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Effects of Halothane, Enflurane, and Isoflurane on Hypoxic Pulmonary Vasoconstriction in Rat Lungs In Vitro

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Rat lungs were ventilated and perfused at a constant rate *in vitro*. The maximal hypoxic pulmonary vasoconstrictor (HPV) response was recorded by measuring the pulmonary artery pressure change when the inspired oxygen concentration was changed from 21% to 3% (with 5.5% carbon dioxide) in the absence of anesthetic vapor.

In different experimental groups, the effects of halothane, enflurane, and isoflurane on HPV were examined. In random order the anesthetics were added to the inspired gas in concentrations of 0.25, 0.5, 1, 1.5, and 2 or 2.5 MAC units. The HPV pressor response to 3% oxygen in the presence of anesthetic agent was expressed as a per cent of the pressure response observed in the absence of anesthetic (R%MAX).

All three agents depressed HPV in a dose-related manner. The concentrations in MAC units at which 50% depression of HPV (ED_{50}) occurred was 0.47, 0.60, and 0.56 for halothane, isoflurane, and enflurane, respectively, and neither the ED_{50} values nor the slopes of these dose response curves were significantly different.

It was concluded that these halogenated general anesthetics inhibit HPV with essentially the same potency. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Hypoxia. Lung: blood flow; vascular resistance.)

HYPOXIC PULMONARY VASOCONSTRICTION (HPV) is a homeostatic mechanism maintaining blood arterial oxygen tension. Abolition of HPV by anesthetic agents has been suggested as a cause of hypoxemia during anesthesia.[1,2] Early studies demonstrated that inhalational agents were inhibitory while injectable agents were not.[3,4] Subsequent reports from *in vivo* studies have suggested that this property of inhalational agents may differ markedly, even among the commonly used halogenated agents.[5-8]

In the present study, the effects of halothane, enflurane, and isoflurane on HPV were compared when the lung conditions were maintained constant *in vitro*.

Methods

The perfused and ventilated rat lung preparation has been described previously[9] and was modified after the method of Hauge.[10] The procedure is summarized below.

Adult, female, Wistar rats (334 ± 9 g; mean ± SE) were anesthetized with 30 mg/kg pentobarbital, intraperitoneally. A tracheostomy was performed and the animals mechanically ventilated. The chest was incised in the midline and the ribs were retracted exposing the heart and lungs. Heparin (200 IU) was injected into the left ventricle. The right ventricle was incised and a T-cannula secured in the pulmonary artery (PA). One inlet to the T delivered perfusate to the lung while the other was connected to a Statham® P 23 transducer to measure the pulmonary artery pressure. Another catheter was inserted into the left ventricle allowing perfusate to return to the reservoir. The lungs and heart were removed from the chest cavity, and were suspended by the tracheal cannula, in a closed, humidified chamber. Perfusate flow was increased to 0.06 $ml \cdot g^{-1}$ body weight $\cdot min^{-1}$ with the mean pulmonary artery pressure at approximately 15–20 cmH_2O. Temperature probes were placed in the perfusate reservoir and the lung chamber.

For the perfusate, 50 ml of blood were obtained from donor rats by means of cardiac puncture. To this was added 1,000 IU of heparin and 10 mg oxycillin. The blood was filtered through a Fenwal PDF-20 micropore filter to remove microaggregates and small clots and was combined with an equal amount of physiologic saline-albumin solution (based on Hansen and Bohr[11]). Previous studies had demonstrated that this mixture consistently provides reproducible HPV responses for periods of at least four hours without formation of edema or deterioration of the responses. The *p*H was measured continuously and adjusted with sodium bicarbonate to maintain a *p*H close to 7.35 units throughout the experiment.

The perfusate was circulated by a Harvard® peristaltic pump from the reservoir to the pulmonary artery and returned to the reservoir again by gravity with the outlet of the left ventricle's cannula 1 cm below the ventricle so that the pressure in the left atrium was zero.

The rat lungs were ventilated with humidified, premixed gases by a Harvard rodent ventilator at a volume of 180 ml/min. The expired gases passed through a mixing chamber with 2 cmH_2O of positive end-expiratory pressure. The lungs therefore were ventilated at more than 10 times the rate at which they were perfused so that the perfusate oxygen tension had little effect on the alveolar oxygen tension.

