

The Actions of General Anesthetic Agents on Tracheal Smooth Muscle

S. W. Fletcher, M.D.,* W. Flacke, M.D.,† M. H. Alper, M.D.‡

The effects of three general anesthetic agents, halothane, diethyl ether, and thiopental, were studied in the guinea-pig tracheal-chain preparation. All three agents by themselves caused relaxation of the chain. In addition, they antagonized the ability of acetylcholine to cause contraction of the chain. The antagonism appears to be nonspecific.

GENERAL ANESTHETIC AGENTS may affect the motor activity of tracheobronchial smooth muscle through effects on afferent receptors, central regulatory mechanisms, autonomic ganglia, events at the neuroeffector junction, or direct effects on the muscle itself.¹ The only extensive study of the direct actions of general anesthetics on isolated tracheobronchial smooth muscle has been that reported by Adriani and Rovenstine in 1943.² Their study, however, was limited to visual observation through a low-power microscope of the responses of isolated bronchial rings to a single concentration of each anesthetic agent.

In 1947, Castillo and deBeer³ described a technique for the quantitative assessment of the effect of drugs on tracheal muscle, utilizing a chain of guinea-pig tracheal rings suspended in an organ bath. This preparation has been used successfully by many investigators in the study of bronchoconstrictor and bronchodilator drugs, in many species.⁴ In the present study, we have used this preparation to observe the direct effects of halothane (Fluothane), diethyl ether and thiopental (Pentothal) on the guinea-pig tracheal chain,

and also to study the antagonism between these drugs and two bronchoconstrictors, acetylcholine and histamine.

Methods

The guinea pig tracheal chain was prepared as described by Castillo and deBeer.³ After the guinea pig was killed by a blow on the head, the trachea was removed and sectioned into rings. A chain of ten rings held together in series by short loops of cotton thread was prepared. The preparation was then suspended in a 10-ml. organ bath at 38° C. and bathed with Krebs-Henseleit solution of the following composition: NaCl 6.89 Gm./l., KCl 0.352 Gm./l., CaCl₂ (anhydrous) 0.143 Gm./l., NaHCO₃ 2.1 Gm./l., glucose 2.0 Gm./l. The bath was oxygenated with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide flowing through a fritted glass filter at the bottom of the bath. The tracheal chain was attached at its upper end to an isotonic frontal lever with 1.2 Gm. tension and ×20 magnification. The responses of the chain to the various drugs were recorded on smoked paper on a kymograph drum moving at a speed of 2 mm./minute. Fresh preparations were used for each experiment and the chains were allowed to equilibrate in the bath for at least 45 minutes before any drugs were introduced. The preparations remained viable and yielded reproducible results for as long as eight hours.

Acetylcholine, sodium thiopental, and histamine were added directly to the bath in known amounts. The volatile anesthetics, halothane and diethyl ether, were vaporized, and their concentrations controlled in the following manner. A vaporizer was constructed from a glass sealing tube (Pyrex® 39580) with a fritted glass filter mounted in the center. The liquid anesthetic was drawn into a gas-tight syringe (Hamilton GS 1001 or 1002) and dripped onto the filter at a fixed rate by

* This work was performed while Dr. Fletcher was a fourth-year student at Harvard Medical School.

† Assistant Professor of Pharmacology.

‡ Assistant Clinical Professor of Anesthesia.

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FIG. 1. Dose-response curve for Ach in the guinea-pig tracheal chain. The ordinate is the contractile response expressed as per cent of maximum response. Each point represents the mean \pm standard error of 32 preparations.

an infusion pump (Harvard Apparatus Co.). The anesthetic was then vaporized by the oxygen/carbon dioxide mixture as it traversed the glass filter. By controlling the rate of gas flow and the speed of the infusion pump, it was possible to deliver constant, reproducible concentrations of the anesthetics to the organ

bath. It was found that the bath solution equilibrated with a given concentration of anesthetic within five minutes and the concentration remained constant thereafter as the gas mixture was bubbled through the bath.

