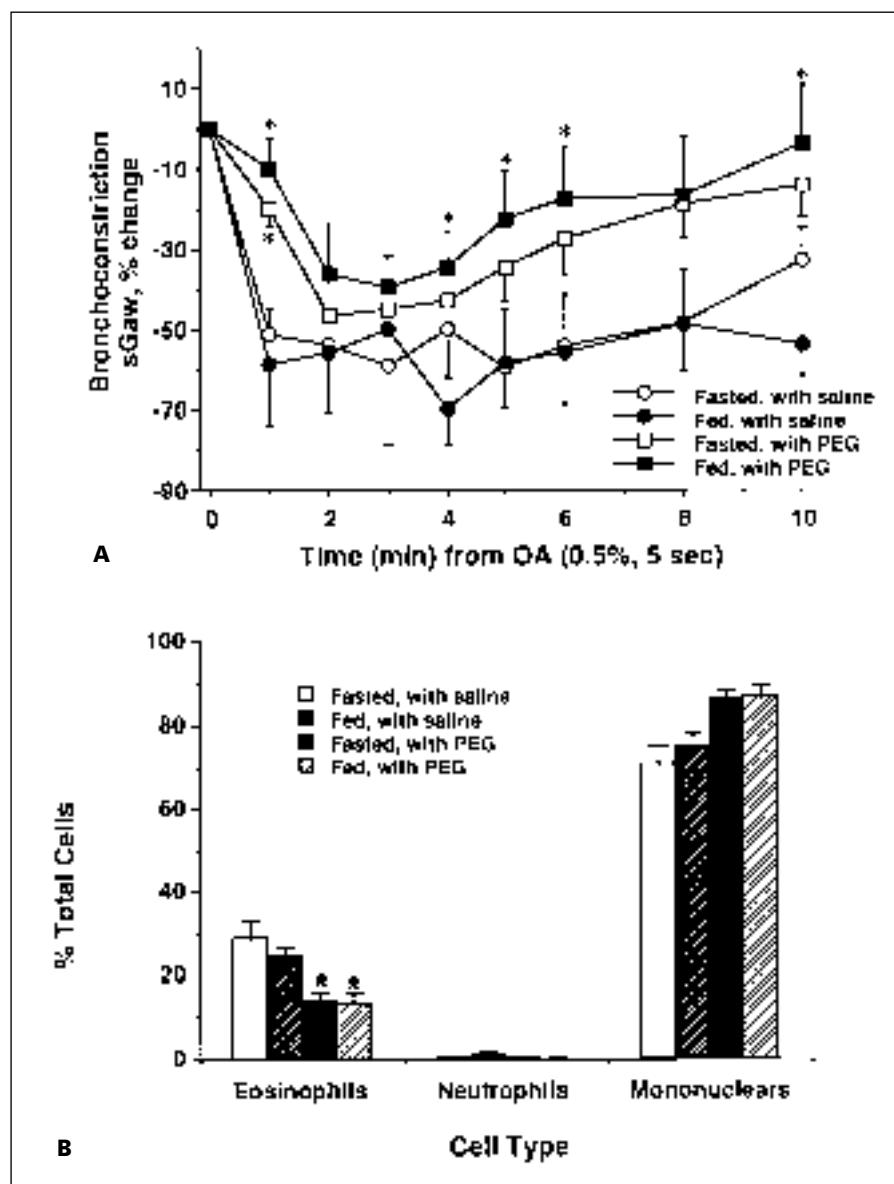
pg/ml and cortisol (fed = 77.0 ± 7.6 μ g/dl; fasted = 85.3 ± 11 μ g/dl) were not significantly different between fasted and fed animals within the PEG treatment regimen (table 1; $p > 0.05$, Student's unpaired t test; $n = 6$). However, plasma norepinephrine concentrations were significantly lower in fasted versus fed, PEG-treated guinea pigs (fasted = 323 ± 60 pg/ml; fed = 592 ± 140 pg/ml; table 1; $p < 0.05$, Student's unpaired t test, $n = 6$). In additional comparisons among the saline and PEG groups, there were higher plasma levels of epinephrine (104% increase) and cortisol (41% increase) in PEG-treated versus saline-treated fed animals (table 1; $p < 0.05$, Student's unpaired t test; $n = 6$). Comparisons of differences in adrenal hormones between fasted, PEG and saline-treated animals were not statistically significant ($p = 0.12$ for epinephrine and $p = 0.21$ for cortisol; ANOVA).

