Evaluation of an In Vitro Degranulation Challenge Procedure for Equine Pulmonary Mast Cells

J.E. Hare, L. Viel, P.D. Conlon, and J.S. Marshall

ABSTRACT

Pulmonary mast cells (PMC) are important components of the inflammatory process in equine allergic lung diseases such as heaves. Very little, however, is known of the degranulation kinetics of these cells and thus, their pathophysiologic role remains largely speculative. The purpose of this study was to develop a repeatable protocol for in vitro equine PMC degranulation. Five mature horses (sex: 2 M, 3 F; age: 8.8 ± 6.5 y), historically free of pulmonary disease and normal on clinical respiratory examination, arterial blood gas analysis, pulmonary mechanics testing and histamine inhalation challenge, were studied. Bronchoalveolar lavage was performed on 4 separate occasions, at least 2 d apart, in a different lung lobe on each occasion. The lavage fluid was concentrated by centrifugation. Cells were resuspended in modified HEPES/Tyrode, assessed for viability by Trypan blue exclusion, and PMC concentration determined. Cell inocula containing 30 000 PMC were incubated with 10−4 to 6 × 10−4 M A23187. Cells were then separated by centrifugation and histamine release (HR) was determined by fluorometric assay. The procedure was readily performed and yielded sufficient PMC for 30 to 60 inocula per lavage. Maximal HR (34.4 ± 16.1%) was obtained with 10−5 M A23187. The degranulation process was largely complete by 20 min but cell lysis was negligible. The challenge was repeatable within horse and produced a mean coefficient of variability of 23.0% following 20 min incubation with 10−5 M A23187. We conclude that equine PMC degranulation can be repeatedly performed in vitro and speculate that this protocol may be useful in further studies on the pathophysiology and treatment of equine allergic lung diseases.

RÉSUMÉ

Les mastocytes pulmonaires (MP) sont une composante importante dans le processus de la réaction pulmonnaire allergique du cheval mais on connaît très peu leur pathophysiologie dans cette maladie. Le but de cette étude est de développer un test in vitro étudiant la dégranulation des MP. Cinq chevaux matures (sexes : 2 M, 3 F; âge : 8,8 ± 6,5 ans) furent exposés à une gueule sanguin normal, une mécanique pulmonaire normale et répondant normalement à l'inhala- tion d'histamine furent utilisés. Quatre lavages bronchoalvéolaires à au moins 2 jours d'intervalle furent pratiqués dans des régions différentes du poumon. Après centrifugation du liquide, les cellules furent mises dans un milieu HEPES/Tyrode et la concentration des MP fut déterminée. Après incu- bation avec du A23187 (10−5 à 6 × 10−5 M), les cellules furent séparées et le relâchement d'histamine fut étudié par fluorométrie. Cette procédure était assez facile à réaliser et permettait de 30 à 60 essais par lavage. Le relâchement maximal d'histamine fut obtenu avec 10−5 M de A23187 après 20 minutes. Les résultats obtenus étaient similaires d'un cheval à l'autre (coefficient de variation 23 %). En conclusion, ce test in vitro semble repro- ductible et pourrait être utile pour l'étude de la pathophysiologie et du traitement des maladies allergiques pulmonaires du cheval.

INTRODUCTION

Heaves is a naturally-occurring respiratory disease of horses characterized by airway obstruction following the inhalation of allergens, usually molds and fungi from improperly cured hay, found in the animals’ environment (1). Pathologically, the disease is characterized by contraction of bronchiolar smooth muscle, mucus hypersecretion, airway edema and inflammatory cell infiltration (2). While neutrophils have been established as the predominant inflammatory cells in the small airways of horses with heaves (2,3), there is evidence to suggest that pulmonary mast cells (PMC) are important effector cells in this condition. Studies on bronchoalveolar lavage (BAL) fluid of horses with heaves have demonstrated significantly reduced PMC counts (4), or a trend towards this effect (5), suggesting that significant PMC degranulation is a feature of the disease. Histamine, a major degranulation product of mast cells, is well known to cause bronchoconstriction in the horse (6,7). McGorum and associates (8) recently reported that, 5 h following environmental challenge, histamine levels in the pulmonary epithelial lining fluid of horses with heaves were elevated as compared to pre-challenge values. Furthermore, work by us (9) and

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others (10,11) has demonstrated the efficacy of the mast cell stabilizer sodium cromoglycate in treating equine allergic lung diseases.

