Airway Reactivity in Ponies with Chronic Airway Disease

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Introduction

Bronchial hyperreactivity to pharmacologic and physical stimuli is a characteristic of human asthmatics.[1-4] Bronchial hyperreactivity can also occur in individuals with hay fever, chronic bronchitis or emphysema and can be induced by exposure of normal human or animal lungs to infectious or toxic agents.[5-8] In these conditions bronchial hyperreactivity is frequently of short duration. In contrast, the hyperreactivity of asthmatics is persistent.

Bronchial hyperreactivity to intravenous administration of histamine was first described in horses with chronic airway disease by *Obel* and *Schmiterlöw* in 1948[9] who primarily investigated horses with signs of respiratory distress. We questioned whether bronchial hyperreactivity was persistent in horses with airway disease or if hyperreactivity waned during periods of clinical remission. To answer these questions we studied a group of ponies with a history of recurrent bouts of COPD precipitated by barn exposure (principal group) and an age- and gender-matched group of ponies with no history of COPD (control group). In this paper we review some of our recent observations from these two groups of animals. Some of the studies have been published previously.[10, 11]

Selection of Animals for Investigation

Ponies with recurrent signs of airway obstruction were obtained through requests for donor animals to practicing veterinarians. We accepted all ponies with chronic or recurrent dyspnea and kept them at pasture for up to 3 months, during which time we expected clinical remission. Ponies failing to go into clinical remission were not used for the study. The remaining ponies were then exposed to a barn environment where stalls were bedded with straw and animals were fed poor quality, and if possible, moldy hay. Animals utilized in subsequent studies developed clinical signs of airway obstruction, increased pulmonary resistance (R_L), decreased dynamic compliance (Cdyn) and hypoxemia in this environment. They were then returned to pasture and again went into clinical remission. Using these criteria, we have a population of animals (principals) which are clinically normal while kept on pasture and develop airway obstruction when housed in a barn environment. We have also acquired a group of normal ponies (controls) which do not develop airway obstruction when exposed to a barn environment. These ponies have been age- and gender-matched with the principal group. To maintain constant environmental stimuli, each pair of ponies (principal and control) is housed and transported together, and studied on the same day. All animals are kept on pasture except during the barn exposure required to produce airway obstruction. During winter, animals have access to gravel-floored sheds in which they are fed a complete pelleted diet, thus preventing exposure to hay dust which can provoke airway obstruction.

The carotid artery is relocated to a subcutaneous site in each pony to facilitate obtaining arterial blood samples. A chronic tracheostoma is also prepared to facilitate intubation for the measurement of lung function. The pony populations are isolated from other horses, being particularly careful to avoid exposure to animals recently acquired from sale barns as these animals will often develop acute infectious respiratory diseases.

Our protocol for the study of airway reactivity is described below. Pairs of ponies (principal and control) are kept at pasture for at least two months prior to the first measurement of lung function and reactivity (designated period A). Ponies are then housed together in a stall, bedded on straw and fed poor quality, dusty, and if possible, moldy hay, until the principal pony develops signs of airway obstruction. Measurements of lung function and reactivity are then repeated (period B). The animals are returned to pasture and measurements are repeated at weekly intervals (periods C, D). Lung function and airway reactivity return to base-line values within two weeks after returning animals to pasture.

Technique to Measure Airway Reactivity

Reactivity to aerosol histamine and methacholine is measured by the following protocol. Arterial blood gas tensions, tidal volume (V_T), respiratory frequency (f), minute ventilation ($\dot{V}_E$), dynamic compliance (Cdyn), and pulmonary resistance (R_L) are measured prior to aerosol exposure. Pleural pressure is measured through an esophageal balloon and flow rates are measured by a Fleisch # 4 pneumotachograph which is attached to a short endotracheal tube inserted through the tracheostomy. Tidal volume, f, $\dot{V}_E$, Cdyn, and R_L are calculated by a pulmonary function computer. Computer-generated values are regularly monitored against calculations from a physiograph recording.