OA-Induced Bronchoconstriction and Eosinophil Influx in Anesthetized Guinea Pigs

In conscious guinea pigs, inhalation of aerosol OA (0.5% for 5 s) substantially reduced airway conductance, measured over the 10 min after exposure (fig. 3). There were no differences overall (as measured by the maximal bronchoconstriction over the 10 min after antigen exposure) or at any single time point of measurement in the bronchoconstrictor response to OA in fasted versus fed animals (fig. 3; $p > 0.05$, Student's unpaired t test, $n = 10-11$, each treatment). When BAL was performed on the same animals 24 h after OA exposure, the percentage of eosinophils recovered from the BAL fluid 24 h after OA exposure ($28.8 \pm 4.1\%$ in fasted animals and $24.3 \pm 2.7\%$ in fed animals) was substantially increased over the eosinophil number recovered from the BAL fluid of guinea pigs which were sensitized, but unchallenged with OA ($6.0 \pm 0.5\%$ of total cells; $p < 0.05$, ANOVA and Fisher's PLSD test, $n = 5$). However, there were no differences between the percentages of eosinophils recovered from the fasted and fed groups (fig. 3; $p > 0.05$; ANOVA and Fisher's PLSD; $n = 10-11$, each treatment).

In another set of animals, OA-induced bronchoconstriction and eosinophil influx was compared between fed and fasted animals which had been pretreated with saline or PEG (1 ml/kg, 1 h prior to OA challenge). There was a significant reduction in the bronchoconstrictor response overall, as well as at selected time points, when comparing PEG-treated animals to saline-treated animals (fig. 4; $p < 0.05$; ANOVA and Fisher's PLSD test; $n = 5-6$). Similarly, in a comparison of feeding versus fasting on OA-induced eosinophilia, there was a significant difference from the other treatment groups only in the PEG-treated animals

Fig. 4. Effect of fasting for 18 h, followed by PEG or saline administration (1 ml/kg 1 h prior to OA challenge), on reductions induced by inhaled, nebulized OA (0.5% for 5 s) in sGaw (**A**) and airway eosinophil influx, as measured by BAL 24 h after OA challenge (**B**) in conscious guinea pigs. Bronchoconstriction results are expressed as percent decrease in sGaw from baseline measured immediately prior to OA challenge; eosinophilia is expressed as percentage of total inflammatory cells recovered in the BAL fluid; $n = 5-6$. * $p < 0.05$, different from corresponding saline treatment group; ANOVA followed by Fisher's PLSD; $n = 5-6$.



(fasted with saline = 29.0 ± 7.4 ; fed with saline = 21.9 ± 4.6 ; fasted with PEG = 13.7 ± 2.0 ; fed with PEG = 13.2 ± 2.6 ; fig. 4; $n = 5-6$, $p < 0.05$, ANOVA and Fisher's PLSD test).

The resting airway tones for the groups, represented by the baseline sGaw [$s^{-1} (cmH_2O)^{-1}$] were not significantly different among the various treatment groups: fasted, no treatment = 0.15 ± 0.03 ; fed, no treatment = 0.17 ± 0.04 ; fasted with saline = 0.16 ± 0.02 ; fed with saline = 0.15 ± 0.02 ; fasted with PEG = 0.12 ± 0.01 ; fed with PEG = 0.10 ± 0.01 (fig. 4, $n = 5-11$; ANOVA and Fisher's PLSD test; $p > 0.05$).

