Comparative responses of bronchial rings to mediators of airway hyperreactivity in healthy horses and those affected with summer pasture-associated obstructive pulmonary disease

Changaram S. Venugopal, BVSc, PhD; Rustin M. Moore, DVM, PhD; Earnestine P. Holmes, BS; Catherine E. Koch, MS; Thomas L. Seahorn, DVM, MS; Ralph E. Beadle, DVM, PhD

Objective—To compare responses of bronchial rings obtained from healthy horses and horses affected with summer pasture-associated obstructive pulmonary disease (SPAOPD) to selected mediators of airway hyperreactivity in vitro.

Sample Population—Bronchial rings from 6 healthy horses and 6 horses affected with SPAOPD.

Procedure—Bronchial rings obtained from each group of horses were mounted in organ baths and attached to force transducers interfaced with a polygraph. After applying 2g of tension, each ring was allowed to equilibrate for 45 minutes in Tyrode's solution at 37 C. Cumulative concentration-response relationships to graded concentrations of selected mediators (10^{-8} to 10^{-4} *M*) were determined and analyzed for significance at each concentration.

Results—Acetylcholine, histamine, 5-hydroxytryptamine, and leukotriene D_4 induced concentration-dependent contractile responses in bronchial rings. Prostaglandin $F_{2\alpha}$ induced weak and inconsistent contractile responses. The other 2 agents, norepinephrine and substance P, did not induce concentration-dependent responses. Considering the overall group-drug effect, acetylcholine, histamine, 5-hydroxytryptamine, and leukotriene D_4 were effective in inducing consistent concentration-dependent contractile responses in both groups. Only 5-hydroxytryptamine and histamine induced significant responses in contractility between groups. The response of bronchial rings from horses with SPAOPD to 5-hydroxytryptamine was significantly greater than those from control horses, whereas the response to histamine was significantly lower. Significant responses were evident at concentrations ranging from 10^{-6} to 10^{-4}*M* for both drugs.

Conclusions and Clinical Relevance—Because the airways of horses with SPAOPD had increased responsiveness to 5-hydroxytryptamine in vitro, treatment modalities using 5-hydroxytryptamine antagonists should be investigated to address this phenomenon. (*Am J Vet Res* 2001;62:259–263).

Summer pasture-associated obstructive pulmonary disease (SPAOPD), a common respiratory disease of horses in the southern region of the United States, is similar to **chronic obstructive pulmonary disease (COPD)**, a common disease in more temperate climates.[1] The etiopathogenesis of SPAOPD is not clearly understood; however, the disease is characterized by airway inflammation and obstruction, which results in mild-to-severe respiratory distress. During periods of airway obstruction, horses develop airway hyperreactivity to inflammatory mediators.[2] In humans with asthma, which is a form of obstructive pulmonary disease, several potent airway inflammatory mediators are released into airway secretions and around the bronchial smooth muscle subsequent to degranulation of mast cells caused by allergic reactions.[2] These mediators are responsible for contraction of smooth muscle in the airways, increased vascular permeability, increased mucus secretion, and damage to airway epithelium.[3] A positive correlation exists between the intensity of airway hyperreactivity and the quantity of chemical mediators available.[2] Commonly accepted airway inflammatory mediators are **histamine (HST)**, bradykinin, **prostaglandin $F_{2\alpha}$ (PGF)**, **leukotriene D_4 (LTD)**, and platelet aggregating factor.[4,5] It has been reported that structural alterations in the smooth muscle of airways may be a possible cause of hyperreactivity in horses with recurrent airway obstruction.[6] Presently, to the authors' knowledge, there is no information regarding airway hyperresponsiveness of SPAOPD-affected horses.

On the basis of this information, we hypothesized that the responsiveness of bronchial rings from healthy and SPAOPD-affected horses to inflammatory mediators would be different in vitro. Therefore, the objectives of the study reported here were to determine and compare responses of bronchial rings obtained from healthy and SPAOPD-affected horses to graded concentrations of **substance P (SP)**, PGF, LTD, HST, **5-hydroxytryptamine (5-HT)**, **acetylcholine (ACh)**, and **norepinephrine (NE)** in vitro.

Materials and Methods

The study was approved by the Louisiana State University Institutional Animal Care and Use Committee.

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From the Equine Health Studies Program, Departments of Veterinary Physiology, Pharmacology, and Toxicology (Venugopal, Moore, Holmes, Koch), and Veterinary Clinical Sciences (Moore, Seahorn, Beadle), School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803. Dr. Seahorn's present address is Hagyard-Davidson-McGee PSC, 4250 Ironworks Pike, Lexington, KY 40511.