Inspired and expired gas samples were drawn through oxygen (IL 407) and carbon dioxide (Goddart Capno-

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graph) analyzers and were returned to the appropriate circuits. Constancy of the airway pressure, measured by a Statham transducer, indicated absence of pulmonary edema. Measurements were continuously recorded on a six-channel Grass® Polygraph.

Study Design

All gas mixtures identified below contained carbon dioxide ($F_{I_{CO_2}}$ = 0.055). The lungs were ventilated for 30 min with an $F_{I_{O_2}}$ of 0.21 to obtain a steady baseline pulmonary artery pressure and were then exposed to an hypoxic mixture ($F_{I_{O_2}}$ = 0.03) a number of times to ensure an equal HPV responsiveness before continuing.

The 18 rat lung preparations were divided into three groups, with one group receiving halothane, another enflurane, and the third isoflurane. MAC for the rat was assumed to be 1.1% for halothane, 2.2% for enflurane, and 1.4% for isoflurane.[12] A Drager® vaporizer in the gas delivery line was adjusted to deliver 0.25, 0.5, 1.0, 1.5, and 2 or 2.5 MAC concentrations of anesthetic, in random order. The delivered concentrations were continuously monitored with a mass spectrometer (Perkin-Elmer® MGA 1100).

Preliminary experiments demonstrated that equilibration of the anesthetic in the perfusate was obtained within 10 min. The lungs therefore were ventilated with the normoxic gas mixture, containing the selected concentrations of anesthetic, for 10 min, the ventilatory gas was then changed to the hypoxic ($F_{I_{O_2}}$ = 0.03) gas mixture which contained the same concentration of anesthetic vapor. The hypoxic stimulus was maintained for six minutes and the pulmonary artery pressure change, airway pressure, and perfusate and inspired (alveolar) anesthetic concentrations were measured. The normoxic gas, containing a different concentration of anesthetic vapor, was restored and the cycle of normoxic/hypoxic exposures repeated for all the MAC concentrations of the agent. The vaporizer was turned off and a final hypoxic response measured after elimination of the anesthetic vapor. To ensure equilibrium and to allow the measurement of the perfusate gas solubility coefficient, during each sequence one of the normoxic gas exposures was prolonged to 20 min and samples of perfusate were obtained at 10 and 20 min. The concentrations of anesthetic contained in the perfusate were measured chromatographically and the calculation of solubility and anesthetic partial pressure followed the method of Butler *et al.*[13]

The relationship between the response and anesthetic dose was analyzed by regression analysis and the curves compared by analysis of covariance. Other statistical tests are identified in the text. The null hypothesis was rejected for $P < 0.05$. The data are shown as mean ± SE.

Results

Lungs from 18 rats were tested. The general conditions did not differ for the three inhalational agents and the data therefore have been combined as follows: *p*H 7.36 ± 0.01; temperature = 36.6 ± 0.1°C; perfusate hematocrit = 18.5 ± 0.6%; and mean airway pressure 3.7 ± 0.1 cmH_2O.

The measurements of perfusate anesthetic partial pressure at 10 and 20 min did not differ significantly (paired *t* test). The perfusate/gas solubility coefficients for the anesthetic agents were 1.73 ± 0.13 for halothane, 1.21 ± 0.13 for enflurane, and 1.13 ± 0.08 for isoflurane. The pulmonary artery pressure response to the hypoxic stimulus was maximal in the absence of anesthetic, and the hypoxic responses in the presence of anesthetic were expressed as a per cent of this maximum (R%MAX). The individual responses with changing anesthetic partial pressure in the perfusate are shown in figure 1, panels "a", for halothane isoflurane and for enflurane. Also shown in figure 1, panels "b", are the dose-response relations calculated by linear regression utilizing Wagner's equation[14] for sigmoid curves. The 95% population confidence intervals are shown as dashed lines. The slopes of these lines do not differ, but the partial pressure of the anesthetics causing a 50% depression of the response (ED_{50}) are 3.83 and 5.96 mmHg for halothane and isoflurane, respectively, and these are significantly ($P < 0.05$) different from the 8.79 mmHg derived for enflurane.

However, in figure 2, these same curves are replotted in sigmoid form after conversion of the abscissa from partial pressure to MAC values. The ED_{50} values in MAC units are 0.47, 0.60, and 0.56 for halothane, isoflurane, and enflurane, respectively, and neither the slopes nor the ED_{50} MAC values differ significantly.