After considerable trial and error, the following system was developed to determine dose responses of the respiratory system to aerosol agonists. Ponies are force-ventilated with ten 3-liter breaths (slightly more than a tidal volume), thus providing a standard volume history, gaining control of ventilation and eliminating sighing subsequent to histamine delivery. The pony is then given six 2-liter breaths containing aerosol. The nebulizer is disconnected from the endotracheal tube and the pneumotachograph is attached to measure lung function.

Transpulmonary pressure, tidal volume and flow rates are recorded for 3 minutes after each aerosol challenge. Because breathing is irregular during the first minute, only data obtained from the second and third minute recordings are utilized. Dynamic compliance and R_L are averaged over this time period. Exactly 3 minutes after the cessation of

the first challenge, another aerosol challenge has begun. The sequence of challenges is air, saline, and histamine diphosphate or methacholine in saline at increasing concentrations. Aerosol challenge is stopped when dynamic compliance decreases below 50 per cent of the value obtained following saline challenge.

Dose response curves of Cdyn, R_L and frequency (f) are plotted as a function of drug dose. By interpolation between points on the dose response curves, the doses of drug required to decrease Cdyn to 65 per cent of the value obtained after saline challenge, and when possible, to double R_L are calculated. These doses are called ED_{65}Cdyn and $ED_{200}R_L$ respectively.[12] Pulmonary resistance in principal and control ponies at a fixed concentration of agonist, e.g. 0.1 mg/ml methacholine is also determined. The latter measurement is less noisy than ED_{65}Cdyn.

Lung Function

Table 1 depicts lung function data from principal and control ponies at the four measurement periods. These data were obtained during our studies of responses to aerosol histamine. At period A there was no significant difference between principal and control ponies in blood gases, f, $\dot{V}_E$, R_L, Cdyn, or V_T normalized for body weight. In other series of experiments, a slightly greater R_L was observed in principal ponies than in the control ponies. Principal ponies developed clinical signs of heaves in 2–18 days (mean 8.6 days) following exposure to the barn environment. Barn exposure (period B) caused no change in lung function in the control group, however barn exposure decreased PaO_2, Cdyn, and V_T and increased R_L and f in the principal group. During the weeks following barn exposure (periods C and D), lung function of principals returned to values observed prior to barn exposure (period A). These data clearly demonstrate that airway obstruction is provoked by exposure to barn dust and its reversibility when ponies are removed from the barn.

Airway Reactivity

Response to Aerosol Histamine

Twelve of the 14 ponies always responded to histamine by decreasing Cdyn. In many ponies, R_L had not doubled when Cdyn was half the base-line value. Therefore, $ED_{200}R_L$ was unable to be calculated in these ponies. An attempt was made to calculate $ED_{150}R_L$ (the dose of histamine to increase resistance by 50 per cent), but the increase in R_L during histamine was less than 50 per cent in principals at period B.[11]

Figure 1 shows mean values of ED_{65}Cdyn at the four measurement periods. At period A, ED_{65}Cdyn did not differ between groups, averaging 0.7-log doses less in the principal ponies than in the control ponies.

At period B, ED_{65}Cdyn decreased significantly in the principal group by 2.5-log doses but was unchanged in the control group. At periods B and C, ED_{65}Cdyn of both the principal and control groups did not differ.

Response to Aerosol Methacholine

Aerosol administration of methacholine consistently decreased Cdyn and in the majority of ponies also increased R_L.[13] However, as was the case during aerosol histamine administration, $ED_{200}R_L$ could not be used as a measure of reactivity because R_L did not consistently double when Cdyn decreased by 35 per cent. The change in R_L and per cent change in Cdyn induced by 0.1 mg/ml methacholine

Table 1: Pulmonary function data of control and principal ponies.

