

Contractile Responses of Guinea Pig Trachea to Oxybarbiturates and Thiobarbiturates

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To determine what mechanisms are involved in barbiturate-induced tracheal constriction and whether a relationship exists between barbiturate structure and the ability of the barbiturate to induce constriction, we compared the effects of thiamylal, thiopental, methohexital, pentobarbital, and phenobarbital at increasing airway tone in an intact guinea pig tracheal preparation in the presence and absence of cyclooxygenase and thromboxane synthetase inhibition. Whole tracheas were suspended between two cannulas in 50-ml tissue baths and perfused at a constant flow rate with Krebs-Henseleit solution. The contractile responses were assessed by measuring the pressure differential between the tracheal inlet and outlet ports. Barbiturates were added to the bath of each trachea, which was washed between each drug. Each drug was added to produce final bath concentrations of 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} , and 3×10^{-3} M. Tracheas were also pretreated with meclofenamate (10^{-6} M) (a cyclooxygenase inhibitor) and UK 37,248 (10^{-8} to 10^{-5} M) and OKY 046 (10^{-9} to 10^{-6} M) (thromboxane synthetase inhibitors), and the thiamylal protocol was repeated. All data were normalized to a concentration of carbachol (2×10^{-6} M) that has been shown to produce maximum constriction in this preparation. Thiamylal and thiopental produced constriction beginning at 10^{-4} M and reached a maximum at 10^{-3} M ($P < 0.0001$). Methohexital, pentobarbital, and phenobarbital did not produce any significant change in airway tone. Pretreatment with meclofenamate (10^{-6} M), UK 37,248 (5×10^{-5} M), and OKY 046 (10^{-6} M) prevented thiamylal-induced tracheal constriction. We conclude that thiobarbiturates, but not oxybarbiturates, constrict guinea pig tracheas in concentrations similar to those achieved *in vivo*. This constriction is mediated by thromboxane. (Key words: Anesthetics, intravenous: thiamylal; methohexital; pentobarbital. Complications: asthma. Prostaglandins: thromboxane. Trachea: constriction.)

BARBITURATES are used as sedatives, as induction agents in anesthesia, and as agents to control increased intracranial pressure. Despite their widespread use, the effects of barbiturates on airway tone and reactivity are understood incompletely.¹ Thiopental, the only barbiturate system-

atically studied, directly constricts some but not all airway smooth muscle preparations.²⁻⁴ A relationship has been suggested between the structure of a barbiturate and its ability to constrict vascular smooth muscle.^{4,5} A similar relationship may exist between barbiturate structure and the ability of barbiturates to constrict airway smooth muscle.

We have been working with an airway preparation in which thiopental consistently produces constriction.³ To determine the structural specificity of barbiturate-induced tracheal constriction, the present study examined the ability of two thiobarbiturates, thiopental and thiamylal, and three oxybarbiturates, methohexital, pentobarbital, and phenobarbital, to produce constriction in the same guinea pig tracheal preparation and to determine the role of the cyclooxygenase product, thromboxane, in the mechanism of contractile action.

Materials and Methods

This study was approved by the animal research committee of The Johns Hopkins University. 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) where it was bathed in a 50-ml tissue bath filled with Krebs-Henseleit solution (millimolar concentrations: NaCl 117.6, KCl 5.36, NaH_2PO_4 10.01, CaCl 2.32, MgSO_4 0.69, NaHCO_3 25, and glucose 11.1) that was kept at 37°C and continuously bubbled with 5% carbon dioxide and 95% oxygen. The lumen of the trachea was perfused from a separate 50-ml Krebs-Henseleit (inner perfusate) bath, also kept at 37°C and bubbled with the same carbon dioxide and oxygen mixture.

Responses of the trachea were monitored with side-hole catheters used to measure pressure at the outlet and pressure at the inlet of the trachea during constant flow. The difference between the outlet and inlet pressures is a direct measure of the degree of tracheal constriction. The flow characteristics of this system have been described in detail.⁶ 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

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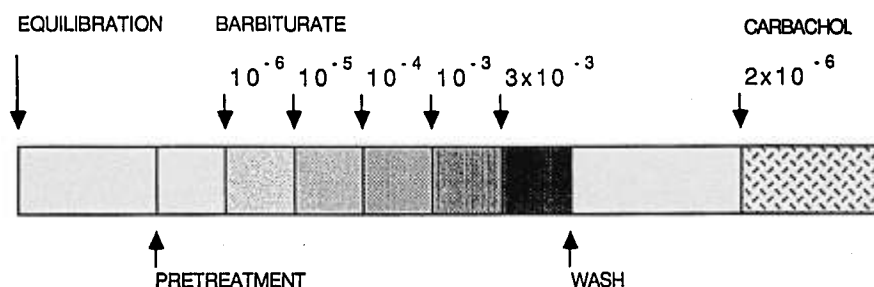


FIG. 1. Experimental design. Tissue was equilibrated and washed for at least 60 min. Tissues were pretreated with UK 37,248.

