Differential effects of thiopental on methacholine- and serotonin-induced bronchoconstriction in dogs

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Background. Thiopental sometimes causes bronchospasm during induction of anaesthesia. In addition, we have reported previously that thiopental produced transient bronchospasm, which was blocked by atropine pretreatment, and worsened histamine-induced bronchoconstriction in dogs. Previous in vitro reports suggest that synthesis of contractile cyclooxygenase products, such as thromboxane A2, may be involved in the mechanism of bronchospasm. However, the in vivo spastic effects have not been defined comprehensively.

Methods. Twenty-seven mongrel dogs were anaesthetized with pentobarbital. Bronchoconstriction was elicited with methacholine (0.5 μg kg⁻¹ min⁻¹; Mch group, n=7) or serotonin (10 μg kg⁻¹ h⁻¹; 5HT group, n=20), and assessed as percentage changes in bronchial cross-sectional area (BCA, basal=100%) using a bronchoscope. In the 5HT group, dogs were subdivided into four groups of five each: S-5HT, I-5HT, 5HT-S and 5HT-A. In the S-5HT and I-5HT groups, 30 min before serotonin infusion dogs were given saline and indomethacin respectively at 5 mg kg⁻¹ i.v. In all groups, 30 min after bronchoconstrictor infusion started, dogs were given thiopental at doses between 0 (saline) and 20 mg kg⁻¹. In the 5HT-S and 5HT-A groups, dogs were given saline or atropine 0.2 mg kg⁻¹ i.v. 5 min after thiopental 20 mg kg⁻¹.

Results. Methacholine and serotonin reduced BCA by about 50 and 40% respectively. Thiopental 20 mg kg⁻¹ increased and decreased BCA by about 20 and 10% in the Mch and 5HT groups respectively. Indomethacin and atropine did not attenuate the potentiation of serotonin bronchoconstriction produced by thiopental.

Conclusion. The present study indicates that thiopental may attenuate or worsen bronchoconstriction induced by muscarinic or serotonin receptor stimulation, respectively. The synthesis of contractile cyclooxygenase products and cholinergic stimulation may not be involved in the contractile effect of thiopental on serotonin bronchoconstriction.

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Several clinical investigations have shown that the incidence of bronchospasm produced by tracheal intubation after induction with thiopental is much higher than that with propofol.1–4 This bronchospasm is mediated by rapidly adapting stretch receptors (irritant receptors), which are units of vagal afferent nerves.5 We have also reported that pretreatment with atropine could completely abolish thiopental-induced bronchospasm in dogs.6 Thus, bronchospasm may be due to thiopental-induced vagal stimulation.

In contrast, we have recently found that thiopental may antagonize M₃ muscarinic receptors, although we had expected agonist or, at least, no antagonist action. Thiopental significantly displaced 1-[N-methyl-³H]scopolamine methyl chloride binding to M₃ \( [pK_i = 4.12 (0.13) \text{[75 mM (19.8 } \mu \text{mol} \text{ l}^{-1}]) ] \), but not M₁ or M₂ receptors. In addition, thiopental \( (10^{-5} \text{ to } 10^{-3} \text{ M}) \) concentration-dependently inhibited the methacholine-stimulated increase in intracellular Ca²⁺ concentration in Chinese hamster ovary cells expressing recombinant human M₃ muscarinic receptors. Previous investigations suggest that at least three subtypes of receptors exist in the human airway: M₁, M₂ and M₃. M₁ receptors are found in airway ganglia and alveolar walls; they facilitate neurotransmission through parasympathetic ganglia and enhance cholinergic reflexes. M₂ receptors are localized on cholinergic postganglionic nerves at the prejunctional level, and inhibit acetylcholine release but counteract β-agonist-induced bronchodilation. M₃ receptors are distributed in airway smooth muscle, submucosal glands and the airway vascular endothelium. Activation of M₃ receptors produces airway smooth muscle contraction via increased phosphoinositide hydrolysis and raised intracellular Ca²⁺ concentration. These data indicate that thiopental should attenuate vagally mediated airway constriction. Thus, there is a discrepancy.

