Comparison of relaxant effects of propofol on methacholine-induced bronchoconstriction in dogs with and without vagotomy


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Propofol has been suggested to have in vivo airway relaxant effects, although the mechanism is still unclear. In this study, we determined whether propofol could antagonize methacholine-induced bronchoconstriction and determined whether vagotomy modifies this relaxant effect. Fourteen mongrel dogs anaesthetized with pentobarbital and pancuronium were assigned to a control group (n=7) and a vagotomy group (n=7). The trachea was intubated with a special endotracheal tube that had a second lumen for insertion of the bronchoscope. Bronchial cross-sectional area, which was monitored continuously through the bronchoscope, was measured with image analysis software. Bronchoconstriction was elicited with methacholine (0.5 μg kg⁻¹ + 5.0 μg kg⁻¹ min⁻¹) until the end of the experiment. Thirty minutes after the start of methacholine infusion, propofol 0, 0.2, 2.0 and 20 mg kg⁻¹ was administered. Changes in bronchial cross-sectional area were expressed as percentages of the basal area. Plasma concentrations of propofol and catecholamine were measured by high-performance liquid chromatography. Maximal inhibition (bronchoconstriction = 0%, baseline = 100%) and IC₅₀ (concentration producing 50% inhibition of maximal effect) produced by propofol was obtained from each concentration-response curve using a curve-fitting program. Methacholine decreased bronchial cross-sectional area to 49.3% (95% confidence interval 38.5–60.1%) and 45.3% (34.8–55.7%) of the baseline value. Propofol 20 mg kg⁻¹ significantly reversed this effect: bronchial cross-sectional area was reduced to 77.8% (66.2–89.6%) and 75.9% (64.0–87.9) in the control and vagotomy groups respectively. The two groups did not differ significantly in the maximal inhibitory effect of propofol [control group, 61.1% (46.3–75.9%), vagotomy group, 64.2% (40.1–88.3%)] or pIC₅₀ [control group 5.03 (4.55–5.51), vagotomy group 4.86 (4.49–5.24)]. Therefore, the relaxant effects of propofol on methacholine-induced bronchoconstriction may not be mediated centrally. Propofol may relax airway smooth muscles directly or through the peripheral vagal pathway.

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Muscarinic receptors play an important role in cholinergic effects on the airway,11 and previous reports suggest that muscarinic receptor subtypes m1, m2 and m3 exist in the human airway.12 The m1 receptors facilitate neurotransmission through parasympathetic ganglia and enhance cholinergic reflexes. Activation of m3 receptors produces contraction of airway smooth muscles. Activation of m2 receptors inhibits the release of acetylcholine and may also counteract the bronchodilation produced by β-agonists.12 Methacholine is a non-selective muscarinic receptor agonist,13 and has been used clinically to quantitate airway responsiveness in asthmatic patients.14

In this study, we determined whether propofol could antagonize the muscarinic receptor-activated bronchoconstriction induced by methacholine. In addition, we compared the relaxant effects in normal dogs with those in vagotomized dogs to determine whether the relaxant effects are centrally mediated.

Methods

Our study protocol was approved by the Animal Care and Use Committee of the University of Hirosaki School of Medicine. Fourteen mongrel dogs (8–12 kg) were anaesthetized with i.v. pentobarbital (30 mg kg−1 + 2.0 mg kg−1 h−1) and assigned to a control group (n=7) or a vagotomy group (n=7). The tracheas were intubated with endotracheal tubes (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. Neuromuscular block was obtained with pancuronium 0.2 mg kg−1 h−1 for mechanical ventilation with oxygen using a volume-controlled respirator (Servo 900C), and end-tidal carbon dioxide was maintained at 4.0–4.5%. The femoral artery was cannulated to monitor arterial blood pressure and for blood sampling. The femoral vein was also cannulated for the insertion of a double lumen catheter, through which fluid and drugs were administered. In the vagotomy group, the vagus nerves were isolated and then cut bilaterally.