In order to better characterize PMC responses to allergen, in vitro degranulation challenge protocols have been developed using PMC harvested by dispersion of surgically acquired lung tissues (12), or by BAL (13). Degranulation challenge procedures have been developed for PMC from several species (13,14,15), and have been a useful tool for elucidating the PMC response to various secretagogues and pharmacologic stabilizers. These experiments have not been performed in the horse, although in vitro degranulation challenges have been developed for equine peripheral blood basophils in an attempt to determine if differences in histamine release occur in horses with heaves as compared to controls (16,17,18). The results of these studies have been equivocal and likely reflect the functional diversity that exists between mast cells and basophils.

The purpose of this study was to develop a repeatable protocol for the demonstration of in vitro equine PMC degranulation in response to a pharmacologic secretagogue.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Five horses (sex: 3 females, 2 geldings; age, mean ± SD: 8.8 ± 6.5 y) were utilized in the study. All were free of historical respiratory disease and did not exhibit clinical signs of airway obstruction following exposure to moldy hay. All horses were normal on complete blood count and plasma fibrinogen assay, arterial blood gas measurement, pulmonary mechanics testing and histamine inhalation challenge (HIC) prior to the commencement of the study period. All animal experiments were performed according to the guidelines established by the Canadian Council on Animal Care (19) and approved by the University of Guelph Animal Care Committee.

ANIMAL HOUSING

Horses were maintained on pasture during the study period and were transported to the Ontario Veterinary College (OVC) Veterinary Teaching Hospital (VTH) for specific experiments during which time they were housed in box stalls, bedded on wood shavings and fed pelleted feed. The ventilation of the stalls was independent of that for the rest of the hospital so that the exposure of the horses to air-borne material from other stalls was minimized.

EXPERIMENTAL PROTOCOL

For all experiments, a full physical examination was performed and a clinical respiratory score was determined for each horse the day following transport to the VTH. Venous blood was obtained for complete blood count (CBC) and fibrinogen assay. Arterial blood was obtained for blood gas, bicarbonate and pH measurement. Pulmonary mechanics measurements were determined and HIC was then performed. Bronchoalveolar lavage was performed at least 48 h following HIC and subsequent BAL were obtained at least 48 h apart in a lung lobe not previously lavaged. No more than 4 BAL were performed in any 1 horse during a single confinement period. In vitro PMC degranulation challenge was carried out on cells isolated from each BAL.

RESPIRATORY EXAMINATION

A physical examination of the respiratory system was performed by 1 clinician on all horses. Horses were assigned a clinical respiratory score as described previously (9).

HEMATOLOGY AND ARTERIAL BLOOD GASES

Blood was obtained by jugular venipuncture into sterile glass tubes containing either ethylenediamine-tetraacetic acid (EDTA), or sodium citrate, for CBC and fibrinogen assays, respectively. The CBC were performed on an automated cell counter (Coulter Counter S-plus, Coulter Electronics of Canada Ltd., Burlington, Ontario) and a manual differential count of 100 leukocytes was performed. Fibrinogen assays were performed on plasma using an automated system (FibroSystem, BBL, Cockeysville, Maryland, USA). Arterial blood was obtained anaerobically by puncture of the carotid artery. Syringes were capped, placed on ice and immediately analysed (Radiometer ABL3, Radiometer Corp., Copenhagen, Denmark) for partial pressures of oxygen (PaO2) and carbon dioxide (PaCO2) and for bicarbonate (HCO3), and pH, all of which were corrected for the rectal temperature of the horse.

PULMONARY MECHANICS TESTING

Pulmonary mechanics testing was performed as described previously (20). Parameters measured included respiratory rate, tidal volume, minute volume, lung resistance, dynamic compliance and maximal change in transpulmonary pressure.

HISTAMINE INHALATION CHALLENGE

The method of histamine aerosol provocation was identical to that described by Hare and Viel (21).