Several in vitro studies \(^{10,11} \) have also shown direct spastic effects of thiopental on tracheal smooth muscle. Lenox and colleagues\(^ {10} \) reported that thiopental produced a concentration-dependent constriction of guinea-pig tracheal smooth muscle, and pretreatment with indomethacin, a cyclooxygenase (COX) inhibitor, inhibited thiopental-induced contraction. Curry and colleagues\(^ {11} \) also reported that thiobarbiturates (thiopental and thiamylal) contracted guinea-pig tracheal smooth muscle, whereas oxybarbiturates (methohexital, pentobarbital and phenobarbital) did not produce any significant changes in airway tone. In addition, as contraction was prevented by pretreatment with meclofenamate (a COX inhibitor), UK27248 and OKY046 (thromboxane synthetase inhibitors), thiobarbiturate-induced contraction may be mediated by thromboxane A₂.

In the present study, we examined whether thiopental would produce differential effects on bronchoconstriction induced by methacholine (a muscarinic receptor agonist) and 5-hydroxytryptamine (5HT). In addition, when thiopental produced bronchoconstriction, we determined whether indomethacin pretreatment and atropine treatment attenuated this response.

**Methods**

Our study protocol was approved by the Animal Care and Use Committee of the University of Hirosaki School of Medicine. Twenty-seven mongrel dogs (8–12 kg) were anaesthetized with an i.v. bolus of pentobarbital 30 mg kg⁻¹ followed by continuous infusion at 2 mg kg⁻¹ h⁻¹, and paralysed with i.v. pancuronium 200 μg kg⁻¹ h⁻¹. The trachea was intubated with a tracheal tube (internal diameter 7.0 mm; Univent tube; Fuji System, Tokyo, Japan) that had a second lumen for the insertion of a superfine fibre-optic bronchoscope. The lungs were mechanically ventilated using a volume-controlled respirator (Servo 900C) with oxygen, and end-tidal carbon dioxide was maintained at 4.0–4.5%. A femoral artery was cannulated to monitor arterial blood pressure and to obtain arterial blood samples. A femoral vein was also cannulated to insert a double-lumen catheter for histamine infusion and administration of fluid and study drugs.

**Imaging and analysis of the airway**

To assess the bronchial smooth muscle tone, the bronchial cross-sectional area (BCA) of the third bronchial bifurcation in the right lung was monitored continuously with a superfine fibre-optic bronchoscope (outside diameter 2.2 mm; AF type 22A; Olympus), as reported previously.\(^ {6} \) Briefly, the image at the third bifurcation was printed out with a video printer (VY-170; Hitachi, Tokyo, Japan) during the end-expiratory pause, and was then taken into a Macintosh computer (Power Macintosh 7100/80 AV; Apple Computer, Cupertino, CA, USA) by a scanner (Scan-Jet 4c; Hewlett-Packard Japan, Tokyo, Japan), and BCA was measured using image analysis software (Macscope 2.56; Mitani, Fukui, Japan). Image processing was performed by an investigator who was blinded to the study protocol.

**Effect of thiopental on methacholine-induced bronchoconstriction**

Seven dogs were allocated to this group (Mch group). Bronchoconstriction was elicited by i.v. infusion of methacholine (0.5 μg kg⁻¹ plus 5 μg kg⁻¹ min⁻¹ until the end of the experiment). Thirty minutes later, when stable bronchoconstriction was achieved, dogs were given i.v. each dose of thiopental: 0 (saline), 0.02, 0.2, 2.0 and 20 mg kg⁻¹, cumulatively. BCA was assessed before and 30 min after the start of methacholine infusion and 5 min after administration of each dose of thiopental. At least 15 min elapsed between administration of each dose.

**Effect of thiopental on 5HT-induced bronchoconstriction**

Twenty dogs were studied in this protocol, and were divided into two groups to examine whether indomethacin pretreatment or atropine treatment would modify the effects of thiopental. Bronchoconstriction was elicited with 5HT (10 μg kg⁻¹ i.v. followed by continuous infusion at 1.0 mg kg⁻¹ h⁻¹ until the end of each experiment). To determine the effects of indomethacin pretreatment, dogs were pretreated with saline (S-5HT group, \( n = 5 \)) or indomethacin 5 mg kg⁻¹ (I-5HT group, \( n = 5 \)) 30 min before
5HT infusion. As previous reports\textsuperscript{13–15} suggest that indomethacin 5 mg kg\textsuperscript{-1} significantly inhibits synthesis of cyclooxygenase products, this dose was used in the present study. We have confirmed previously that bronchoconstriction was stable during the experiment.\textsuperscript{16} Thirty minutes after the start of 5HT infusion, thiopental i.v. 0 (saline), 0.02, 0.2, 2.0, 20 mg kg\textsuperscript{-1} was given cumulatively. BCA was assessed before (basal) and 30 min after the start of 5HT infusion and 5 min after each dose of thiopental. Another 10 dogs were studied to determine the effects of atropine treatment. Thirty minutes after the start of 5HT infusion, thiopental 20 mg kg\textsuperscript{-1} was given i.v., and 5 min later atropine 0.2 mg kg\textsuperscript{-1} (5HT-A group, \(n=5\)) or saline (5HT-S group, \(n=5\)) was given. BCA was assessed before and 30 min after the 5HT infusion started and 5 min after administration of thiopental and atropine or saline.