The cross-sectional area of third bronchial bifurcation in the right lung was monitored via a superfine fibre-optic bronchoscope (outside diameter 2.2 mm, AF type 22A; Olympus, Tokyo, Japan) to assess bronchial tone, as reported previously.5 15–18 Briefly, the image of the third bifurcation was printed with a video printer (VY-170; Hitachi, Tokyo, Japan) during the end-expiratory pause, and was then loaded into a Macintosh computer (Power Macintosh 7100/80 AV, Apple Computer, CA, USA) via a scanner (Scan-Jet 4c; Hewlett Packard, CO, USA) to measure bronchial cross-sectional area with image analysis software (MacScope 2.56; Mitani, Fukui, Japan). Image processing was performed by an investigator who was blinded to the study protocol.

Bronchoconstriction was elicited by i.v. infusion of methacholine (0.5 μg kg−1 + 5 μg kg−1 min−1) until the end of the experiment. Initially we confirmed that this bronchoconstriction could be fully reversed by atropine. Thirty minutes after the start of methacholine infusion, the following doses of propofol were given at 10 min intervals in the order indicated: 0 (saline), 0.2, 2.0, 20 mg kg−1. Bronchial cross-sectional area was measured before and 30 min after methacholine infusion started and 5 min after each propofol dose. Bronchial cross-sectional areas are presented as percentages of basal.

Arterial blood samples (6 ml) were centrifuged immediately at 3000 r.p.m. for 10 min at −10°C to separate plasma, which was then frozen at −70°C until measurement of catecholamines and propofol concentrations by high-performance liquid chromatography.19 20 The lower detection limits were 15 pg ml−1 for norepinephrine, 20 pg ml−1 for epinephrine and 100 ng ml−1 for propofol. The intra-assay coefficient of variation was 3.3% for norepinephrine, 6.0% for epinephrine and 2.9% for propofol.
**Statistical analysis**

All data are expressed as mean (95% confidence interval). Data were analysed by repeated measures analysis of variance followed by Fisher’s protected least significant difference test using StatView II on a Macintosh computer. \( P < 0.05 \) was considered significant. To obtain a concentration–response curve for propofol-induced relaxation, relaxation was expressed as a percentage: peak constriction by methacholine=0% and full relaxation (baseline)=100%. The sigmoid concentration–response curve was fitted using GraphPad Prism 1.03, and the maximal inhibition and \(-\log\) of the concentration \((\mu M)\) producing 50% inhibition of maximal effect (pIC\(_{50}\)) of propofol were obtained from each concentration–response curve.

**Results**

Plasma propofol concentration 5 min after 0.2, 2.0 and 20 mg kg\(^{-1}\) of propofol i.v. was 1.50 (0.69–2.30), 5.31 (4.52–6.10) and 126.0 (92.6–159.3) \(\mu M\) respectively in the control group and 0.95 (0.73–1.18) and 3.90 (3.35–4.46) and 125.2 (107.3–143.1) \(\mu M\) in the vagotomy group. Plasma concentration after propofol 2.0 mg kg\(^{-1}\) i.v. was significantly different between groups \((P<0.01)\).

Methacholine decreased the bronchial cross-sectional area to 49.3 (38.5–60.1) and 45.3 (34.8–55.7)% of the basal bronchial cross-sectional area in the control and vagotomy groups respectively. In the control group, propofol 2.0 and 20 mg kg\(^{-1}\) significantly increased bronchial cross-sectional area, to 61.2 (49.4–73.1) and 77.8 (66.2–89.6)% of basal respectively (Fig. 1A). Similarly, in the vagotomy group the bronchial cross-sectional area was significantly increased to 75.9 (64.0–87.9)% of basal after propofol 20 mg kg\(^{-1}\) but not significantly after 2.0 mg kg\(^{-1}\) (Fig. 1A).