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 the distal to the proximal end (carina to glottis). The transmural pressure of the trachea was maintained at 0 ± 0.5 cmH₂O by changing the height of the inner perfusate reservoir, and the baseline value for the outlet-inlet pressure difference was set between 0.5 and 1 cmH₂O by adjusting the flow rate of the inner perfusate. The flow rate was maintained between 25 and 35 ml/min. Once this adjustment was made, the flow rate was kept constant throughout the experiment.

SOLUTIONS AND DRUGS

All drugs were placed in solution just before each experiment. Carbachol chloride (Sigma, St. Louis, MO), pentobarbital sodium (Sigma), thiamylal sodium (Parke Davis, Morris Plains, NJ), thiopental sodium (Abbott, Chicago, IL), methohexital sodium (Eli Lilly, Indianapolis, IN), UK 27,248 (Pfizer, New York, NY), meclofenamate sodium (Parke Davis, Ann Arbor, MI) and OKY 046 hydrochloride (Kissei, Matsumoto, Japan) were dissolved in distilled water, and phenobarbital sodium (Sigma) was dissolved in 10% ethanol and distilled water.

EXPERIMENTAL DESIGN

The experimental protocol is shown in figure 1. Tracheas were allowed to equilibrate for 60–90 min. Thiamylal ($n = 30$), thiopental ($n = 6$), methohexital ($n = 11$),

pentobarbital ($n = 5$), or phenobarbital ($n = 6$) was cumulatively added to the outer bath of each preparation in concentrations ranging from 10^{-6} to 3×10^{-3} M. After the addition of each barbiturate concentration, a period of 10–20 min was allowed for the outlet-inlet pressure difference to stabilize. After the final barbiturate concentration, the preparation was washed for 60–90 min, and carbachol (2×10^{-6} M) was added to the outer bath. This concentration has been shown previously to produce near maximal constriction in this preparation.³ The cyclooxygenase inhibitor sodium meclofenamate (10^{-6} M; $n = 6$) or either of the thromboxane synthetase inhibitors UK 37,248 (5×10^{-5} M; $n = 6$) or OKY 046 (10^{-6} M; $n = 4$) were added to the external bath. The preparation allowed to equilibrate for 15 min and then treated with cumulative concentrations of thiamylal. The tissue was washed for 1 h and constricted with carbachol as above. The concentrations of UK 27,248 and OKY 046 used were determined in preliminary studies.

DATA ANALYSIS

Barbiturate effects on tracheal tone are expressed as a percent of carbachol constriction produced in the same trachea. Results are presented as means \pm standard error of the mean. Comparisons within each treatment group were analyzed with one-way analysis of variance and Fisher's multiple comparison test. Comparisons between treatment groups were analyzed with the Kruskal-Wallis statistic. $P < 0.05$ was considered significant.

Results

A representative tracing of thiamylal-induced constriction is shown in figure 2. Thiamylal and thiopental each

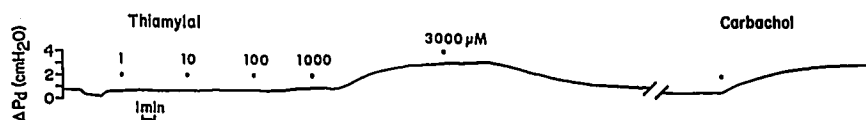


FIG. 2. A representative tracing of thiamylal effects on guinea pig trachea. The difference between pressure at the outlet of the trachea and pressure at the inlet is a direct measure of the degree of tracheal constriction.