**Plasma catecholamine measurement**

Blood samples (6 ml) were taken through the arterial line 5 min after each dose of thiopental and were immediately centrifuged at 3000 rpm for 10 min at \(-10^\circ\text{C}\) to separate plasma, which was then frozen at \(-70^\circ\text{C}\) until assay. Plasma epinephrine and norepinephrine concentrations were determined by high-performance liquid chromatography with electrochemical detection. Twenty microlitres of acidified ( perchloric acid) sample was injected onto a reverse-phase column (C18, 4.6 \(\times\) 150 mm). Monoamines were separated using a mobile phase buffer consisting of 0.05 M NaH\textsubscript{2}PO\textsubscript{4}, 0.05 M CCl\textsubscript{3}COOH, 0.7 mM CH\textsubscript{3}(CH\textsubscript{2})\textsubscript{11}OSO\textsubscript{3}Na, 0.02 mM EDTA2Na; 85: acetonitrile 10: methanol 5, pH 3.4, at a flow rate of 1 ml min\textsuperscript{-1} at 40°C, and quantified using an electrochemical detector at 300 mV (optimum voltage for oxidation). The intra-assay coefficient of variation for epinephrine and norepinephrine was 3.31 and 2.93% respectively. The lower limit of detection for epinephrine and norepinephrine was 9 and 12.5 pg ml\textsuperscript{-1} respectively.

**Statistical analysis**

All data are expressed as mean (SD). BCA is presented as the percentage of basal. Statistical analysis was by one-way or two-way repeated measures analysis of variance followed by the Student–Newman–Keuls test using SigmaStat for Windows (Jandel Scientific Software, Chicago, IL, USA), with \(P<0.05\) considered significant.

**Results**

**Effects of thiopental on methacholine-induced bronchoconstriction**

Methacholine infusion reduced the basal BCA by 49.2 (15.8)%. Thiopental 2 and 20 mg kg\textsuperscript{-1} significantly reversed methacholine-induced bronchoconstriction in a dose-dependent fashion (Fig. 1). Plasma concentrations of epinephrine and norepinephrine decreased significantly after administration of thiopental 2.0 and 20 mg kg\textsuperscript{-1} respectively (Table 1).

**Effects of thiopental on 5HT-induced bronchoconstriction**

5HT infusion reduced basal BCA by 41 (8.0), 39.4 (10.9), 35.6 (9.1) and 38.2 (8.2)% in the S-5HT, I-5HT, 5HT-S and 5HT-A groups respectively. Thiopental 20 mg kg\textsuperscript{-1} significantly worsened 5HT-induced bronchoconstriction in all 5HT groups. However, there was no significant difference between the S-5HT and I-5HT groups (Fig. 2). In addition, atropine did not attenuate the thiopental effect compared with saline (Fig. 3).

Plasma concentrations of catecholamines decreased significantly after thiopental 20 mg kg\textsuperscript{-1} (Table 2).

![Fig 1](https://academic.oup.com/bja/article-abstract/91/3/379/297424/379) **Relaxant effects of thiopental on methacholine (Mch)-induced bronchoconstriction.** Pre=before bronchoconstrictor infusion started; Mch30=30 min after Mch infusion started. *\(P<0.05\); **\(P<0.01\) vs Mch30. Values are mean and SD.

