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Inhalational Anesthesia and Histamine Release during Bronchospasm

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The influence of inhalational anesthetics on histamine released during bronchospasm induced by *Ascaris* antigen aerosol was studied in Basenji-Greyhound (BG) dogs. Five BG dogs were anesthetized with thiopental and halothane on separate occasions and challenged with *Ascaris* antigen aerosol for five minutes. Pulmonary resistance (R_L), dynamic compliance (C_{dyn}), and arterial plasma histamine levels were measured over 30 min following antigen exposure.

Prior to antigen challenge, R_L, C_{dyn}, and histamine levels were similar in thiopental- and halothane-anesthetized dogs. The peak change in R_L and C_{dyn} occurred 10 min after the start of antigen challenge. R_L increased 3.0 ± 0.4-fold (mean ± SEM) in thiopental-anesthetized dogs as compared with 1.6 ± 0.2-fold in halothane-anesthetized dogs ($P < 0.05$). C_{dyn} decreased to a similar extent in both groups, with maximal decreases of 0.53 ± 0.08 and 0.53 ± 0.09 times the prechallenge value for thiopental- and halothane-anesthetized dogs, respectively. Plasma histamine levels peaked at 5 min. Plasma histamine increased to 8.0 ± 5.0 ng/ml in thiopental-anesthetized dogs compared with 10.7 ± 4.7 ng/ml in halothane-anesthetized dogs. Histamine levels were not significantly different during or after antigen challenge between thiopental- and halothane-anesthetized dogs.

Halothane, but not thiopental, significantly attenuated the increase in R_L provoked by antigen challenge. However, arterial plasma histamine levels were similar during both anesthetics. Therefore, the authors conclude that the protective effect of halothane on airways was not due to an inhibition of release of histamine from mast cells. (Key words: Allergy: asthma. Anesthetics, intravenous: thiopental. Anesthetics, volatile: halothane. Histamine. Lung: bronchospasm; compliance.)

POTENT INHALATIONAL ANESTHETICS are known to be bronchodilators. Halothane,[1,2] isoflurane,[2] and enflurane[1] attenuate bronchospasm induced by *Ascaris* antigen aerosol in the Basenji-Greyhound (BG) dog model of asthma. The mechanisms by which these inhalational anesthetics attenuate bronchospasm are not understood entirely. Some possible mechanisms include depression of parasympathetic-mediated irritant reflexes, direct relaxation of airway smooth muscle, and inhibition of release of bronchoactive mediators.

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Previous studies have shown that potent inhalational anesthetics block a wide variety of airway reflexes.[3,4] Clinically useful concentrations of inhalation anesthetics produce only a small direct effect on constricted airway smooth muscle *in vivo.*[2] The influence of inhalation anesthetics on mediator release during bronchospasm has not been reported previously.

Pulmonary mast cells contain a variety of chemical mediators that are thought to play a vital role in the pathogenesis of asthma and immediate hypersensitivity reactions.[5,6] Histamine, eosinophilic chemotactic factor and neutrophilic chemotactic factor are stored preformed in granules in mast cells and basophils and are released simultaneously with degranulation. Prostaglandins and slow-reacting substances (now called leukotrienes) are newly synthesized after activation of mast cells and possibly other cells. We have shown previously that plasma histamine levels increase following aerosol challenge with *Ascaris* antigen in the Basenji-Greyhound dog model of asthma.[7] Therefore, using histamine as a marker of mediator release from pulmonary mast cells, we can determine if clinically usable concentrations of halothane inhibit mediator release from mast cell granules during bronchospasm.

Methods

Five Basenji-Greyhound dogs, 3–5 years old and weighing 20–22 kg, were employed in these studies. All dogs were either natively allergic or previously sensitized to aerosolized *Ascaris* antigen by repeated challenge.

Unpremedicated dogs were anesthetized in the standing position supported by a sling. After induction of anesthesia with intravenous thiopental 12 mg/kg, the dogs were paralyzed with succinylcholine, 0.75 mg/kg, intubated with a 9.0-mm cuffed endotracheal tube, and ventilated manually with 100% oxygen. An esophageal balloon (Dynasciences, Blue Bell, Pennsylvania) was placed in the esophagus and positioned at the point where recorded end-expiratory pressure was lowest. The balloon contained 0.8 ml air. A separate catheter connected to suction was placed in the esophagus to keep it empty of air and liquid. Each dog then was placed in the supine position, and a 21-gauge catheter was placed in the femoral artery for blood sampling and arterial pressure measurement. The electrocardiogram was monitored with a

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Tektronix® 410 monitor (Beaverton, Oregon). The dogs were ventilated with a piston-type ventilator (Harvard Apparatus, Millis, Massachusetts) set to deliver a tidal volume of 400 ml at a frequency of 14/min.

