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Halothane Inhibits the Microbicidal Oxidative Activity of Pulmonary Alveolar Macrophages

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The effect of clinical concentrations of halothane on the microbicidal oxidative activity of pulmonary alveolar macrophages (PAM) was investigated. PAM oxidative activity [generation of the microbicidal oxidative intermediates hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$), and superoxide anions (O_2^-)] was assessed using luminol and lucigenin chemiluminescence (CL). Whereas luminol CL is an indicator of oxidative activity due to H_2O_2, $OH^{\cdot}$, or O_2^-, lucigenin CL provides an ultrasensitive measurement of O_2^- generation. The use of both chemoluminigenic probes thus enables a detailed analysis of PAM oxidative function. Exposure of PAM to 3, 2, and 1% halothane vaporized in air significantly inhibited both luminol (23–46%) and lucigenin (30–51%) CL responses, $P < 0.01$. Halothane-treated PAM exposed to air recovered to the extent that their luminol CL responses were significantly greater than control (no halothane) experiments. Lucigenin reaction mixtures given halothane then air showed less inhibition than PAM treated with halothane only. These results suggest that 1) the generation of O_2^- and to a lesser extent other oxidative metabolites are decreased following halothane exposure, and 2) this inhibition is reversible. (Key words: Anesthetics, volatile: halothane. Lung: pulmonary macrophages; microbicidal activity.)

PULMONARY ALVEOLAR MACROPHAGES (PAM) are the first phagocytic cells to encounter airborne pathogenic microorganisms. Following inhalation such organisms are engulfed rapidly and killed by PAM.[1,2] The mechanism(s) responsible for this microbicidal activity in PAM is dependent in part on the production and interaction of highly reactive oxygen species such as superoxide anions (O_2^-).[3–6] That such oxidative species are critical to host defenses is documented in patients whose PAM are unable to generate O_2^-. These patients are highly susceptible to a variety of pulmonary infections.[7,8]

Halothane, a frequently used inhalational anesthetic, recently has been shown to significantly depress *in vivo* lung bactericidal activity.[9] Because no effect of halothane was seen on the physical clearance of bacteria by ciliated respiratory epithelial cells,[10] the reduced bacterial killing was ascribed to altered PAM function.[9] The present study has investigated the effect of halothane on the oxidative microbicidal activity of PAM. The results suggest that the generation of microbicidal oxidative species is reversibly inhibited by clinically relevant concentrations of halothane.

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Materials and Methods

ISOLATION OF PAM

PAM were isolated from Sprague-Dawley rats weighing 200 to 250 g immediately after intravenous administration of 2 mg succinylcholine. PAM were obtained in average yields of 1 to 2×10^7 cells per animal by bronchial lavage using 30 ml of normal saline with 10 units per milliliter of heparin and adjusted to the desired concentration in Hanks buffer, *p*H 7.4, as described previously.[11] The lavage fluid contained 60 to 80% mature macrophages, 15 to 35% immature macrophages, and less than 2% neutrophils as determined by microscopy of Wright-stained smears. Trypan blue exclusion revealed a greater than 98% viability of the lavagate cells.

ANALYSIS OF PAM OXIDATIVE ACTIVITY BY LUMINOL AND LUCIGENIN CHEMILUMINESCENCE (CL)

Luminol (5-amino-2, 3-dihydro-1, 4-phtalazinedione, Sigma Chemical Co., St. Louis, Missouri) enhanced CL is a highly sensitive indicator of overall oxidative activity of alveolar macrophages,[12,13] hydrogen peroxide (H_2O_2), O_2^-, and hydroxyl radicals ($OH^{\cdot}$), and correlates with bactericidal function.[14,15] In order to determine more specifically the effect of halothane exposure on PAM oxidative activity, another chemoluminigenic probe, lucigenin (10,10′-dimethyl-9,9′biacridinium dinitrate, Sigma) was used also. Lucigenin has been shown to be a sensitive indicator of O_2^- generation by PAM where nanomolar quantities of superoxide dismutase inhibit the lucigenin CL response.[16]

The CL reaction mixture consisted of 1×10^5 PAM, 400 mM luminol or lucigenin, and Hanks buffer in total volume of 2 ml. CL was initiated with 1 nM (final concentration) phorbol myristate acetate (Sigma). The CL assay was performed exactly as described previously.[17] The effect of halothane on the luminol or lucigenin CL response was determined by calculating the per cent inhibition of the air exposed CL response as follows:

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$$\text{Per cent inhibition of air exposed CL (luminol or lucigenin)} = \frac{\text{Integrated area from air exposed luminol or lucigenin CL} - \text{Integrated area from halothane exposed luminol or lucigenin CL}}{\text{Integrated area from air exposed luminol or lucigenin CL response}} \times 100$$

The emission spectrum of luminol and lucigenin CL falls within the range of 350 to 600 nm.[18,19] Halothane is a clear colorless liquid that was not found to absorb light in this wavelength range. Thus, the lowered CL responses observed following halothane exposure are probably not the result of a quenching of light emission from oxidized luminol or lucigenin.