**Table 1** Changes in plasma catecholamine levels 5 min after i.v. thiopental in the Mch group. Data are mean (sd); \(n=7\). Mch30=30 min after methacholine infusion started. *\(P<0.05\); **\(P<0.01\) vs Mch30

<table>
<thead>
<tr>
<th>Catecholamines (pg ml\textsuperscript{-1})</th>
<th>Mch30\textsuperscript{0}</th>
<th>Thiopental (mg kg\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (saline) 0.02 0.2 2.0 20</td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>554 (285)</td>
<td>551 (146) 458 (301) 437 (275) 364 (298)** 158 (103)**</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>429 (211)</td>
<td>413 (201) 381 (185) 367 (164) 407 (190) 281 (105)**</td>
</tr>
</tbody>
</table>
Discussion

The present study showed that thiopental significantly attenuated bronchoconstriction induced by i.v. methacholine, which stimulates muscarinic receptors. We recently reported that thiopental significantly displaced l-[N-methyl-3H]scopolamine methyl chloride binding to M₃ receptors and inhibited the methacholine-stimulated increase in intracellular Ca²⁺ concentration in Chinese hamster ovary cells expressing recombinant human M₃ muscarinic receptors. In addition, several reports showed that thiopental inhibits acetylcholine-induced contraction of guinea-pig tracheal smooth muscle. Thus, thiopental may attenuate methacholine-induced bronchoconstriction by inhibiting M₃ receptors.

In the present study, thiopental worsened 5HT-induced bronchoconstriction. Similarly, Gross and Abel found that less than 3×10⁻⁴ M thiopental concentration-dependently potentiated 5HT-induced contraction of the rabbit basilar artery, whereas more than 3×10⁻⁴ M thiopental dose-dependently relaxed the artery. Introna and colleagues also reported that thiopental per se caused coronary constriction and 5HT possibly potentiated this constriction.

Regarding airway smooth muscle, Lenox and colleagues reported that thiopental per se contracted guinea-pig tracheal smooth muscle, which was completely inhibited by indomethacin 10⁻⁵ M. Moreover, Curry and colleagues showed that thiobarbiturates, but not oxybarbiturates, produced tracheal smooth muscle contraction directly. This may have been due to the production of thromboxane A₂, as UK37248 5×10⁻⁵ M and OKY046 10⁻⁶ M, which are thromboxane synthetase inhibitors, inhibited contraction. Ouedraogo and colleagues also showed that indomethacin 10⁻⁵ M partially blocked thiopental-induced contraction of human bronchial smooth muscle. Thus, we tested if indomethacin pretreatment could attenuate the potentiation of 5HT-induced bronchoconstriction produced by thiopental. However, in the present study the thiopental-induced potentiation did not differ between saline and indomethacin pretreatment. Thus, the potentiation may not be due to synthesis of a contractile cyclooxygenase product of arachidonic acid metabolism.

We reported previously that bronchoconstrictive effects of thiopental could be inhibited by atropine. Several clinical investigations also suggest that thiopental may increase vagal tone. However, in the present study, atropine

![Fig 2](https://example.com/fig2.png)

**Fig 2** Effects of thiopental on 5HT-induced bronchoconstriction after saline (S-5HT) or indomethacin (I-5HT) pretreatment. Pre=before bronchoconstrictor infusion started; 5HT30=30 min after 5HT infusion started. *P<0.05 vs 5HT30. Values are mean and sd.

![Fig 3](https://example.com/fig3.png)

**Fig 3** Aggravating effects of thiopental on 5HT-induced bronchoconstriction with saline (5HT-S) or atropine (5HT-A) treatment. Pre=before bronchoconstrictor infusion started; 5HT30=30 min after 5HT infusion started. **P<0.01 vs 5HT30. Values are mean and sd.

<table>
<thead>
<tr>
<th>Group</th>
<th>Catecholamine (pg ml⁻¹)</th>
<th>5HT30’</th>
<th>Thiopental (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 (saline) 0.02 0.2 2.0 20</td>
</tr>
<tr>
<td>S-5HT (n=5)</td>
<td>Norepinephrine 184 (107)</td>
<td>188 (125)</td>
<td>155 (109) 160 (109) 159 (118) 105 (81)**</td>
</tr>
<tr>
<td></td>
<td>Epinephrine 49 (42)</td>
<td>50 (40)</td>
<td>39 (31) 46 (44) 28 (22) 24 (6)*</td>
</tr>
<tr>
<td>I-5HT (n=5)</td>
<td>Norepinephrine 161 (93)</td>
<td>175 (82)</td>
<td>170 (129) 183 (111) 196 (136) 65 (33)**</td>
</tr>
<tr>
<td></td>
<td>Epinephrine 190 (203)</td>
<td>254 (257)</td>
<td>233 (241) 235 (245) 221 (219) 54 (43)**</td>
</tr>
</tbody>
</table>

