

Mechanism of Thiopental-Induced Constriction of Guinea Pig Trachea

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The authors studied the effects of thiopental on baseline airway tone in intact guinea pig tracheas using a preparation where the epithelial (inside) and serosal (outside) surfaces were isolated. Whole tracheas were excised, cannulated, and mounted in 50-ml tissue baths. The serosal and epithelial surfaces were perfused *via* separate circuits with Krebs-Henseleit solution. All data were expressed as a percent of constriction produced by 2×10^{-6} M carbachol (a concentration that elicited a 90 + % of maximal constriction). Thiopental elicited a dose-dependent constriction in all 25 tracheas. Increases in tone were first seen at 10^{-5} M ($14.3 \pm 1.84\%$; mean \pm SEM) and reached a peak at 10^{-3} M ($29 \pm 3.16\%$; $P < .0001$). Responses to thiopental were similar when the epithelium was removed, when thiopental was added to the inner perfusate, and when tracheas were pretreated with 10^{-5} M pyrilamine. Constriction was entirely inhibited by pretreatment with indomethacin 10^{-5} M. The authors conclude that thiopental, at concentrations in the clinical range, causes a reproducible dose-dependent constriction of guinea pig trachea. This effect is mediated by constrictor prostaglandins. (Key words: Anesthetics, intravenous: thiopental. Antihistamines. Asthma. Lung, trachea: constriction. Prostaglandins.)

BRONCHOSPASM is a dramatic and frightening event that occurs in the operating room. The effect of barbiturates on the incidence of such bronchospasm has not yet been established. Since thiobarbiturates are widely used as anesthetics, as sedatives, and to control increased intracranial pressure, this issue requires clarification. Two major textbooks of anesthesia state that thiopental is contraindicated for use in patients with a history of reactive airway disease^{1,2} and another is equivocal.³ The literature contains many opinions and little fact. Retrospective studies show either an association between the use of thiopental and bronchospasm^{4,5} on induction or no association at all.⁶

In vitro data have also shown conflicting results with studies complicated by the use of tissue from different segments of the airway, difficulty in quantitating the amount of airway tone, species variability, and large ranges in thiopental doses.

We therefore studied the effect of the thiobarbiturate thiopental on baseline airway tone in intact guinea pig tracheas. In this paper we present evidence for dose-dependent thiopental-induced tracheal constriction at clinically used concentrations that appears to be a function of prostanoid production.

Methods

This study was approved by the animal research committee of The Johns Hopkins University. As previously described by our laboratory,⁷ male Hartley guinea pigs (400–600 g; Charles Rivers, Wilmington, MA) were killed by cervical dislocation. The tracheas were immediately excised and dissected free of surrounding tissue. The entire trachea, from carina to glottis, was mounted in a perfusing circuit (fig. 1) while bathed in a 50-ml tissue bath filled with Krebs-Henseleit (KH) solution (NaCl 117.6 mM, KCl 5.36 mM, NaH_2PO_4 1.01 mM, CaCl 2.32 mM, MgSO_4 0.69 mM, NaHCO_3 25 mM, glucose 11.1 mM) that was kept at 37° C and continuously bubbled with 5% CO_2 and 95% O_2 . The lumen of the trachea was perfused from a separate 50-ml KH (inner perfusate) bath, also kept at 37° C and bubbled with the same CO_2 and O_2 mixture. Responses of the trachea were monitored with side-hole catheters used to measure pressure at the inlet (P_{in}) and outlet (P_{out}) of the trachea during constant flow. The difference between P_{out} and P_{in} (Pd) is a direct measure of the degree of tracheal constriction. The flow characteristics of this system have been previously described in detail.⁸ In brief, at the flow rates used, the system is best approximated by laminar flow through a circular tube containing a cylindrical central core. Whereas Poiseuille's formula for laminar flow through an unobstructed tube relates resistance to the inverse of the radius to the fourth power, the addition of the central core actually makes the system more sensitive in that resistance varies with the inverse of the radius to the fifth power. Tracheas were mounted so as to direct flow from distal to proximal end (carina toward glottis). The transmural pressure of the trachea was maintained at 0 ± 0.5 cm H_2O and baseline Pd was set between 0.5 and 1 cm H_2O . This was accomplished by changing the height of the inner perfusate reservoir and adjusting the flow rate of the inner perfusate, respectively. The flow rate was between 25 and 35 ml/min. Once this adjustment was made, flow rate was kept constant throughout the experiment. Separate exposure of the inner epithelial side or