Discussion

This comparison of the effect of halothane, enflurane, and isoflurane on HPV has demonstrated that all three are inhibitory and when the doses are expressed in MAC units, the dose-response curves are essentially the same with an ED_{50} of approximately 0.5 MAC units. The effect of the inhalational agents on HPV therefore parallels their anesthetic potency and does not support the concept that there are differences in their specific actions on HPV.

The sensitivity of the *in vitro* model to hypoxia and anesthetic agents may differ from the *in vivo* situation, but it is unlikely that the response in the rat is fundamentally different from that of other species.[15] Previous work with animal models *in vivo* and *in vitro* generally have demonstrated inhibition of HPV by inhalational agents, while injectable agents have not had this effect.

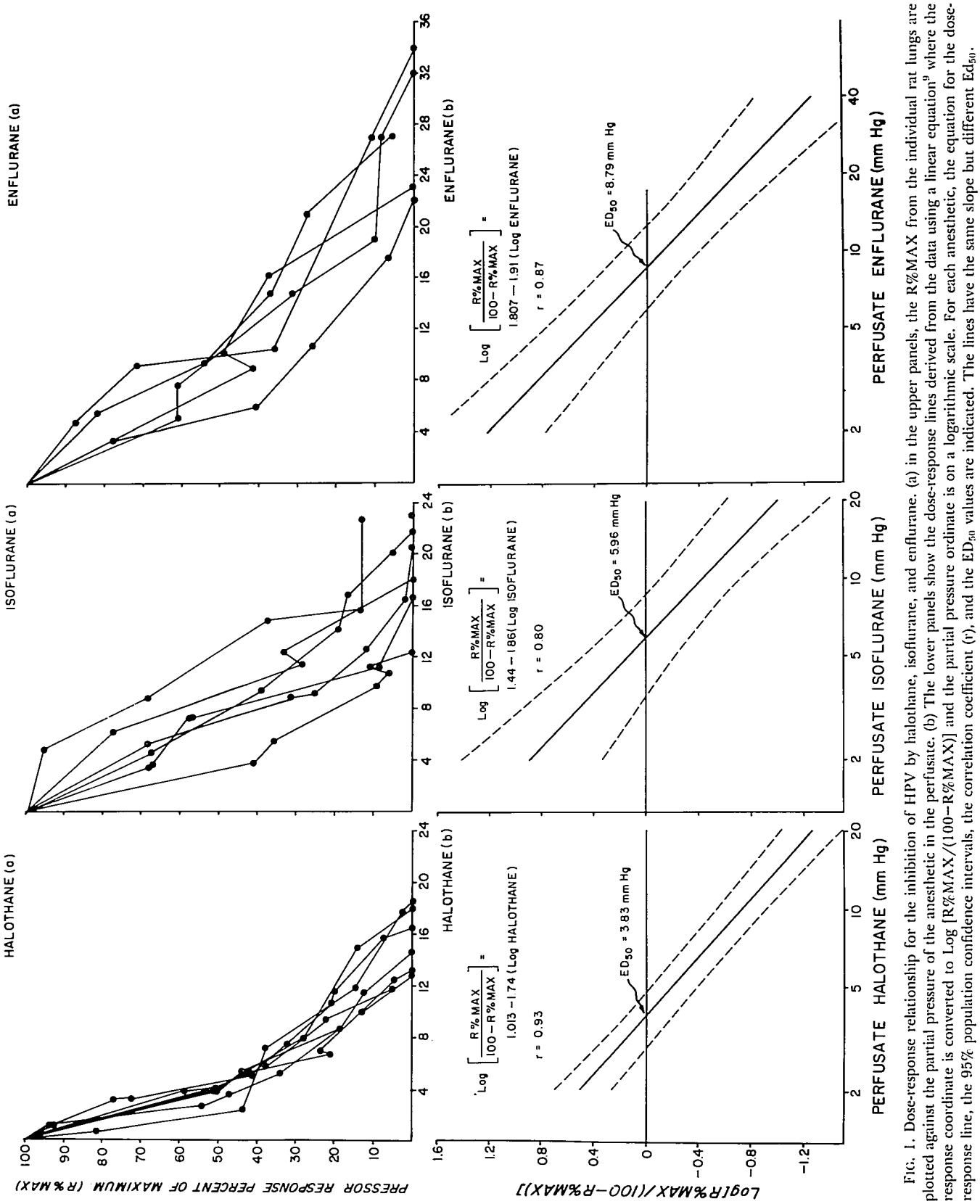

FIG. 1. Dose-response relationship for the inhibition of HPV by halothane, isoflurane, and enflurane. (a) in the upper panels, the R%MAX from the individual rat lungs are plotted against the partial pressure of the anesthetic in the perfusate. (b) The lower panels show the dose-response lines derived from the data using a linear equation[9] where the response coordinate is converted to Log [R%MAX/(100−R%MAX)] and the partial pressure ordinate is on a logarithmic scale. For each anesthetic, the equation for the dose-response line, the 95% population confidence intervals, the correlation coefficient (r), and the ED_{50} values are indicated. The lines have the same slope but different Ed_{50}.