Discussion

The purpose of this study was to evaluate the effect of fasting guinea pigs for 18 h on the bronchoconstrictor and eosinophilic response to OA and on circulating catecholamines and cortisol levels in a variety of oral pretreatment regimens. The following observations were noted: (1) fasted animals had lower bronchoconstrictor responses to intravenous OA than fed animals; (2) corresponding to these reductions in airway responses to OA, fasted animals had significantly higher epinephrine and cortisol levels; (3) norepinephrine levels were generally lower in fasted versus

fed groups; (4) in the PEG treatment group there were substantially higher catecholamine and cortisol levels compared to either controls or the saline-treated animals, although there were no clear differences in either airway responsiveness to antigen or plasma levels of adrenal hormones between fed and fasted animals within the PEG treatment regimen, and (5) in the conscious guinea pig model, there were no clear differences in the airway or eosinophilic response to OA in fed versus fasted animals. However, oral pretreatment with PEG significantly reduced both the OA-induced bronchoconstriction and the resultant airway eosinophilia.

The present results suggest that alterations of feeding schedules as well as the particular vehicle employed may significantly alter the response to antigen in the guinea pig lung. Differences in the responsiveness to OA stimulation between fasting and non-fasting animals were more evident in anesthetized than in conscious animals. The effect of the anesthesia, including possible changes in body temperature, may potentiate the effects of fasting on adrenal hormone release.

The administration of PEG appeared to have some effect on adrenal hormonal levels in the plasma, regardless of the fed state of the animal. PEG as a pure solvent mixes with any aqueous phase with an exothermic reaction. Therefore, administration of 100% PEG may actually be initiating an exothermic reaction within the stomach of the animal. Although PEG is still employed in this laboratory as an excellent drug solvent, after initial solubilization of a drug, we now add a minimum of 5% aqueous phase to release the 'heat of solution' before administering it to an animal. In addition, PEG is a preservative. Because catecholamines are broken down by oxidative processes, there is the potential that PEG may reduce the catabolism of circulating catecholamines, thereby increasing their concentration in the plasma.

Several laboratories, including our own, have demonstrated an enhanced airway responsiveness to exogenous spasmogens or antigen after treatment with β -adrenoceptor antagonists [15, 17, 19, 27, 28]. It has been suggested that this increase in responsiveness is due to a decrease in the adrenergic tone generated by circulating catecholamines acting at β_2 -adrenoceptors [29, 30]. Other studies have shown an increase in airway reactivity to selected spasmogens [31] or antigen [17, 19] in adrenalectomized animals. This enhanced reactivity correlates with a reduced level of circulating catecholamines [17, 19].

Although increased plasma epinephrine concentrations are associated with fasting or PEG treatment in the present study, one must also consider the potential impact of the

changes in norepinephrine and cortisol, both of which may have a regulatory role on the antigen-induced response. The inhibitory effect of cAMP, the second messenger involved in β -adrenoceptor activation on the suppression of mast cell degranulation, is well documented [32, 33]. In addition, Weinrich and Udem [34], demonstrated an increased synaptic activity as a result of antigenic stimulation of sympathetic ganglia in the guinea pig, suggesting that immunologic activation of mast cells can directly potentiate neurotransmission of sympathetic ganglia.

Stress is an important risk factor in asthma. It has been widely accepted that stress may predispose an individual to an asthmatic attack. However, it has been recently reported that acute stress may have a brief protective effect on responses to antigen, and chronic stress may be a more important risk factor [26, 27]. The present studies may parallel the effects of acute stress in human reactions to antigen. Stress in laboratory animals results in stimulation of the pituitary-adrenal axis leading to altered circulating levels of catecholamines and cortisol [20–25].

Based on the present findings, we speculate that the reduced responsiveness to antigen in fasted versus fed animals may result from food-restriction-induced, stress-related release of epinephrine and cortisol from the adrenal glands which act to stabilize mast cells from antigen-induced degranulation or reduce smooth muscle responsiveness to inflammatory cell products. In addition, the results suggest that oral dosing with 100% PEG has the potential to further enhance this phenomenon. These findings, coupled with previous studies in our laboratory employing adrenalectomy and β -adrenoceptor antagonists, provide further evidence of a didactic interplay between circulating adrenal hormones and reactivity to antigen in the guinea pig.

In comparing fed and fasted animals pretreated orally with saline, the results in airway responsiveness to OA as well as plasma levels of circulating adrenal hormones, mirrored the results in the previous experiment in which the animals had received no oral pretreatment before anesthesia and surgery prior to intravenous OA challenge.

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