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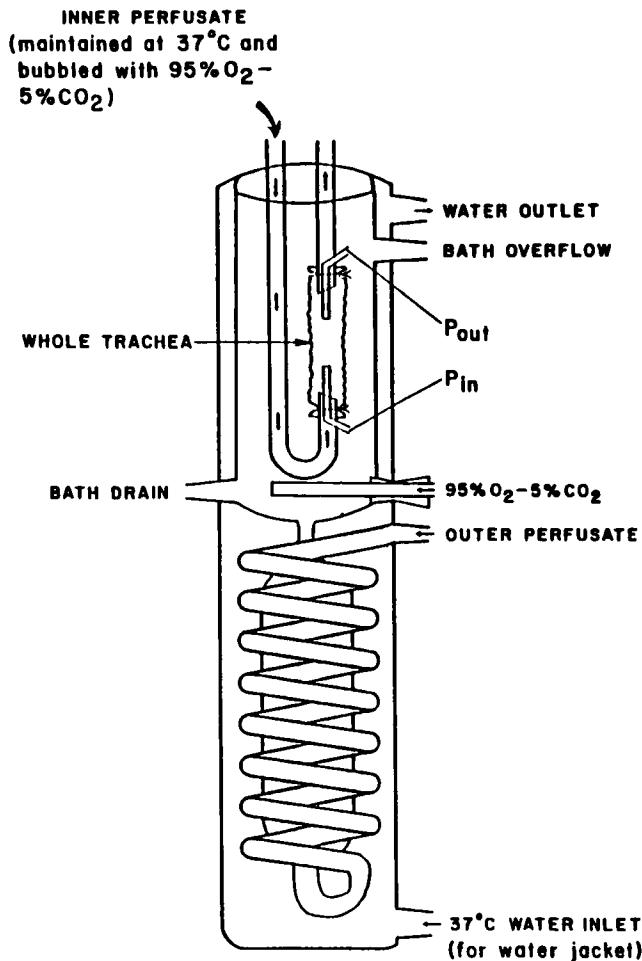


FIG. 1. Schematic presentation of measurement system. P_{in} , pressure at inlet of trachea; P_{out} , pressure at outlet of trachea. $P_d = P_{in} - P_{out}$. Reproduced with permission from Munakata *et al.*⁷

the outer serosal side of the trachea was accomplished by adding drugs to the inner perfusate or outer bathing KH solution.

During some experiments, a pH probe (PHM 82 Radiometer America, Cleveland, OH) was placed in the outer bath and pH was measured continuously.

Removal of epithelium was accomplished by drawing a tapered 0.5 × 10-cm strip of cloth through the trachea in a rotary fashion twice in each direction. Representative tracheas were fixed in 10% buffered formalin and examined histologically for epithelium removal and smooth muscle damage.

SOLUTIONS AND DRUGS

All drugs were placed in solution just prior to each experiment. Thiopental sodium, pyrilamine maleate, and carbachol chloride were added to distilled water to make stock solutions. Indomethacin was made into a 5×10^{-5} M stock solution by dissolving it in ethanol and 10 μ l were

added to the 50-ml bath. Concentrations of drugs noted were the final concentration in the inner perfusate or outer solution. All drugs were purchased from Sigma Chemical Company, St. Louis, Missouri, except thiopental which was obtained from Abbott Labs, North Chicago, Illinois.