	Measurement Period			
	A	B	C	D
	Control Ponies			
PaO_2, Torr	92 ± 6	89 ± 6	82 ± 5	84 ± 6
$PaCO_2$, Torr	35 ± 2	38 ± 1.3	39 ± 2	40 ± 1
Cdyn, 1 · cmH_2O^{-1}	0.80 ± 0.14	0.80 ± 0.19	0.77 ± 0.18	0.77 ± 0.15
R_L, $cmH_2O \cdot 1^{-1} \cdot s^{-1}$	0.96 ± 0.18	0.89 ± 0.20	0.88 ± 0.14	0.83 ± 0.13
V_T/BW, ml · kg^{-1}	9.8 ± 1.3	8.0 ± 0.5	9.1 ± 0.6	9.8 ± 0.8
$\dot{V}_E$/BW, ml · min^{-1} · kg^{-1}	166 ± 23	168 ± 26	192 ± 31	129 ± 7
f, min^{-1}	20.5 ± 5.6	20.7 ± 2.5	21.5 ± 3.3	14.7 ± 1.2
	Principal Ponies			
PaO_2, Torr	87 ± 4	68* ± 5	81 ± 4	84 ± 4
$PaCO_2$, Torr	40 ± 1	41 ± 1	41 ± 1	41 ± 1
Cdyn, 1 · cmH_2O^{-1}	0.78 ± 0.12	0.28*† ± 0.06	0.57 ± 0.10	0.67 ± 0.13
R_L, $cmH_2O \cdot 1^{-1} \cdot s^{-1}$	1.20 ± 0.22	3.79*† ± 0.85	2.08 ± 0.47	1.31 ± 0.40
V_T/BW, ml · kg^{-1}	12.7 ± 1.1	9.0* ± 0.6	10.3 ± 0.3	10.7 ± 0.9
$\dot{V}_E$/BW, ml · min^{-1} · kg^{-1}	134 ± 15	176 ± 18	156 ± 15	146 ± 17
f, min^{-1}	10.4 ± 0.8	19.6* ± 1.9	15.3 ± 1.6	15.0 ± 3.2

Values are means ± SE. A, after two months on pasture; B, after barn housing; C, after one week on pasture; D, after 2 weeks on pasture. Cdyn, dynamic compliance; BW, body weight; R_L, pulmonary resistance; V_T, tidal volume; $\dot{V}_E$, minute ventilation; f, respiratory frequency. *, significantly different from A. †, significantly different from control group. Modified from reference 11.

($\Delta R_L 0.1$ and Δ % Cdyn 0.1 respectively) was calculated. These measures of reactivity and ED_{65}Cdyn are tabulated in Table 2. Reactivity of principal and control groups of ponies did not differ at period A. At period B principal ponies were hyperreactive to the aerosol administration of methacholine. Reactivity waned at periods C and D.

Responses to Citric Acid Aerosol

The protocol for delivery of citric acid is described below. The ultrasonic nebulizer containing saline was attached to the endotracheal tube through a non-rebreathing valve and the pony was allowed to spontaneously breathe saline aerosol for 10 minutes. The pneumotachograph was reattached and data were recorded every two minutes for 10 minutes. The delivery of an aerosol of 10% citric acid was then given for 10 minutes and data were recorded every two minutes for 10 minutes and at 5-minute intervals from 10 to 30 minutes. There was no effect on Cdyn in either group following the inhalation of either saline or citric acid. Saline and citric acid did not cause a change in R_L of control ponies at any measurement period. In the principal group, the R_L response to citric acid was highly variable in both magnitude and time of occurrence. The change in R_L in response to citric acid was greatest at period B and declined significantly at periods C and D.[13]

Response to Intravenous Histamine

Histamine dose-response curves were generated in the following manner. Saline or histamine solutions were each infused intravenously for 3 minutes. Transpulmonary pressure, V_T and flow rates were recorded during the last 2 minutes of each infusion period. The Cdyn, R_L and f calculated were the average of at least 10 breaths. The sequence of challenge exposure was saline solution (3.82 ml/min) followed by histamine (500 μg of histamine diphosphate/ml) at an infusion rate of 0.0764 ml/min. Subsequently, histamine infusion rates were approximately doubled until Cdyn decreased by more than 50 per cent of the value obtained after saline challenge.[10]

There was no significant difference between the principal and control groups of ponies in the airway response to IV histamine administration at period A. Barn exposure (period B) or pasture housing (periods C and D) did not change the log ED_{65}Cdyn in control ponies. However, barn exposure decreased the log ED_{65}Cdyn in principal ponies. One and 2 weeks after return to pasture (periods C and D), the log ED_{65}Cdyn of principal ponies returned to the value observed before barn exposure.