Exposure of CL Reaction Mixtures to Halothane

The CL reaction mixtures were exposed to halothane exactly as described previously[17,20] using an air-tight chamber and air-vaporized halothane. Halothane concentrations were monitored at the chamber exit port using a Hewlett Packard® 5730 A gas chromatograph (50/80 poropak Q glass column) with a 3380S integrator. As shown previously[20] the air–halothane mixture did not significantly alter the *p*H, P_{CO_2}, or P_{O_2} of the Hanks buffer as compared with buffer exposed to air only.

Determination of the Partition between Halothane and Hanks Buffer

The partition coefficient between halothane and Hanks buffer was determined previously at this laboratory and found to be 1.74 ± 0.2 at 24° C.[20]

Statistical Analysis

Means, standard deviations, and one-way analysis of variance were determined in experimental studies as indicated.

Results

Effect of Halothane on the Luminol CL Response of PAM

The effect of a 60-min exposure of air-vaporized halothane in concentrations from 0.5 to 3% on PAM luminol CL is shown in figure 1. Significant inhibition of luminol CL was seen with 3, 2, and 1% halothane, with 46, 40, and 23% inhibition, respectively ($P < 0.01$). A 30-min air exposure after halothane treatment resulted in a luminol CL response greater than control experiments (no halothane, air exposure only). This stimulatory CL response was highest with 0.5% halothane (43% greater than control, $P < 0.001$) and lowest with 3% halothane (12% greater than control, $P < 0.01$).

Effect of Halothane on the Lucigenin CL Response of PAM

The effect of a 60-min halothane exposure on PAM lucigenin CL is shown in figure 2. Significant inhibition of CL was seen with 3, 2, and 1% halothane with 51, 44, and 30% inhibition, respectively ($P < 0.01$). A 30-min air exposure after halothane treatment resulted in significantly less inhibition at all halothane concentrations.

Discussion

PAM are an integral part of the lung's defenses against pathogenic microorganisms. The killing of potentially harmful microorganisms by PAM is achieved in part by the generation of highly reactive and microbicidal oxygen species such as $OH^{\cdot}$, H_2O_2, and O_2^{-}.[3,6] These oxidative metabolites may interact with themselves or non-oxidative microbicidal systems like lysozyme ultimately resulting in efficient killing of the mi-

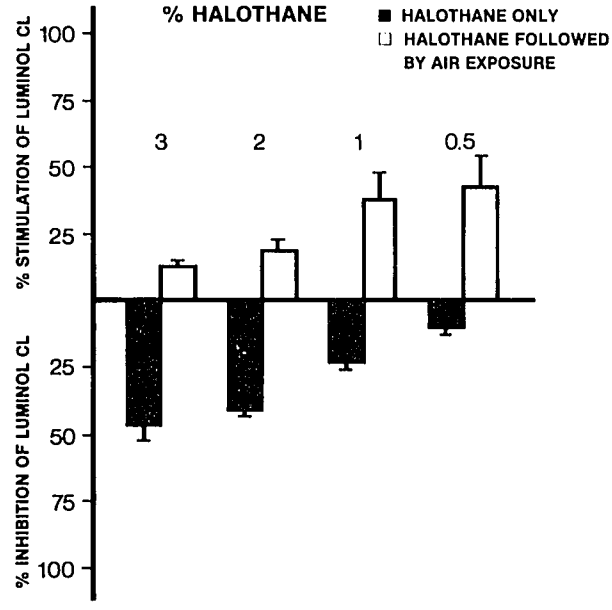

FIG. 1. Effect of a 60-min exposure of halothane on PAM luminol CL. PAM CL responses following treatment with 3, 2, and 1% halothane were significantly different from control (no halothane, air exposure only) luminol CL responses, $P < 0.01$, one-way analysis of variance (ANOVA). Luminol CL responses after halothane then air exposure were significantly higher than control CL responses at all halothane concentrations, $P < 0.01$, ANOVA.