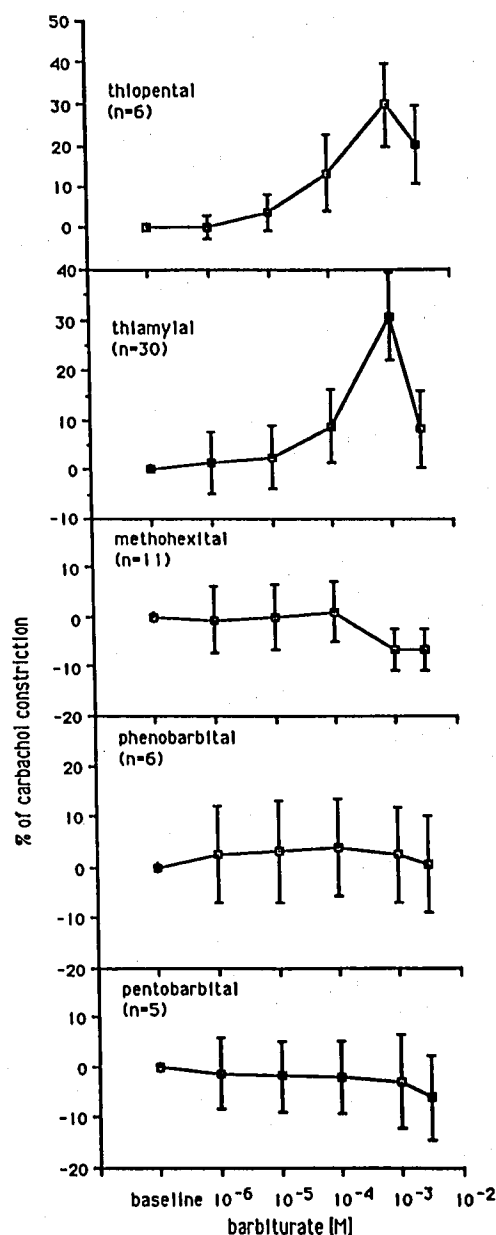


FIG. 3. Barbiturate-induced constriction of guinea pig trachea. Data are expressed as percent of carbachol (2×10^{-6} M) constriction. Thiamylal- and thiopental-induced constrictions (10^{-3} M and 10^{-4} M, respectively) were significantly greater than baseline ($P < 0.0001$). Each point represents the mean \pm SEM. Between groups comparison was significant at $P < 0.0001$.

produced significant dose-related constriction at 10^{-4} and 10^{-3} M ($P < 0.0001$) and initial constriction followed by a reversal of the constriction at 3×10^{-3} M (fig. 3). Methohexital, phenobarbital, and pentobarbital did not produce constriction in this preparation (fig. 3). Carbachol (2×10^{-6} M), however, subsequently constricted all prep-

arations, producing changes in the outlet-inlet pressure difference ranging from 1.68 to 3.12 cmH₂O.

Pretreatment with sodium meclofenamate (10^{-6} M), a cyclooxygenase inhibitor, completely prevented thiamylal-induced constriction. Pretreatment with OKY 046 (10^{-6} M) or UK 37,248 (5×10^{-5} M) (thromboxane inhibitors) also completely inhibited thiamylal-induced constriction (fig. 4). In preliminary studies, UK 37,248 10^{-8} M had no effect on constriction induced by thiamylal 10^{-3} M, whereas UK 37,248 10^{-7} and 10^{-6} M inhibited this constriction by 50 and 65%, respectively. Similarly, OKY 046 10^{-9} M did not inhibit constriction by thiamylal 10^{-3} M, whereas OKY 046 10^{-8} and 10^{-7} M inhibited this constriction by 60 and 84%, respectively.

Discussion

This study demonstrates that the thiobarbiturates thiamylal and thiopental in concentrations similar to those achieved in serum during clinical use⁷ produced significant constriction of intact guinea pig trachea. In contrast, the oxybarbiturates methohexital, phenobarbital and pentobarbital did not produce any significant constriction in this same preparation. Thiopental, the thiobarbituric analogue of pentobarbital, has been shown previously in this preparation to produce a constriction similar,³ both quantitatively and qualitatively, to that produced by thiamylal. These results suggest that barbiturate-induced constriction of guinea pig trachea is a function of structure and is a property, in general, of thiobarbiturates but not oxybarbiturates.

Both thiamylal and thiopental also have been shown to produce constriction of vascular smooth muscle, whereas a variety of oxybarbiturates, including both pentobarbital

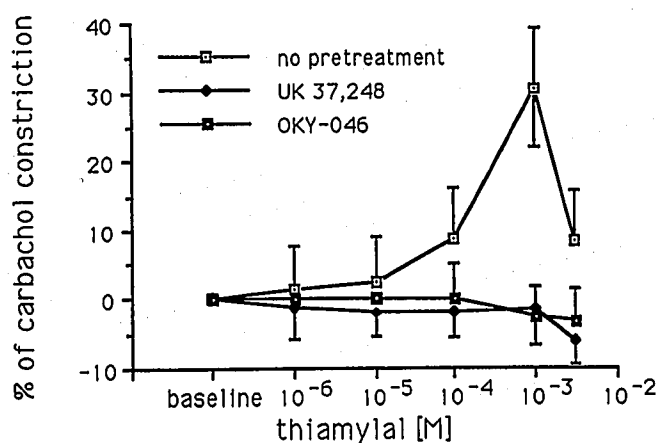


FIG. 4. Comparison of guinea pig tracheal constriction to thiamylal in the presence and absence of pretreatment with UK 37,248 (5×10^{-5} M) and OKY 046 (10^{-6} M).