In each experiment, the concentration of halothane or ether in the bath solution was measured by gas chromatography according to a method previously described.⁵ Briefly, samples from the bath were extracted with *n*-heptane and aliquots of the extract analyzed in an F and M Model 720 gas chromatograph equipped with a Tide[®] column and a thermal-conductivity detector cell. A calibration curve was constructed daily, using known amounts of the unknowns.

In the experiments on the effects of the anesthetics alone, the chain was exposed to a given concentration of the agent for ten minutes, at which time the response was maximal. The bath was then washed twice with fresh

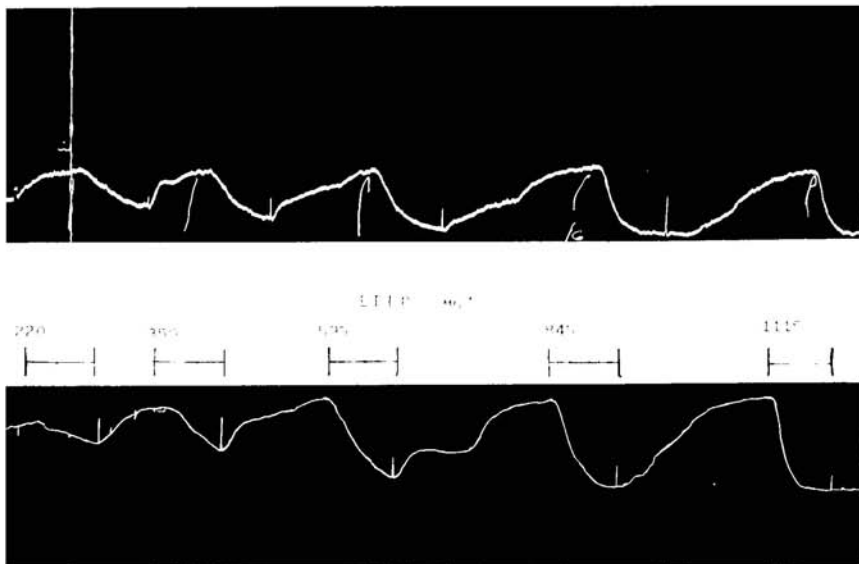


FIG. 2. Effect of ether on the guinea-pig tracheal chain. Two chains were observed simultaneously and exposed to each concentration of ether for 10 minutes followed by a double washing. Note the complete recovery of the chain after each exposure and washing. Downward deflection of the lever represents relaxation of the chain.

Krebs-Henseleit solution, with prompt recovery of the preparation.

In the experiments with acetylcholine, a dose-response curve was first determined with the chain exposed to each concentration of acetylcholine for five minutes, followed by a double wash. The effect at five minutes was near maximum and therefore was selected arbitrarily as the response to a given concentration. To study the interaction of the anesthetics and acetylcholine, the anesthetic was administered for ten minutes to elicit a maximal response. The test dose of acetylcholine was then added to the bath and the response after five minutes was recorded. The bath was then washed twice and the next set of observations was made. In any single preparation about 20 such determinations were made. At the end of each experiment the dose-response curve to acetylcholine was repeated to ensure the validity of the observations. The data thus obtained were analyzed as described in the section of results.

Results

ACETYLCHOLINE

The addition of acetylcholine to the organ bath resulted in contraction of the tracheal chain. A dose-response curve was constructed from 32 preparations (fig. 1). The preparation was very sensitive to acetylcholine, with a threshold response at a concentration of 10^{-14} M and a maximal contraction at 10^{-9} M. The ED_{50} of acetylcholine was 5.5×10^{-12} M.

ANESTHETICS

In eight experiments with each of the three anesthetics, halothane, ether, and thiopental by themselves all caused relaxation of the tracheal chain. A typical experiment with ether is shown in figure 2. The lowest concentration of ether which caused a discernible relaxation of the chain was approximately 200 mg./100 ml., that of halothane 75 mg./100 ml., and that of thiopental 60 mg./100 ml. In no experiment did any of the anesthetics cause contraction of the tracheal chain.