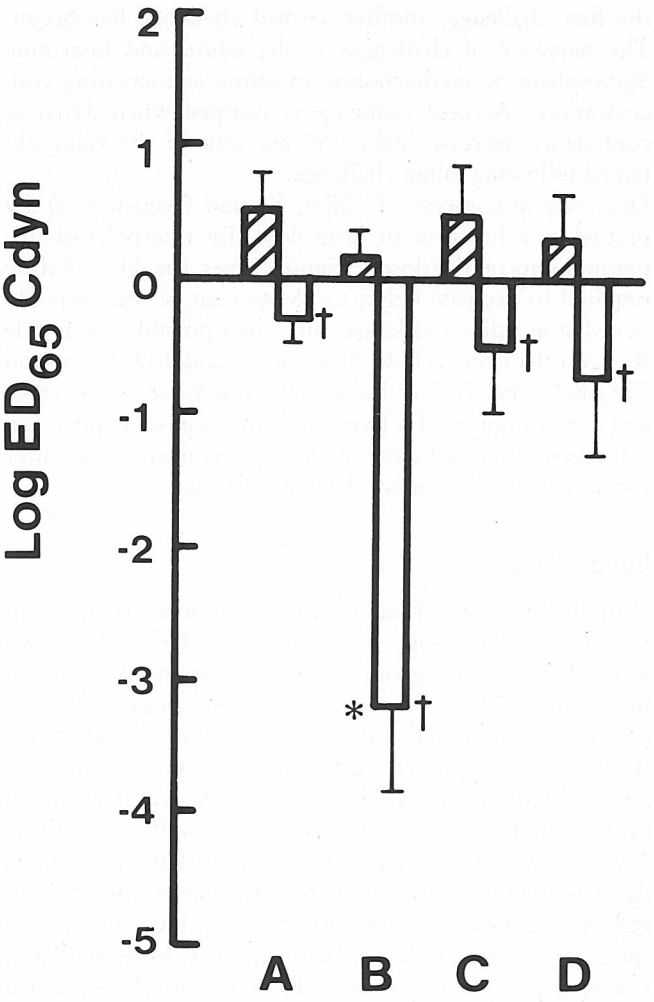

Figure 1. Histamine dose required to reduce dynamic compliance to 65 % of base line (ED_{65}Cdyn) in principal (unshaded) and control (shaded) ponies at periods A, B, C and D. Histamine dose is expressed as log histamine base. † Significant difference between principal and control ponies. * Significant difference from period A. Reproduced with permission from the Journal of Applied Physiology.

		Measurement Period			
		A	B	C	D
Log ED_{65}Cdyn	Control	−.03 ± .2	.49 ± .13	.46 ± 19	.67 ± .17
	Principal	.15 ± .06	−1.30 ± .32*†	−.07 ± .37	.62 ± .09
$\Delta R_L 0.1$	Control	.03 ± .05	.02 ± .05	−.06 ± .05	.02 ± .09
	Principal	.19 ± .22	1.63 ± .61*†	.59 ± .25	−.05 ± .11
Δ%Cdyn0.1	Control	−8.5 ± 5.7	8.1 ± 8.6	1.6 ± 8.6	−7.2 ± 7.8
	Principal	−11.7 ± 4.5	−43.9 ± 9.2	−16.1 ± 6.8	17.5 ± 14.4

Table 2: Reactivity of principal and control pony airways to aerosol methacholine. Log ED_{65}Cdyn = logarithm of the dose of methacholine which decreases dynamic compliance to 65% of base-line value, Δ RL 0.1 = change in pulmonary resistance induced by 0.1 mg/ml methacholine, Δ%Cdyn0.1 = per cent change in dynamic compliance induced by 0.1 mg/ml methacholine. * = significantly different from A. † = significantly different from control group.

Discussion

These investigations of airway reactivity to aerosols of histamine, methacholine and citric acid and to intravenous administration of histamine clearly demonstrate that ponies with a history of recurrent airway obstruction (the principal group) do not have hyperreactive airways when in clinical remission. While being maintained on pasture (period A), reactivity of principal and control groups of ponies is identical. Airway hyperreactivity develops in principal ponies when housed in a barn and when clinical and physiological signs of obstructive lung disease develop. When animals are returned to pasture (periods C and D), airway reactivity wanes.