Transpulmonary pressure (P_L) was measured with a differential pressure transducer (Hewlett-Packard 270) connected to the esophageal balloon and to a needle inserted in the endotracheal tube. Airflow ($\dot{V}$) was measured with a pneumotachygraph (Hewlett-Packard 2100) and a differential flow transducer (Hewlett-Packard 47304A). Pressure and flow signals were recorded with a Hewlett-Packard 47601 recorder. End-tidal gases were measured using a Perkin-Elmer 1100 mass spectrometer (Pomona, California).

Dynamic pulmonary compliance (C_{dyn}) was calculated by dividing the tidal volume by the absolute difference in transpulmonary pressure between points of zero flow. Pulmonary resistance (R_L) was calculated according to the method of Von Neergaard and Wirz[8,9] by dividing P_L minus elastic recoil pressure by $\dot{V}$ at midtidal volume. Apparatus resistance, determined by ventilating a mechanical analog with known parameters, was subtracted from the resulting value to give R_L. R_L and C_{dyn} were reported as a mean from five consecutive breaths.

In control studies, after the initial dose of thiopental, additional increments of thiopental 2 mg/kg were administered as needed, usually at 15-min intervals. For halothane studies, halothane was administered until a steady state end-tidal concentration of 1.5 MAC was established, approximately 45 min after initiating halothane anesthesia. The MAC value of halothane in the dog was taken to be 0.87%.[10]

Each of the five BG dogs received two separate antigen challenges, one during thiopental anesthesia and one during halothane anesthesia. The studies in each dog were done in random order at least 1 week apart. Each dog served as its own control. A purified extract of *Ascaris* antigen was prepared by Sephadex filtration according to the methods of Mackler *et al.*[11] Aerosol solutions of *Ascaris* antigen (3 μg protein/ml) were prepared in distilled water and delivered by a Hudson 3000 nebulizer (Temecula, California). The nebulizer delivered aerosol particles with a median diameter of 5.7 μm. The nebulizer was driven by compressed O_2 (4 l/min at 20 psi) and was inserted between the inspiratory limb of a circle anesthesia system and the endotracheal tube. *Ascaris* antigen (0.3–0.4 ml/min) was delivered for five minutes while ventilation was controlled manually. To avoid residual contamination, the entire anesthesia circuit was changed after completion of the aerosol challenge. P_L and $\dot{V}$ were measured continuously before and after antigen challenge. R_L and C_{dyn} were calculated from measurements made just prior to antigen challenge, immediately following antigen challenge, and at 5-min intervals for 30 min.

All dogs were placed in the supine position to facilitate placement of the femoral artery catheter and subsequent arterial blood sampling. Although the absolute values for R_L and C_{dyn} may vary from prone to supine position, the magnitude of the changes is consistent if the esophageal balloon is positioned properly.[12] Therefore, our airway response data are expressed as absolute values and as the ratio of postchallenge to prechallenge values.

Five milliliters of arterial blood were collected for determination of histamine concentration. Samples were collected immediately before aerosol challenge and 1, 2, 3, 4, 5, 7, 10, 15, and 30 min after the start of aerosol challenge. Blood was collected in polypropylene tubes with preservative-free heparin, 10 units/ml whole blood, and placed immediately on ice. The plasma was separated by centrifugation at 800 × *g* for 20 min, transferred to plastic tubes, and frozen at −70° C until assayed. Samples were coded and analyzed without knowledge of the anesthetic used.

Plasma histamine was determined by the double isotope radioenzymatic method of Shaff and Beaven[13] using rat kidney histamine N-methyltransferase. The ^{14}C radiolabeled methyl donor, S-adenosyl-L-methionine, was purchased from Amersham (Arlington Heights, Illinois). Confirmation of histamine specificity in plasma samples was obtained by complete degradation of measurable histamine by histaminase 8 mg/ml (diamine oxidase, Sigma, St. Louis, Missouri) for 30 min at 37° C. In addition, histamine standard curves were run in the presence of 75–90% concentrations of dog plasma. These curves varied less than 5% from standard curves generated in buffer. In repeat studies in the same animal, plasma histamine levels varied by less than 1 ng.