EXPERIMENTAL DESIGN

The experimental design is shown in figure 2. Tracheas were allowed to equilibrate for a minimum of 1 h. In some experiments, tracheas were pretreated with either pyrilamine (10^{-5} M) or indomethacin (10^{-5} M) as noted. Following a dose of thiopental, 10–20 min was allowed for P_d to stabilize before subsequent doses were administered. After thiopental doses and before exposure of the trachea to carbachol, tissues were washed for a minimum of 1.5 h.

DATA ANALYSIS

All data were expressed as a percent of constriction produced by 2×10^{-6} M carbachol in the outer bath (a concentration that reproducibly elicits a maximal constriction in this preparation). Results were presented as mean \pm SE and were analyzed by a sign test with $P < 0.01$ considered significant.

Results

CARBACHOL DOSE RESPONSE

Two representative carbachol dose response curves are presented in figure 3. Carbachol 2×10^{-6} M produced a maximal contraction of the guinea pig trachea in our preparation. All subsequent data were expressed as a percent of contraction elicited by this dose of carbachol.

THIOPENTAL-INDUCED CONSTRICTION

A representative tracing of thiopental-induced constriction is shown in figure 4. Thiopental produced dose-related tracheal constriction from 10^{-5} to 10^{-3} M. Thiopental 3×10^{-3} M produced an initial constriction fol-

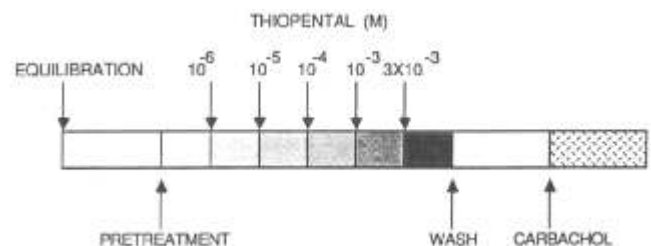


FIG. 2. Experimental design. Tissue was equilibrated and washed for at least 1 h. Tissues were pretreated with either pyrilamine (10^{-5} M) or indomethacin (10^{-5} M).

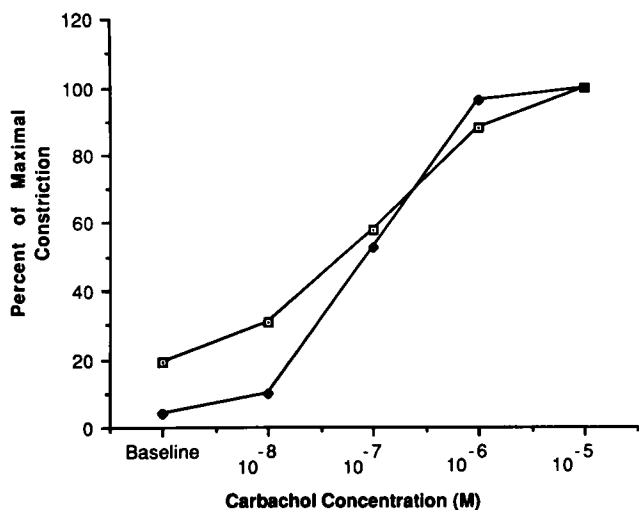


FIG. 3. Two representative carbachol dose response curves.

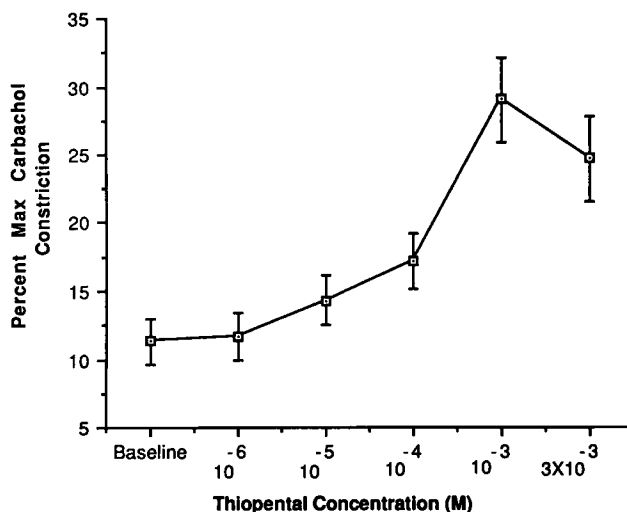


FIG. 5. Thiopental-induced constriction of guinea pig tracheas. Data are expressed as percent of carbachol (2×10^{-6} M) constriction. Concentrations 10^{-5} M and above resulted in constriction significantly greater than baseline ($P < 0.01$). Each point represents a mean \pm SEM of 25 tracheas.