In our studies, the $ED_{65}Cdyn$ is a consistent measure of airway reactivity. It is equivalent to $PC_{35}Cdyn$ reported by *Klein.*[14] The location of $ED_{65}Cdyn$ is determined by connecting points on the dose response curves. *Klein* fits a linear regression to the dose response curve to obtain $PC_{35}Cdyn$. $ED_{65}Cdyn$ is a useful index of reactivity because a 35 per cent decrease in Cdyn exceeds the daily variability in Cdyn. In addition an $ED_{65}Cdyn$ dose of histamine and methacholine do not alter respiratory frequency. An increase in respiratory frequency would seriously compromise the interpretation of any changes in dynamic compliance.

Consistent use of $ED_{200}R_L$ as a measure of reactivity has been impossible. Decreases in Cdyn are not consistently accompanied by increases in R_L in response to histamine or methacholine. Presumably this reflects a predominant narrowing of peripheral airways in response to these agonists. The lack of increase in R_L occurred more frequently in response to histamine than in response to methacholine suggesting that in the horse, as in other species, histamine receptors may predominate in peripheral airways whereas muscarinic receptors are distributed throughout the tracheobronchial tree.[15,16]

In the study of reactivity to methacholine, the changes in R_L and Cdyn in response to 0.1 mg/ml methacholine were calculated in all ponies. A similar analysis of the response to aerosol histamine was not possible because of the extreme hyperreactivity of some ponies to histamine. The change in R_L in response to 0.1 mg/ml methacholine appears to be the best measure of airway reactivity in ponies. Normal ponies and ponies in clinical remission from airway obstructive disease did not increase R_L in response to this methacholine dose but hyperreactive principal ponies increased R_L significantly (Table 2).

In all studies in which airway hyperreactivity occurs concomitantly with airway obstruction, decreased base-line airway caliber must be considered as a cause of hyperreactivity. This is especially true in our principal ponies in which hyperreactivity and airway obstruction both occur at period B. We have found only weak correlations between measurements of airway reactivity and either R_L or Cdyn. Furthermore, examination of data from individual principal and control ponies (Figure 2) shows that changes in $ED_{65}Cdyn$ were not always associated with changes in R_L or Cdyn, nor were changes in R_L and Cdyn always associated with changes in $ED_{65}Cdyn$. The R_L of principal ponies at period A was greater than R_L of controls in the me-