ANESTHETICS AND ACETYLCHOLINE-INDUCED CONTRACTION

Once it had been determined that no qualitative difference could be observed among the

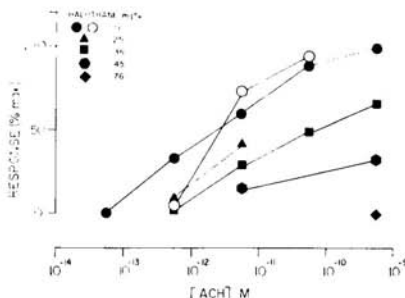


FIG. 3. Response of tracheal chain to Ach at various concentrations of halothane. Closed circles: control dose-response curve to Ach. Open circles: response to Ach upon termination of exposure to halothane. Results of a single experiment (see text).

anesthetics as such in their effects on the tracheal chain, we proceeded to investigate their effects on contraction induced by acetylcholine.

HALOTHANE

Figure 3 depicts the results of a typical experiment dealing with the interaction of acetylcholine and halothane. With increasing concentrations of halothane in the bath, the response curve to acetylcholine was shifted progressively to the right and the slope depressed. Note also the virtually complete recovery of the response of the tracheal chain to acetylcholine at the end of the experiment (open circles).

In order to analyze the results of all of the experiments, the data for each anesthetic were handled in the following manner. (A detailed presentation of the results is included only for halothane.) As a first step, all of the responses to each of four concentrations of acetylcholine (given in table 1) were plotted (as per cent of control response) against the concentration of anesthetic on a log-probit scale. Figure 4 depicts such a plot of the responses to a concentration of 5.5×10^{-12} M acetylcholine (control ED_{50}) with various concentrations of halothane in the organ bath. Antagonism to acetylcholine first was observed at a halothane concentration of about 10 mg./100 ml., a concentration well below the lowest concentration of halothane (75 mg./100 ml.) that by itself

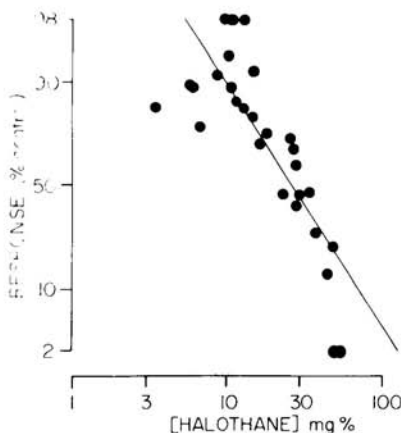


FIG. 4. Log-probit plot of response to 5.5×10^{-12} M acetylcholine at various concentrations of halothane with line of regression drawn in. Calculated ED_{50} of halothane is 25 mg./100 ml. (see text).

caused relaxation of the chain. Complete abolition of the response was seen at a concentration of somewhat less than 100 mg./100 ml. The concentration of halothane which resulted in a 50 per cent inhibition of the response to this dose of acetylcholine was 25 mg./100 ml., as calculated from the regression line in the figure.

The results of identical calculations for all doses of acetylcholine in 16 experiments are shown in table 1 and in figure 5. It is apparent that increasing concentrations of halothane were required to reduce by 50 per cent the responses of the tracheal chain to increasing concentrations of acetylcholine.

TABLE 1.

Concentration of Acetylcholine (M)	Control Response (% of Maximum)	ED_{50} of Halothane* (mg./100 ml.)
5.5×10^{-13}	19	19.5
5.5×10^{-12}	48	25
5.5×10^{-11}	84	30
5.5×10^{-10}	99	33

* Concentration of halothane which reduced the response to a given concentration of ACh by 50 per cent (calculated from line of regression, fig. 4). Figure 5 is a graphic representation of the data in this table.