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Two groups of horses, 6 healthy (control) and 6 affected with SPAOPD, were used. Control horses had no evidence of abnormalities of the cardiopulmonary system.

Grouping of horses—All horses were acquired by donation and were destined to be euthanatized. The SPAOPD-affected horses had histories of recurring signs of obstructive pulmonary disease that developed after exposure to pasture during the summer months. None of these horses received medications within 7 days prior to assessment. During assessment, these horses were assigned a **clinical score (CS)** for SPAOPD. The CS was determined by the following algorithm[7,8]:

$$CS = \left[\frac{(\text{medial nostril flare} + \text{lateral nostril flare})}{2}\right] + \text{abdominal lift}$$

Each of the variables in the formula was scored from 0 to 4. A score of zero indicated the nostril had little movement, and the ventral flank had little or no movement. A score of 4 indicated the nostril remained maximally flared throughout the respiratory cycle, and abdominal lift resulted in a heave line extending cranially to the 5th intercostal space. Thus, the maximum CS was 8. To be included in the SPAOPD-affected group, in addition to a history of recurrent obstructive pulmonary disease following exposure to summer pasture, horses had to have a CS of 4 or greater, a change in **pleural pressure (Ppl)** > 15 cm H_2O, abnormal lung sounds on auscultation of the thorax, and no evidence of bacterial infection on the basis of results of CBC and fibrinogen concentration, cytologic analysis, and bacteriologic culture of **bronchoalveolar lavage (BAL)** fluid.[9]

Intrapleural pressure was measured indirectly by use of an esophageal balloon[a] secured over the end of a catheter connected to a pressure transducer[b] interfaced with a physiograph.[c] A 10-cm long 3.5-cm circumference balloon was placed over the end of a 2-cm long 2-mm internal diameter cannula. The balloon was inserted through a lubricated nasogastric tube that was passed into the rostral portion of the esophagus. Once the esophageal balloon was located between the heart and the diaphragm, the nasogastric tube was removed. The balloon was inflated with 1.5 ml of air, and 3 measurements were recorded. Changes in esophageal pressure (peak inspiratory minus peak expiratory pressures) during tidal breathing measured with this system reflected changes in Ppl.[9,10] Healthy horses had clinical scores < 2.0, changes in Ppl < 10.0 cm H_2O, normal bronchiovesicular sounds on auscultation of the thorax during a period of rebreathing of CO_2, and no abnormalities in the BAL fluid.

Tissue collection—Bronchial tissues were obtained immediately after euthanasia, which was performed by administration of an overdose of pentobarbital sodium[d] (100 mg/kg of body weight, IV). Bronchial rings were prepared according to previously described methods for preparation of blood vessels.[11,12] Bronchial rings (4 mm wide) obtained from airways (4th to 7th generation bronchi) of the right lung lobe were prepared from all horses. Bronchial rings were placed in 10 ml organ baths; 1 side of the ring was fixed to the floor of the bath, and the other end was attached to a force transducer[e] interfaced with a polygraph.[c] The bath was filled with Tyrode's solution maintained at 37 C by a circulating water bath and was continuously oxygenated with a gas mixture of 95% O_2 and 5% CO_2. An initial tension of 2 grams was applied to the rings to mimic the airway tone observed under conditions in vivo. The rings were allowed to equilibrate for 45 minutes, and the solution was gently replaced with fresh warm Tyrode's solution at 15-minute intervals. After each solution change, tension was reapplied to maintain 2 grams of tension except after the last solution change. After a 45-minute equilibration period, graded concentrations of the mediators (ranging from 10^{-10} to 10^{-4} *M*) were added at 1-minute intervals to determine the cumulative **concentration-response (CR)** relationship.[13] Separate rings were used for each mediator.

Selection of mediators—On the basis of information available in the literature, 7 potential contractile mediators were chosen for their ability to induce contractile responses on airways in mammals. Acetylcholine,[f] a parasympathetic neurotransmitter, was chosen because of its role in causing bronchoconstriction.[14] Norepinephrine,[f] a sympathetic neurotransmitter, was selected because of its potential role in enhancing airway hyperresponsiveness via stimulation of α-adrenergic receptors, especially when β-adrenergic receptors (which cause bronchodilation) are suppressed or nonfunctional because of disease.[15] The remaining 5 agents selected for the study were SP,[f] PGF,[f] LTD,[g] HST,[f] and 5-HT,[f] which (directly or indirectly) are known to be associated with airway hyperreactivity in various species.[5] These agents were dissolved following manufacturers' recommendations and diluted in Tyrode's solution to obtain the appropriate concentrations. Responses for each concentration of the mediator were recorded on a polygraph chart recorder.[c] To account for differences in size of the bronchial rings, contractile responses were expressed as milligram of tension per milligram of dry weight of the tissues. The tissues were weighed immediately after the experiment, using an analytic scale, and were allowed to dry at room temperature (20 to 22 C) until no further weight loss was evident. This dry weight was recorded as the final dry weight for each tissue.