lowed by a relaxation that in some instances returned to baseline. Figure 5 summarizes the responses to increasing thiopental doses as a percent of carbachol constriction in 25 tracheas. No significant increase in tracheal constriction was found at 10^{-6} M thiopental but from 10^{-5} M ($P = 0.003$) to 10^{-3} M significant increases were seen ($P = 0.0001$). At thiopental 10^{-3} M this averaged 30% of maximal response to carbachol. Although on the average some amount of relaxation occurred at 3×10^{-3} M, the degree of tracheal constriction was still well above baseline.

EFFECT OF EPITHELIUM

To determine whether thiopental-induced constriction was influenced by the epithelium, thiopental effects were compared when administered to either the epithelial or serosal surfaces. A reproducible dose-dependent constriction was observed with thiopental given to either the epithelial or serosal surfaces (fig. 6). Although there was a small shift in baseline tone with epithelium removal, thiopental produced similar dose-dependent constriction in the presence and absence of epithelium (fig. 7). Histologic analysis of representative tracheas confirmed the absence of epithelium without smooth muscle damage.

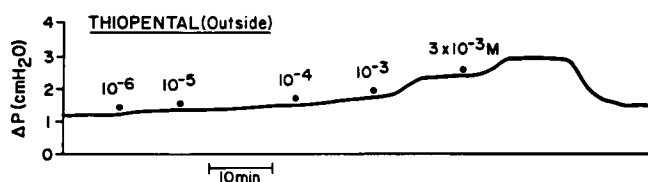


FIG. 4. A representative tracing of thiopental effects on guinea pig trachea. $P_{out} - P_{in} = Pd$.

PRETREATMENT WITH CYCLOOXYGENASE AND HISTAMINE ANTAGONISTS

As can be seen in figure 8, pretreatment with pyrillamine (10^{-5} M) did not inhibit the constrictor response of the trachea to thiopental. In contrast, indomethacin 10^{-5} M pretreatment totally abolished the constrictor effects of thiopental (fig. 9). Indomethacin itself produced a small decrease in baseline airway tone in the preparation.

Thiopental 10^{-6} M to 10^{-3} M resulted in no significant alteration in the pH of the bath solution.

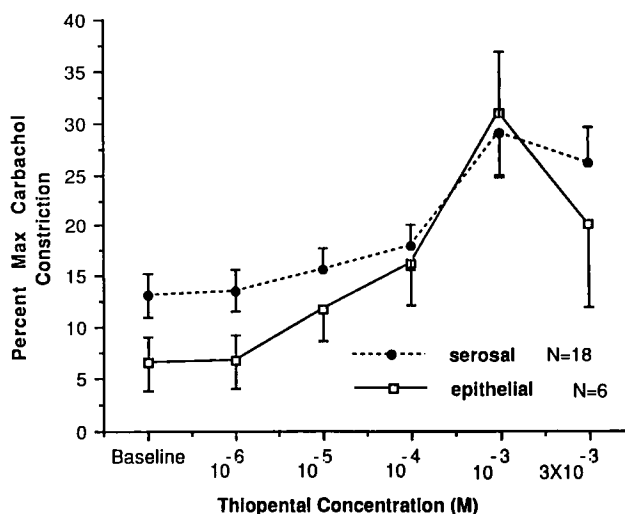


FIG. 6. Comparison of guinea pig tracheal constriction when thiopental was placed in outer/serosal bath or inner/epithelial bath.