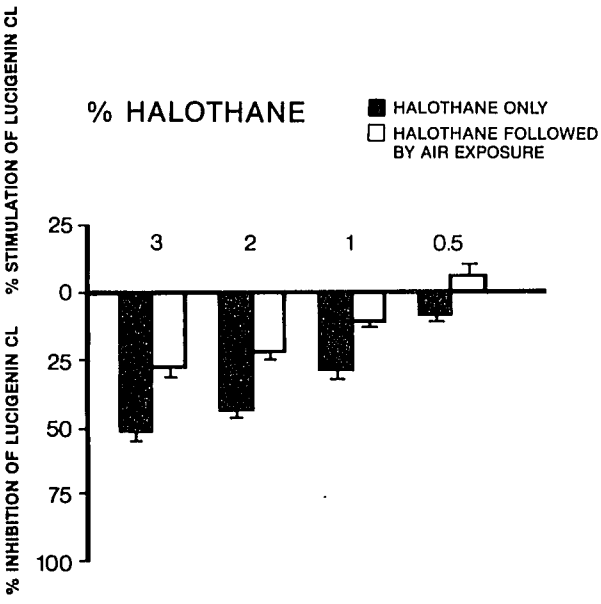

FIG. 2. Effect of a 60-min exposure of halothane on PAM lucigenin CL. PAM CL responses following treatment with 3, 2, and 1% halothane were significantly different from control (no halothane, air exposure only) lucigenin CL responses, $P < 0.01$, ANOVA. Lucigenin CL responses after halothane then air exposure had significantly less inhibition than halothane treated reaction mixtures, $P < 0.01$, ANOVA.

croorganisms.[3,6,21] An impairment of these destructive processes in PAM by frequently used inhalational anesthetics is thus of obvious importance.

In the current study, luminol and lucigenin were used as chemoluminigenic probes to analyze the oxidative activity of PAM following halothane exposure. Whereas luminol CL is indicative of PAM generation of H_2O_2, $OH^{\cdot}$, and O_2^-,[12,13] lucigenin CL is primarily and perhaps only a sensitive indicator of O_2^- production.[16] O_2^- are necessary for the final production of $OH^{\cdot}$, H_2O_2, and other reactive oxidative microbicidal intermediates such as hypohalite ions.[5,22] Luminol CL therefore can provide an overview of oxidative processes with lucigenin CL enabling a partial dissection of the oxidative response.

The contribution of O_2^- to luminol Cl is large, as exemplified in the low luminol CL responses in cells from patients who cannot generate O_2^-.[23] Both luminol and lucigenin CL responses were found to be inhibited in a dose-dependent manner by halothane. The decrease in luminol CL seen with increasing halothane concentrations thus may be explained partly by reduced O_2^- production. The mechanism for this lowered oxidative activity in PAM following halothane exposure may result from impaired protein mobility in the PAM plasma membrane. Such mobility may be necessary for the activitation of plasma membrane bound oxidative enzymes that generate O_2^- (NADPH:O_2 oxidoreductase). It is also possible that the NADPH:O_2 oxidoreductase is inactivated directly by halothane interaction.

The concentration of air-vaporized halothane required to significantly depress CL in the present study was 1%. The minimal alveolar concentration (MAC) of halothane is 0.77%[24] with 1% halothane being equal to about 1.3 MAC. In consideration of the 1.74 partition coefficient between halothane and Hanks buffer, in which the PAM were suspended, the volume per cent halothane in the CL reaction mixture would be 1.74%. Interestingly, exposure of PAM to 0.5 to 3% halothane and then air for 30 minutes resulted in a luminol CL response which was greater than control (no halothane, air only) experiments. A similar result has been reported with N_2O-halothane and human neutrophils.[17] The explanation proposed to explain this increase in oxidative activity (as measured by luminol CL) was a pertubation of the neutrophil plasma membrane elicited by removal of the anesthetic mixture.[17] A similar mechanism also may be operative in the luminol CL response of halothane exposed PAM. These results suggest that 1) the enhanced luminol CL response is due primarily to oxidative metabolites other than O_2^- or 2), the removal of halothane from PAM plasma membranes may not be a significant stimulus for O_2^- generation.

Exposure of mice to 1 and 2 MAC of halothane for four hours, followed by a one-hour recovery and challenge with aerosols of *Staphlycoccus aureus* was shown recently to significantly depress lung *in situ* bactericidal activity.[9] In a somewhat similar study by Goldstein *et al.*,[25] no inhibition of lung *in situ* bactericidal activity was seen when mice were challenged with bacteria and then given halothane. In both reports no depression of physical mechanisms of bacterial removal in the lungs via ciliated epithelial cells was seen. The results of the current study provide support for the former work[9] in that a depression of microbicidal activity followed halothane exposure and was not as evident if the anesthetic treated cells were given air.

In conclusion, these results suggest that PAM microbicidal oxidative activity may be inhibited by clinically relevant concentrations of halothane. However, this inhibition is temporal and may only occur during anesthetic exposure.

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