BAL AND SAMPLE PROCESSING

Horses were sedated with either xylazine 0.5 mg/kg IV or romifidine 0.05 mg/kg IV. Bronchoscopy and BAL were performed as described previously (9). Three subsequent BAL were performed no closer than 48 h apart and involved the instillation and subsequent aspiration of up to 1000 mL of physiologic saline in 250 mL aliquots in order to maximize cell harvest. The volume of recovered fluid was standardized at 400 mL. All 4 BAL were performed in different lung lobes.

PROCESSING OF BAL FLUID

The lavage fluid was kept on ice until processing. Concentrations of nucleated cells in the BAL fluid were measured with an automated cell counter (Coulter Counter ZM, Coulter Electronics of Canada Ltd., Burlington, Ontario). Ten mL of lavage fluid were centrifuged (200 g for 10 min) and the supernatant removed, boiled for 10 min and frozen at −20°C for quantitation of supernatant histamine concentration; this value representing the amount of histamine present in the fluid and released by the PMC. Additionally, 10 mL of uncentrifuged lavage fluid were boiled for 10 min and then stored at −20°C for eventual quantitation of lysate histamine concentration; this parameter was the amount of released histamine (supernatant histamine concentration) plus the amount of histamine contained within the BAL PMC population.
BAL histamine concentrations were determined by radioenzymic assay (22).

**SLIDE PREPARATION, FIXATION AND STAINING**

For each BAL, 2 slide preparations for PMC quantitation were prepared by cytocentrifugation, and either air dried or fixed in Carnoy's solution and then stained with a modified Wright's stain using an automated stainer (Hema-Tek Slide Stainer, Miles Laboratories Ltd., Rexdale, Ontario), or toluidine blue, respectively. Cell differentials were performed on 500 consecutive nucleated cells. Calculation of cell concentrations in BAL fluid was obtained by multiplying the cell differential percentage by the total nucleated cell count.

**PMC DEGRANULATION CHALLENGE**

Fluid obtained by BAL was decanted into eight 45 mL polypropylene centrifuge tubes and kept on crushed ice for transport to the laboratory. The tubes were then centrifuged (200 × g, 4°C) for 10 min. Supernatant fluid was decanted and 0.5 mL of HEPES/Tyrode buffer solution (4°C) was added. A 100 μL aliquot was diluted in 900 μL of HEPES/Tyrode buffer and 1.0 mL 0.4% trypan blue and allowed to incubate at room temperature for 5 to 10 min. Cell viability and concentration were determined using a hemocytometer. A further 100 μL aliquot was diluted in 900 μL HEPES/Tyrode buffer and a cytocentrifuge preparation was made from 200 μL of this solution. The slides were air-dried, stained with a modified Wright's stain (Hema-Tek Slide Stainer, Miles Laboratories, Rexdale, Ontario) and the PMC were counted using 1000x magnification and expressed as a percentage of 500 cells. The viable PMC concentration of the initial suspension was then determined by multiplying the percentage of PMC by the total viable cell concentration as determined by hemocytometer count. The cell suspension was then diluted with an appropriate concentration of 4°C HEPES/Tyrode buffer to yield a cell suspension containing 1 × 10^5 PMC/mL. The cell suspension and the incubation media were warmed in a 37°C water bath for 3 min before the addition of an aliquot of 3 × 10^4 PMC (300 μL of cell suspension) to polypropylene tubes containing 700 μL of incubation medium.

Following incubation, the tubes were centrifuged (200 × g, 4°C, 7 min). Supernatant was then separated from the cell pellet and placed in polypropylene tubes. Tubes containing cell pellets were then boiled for 10 min to release intracellular histamine and inactivate histaminase. Volumes of 0.5 mL and 1.5 mL of HEPES/Tyrode buffer were added to tubes containing supernatant and cell pellets, respectively, and fluorometric histamine assay was performed on all tubes.