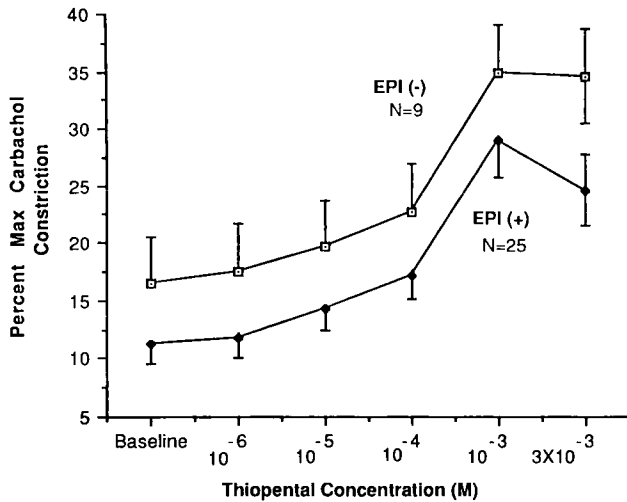


FIG. 7. Thiopental-induced constriction of epithelial-denuded tracheas (EPI (-)) and tracheas with epithelium intact (EPI (+)).

Discussion

In 1943 Adriani and Rovenstine⁹ observed that human, rat, and dog bronchi constricted when exposed to sodium thiopental. In contrast, Fletcher *et al.*¹⁰ found relaxation and Edney and Downes¹¹ found no effect while we found dose-related constriction at low concentrations and relaxation at high concentrations of thiopental in guinea pig airway tissues. Most of these apparent discrepancies can be explained by the different species studied, by the state of pre-existing airway tone, and by the concentration of thiobarbiturate used. Adriani and Rovenstine^{9,12} exposed sections of human, rat, and dog bronchi to high doses of thiopental (10 mg/100 ml) while directly observing airway caliber under a dissecting microscope. Immediate constriction was seen in all species and was estimated to be

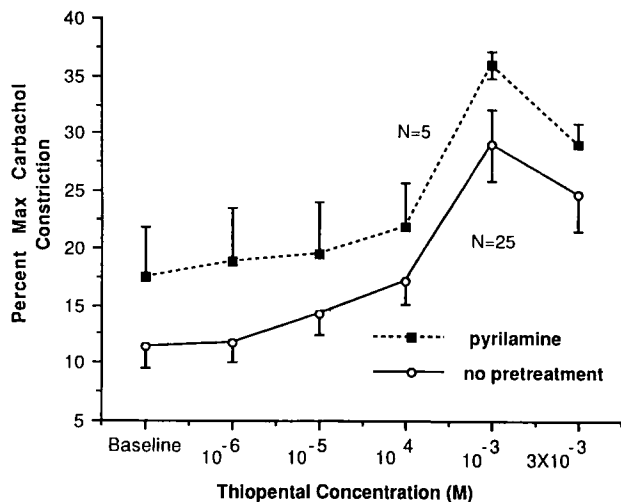


FIG. 8. Thiopental-induced constriction of guinea pig tracheas untreated and pretreated with the H-1 receptor antagonist pyrillamine (10^{-5} M).

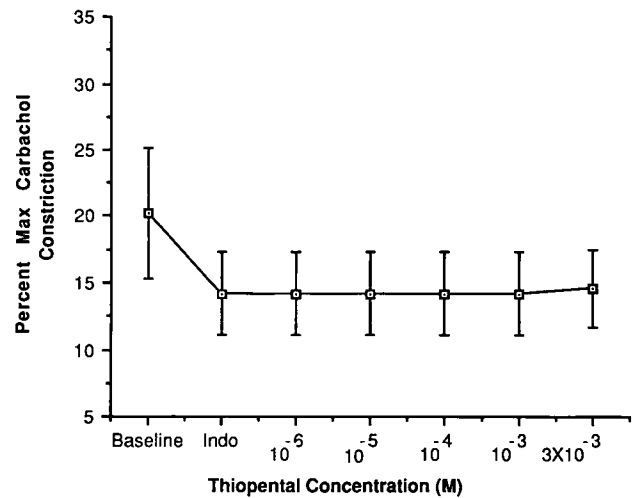


FIG. 9. Inhibition of thiopental-induced tracheal constriction by indomethacin 10^{-5} M.