R_L, C_{dyn}, and plasma histamine levels were compared at each time point during thiopental and halothane anesthesia using a paired Student's *t* test. Analysis of these data has been controlled for a Type 1 (alpha) error at 0.05 and a Type 2 (beta) error at 0.1.[14]

Results

Prior to *Ascaris* antigen challenge, R_L and C_{dyn} were similar in thiopental- and halothane-anesthetized dogs. In thiopental-anesthetized dogs, prechallenge values of R_L averaged 2.9 ± 0.5 $cmH_2O \cdot l^{-1} \cdot s$ (mean ± SEM) and values of C_{dyn} averaged 70 ± 7 ml/cmH_2O. In the same dogs anesthetized with halothane, prechallenge values of R_L averaged 3.3 ± 1.1 $cmH_2O \cdot l^{-1} \cdot s$ and values of C_{dyn} averaged 88 ± 14 ml/cmH_2O.

Maximal changes in R_L and C_{dyn} occurred 10 min after the start of *Ascaris* antigen challenge (fig. 1). R_L increased 3.0 ± 0.4-fold at 10 min in thiopental-anesthetized dogs compared with 1.6 ± 0.2-fold in halothane-anesthetized dogs ($P < 0.05$). C_{dyn} decreased to a similar extent during

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halothane and thiopental anesthesia (0.53 ± 0.08 and 0.53 ± 0.09 times the prechallenge value, respectively).

Prior to *Ascaris* antigen challenge, arterial plasma histamine levels were similar in both groups. Plasma histamine levels averaged 0.6 ± 0.4 ng/ml in thiopental-anesthetized dogs and 1.6 ± 1.3 ng/ml in halothane-anesthetized dogs. In both groups, plasma histamine levels began increasing three minutes after the start of antigen challenge with peak concentrations detected five minutes after the start of antigen challenge. There was no significant difference in plasma histamine levels at any given time in dogs anesthetized with thiopental compared with levels in dogs anesthetized with halothane (fig. 1).

Discussion

Several mediators may play important roles in bronchospasm. Histamine is a mediator released from activated mast cells and serves as a marker for release of other mast cell mediators. In this study we examined the relationship between plasma histamine and pulmonary resistance to determine if halothane attenuated bronchospasm by inhibiting mediator release. Our results show that halothane significantly attenuated the increase in pulmonary resistance produced by antigen challenge but did not decrease histamine release induced by antigen challenge.

Mast cell granules contain several preformed mediators that are released in response to antigen bridging of surface immunoglobulin E(IgE) molecules.[15] Preformed mediators include histamine, eosinophilic chemotactic factors of anaphylaxis, and neutrophil chemotactic factors. Mast cell activation stimulates degranulation and release of these substances. In addition, mast cell activation initiates arachidonic acid metabolism at the cell membrane, which results in the generation of numerous other mediators. Mediators derived from arachidonic acid include the prostaglandins and leukotrienes. $PGF_{2\alpha}$, PGD_2, and thromboxane B_2 are bronchconstrictors, while PGE_2 and PGI_2 are bronchodilators.[16] Leukotrienes C_4, D_4, E_4, and F_4 (previously referred to as slow reacting substance of anaphylaxis) are potent bronchoconstrictors.[16]

Despite this myriad of mediators released or synthesized when mast cells are activated by exposure to an allergen, we have measured only one mediator in this study. Our reasons for choosing to measure histamine are threefold. First, histamine is well established as the marker of mast cell degranulation in *in vivo* and *in vitro* studies, since histamine release is associated with elevated serum levels of other substances contained in the mast cell granules.[17] Secondly, a sensitive and specific assay to measure plasma histamine is readily available.[13] Thirdly, assays sensitive and specific enough to measure levels of most other mediators in plasma are not available at present. Bioassays for these mediators are semiquantitative at best.