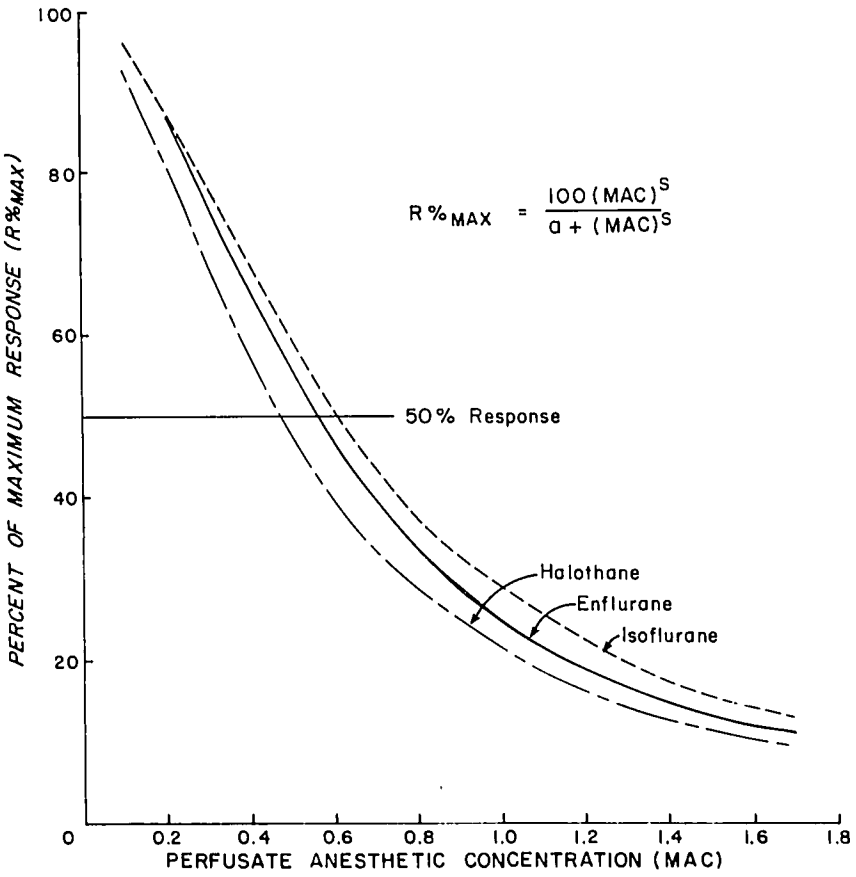

FIG. 2. Combined dose-response curves for inhibition of HPV by halothane, isoflurane, and enflurane. The regression equations derived in figure 1 for each anesthetic agent were used to generate these curves relating the R%MAX response to the concentration of the anesthetic agent expressed in MAC units. The sigmoid form of Wagner's equation is shown and for halothane, isoflurane, and enflurane, respectively, the values for "a" are 3.035, 2.535, and 3.697 and for "s" are −1.914, −1.857, and −1.737. The curves do not differ significantly in slope or ED_{50} MAC and for each, $r > 0.82$.

Several of the *in vivo* studies have observed a greater inhibition with some inhalational agents than with others, but for a particular anesthetic in animals and in humans the results have been contradictory both between different laboratories[4,16,17] and for the same laboratory at different times.[4,6]

However, the measured outcome of HPV *in vivo*, whether calculated as pressure, flow, or shunt changes, is modified by secondary variables, particularly hemodynamic and pulmonary mechanical factors.[10] We hypothesize, therefore, that the inhalational anesthetics have an inhibitory action on HPV probably exerted directly on the pulmonary vascular smooth muscle[18] but that this action may be obscured *in vivo* by the influence of secondary variables altered by the anesthetic administration,[19] posture,[20] or surgical manipulation.[21]

As part of a systematic examination of this hypothesis, the present studies confirm the basic similarity of the action of inhalational agents on HPV, when secondary variables are controlled.

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