as high as 25% of the luminal diameter in the rat. The three human airways in the study⁹ were obtained from surgical specimens. No medical histories of these patients were included. Fletcher *et al.*¹⁰ studied the effects of thiopental on guinea pig tracheal chains already precontracted with acetylcholine. The concentrations used were very high (60 mg/100 ml). Although Edney and Downes¹¹ did not demonstrate a constrictor effect of thiopental in guinea pig tracheal chains, they did find that thiopental in concentrations used in our study produced constriction in both rabbit aorta and jejunum.

Explanations by which thiopental constricts airways include stimulation of cholinergic nerves in the tissue preparation, direct effects of thiopental on the smooth muscle itself, release of histamine from tissue mast cells, or release of other constrictor substances from the airway epithelium or from other cells in the airway. It is unlikely that the airway constriction after thiopental resulted from stimulation of cholinergic nerves because barbiturates, in a dose-dependent manner, depress rather than stimulate cholinergic nerves.¹³⁻¹⁶

Thiopental releases histamine from human skin mast cell preparations.¹⁷ It is possible that histamine released from lung mast cells in the preparation constricted the airway by stimulating histamine-1 receptors. This is unlikely because the histamine-1 receptor antagonist pyrillamine in concentrations that prevent histamine-induced constriction of the trachea¹⁸ did not prevent the constrictor effects of thiopental.

It is not surprising that thiopental did not release histamine from lung mast cells. Mast cells from different tissues are functionally heterogeneous and lung mast cells cannot be triggered to release histamine by morphine, whereas skin mast cells release histamine when triggered by morphine.¹⁹

It is also unlikely that thiopental directly constricted the smooth muscle itself, as the cyclooxygenase blocker indomethacin totally abolished the constrictor effects of the thiopental. This suggests that thiopental releases a cyclooxygenase product that constricts the airway.

Our preparation allows us to evaluate the role of the airway epithelium as the source of the constrictor because we are able to isolate the luminal from the serosal surface.⁷ However, the similar effects of thiopental when administered to either the luminal or serosal surface and the similar effects in the presence and in the absence of the epithelium suggests that the epithelium is not a likely source of the constrictor prostaglandin.

Prostaglandin E₂ (PGE₂), prostaglandin F_{2α} (PGF_{2α}), thromboxane (TXB), and prostaglandin D₂ (PGD₂) will all constrict guinea pig airway and are possible candidates. Orehek *et al.*²⁰ have shown that PGF_{2α} largely regulates baseline airway tone in guinea pigs and that contraction is modulated by both it and PGE₂. Moreover, Coleman and Kennedy²¹ found that PGE₂ caused either constriction or relaxation, depending on concentration and resting tone of the airway. McKenniff *et al.*²² have recently shown that in guinea pig airway as well as in human lung strips the constriction by PGE₂ and PGF_{2α} is mediated by TXB receptors. It is unlikely that the constriction by thiopental is the result of change in pH as the concentrations of thiopental used in this study produce no significant alteration of the pH.

The guinea pig trachea was chosen for this study because the guinea pig, like the human, and unlike the dog has resting airway tone. This allows us to evaluate the effects of thiopental in the absence of exogenous precontraction. Moreover, this system, using a constant flow through an intact trachea, allows quantitation of muscle shortening analogous to that which must occur *in vivo*.

In conclusion, thiopental, at doses in the clinical range,²³ causes a reproducible dose-dependent constriction of the whole guinea pig trachea. This response is not dependent upon tracheal epithelium and is unrelated to histamine release, but is likely related to the release of cyclooxygenase products by thiopental. Studies using human airways and prospective controlled clinical trials are needed to determine if thiopental produces an increased incidence of bronchospasm in the operating room in patients with reactive airway disease.

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