Table 2 Changes in plasma catecholamine levels 5 min after i.v. thiopental in the S- and I-5HT groups. Data are mean (sd), S-5HT=pretreatment with saline; I-5HT=pretreatment with indomethacin. *P<0.05; **P<0.01 vs 5HT30'.
did not attenuate the spastic effects of thiopental. In the previous study the bronchoconstrictive effect of thiopental was transient and BCA returned to baseline within 3 min, whereas in the present study the results for the 5HT-S group suggest that potentiation of 5HT-induced bronchoconstriction by thiopental may be maintained from 5 to 10 min after i.v. thiopental. Therefore, the mechanism of the spasitic effect of thiopental on 5HT-induced bronchoconstriction may be different from that of the effect on basal or histamine-induced bronchoconstriction. In addition, cholinergic stimulation may not be involved in aggravating the effects of thiopental on 5HT-induced bronchoconstriction.

In vascular smooth muscle, several reports suggest that the contractile effect of thiopental may be due to increases in Ca\(^{2+}\) release from the sarcoplasmic reticulum and extracellular Ca\(^{2+}\) influx. Moriyama and colleagues reported that thiobarbiturates (10\(^{-5}\) to 10\(^{-4}\) M) caused further contraction of canine cerebral and mesenteric arterial smooth muscle induced by prostaglandin F\(_2\alpha\), and the contractile effect of thiobarbiturates may be due to enhancement of Ca\(^{2+}\) influx via receptor-operated Ca\(^{2+}\) channels. Mousa and colleagues found that thiopental-induced contraction of rat aortic smooth muscle was reduced but not abolished in Ca\(^{2+}\)-free solution. In addition, the contraction was abolished completely with thapsigargin depletion of the sarcoplasmic reticulum Ca\(^{2+}\) store. Henkell and colleagues reported that thiopental contracted rat aortic rings only in the presence of extracellular Ca\(^{2+}\) and the contractile effect was not inhibited by the L-type Ca\(^{2+}\) antagonists verapamil and diltiazem. In addition, they found that thiopental abolished vasocontractile effects of either phenylephrine or caffeine in Ca\(^{2+}\)-free solution and augmented intracellular Ca\(^{2+}\) concentration in cultured smooth muscle cells incubated in both the presence and absence of Ca\(^{2+}\). Similarly, Tsuji and Chiba reported that thiopental-induced constriction of perfused canine basilar arteries was only slightly suppressed by diltiazem pretreatment, whereas diltiazem depressed potassium chloride-induced constriction. Thus, similar mechanisms, by which thiopental could increase both Ca\(^{2+}\) release from the sarcoplasmic reticulum and Ca\(^{2+}\) influx that may be independent of L-type Ca\(^{2+}\) channels, may contribute to thiopental-induced airway smooth muscle constriction.

Thiopental inhibits sympathetic neural outflow. Sympathetic influence on airway tone is mainly dependent on circulating catecholamines as direct sympathetic neural supply to the lung is limited. Reduction in endogenous epinephrine by i.v. thiopental might thus worsen 5HT-induced bronchoconstriction. However, we reported previously that thiopental increased basal airway smooth muscle tone and worsened histamine-induced bronchoconstriction with no changes in plasma epinephrine. In the present study, thiopental antagonized methacholine-induced bronchoconstriction with a reduction in plasma epinephrine, the reduction of which was similar to that in the 5HT groups. In addition, as plasma epinephrine was within the normal range before i.v. thiopental, the reduction may not affect the tone strongly. Therefore, it is unlikely that the sympatholytic effects of thiopental could potentiate bronchospasm in the present study.

In conclusion, the present study indicates that thiopental may attenuate bronchoconstriction induced by muscarinic but not 5HT receptor stimulation. Synthesis of a contractile cyclooxygenase product of arachidonic acid metabolism in response to thiopental may not contribute significantly to the potentiation of 5HT-induced bronchoconstriction. As in our previous report we also observed that thiopental increased airway smooth muscle tone with and without histamine infusion, thiopental should be used with care in patients with hyper-reactive airways.

References
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