DIETHYL ETHER

In eight experiments ether also antagonized the response of the chain to acetylcholine, but not until it reached a threshold concentration of about 200 mg./100 ml. This was also the lowest concentration of ether that by itself caused relaxation of the chain. Complete abolition of the response to acetylcholine was observed at an ether concentration of 700 mg./100 ml. Figure 6 depicts graphically the concentrations of ether required to reduce by 50 per cent the responses to the four test concentrations of acetylcholine. Again, there is a slight increase in the ED_{50} of ether with increasing concentrations of acetylcholine, with a range of 350 to 450 mg./100 ml. ether in the bath.

SODIUM THIOPENTAL

Like halothane and ether, thiopental antagonized acetylcholine-induced contraction of the guinea-pig tracheal chain in eight experiments. First apparent at concentrations of thiopental as low as 6 mg./100 ml., there was complete abolition of the response at a concentration of 60 mg./100 ml. Thiopental in a concentration of 45 mg./100 ml. resulted in a 50 per cent

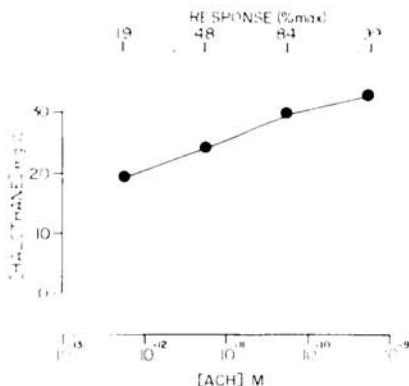


FIG. 5. ED_{50} of halothane (concentration required to reduce response to given concentration of ACh by 50 per cent) plotted against concentration of ACh (lower scale) and control response to ACh (top scale). The regression was significant at the 95 per cent probability level for all four points. These data are summarized in table 1. See text for further explanation.

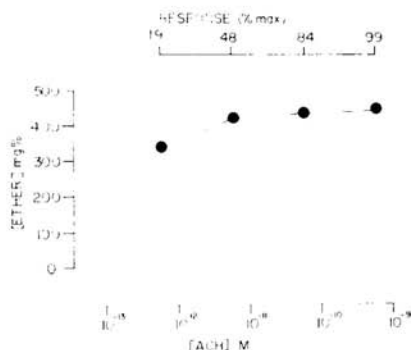


FIG. 6. Antagonism of ACh by diethyl ether. See legend for figure 5. Regression was significant at 95 per cent level for 5.5×10^{-12} M and 5.5×10^{-11} M ACh.

reduction of the contractile response to the ED_{50} of acetylcholine. Figure 7 demonstrates that, as with halothane and ether, the antagonism is concentration-dependent, higher concentrations of thiopental being required to produce the same degree of antagonism as the concentration of acetylcholine increases.

HISTAMINE AND PROPRANOLOL

In three experiments we observed that halothane in the same range of concentrations that antagonized acetylcholine also antagonized histamine-induced contraction of the tracheal chain. In two additional experiments the ability of halothane to relax the tracheal smooth muscle was not affected by the prior addition of the sympathetic beta-blocking drug, propranolol, in a concentration of 10 mg./ml., a concentration which completely abolished the response of the chain to isoproterenol (10^{-7} M).

Discussion

In these experiments with the guinea-pig tracheal chain, halothane, ether and thiopental by themselves, at all concentrations, produced relaxation of the chain. These results thus agree only in part with observations of Adriani and Rovenstine² with regard to ether. They found bronchodilation at one concentration with ether (1:10,000), but at a higher concentration (1:1,000) ether produced bronchoconstriction which was enhanced by physostigmine, pre-

sumably acting by preventing the hydrolysis of acetylcholine. In the case of thiopental, Adriani and Rovenstine reported a prompt bronchoconstriction, prevented by atropine and enhanced by physostigmine. In our experiments, we observed only an antagonism between acetylcholine and thiopental over a wide range of concentrations. These differences may be related to species differences, since Adriani and Rovenstine used preparations from rat, dog and man, and also to the major differences in techniques of experimentation.