Statistical analyses—Responses for each concentration of the agent were analyzed by use of a mixed-model ANOVA with a factorial arrangement of treatments. Preplanned post-hoc comparisons of the least square means were performed by use of paired *t*-tests. Values of $P \leq 0.05$ were considered significant for all tests.

Results

Of the 7 agents used in this study, only ACh, HST, 5-HT, and LTD were effective in inducing contractile responses in a concentration-dependent manner in bronchial rings from both groups of horses.

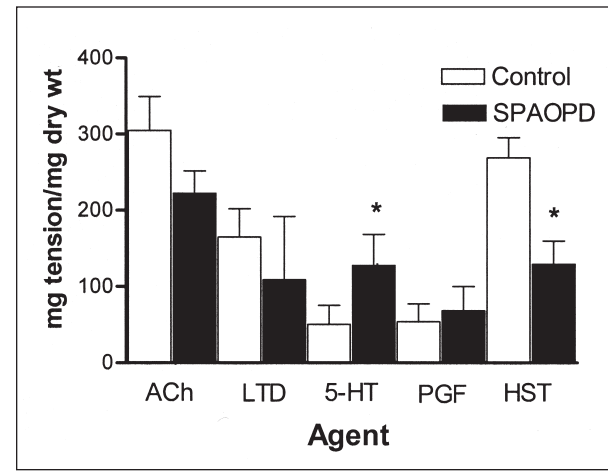

Figure 1—Mean (± SEM) contractile responses (mg of tension/mg of dry tissue weight) of bronchial rings obtained from healthy horses and those affected with summer pasture-associated obstructive pulmonary disease (SPAOPD) at 10^{-5} *M* concentrations of acetylcholine (ACh), leukotriene D_4 (LTD), 5-hydroxytryptamine (5-HT), prostaglandin $F_{2\alpha}$ (PGF), and histamine (HST). *Indicates significantly ($P \leq 0.05$) different between groups.

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Norepinephrine did not cause a contractile response, even at the highest bath concentration, in either group. The contractile response induced by SP was weak and inconsistent and was not different between groups. The CR relationship of LTD at concentrations higher than 10^{-5} *M* could not be determined because of the expense involved in preparing higher concentrations of LTD. The PGF caused a weak and inconsistent contractile response in some bronchial rings regardless of the group. Considering the overall group-drug effect, the contractile response caused by PGF was more noticeable (at 10^{-5} *M*) in bronchial rings from SPAOPD-affected horses than those from healthy horses (**Fig 1**).

The overall comparison of these agents between groups (SPAOPD-affected vs healthy) indicated the only drugs that caused a significant difference in the CR relationship between the 2 groups were 5-HT and HST. Contractile responses of bronchial rings from SPAOPD-affected horses to 5-HT at concentrations of 10^{-6}, 10^{-5}, and 10^{-4} *M* were significantly increased (**Fig 2**), whereas contractile responses to HST at these concentrations were significantly decreased (**Fig 3**).

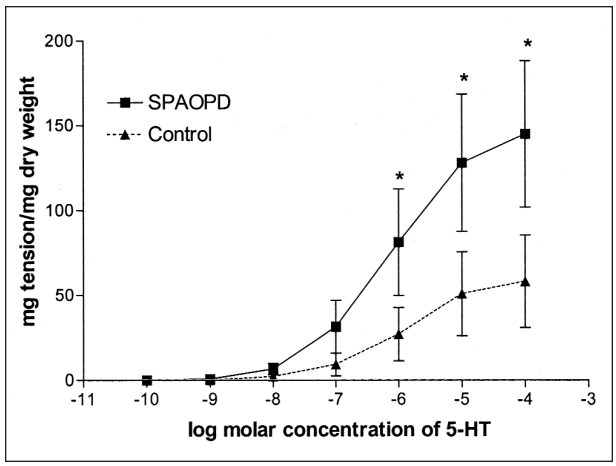

Figure 2—Mean (± SEM) contractile responses (mg of tension/mg of dry tissue weight) of bronchial rings obtained from healthy horses and those affected with SPAOPD to graded concentrations (10^{-10} to 10^{-4} *M*) of 5-HT. *See* Figure 1 for key.