**FLUOROMETRIC HISTAMINE ASSAY**

Histamine was assayed by a fluorometric method as described previously (23). Briefly, samples consisting of 1.5 mL HEPES/Tyrode buffer were thawed and 110 μL of 25% trichloroacetic acid was added. Tubes were vortexed and allowed to stand for 10 min. Tubes were then centrifuged (500 × g, 10 min) and the supernatant decanted into polystyrene tubes. Following this, 110 μL of 3N NaOH followed by 50 μL of 1% o-phthalaldehyde in methanol were added. Exactly 4 min after the addition of o-phthalaldehyde, the reaction was quenched with 175 μL of 4N HCl. Tubes were centrifuged (800 × g, 5 min) and fluorescence was determined at an excitation of 360 nm and an emission of 440 nm. Appropriate histamine standards (1 ng/mL to 400 ng/mL) were assayed concurrently and sample histamine concentrations were determined by comparison with the standard curve. Pilot studies with the assay had determined it to have an inter- and intra-assay coefficient of variation of <10% and a sensitivity of approximately 5 ng/mL.

**CALCULATION OF PMC HISTAMINE RELEASE**

Pulmonary mast cell histamine release (HR) was expressed as the percent of total PMC histamine and was determined by the following equation:

\[ \%HR = \frac{(F_{sup} - F_{blank})}{(F_{sup} - F_{blank,2})} \times 100\% \]

where: \( F_{sup} \) is fluorescence of the supernatant; \( F_{blank} \) is fluorescence of the supernatant blank; \( F_{blank,2} \) is fluorescence of the pellet blank. Supernatant blanks consisted of HEPES-Tyrode buffer and secretagogue at the same concentration as the supernatant. Pellet blanks consisted of HEPES-Tyrode buffer only. Spontaneous PMC HR was defined as the percent release induced by incubation of cells with the diluent control. In all experiments, this value was less than 10% and was usually less than 5%.

**A23187 PREPARATION**

The calcium ionophore A23187 was used as the degranulating agent. The agent was prepared as a stock solution of 1 × 10^{-3} M in 99.5% dimethylsulfoxide (DMSO). The stock solution was stored at −20°C until needed. It was diluted to its final concentration in HEPES-Tyrode buffer.

**HISTAMINE RELEASE EXPERIMENTS**

The time course of histamine release (HR) was determined by incubating cell preparations with 1 × 10^{-3} M A23187, a concentration known to induce PMC degranulation (13,14), for 1, 5, 10, 20, and 40 min.

The calcium requirement for HR was determined by incubating cell preparations for 20 min with 1 × 10^{-3} M A23187 in modified HEPES/Tyrode buffer containing either 2 mmol/L EDTA, or 2, 4, 6, or 10 mmol/L Ca^{2+}.

Cell viability following HR was studied by incubating 3 cell preparations for 20 min with 1 × 10^{-3} M A23187. One preparation was used to determine HR as described above. Cell viability in the 2nd preparation was determined by percent uptake of trypan blue as compared to a control preparation. Cell viability in the 3rd preparation was determined by measurement of supernatant lactate dehydrogenase (LDH) concentration after centrifugation (200 × g, 4°C, 7 min) using a biochemical analyzer (DACOS, Coulter Electronics of Canada Ltd., Burlington, Ontario). Pilot studies indicated that 1 × 10^{-3} M A23187 did not interfere with LDH analysis.

Following these initial experiments, the dose responses to A23187 were determined by incubating cell preparations for 20 min in concentrations of 1 × 10^{-8} to 6 × 10^{-3} M A23187.
TABLE I. Respiratory score, pulmonary function and histamine inhalation challenge parameters of normal horses (n = 5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory score</td>
<td>1.2 ± 1.1</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>98 ± 7</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>44 ± 2</td>
</tr>
<tr>
<td>f (bpm)</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>V̇(L)</td>
<td>6.1 ± 1.1</td>
</tr>
<tr>
<td>V̇m (L/min)</td>
<td>86 ± 26</td>
</tr>
<tr>
<td>Rₗ (cmH₂0/L/s)</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>Cdyn (L/cmH₂0)</td>
<td>3.0 ± 2.0</td>
</tr>
<tr>
<td>ΔṖw (cmH₂0)</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>PC35Cdyn (mg/mL)</td>
<td>27 ± 7</td>
</tr>
</tbody>
</table>

PaO₂: partial pressure of arterial oxygen; PaCO₂: partial pressure of arterial carbon dioxide; f: respiratory rate; V̇: tidal volume; V̇m: minute volume; Rₗ: lung resistance; Cdyn: dynamic compliance; ΔṖw: maximal change in transpulmonary pressure; PC35Cdyn, concentration of histamine effecting a 35% drop in Cdyn from baseline. Values are mean ± SD.