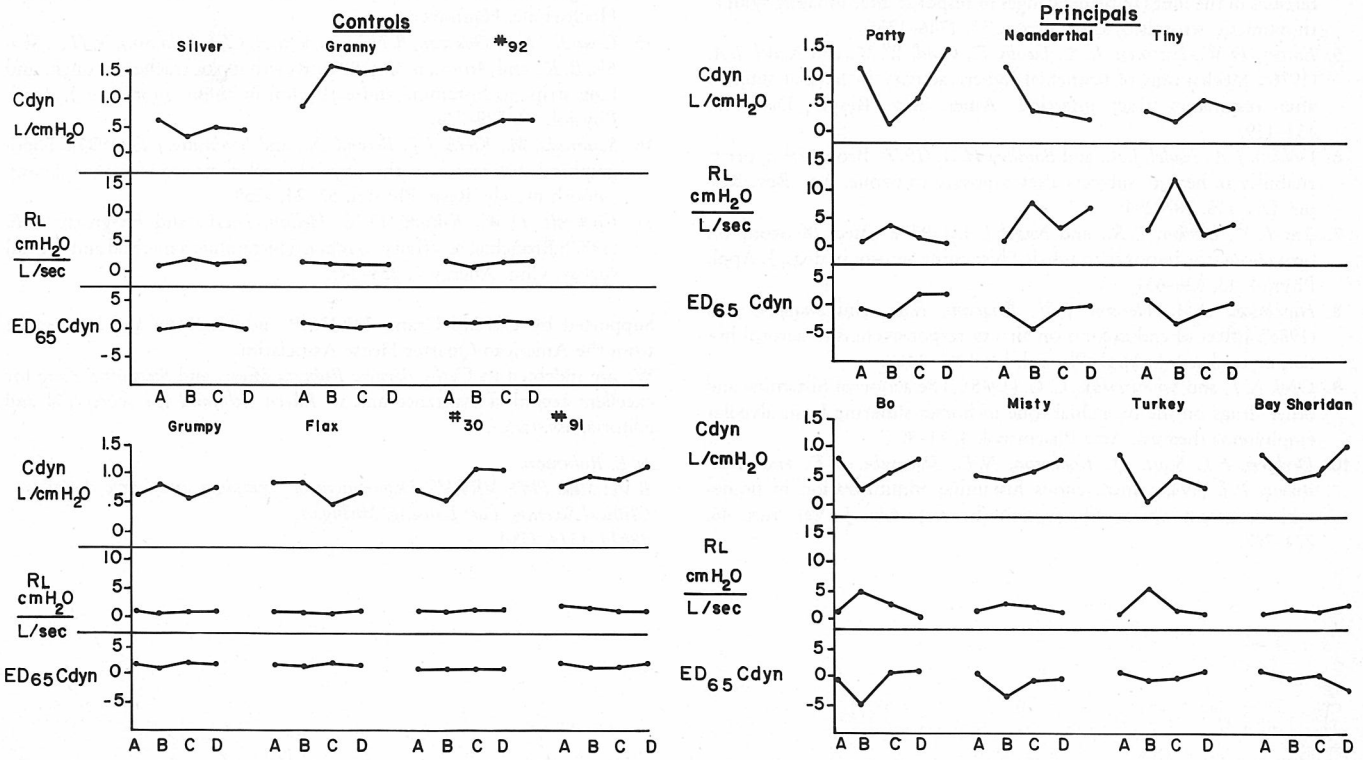

Figure 2. Dynamic compliance (Cdyn) and pulmonary resistance (R_L) measured after aerosol saline exposure and dose of histamine required to reduce Cdyn to 65 % of base-line value ($ED_{65}Cdyn$) in each pony at each measurement period. Reproduced with permission from the Journal of Applied Physiology.

thacholine study, but measurements of reactivity did not differ. For these reasons, it is believed that the base-line airway caliber is not the principal determinant of airway reactivity in ponies with airway disease.

The response to citric acid is consistent with our observations of hyperreactivity to aerosol methacholine and intravenous and aerosol histamine occurring only during periods of acute airway obstruction. Curiously, unlike the response to methacholine, which usually involved both an increase in R_L and decrease in Cdyn, the response to citric acid was characterized by a change in R_L unaccompanied by a decrease in Cdyn, suggesting the response was occurring primarily in central airways. At present it is unknown if this primarily central airway response is a result of the type of aerosol delivery or a local mechanism of response. However, this increase in R_L suggests hyperreactivity occurs in central airways even though lesions of chronic airway disease are usually described in peripheral airways.

Klein[14] classified airway reactivity of horses using a scale established by *Cockroft,* et *al.*[17] for human lungs. Normal reactivity is classified by *Klein* as a PC_{35}Cdyn (ED_{65}Cdyn) greater than 8 mg/ml histamine dihydrochloride (4.8 mg/ml histamine base, log PC_{35}Cdyn = .68). Normal ponies in our study had a log ED_{65}Cdyn between 0 and 1 and several would have been classified by *Klein* as marginally hyperreactive. Several of the principal ponies would have been classified as moderately hyperreactive even during clinical remission. However, a statistically significant difference between groups at period A could not be shown. When our principal ponies had acute exacerbations of airway obstruction (period B), the majority would be classified by *Klein* as severely hyperreactive (log ED_{65}Cdyn less than −1.12). Despite these differences in classification of reactivity, overall observations agree with those of *Klein* that airway hyperreactivity is most severe in ponies or horses with clinical signs of airway obstruction.