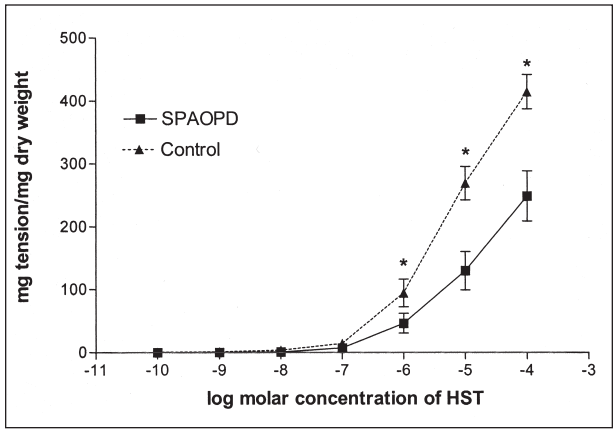

Figure 3—Mean (± SEM) contractile responses (mg of tension/mg of dry tissue weight) of bronchial rings obtained from healthy horses and those affected with SPAOPD to graded concentrations (10^{-10} to 10^{-4} *M*) of HST. *See* Figure 1 for key.

Discussion

Results of the present study revealed important differences between airway responsiveness of healthy and SPAOPD-affected horses to selected mediators of airway hyperreactivity. The agents that induced a consistent contractile response caused the bronchial rings of both groups to contract in a concentration-dependent manner. The contractile response of bronchial rings of SPAOPD-affected horses to 5-HT was greater than that of bronchial rings in healthy horses, whereas the contractile response to HST was lower in bronchial rings of SPAOPD-affected horses than that of healthy horses.

The interaction between drug molecules and tissue receptors leads to the formation of a drug-receptor complex, which triggers a series of changes in smooth muscle cell membrane potential that eventually lead to a tissue response.[16] Modulation of the drug-receptor complex could lead to a change in responsiveness of an animal. Such a condition may result from changes in the muscle such as hypertrophy, the production, release, and bioavailability of the mediator, or a change in the affinity and efficacy of the tissue's receptors.[16] In our study, tissue responses were normalized as milligram of tension per milligram of dry tissue weight, which should account for the effects of smooth muscle hypertrophy. Likewise, in a study performed in vitro, production or release of the drug (mediator) is not relevant because a known amount of a specific mediator is added directly to the bath. However, bioavailability of free drug at the receptor level could be affected in disease states by increased or decreased production of metabolizing enzymes or by a change in the drug transporting proteins, thereby leading to a change in responsiveness.[16] Although it has been reported that functional changes can develop in the bronchial smooth muscles of COPD-affected horses, histologic examination of such tissues did not reveal or detect structural changes.[17] In the present study, we detected subtle functional changes in airway smooth muscle in vitro, which suggests that in disease conditions these subtle changes may be caused by a change in receptor affinity or efficacy.

Airway tone in an animal can be considered as the sum of interactive mechanisms between excitatory and inhibitory factors.[8] Under normal physiologic conditions, parasympathetic innervation principally regulates airway smooth muscle tone. Yu et al[18] observed that isolated trachealis muscle strips from horses with heaves were hyporesponsive to exogenous muscarinic agonists. Those authors suggested that this may be caused by excessive release of ACh, leading to down-regulation of muscarinic receptors during COPD. In our study, although effective, ACh failed to induce a significant difference in airway responsiveness between bronchial rings from healthy and SPAOPD-affected horses. Our findings agree with the findings of Broadstone et al,[19] who also did not observe significant differences in responsiveness of smooth muscles of the trachea and smaller bronchi to ACh in COPD-affected horses.

Obstructive pulmonary disease is fundamentally an inflammatory disease.[17] Additionally, it has been

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suggested that COPD has an allergic component, and it is possible that HST is involved in the disease process.[2] In the present study, bronchial rings from SPAOPD-affected horses had a significantly lower contractile responsiveness to HST, compared with those from healthy horses. Although HST is typically present in all tissues, the primary sources of free HST in the lung are mast cells and basophils, which play a major role in the pathogenesis of several pulmonary inflammatory conditions in humans and animals, including COPD.[20] McGorum et al[21] reported there was a significantly higher concentration of HST in the pulmonary epithelial lavage fluid obtained from COPD-affected horses, suggesting an IgE-mediated hypersensitivity pathogenesis. Airways of COPD-affected horses are constantly exposed to HST before it is metablolized and eliminated by the liver and kidneys.[22] Exposure of tissues to an increased amount of HST may lead to desensitization or tachyphylaxis, resulting in a decreased immune response.[23] Although the mechanism of tachyphylaxis is not clearly understood, a general downregulation of tissue receptors is believed to be one of the factors responsible for this phenomenon.[24] Release of HST, via degranulation of mast cells, may cause bronchoconstriction in the initial stages of SPAOPD. However, this may later be followed by homeostatic tissue changes leading to downregulation of HST receptors in smooth muscle cells of the airways, which subsequently results in decreased contractile responsiveness to HST, as was detected in our study.