TABLE II. Bronchoalveolar lavage cytology parameters of normal horses (n = 5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nucleated cells (× 10⁶/L)</td>
<td>340 ± 110</td>
</tr>
</tbody>
</table>

Wright-Giemsa stain

Differential (%)

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophage</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>15 ± 7</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>1.6 ± 1.1</td>
</tr>
<tr>
<td>PMC</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>6.7 ± 5.7</td>
</tr>
<tr>
<td>Epithelial</td>
<td>0.5 ± 1.2</td>
</tr>
</tbody>
</table>

Numbers (× 10⁶/L)

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophage</td>
<td>250 ± 70</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>49 ± 22</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>5.5 ± 4.6</td>
</tr>
<tr>
<td>PMC</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>26 ± 28</td>
</tr>
<tr>
<td>Epithelial</td>
<td>1.5 ± 3.5</td>
</tr>
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</table>

Toluidine blue stain

<table>
<thead>
<tr>
<th>PMC (%)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMC (× 10⁶/L)</td>
<td>4.4 ± 1.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD

STATISTICAL ANALYSIS

Descriptive statistics for clinical score, hematology, pulmonary function parameters, PC35Cdyn, BAL cytology and HR data were determined using a commercially available software program (Statistix 4.1, Analytical Software, St. Paul, Minnesota, USA).

RESULTS

All procedures performed on the horses were well tolerated and without adverse effects. The characteristics of the study horses with regard to clinical respiratory score, pulmonary function testing, PC35Cdyn, and BAL cytology are presented in Tables I and II. All horses were within normal limits for both the CBC and the fibrinogen assay. Following processing for the degranulation challenge, cells were greater than 90% viable based on trypan blue exclusion. Each BAL yielded sufficient PMC for 40 to 60 inocula. Spontaneous HR was 8.6 ± 0.8 % (mean ± SD). Calculated histamine content per 104 PMC was 49.0 ± 6.5 ng.

Histamine release was largely complete by 10 to 20 min (Figure 1). The HR was calcium-dependent and supplementation with additional calcium (above 2 mmol/L) did not augment HR (Figure 2). The procedure was repeatable within horse and...
demonstrated a mean coefficient of variability of 23%.

The HR observed in response to \(1 \times 10^{-5}\) M A23187 was not secondary to cell lysis. Significant LDH release did not occur concurrently with HR (Figure 3). Furthermore, cell viability following A23187-induced HR, as assessed by trypan blue exclusion, was not significantly different from control (95 ± 3% vs. 94 ± 4% respectively, mean ± SD). A dose dependent HR was observed in response to A23187, with maximal release occurring at \(6 \times 10^{-6}\) M A23187 (Figure 4).

**DISCUSSION**

The divalent cation-selective ionophore A23187 was selected for use in this study for several reasons. First, this agent is well known to induce PMC HR (12,13,14,24,25). Second, degranulation in response to A23187 is observed in PMC from diverse anatomic locations such as lung, skin, adenoids and colon (12). Third, A23187-induced HR is non-immunologic since it is not associated with cell surface receptors (26), and, therefore induces HR in a relatively non-selective fashion. Fourth, the HR associated with this agent requires a viable PMC population and is not associated with significant cell lysis (27).