Klide, in 1966,⁶ observed a decrease in airway resistance of the dog's lung during halothane anesthesia, which he ascribed to stimulation by halothane of beta receptors in the tracheobronchial smooth muscle. Our preliminary results, in the guinea pig, do not support this conclusion, since the relaxant effect of halothane was not antagonized by propranolol at all. From our data, then, we conclude that halothane, ether and thiopental all have direct relaxant effects on tracheobronchial smooth muscle.

In addition, all three anesthetics antagonized the ability of acetylcholine to contract the tracheal chain. The antagonism appears to be of a nonspecific type. This conclusion is supported by several observations. First,

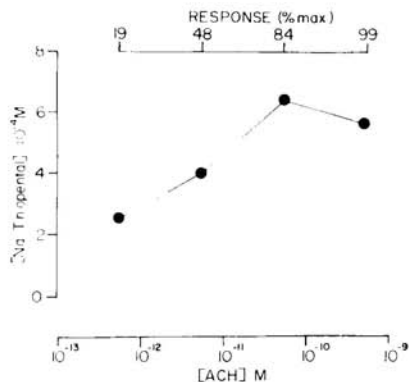


FIG. 7. Antagonism of ACh by thiopental. See legend for figure 5. Regression was significant at 95 per cent level for 5.5×10^{-12} M and 5.5×10^{-11} M.

the anesthetics themselves caused relaxation. Second, in a few experiments, contractions induced by histamine, an agonist unrelated to acetylcholine, were also antagonized by halothane. Finally, the analysis of the data as presented above does not conform to any of the models of specific, pharmacologic antagonism.

In every case, the antagonism to acetylcholine was of the non-surmountable variety in that, above a certain concentration of the anesthetic, the response to acetylcholine was abolished completely. Further, the concentration-response curves for the antagonism of the various anesthetics to acetylcholine were not parallel. With halothane and thiopental, the initial effects were observed at concentrations of 10 mg./100 ml. and 6 mg./100 ml., respectively (concentrations well below those required to cause direct relaxation of the chain). Maximal antagonism occurred at about ten times the threshold concentration of the anesthetics. By contrast, in the case of ether, antagonism to acetylcholine first was observed at a concentration of 200 mg./100 ml., and was maximal at 700 mg./100 ml., a ratio of only 3.5 to 1. These results suggest that the antagonism to acetylcholine by these anesthetic agents is not the result of interaction at a specific tissue receptor.

In attempting to assess the significance of these results, several points may be pertinent, aside from the obvious difficulty in transferring results from the guinea pig to other species and from isolated tissue to airways with intact innervation and circulation. Ether had no effect at all in this preparation until concentrations well above the lethal range in man were reached. This suggests that any bronchodilating action of ether is probably secondary to its other actions. In the case of thiopental, antagonism to acetylcholine was observed at concentrations in the range of 6 mg./100 ml., again higher than those usually seen clinically. Still, it is of interest to note that in 1957, Bernstein *et al.*⁷ reported that, in dogs, thiopental prevented the bronchoconstrictor effect of electrical stimulation of the distal and of the severed cervical vagus nerve. Blood levels of thiopental were not measured in those experiments, but the data suggest that the blood

levels must have been considerably lower than in our experiments.

Unlike ether and thiopental, halothane caused relaxation of the tracheal chain and antagonized the constrictor effects of acetylcholine at concentrations as low as 10 mg./100 ml., well within the clinical range. It is possible, therefore, that this direct effect of halothane may be manifest during clinical anesthesia and may contribute in part to the apparent beneficial aspects of halothane anesthesia in patients with bronchospastic disease.⁸

The guinea-pig tracheal-chain preparation proved to be a reliable research tool and yielded reproducible data. Further investigations with other agonists (e.g., norepinephrine, isoproterenol, histamine) and antagonists (e.g., propranolol) in preparations from human lung should be carried out to permit a more definitive appraisal of the importance of the direct effects of anesthetics on tracheobronchial smooth muscle.

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