Another important finding of the present study was that 5-HT enhanced smooth muscle tone in bronchial rings of SPAOPD-affected horses. Although the findings from a study performed in vitro may not project similarly to conditions in vivo, our results indicated there may be a role for 5-HT in mediating bronchoconstriction in SPAOPD-affected horses. The cumulative CR relationship of the bronchial rings from SPAOPD-affected horses revealed there was an enhanced responsiveness to 5-HT at concentrations of 10^{-6} *M*, 10^{-5} *M*, and 10^{-4} *M*. In the lung, 5-HT normally causes bronchoconstriction.[25] It is produced in the gastrointestinal tract, reaches the liver via the portal circulation, and is effectively inactivated to 5-hydroxyindolacetic acid. However, 5-HT, which escapes hepatic metabolism, reaches the lung where it is effectively removed by uptake into pulmonary endothelial cells, a process that is controlled by a sodium-dependent carrier mechanism.[22] It has been suggested that 5-HT may be involved with nonadrenergic noncholinergic excitatory neurotransmission in various species.[26,27] Thus, the present study poses the question, how can a natural endogenous mediator such as 5-HT have an enhanced effect on airways of SPAOPD-affected horses? There is no straightforward answer to this question. To our knowledge, a possible involvement of 5-HT in the pathogenesis of either SPAOPD or COPD has not been reported.

Although speculative, a logical possible explanation for the role of 5-HT in the development of SPAOPD is that during the initial stages of disease, HST most likely contributes to the inflammatory process by causing an increase in pulmonary blood flow via its vasodilatory actions. We speculate that this increased blood flow may enhance the elimination of 5-HT by increased uptake in the lung and possibly by degradation in the liver.[22] Because 5-HT modulates bronchial tone, decreased bioavailability of 5-HT at the receptor level in disease states could induce upregulation of 5-HT receptors in the airways as an expected homeostatic response. In the present study, the bronchial rings from SPAOPD-affected horses (with upregulated 5-HT receptors) could have enhanced responsiveness when exposed exogenously to 5-HT. This up regulation of 5-HT receptors in recurrent airway obstruction disorders may explain some of the clinical signs observed in SPAOPD-affected horses, such as airway obstruction and bronchospasm.

In our study, neither NE nor SP caused a contractile response in the bronchial rings of either group. Norepinephrine would be expected to cause contraction of bronchial smooth muscle only if the β-adrenergic receptors, which are the predominant receptors for bronchodilation, [28] were blocked or damaged by the disease, thus facilitating stimulation of α-adrenergic receptors.[15] This idea prompted Szentivani et al[29] to hypothesize that an inborn β-adrenergic receptor blockade is the progenitor of asthma in humans. The inability of NE to induce a contractile response in our study suggests that β-adrenergic receptors are not damaged or deactivated in the airways of SPAOPD-affected horses. This finding agrees with that of Scott et al,[30] who reported there was no deficiency of β-adrenergic receptor function in the airways of COPD-affected ponies. Although SP receptors are reported to be present in the periphery of the lungs of horses,[31] SP did not induce consistent bronchoconstriction in our study. Prostaglandin $F_2\alpha$ induced weak and inconsistent contractile responses of the bronchial rings of both healthy and SPAOPD-affected horses. However, the contractile response of bronchial rings to PGF in SPAOPD-affected horses , although not significant, was greater than the response observed in bronchial rings of the healthy horses. Previous reports suggest that bronchodilatory prostaglandins play a role in airway homeostasis in advanced stages of SPAOPD.[8]

[a]Esophageal balloon, AE Medical, Farmingdale, NJ.
[b]Statham Model P50 pressure transducer, Statham Instruments, Hato Ray, Puerto Rico.
[c]Model 7D polygraph, Grass Medical Instruments, Quincy, Mass.
[d]Beuthanasia, Schering-Plough Animal Health Corp, Kenilworth, NJ.
[e]Grass model FT.03 force transducer, Grass Medical Instruments, Quincy, Mass.
[f]Sigma Chemical Co, St Louis, Mo.
[g]Cayman Chemical Co, Ann Arbor, Mich.

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