References

1. *Curry, J.J.* (1947): Comparative action of acetyl-beta-methylcholine and histamine on the respiratory tract in normal patients with hay fever, and subjects with bronchial asthma. J. Clin. Invest. 26, 430–438.
2. *Orehek, J., Gayrard, P., Smith, A.P., Grimaud, C.*, and *Charpin, J.* (1977): Airway response to carbachol in normal and asthmatic subjects. Am. Rev. Respir. Dis. 115, 937–943.
3. *Wells, R.E., Walker, J.E.C.*, and *Hickler, R.B.* (1960): Effects of cold air on respiratory air flow resistance in patients with respiratory tract disease. N. Engl. J. Med. 263, 268–273.
4. *Dubois, A.B.*, and *Dautrebande, L.* (1958): Acute effects of breathing inert dust particles and of carbachol aerosol on the mechanical characteristics of the lungs in man: changes in response after inhaling sympathomimetic aerosols. J. Clin. Invest. 37, 1746–1755.
5. *Empey, D.W., Laitinen, L.A., Jacobs, L., Gold, W.M.*, and *Nadel, J.A.* (1976): Mechanisms of bronchial hyperreactivity in normal subjects after respiratory tract infection. Amer. Rev. Respir. Dis. 113, 131–139.
6. *Golden, J.A., Nadel, J.A.*, and *Boushey, H.A. (1978):* Bronchial hyperirritability in healthy subjects after exposure to ozone. Am. Rev. Respir. Dis. 118, 287–294.
7. *Lee, L.Y., Bleeker, E.R.*, and *Nadel, J.A.* (1977): Effect of ozone on bronchomotor response to inhaled histamine aerosol in dogs. J. Appl. Physiol. 43, 626–631.
8. *Hutchison, A.A., Henson, J.M., Brigham, K.L.*, and *Snapper, J.R.* (1983): Effect of endotoxinn on airway responsiveness to aerosol histamine in sheep. J. Appl. Physiol. 54, 1463–1468.
9. *Obel, N.J.*, and *Schmiterlöw, C.G.* (1948): The action of histamine and other drugs on the bronchial tone in horses suffering from alveolar emphysema (heaves). Acta Pharmacol. 4, 71–80.
10. *Derksen, F.J., Scott, D., Robinson, N.E., Slocombe, R.F.*, and *Armstrong, P.J.* (1985): Intravenous histamine administration in ponies with recurrent airway obstruction (heaves). Am. J. Vet. Res. 46, 774–777.
11. *Derksen, F.J., Robinson, N.E., Armstrong, P.J., Stick, J.A.*, and *Slocombe, R.F.* (1985): Airway reactivity in ponies with recurrent airway obstruction (heaves). J. Appl. Physiol. 58, 598–604.
12. *Snapper, J.R., Drazen, J.M., Loring, S.H., Schneiden, W.*, and *Ingram, R.H.* (1978): Distribution of pulmonary responsiveness to aerosol histamine in dogs. J. Appl. Physiol. 44, 738–742.
13. *Armstrong P.J., Derksen, F.J., Slocombe, R.F.*, and *Robinson, N.E.* (1985): Airway responses to aerosol methacholine and citric acid in ponies with recurrent airway obstruction (heaves). Submitted to Am. Rev. Respir. Dis.
14. *Klein, H.J.* (1984): Der Histamininhalationsprovokationstest zur Bestimmung der unspezifischen Reagibilität der Atemwege beim Pferd. Inauguraldissertation, Doctor Medicinae Veterinariae. Tierärztliche Hochschule, Hannover.
15. *Chand, N., Dhawan, B.N., Sriemal, R.C., Rahmani, N.H., Shukla, R.K.*, and *Altura, B.M.* (1980): Reactivity of trachea, bronchi and lung strips to histamine and carbachol in rhesus monkeys. J. Appl. Physiol. 49, 729–734.
16. *Souhrada, M., Klein, J.J., Berend, N.*, and *Souhrada, J.F.* (1983): Topographical differences in the physiological response of canine airway smooth muscle. Resp. Physiol. 52, 245–258.
17. *Cockroft, D.W., Killian, D.N., Mellon, J.J.A.*, and *Hargreave, F.E.* (1977): Bronchial reactivity to inhaled histamine: a method and clinical survey. Clin. Allergy 7, 235–243.

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