Mast cell degranulation induced by A23187 occurs as a result of calcium transport across the cell membrane and down the concentration gradient from the extracellular space into the cytosol (26). In the present study, the maximal A23187-induced HR was 40.6 ± 5.6% (mean ± SD) of the total histamine available. These data compare favourably with BAL-obtained PMC from normal dogs (41.5%) (14), and from 1 human study (50%) (13) but were considerably higher than in the human study of Casolaro and coworkers (19 ± 5.3%) (24). In the latter study, however, PMC were incubated for 45 min (compared to 11 to 20 min for the other studies) which resulted in a higher rate of spontaneous HR. In addition, the dose response curve generated by the work of Casolaro and associates appeared to be climbing at the maximal dose of A23187 studied (approximately 1.5 \(10^{-6}\) M) and was below that demonstrated to cause maximal HR by Wardlaw and colleagues (13). The observation that maximal HR occurred at approximately \(6 \times 10^{-6}\) M in the present work is in agreement with previous work where the peak response in dogs was obtained at \(3 \times 10^{-6}\) M (14) and in humans at \(5 \times 10^{-6}\) M (13). The waning of the mast cell response to A23187 at concentrations higher than \(6 \times 10^{-6}\) M has been previously noted (13,14) and may be a consequence of over-saturation of the adenylyl-cyclase pathway by calcium or, possibly, A23187-mediated stimulation of mast cell inhibitory agents from other inflammatory cells.
The demonstration that HR was not associated with either cell membrane rupture (as evidenced by the lack of significant release of the cytoplasmic marker LDH) or impairment of metabolic function (as evidenced by trypan blue dye exclusion) suggests that the process is not cytolytic. Further evidence that the HR is a result of degranulation was obtained from the time course of this phenomenon. The observation that HR was largely complete by 10 to 20 min following addition of A23187 is similar to the time course of degranulation observed in human BAL-derived PMC following challenge with anti-IgE (13,28) and equine basophils challenged with anti-Fab (17).

The $\alpha_1$ adrenergic agonist used as sedatives in this trial may have influenced the magnitude of HR. Studies on this are conflicting, however. Clonidine, a predominantly $\alpha_2$ adrenergic agonist, has been demonstrated to enhance IgE-mediated 14C-serotonin release from rat mast cells (29) and to inhibit allergen induced histamine release from human lung slices (30). Nevertheless, the washing of the cell preparation prior to A23187 challenge probably mitigated the effect, if any of this stimulation. Furthermore, calcium-mediated stimulation of the adenylyl-cyclate pathway — the mechanism of calcium ionophore-induced HR — can occur independently of other receptor-mediated degranulation stimuli.

The reproducibility of the degranulation challenge was similar to that described by Sommerhoff and associates (14) who reported the A23187-induced PMC HR from a single dog examined on 5 different occasions as 43.8 ± 3.3% (mean ± SE), which results in a coefficient of variation of approximately 17%. This value is similar to the average coefficient of variation reported here.

Although the present study represents original work with regard to the HR of equine PMC, Kings and de Weck (16) examined the effect of different secretagogues on HR by equine peripheral blood basophils. These workers found that equine basophils were unresponsive to A23187 in the narrow range of $10^{-7}$ to $8 \times 10^{-6}$ M. The significance of this finding was not discussed by the authors and, although it is tempting to cite inherent differences in mast cell and basophil responses to secretagogues as a possible reason, work in humans would suggest that basophils are up to 3 times more sensitive than PMC to this agent (24).

The horses utilized in this experiment were free of historical respiratory disease and fulfilled the clinical, functional and cytologic definitions of respiratory normalcy as determined by this laboratory and by other workers (2,3,20,31). This study demonstrated that the PMC recovered by BAL from normal horses have the capacity to release approximately 20 ng of histamine per 10^6 PMCs. In other words, given that approximately $2.6 \times 10^6$ PMCs were recovered per 200 mL lavage, the potential HR by the cells of a single 3rd or 4th generation bronchus approaches 5–6 $\mu$g. Given that histamine concentrations of approximately 0.5 mg/mL were demonstrated by Broadstone and colleagues (32) to induce optimal contraction of 3rd generation bronchial smooth muscle, and that McGorum and co-workers (39) showed that the pulmonary lining fluid component of equine BAL comprises no more than 1% of the lavage volume, it can be speculated that equine PMC HR has a potential biologic significance in equine respiratory homeostasis.

In conclusion, an in vitro calcium ionophore-responsive degranulation challenge procedure for equine BAL-derived PMC is described. The protocol demonstrated the non-lytic, calcium-dependent release of histamine in a dose-dependent manner within 10 to 20 min of incubation with ionophore. The model was comparable to those described for PMC of other species and, in addition, was repeatable within horse. Thus, there is strong evidence supporting the use of this in vitro model for the investigation of PMC function in equine allergic respiratory diseases.

ACKNOWLEDGMENTS

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