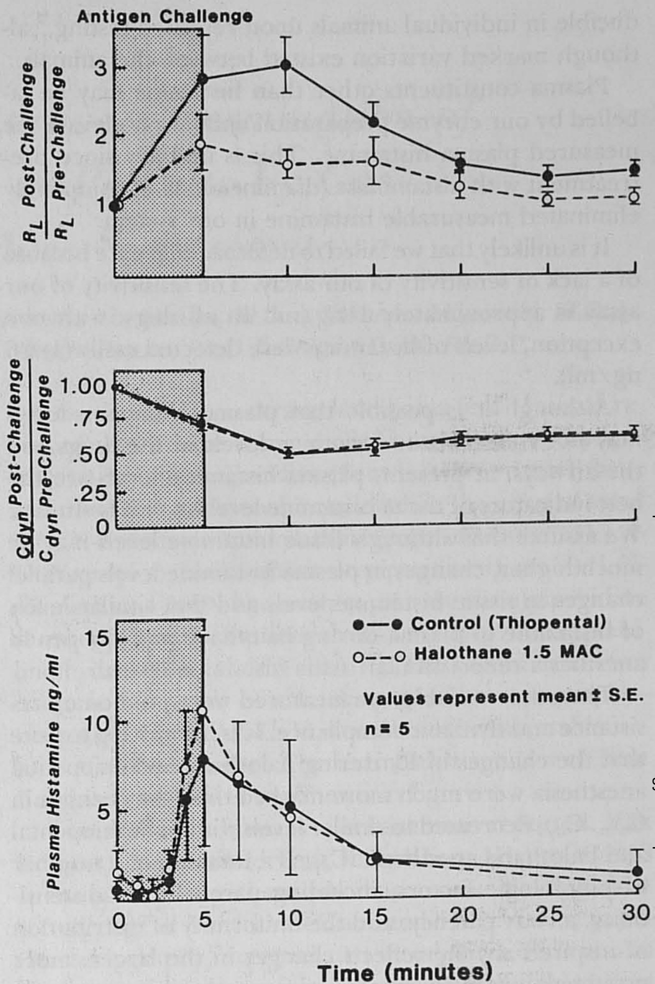

FIG. 1. Increase in R_L, decrease in C_{dyn}, and plasma histamine levels prior to and following *Ascaris* antigen challenge during thiopental and halothane anesthesia.

We found that *Ascaris* antigen-induced histamine levels were similar during thiopental and halothane anesthesia although pulmonary resistance was decreased during halothane anesthesia. A preliminary explanation of these results is that halothane anesthesia does not change mast cell activation and degranulation induced by antigen challenge compared with thiopental anesthesia. Therefore, other mechanisms during halothane anesthesia must be responsible for the attenuated airway response. Before this interpretation is accepted, several other possibilities must be considered.

It is unlikely that we failed to show a difference in histamine levels because of our small sample size. In the five animals studied, histamine levels were identical in two dogs during halothane and thiopental anesthesia, slightly less during thiopental anesthesia in one dog, and slightly greater during thiopental anesthesia in two dogs. The time course and histamine levels were very repro-

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ducible in individual animals upon repeated testing,[7] although marked variation existed between the animals.

Plasma constituents other than histamine may be labelled by our enzyme preparation and falsely elevate the measured plasma histamine. This is unlikely since pretreatment with histaminase (diamine oxidase) completely eliminated measurable histamine in our system.

It is unlikely that we failed to detect a difference because of a lack of sensitivity of our assay. The sensitivity of our assay is approximately 1 ng/ml. In all dogs, with one exception, levels of histamine were detected easily (1–26 ng/ml).

Although it is possible that plasma histamine levels may not reflect tissue histamine levels in the lungs and the airways, at present, plasma histamine levels are the best indicators of tissue histamine levels in *in vivo* studies. We assume that although tissue histamine levels may be much higher, changes in plasma histamine levels parallel changes in tissue histamine levels and that similar levels of histamine in plasma during halothane and thiopental anesthesia reflect similar tissue histamine levels.

The other variables we measured were pulmonary resistance and dynamic compliance. It is interesting to note that the changes in R_L during halothane and thiopental anesthesia were much more marked than the changes in C_{dyn}. C_{dyn} decreased to similar levels with both thiopental and halothane anesthesia. C_{dyn} is a function of a number of physiologic factors, including parenchymal distensibility, airway patency, and the uniformity of distribution of inspired air. R_L reflects changes in the larger, more proximal airways where parasympathetic innervation predominates. The attenuation of changes in R_L but not C_{dyn} is consistent with the view that the major influence of inhalational anesthetics is on the larger airways through depression of vagally mediated reflexes.

In conclusion, halothane (1.5 MAC) significantly attenuated the increase in R_L provoked by antigen aerosols compared with thiopental. However, arterial plasma histamine levels increased to a similar extent in both groups. Therefore, we conclude that the protective effect of halothane on airways is due to mechanisms other than inhibition of release of histamine from mast cells.

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