and secobarbital, the oxybarbituric acid analogue of thiamylal, have been shown not to produce this constriction.^{4,5} Edney and Downes⁴ showed constriction of both vascular and airway smooth muscle with a subset of convulsant oxybarbiturates which contain benzyl, alkoxy, or branched alkene radicals. However, contractions produced in rabbit aortic strips by the convulsant oxybarbiturates differed both qualitatively and quantitatively from those produced by thiopental. This suggests that convulsant oxybarbiturate-induced constriction and thiobarbiturate-induced constriction of vascular tissue operate through different mechanisms.

Thiobarbiturates may produce tracheal constriction through either an intermediate product or by a direct effect on the airway smooth muscle. Thiobarbiturates, but not oxybarbiturates, release histamine from human cutaneous mast cells, and histamine constricts tracheal smooth muscle.⁸ However, histamine-1-receptor blockade with pyrilamine did not attenuate thiopental-induced tracheal constriction,³ suggesting that thiobarbiturate-induced tracheal constriction is not related to histamine release from mast cells in this preparation.

Pretreatment of tracheal tissues with the cyclooxygenase inhibitors indomethacin³ and meclofenamate attenuated thiobarbiturate-induced tracheal constriction. Both indomethacin and meclofenamate inhibit conversion of arachidonic acid to a variety of metabolites that have significant effects on airway smooth muscle tone.⁹ Thromboxane A₂, prostaglandin (PG) F_{2α}, and PGD₂ produce constriction, whereas PGI₂ produces relaxation and PGE₂ produces either relaxation or constriction depending on the contractile state of the tissue.⁹ Our previous results with indomethacin³ as well as our present results with meclofenamate suggest that a contractile cyclooxygenase product of arachidonic acid metabolism is necessary for thiobarbiturate-induced tracheal constriction.

In the present study, pretreatment with either of the two selective yet structurally dissimilar thromboxane synthetase inhibitors UK 37,248 and OKY 046 eliminated thiamylal-induced tracheal constriction. It is unlikely that this effect was due to inhibition of cyclooxygenase, since this is reported to occur at concentrations several orders of magnitude greater than those used in the present study.^{10,11} Thus, the present results strongly suggest that constriction of tracheal smooth muscle induced by thiobarbiturate is dependent upon the production of thromboxane A₂.

Thromboxane A₂ is produced by many tissues in the intact trachea, including epithelium, cartilage, fibroblasts, airway smooth muscle, macrophages, residual platelets, and other blood-borne cells. Since removal of the epithelium had no effect on thiopental-induced constriction,³ it is an unlikely source of thromboxane A₂ in this preparation.

Thromboxane A₂ may produce its constrictor actions directly by acting on receptors on the tracheal smooth muscle or indirectly by facilitating the action of other constrictor agents. Thromboxane A₂ directly constricts tracheal smooth muscle preparations by activating a subset of PG receptors known as TP receptors.¹² PGF_{2α} produces constriction of tracheal as well as vascular smooth muscle by activating the same TP receptor.¹³ Moriyama *et al.*¹⁴ have recently shown that thiamylal and thiopental constrict vascular smooth muscle that has been pretreated with indomethacin and partially precontracted with PGF_{2α}, but these thiobarbiturates did not constrict vascular tissue partially precontracted with potassium chloride. These results suggest that thromboxane A₂ may act indirectly to facilitate thiobarbiturate-induced smooth muscle contraction. Alternatively, thromboxane synthetase inhibitors have been shown to shift substrate utilization; this shift may increase the production of cyclooxygenase products,¹² which can relax tracheal smooth muscle. This, however, is unlikely, because cyclooxygenase inhibition itself blocks tracheal constriction.

In summary, the present study examined the effects of five barbiturates on smooth muscle tone in guinea pig tracheal preparations and found that thiobarbiturates, but not oxybarbiturates, produced constriction. This constriction was attenuated by pretreatment with two thromboxane synthetase inhibitors, indicating the involvement of thromboxane A₂ in the mechanism of this effect. These results suggest similarities between vascular and airway smooth muscle with regard to the modulatory influences of thiobarbiturates and oxybarbiturates. Studies using airway tissue from asthmatic humans and prospective clinical trials with oxybarbiturates and thiobarbiturates are needed to elucidate the clinical importance of these findings in asthmatic humans.

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