Maximal inhibition of methacholine-induced bronchoconstriction by propofol in the control and vagotomy groups was 5.03 (4.55–5.51) (mean 9.3 \(\mu M\)) and 4.86 (4.49–5.24) (13.8 \(\mu M\)) respectively. There were no significant differences in maximal inhibition and IC\(_{50}\) between groups (Fig. 1B).

Plasma catecholamine concentration was significantly reduced after propofol 20 mg kg\(^{-1}\) i.v. in both groups, and the reduction in plasma catecholamines did not differ between groups (Table 1).

**Discussion**

We have reported previously that the relaxant effect of propofol may be due to vagolysis.\(^5\) In the present study, as the spasmolytic effects of propofol did not differ between vagotomized and intact dogs, the spasmolytic effects of propofol on methacholine-induced bronchoconstriction may not be mediated centrally. Therefore, the relaxation could be due to inhibition of the peripheral vagal motor pathway or to direct inhibition. In addition, Brown and Wagner\(^2\) reported that bronchoprotective effects of propofol in vivo as a local infusion via the bronchial artery attenuated vagal nerve stimulation-induced bronchoconstriction.

Figure 1B shows that the in vivo relaxant effect of propofol is concentration-dependent, and the clinical concentration (about 30 \(\mu M\)\(^2\) may reverse methacholine-induced bronchoconstriction by 45%. Azizkhan and colleagues\(^2\) reported that tracheal cross-sectional area in respiratory symptomatic paediatric patients with a mediastinal mass was decreased by 35–93% of the normal tracheal dimensions. Huysmans and colleagues\(^2\) studied the effectiveness of radioiodine therapy in patients with a large goitre compressing the trachea. They found that the tracheal cross-sectional area of the smallest lumen increased by 36% after therapy, and clinical symptoms such as dyspnoea and inspiratory stridor improved in eight of 12 patients. Therefore, 45% relaxation by clinical concentrations of propofol in the present study may be clinically significant.

In this study, propofol 2 mg kg\(^{-1}\) significantly reversed bronchoconstriction in the control group, but the reversal effect was not significant in the vagotomy group. However, plasma propofol concentration after 2 mg kg\(^{-1}\) i.v. was significantly higher in the control group than in the vagotomy group. In addition, as described above, there were no significant differences between the groups in...
maximal inhibition and IC_{50}. Therefore, vagotomy may not alter the relaxant effects of propofol, although we cannot explain the difference in plasma propofol concentration.

Direct relaxant effects of propofol should also be considered. As methacholine predominantly activates muscarinic receptors rather than nicotinic receptors, it is possible that propofol inhibits muscarinic receptor responses directly. Similarly, we reported previously that propofol concentration-dependently shifts the concentration–response curve of carbachol-induced contraction of guinea-pig tracheal smooth muscle. However, using a radioligand binding assay, Lin and colleagues reported that propofol (<100 μM) may not interact with muscarinic receptors in airway smooth muscles, although propofol 300 μM significantly decreased the binding affinity of [3H]N-methyl-scopolamine to muscarinic receptors. It was also reported that propofol reduced the intracellular Ca^{2+} concentration in airway smooth muscle cells by inhibition of Ca^{2+} influx and of Ca^{2+} release from internal stores. Similarly, Yamakage and colleagues showed that more than 10 μM of propofol significantly reduced Ca^{2+} current through voltage-sensitive Ca^{2+} channels in the airway smooth muscle cells. These data suggest that inhibition of Ca^{2+} mobilization by propofol may be involved in the direct relaxant effects.

Pancuronium was infused to prevent spontaneous respiratory bucking in the present study. As pancuronium has been reported to inhibit m2 rather than m1 or m3 receptors, pancuronium may antagonize activation of m2 receptors by methacholine. Although m2 activation causes a reduction in acetylcholine release, the main mechanism of methacholine-induced bronchoconstriction is via m3 activation. In addition, although m2 receptors also counteract β-agonist-induced relaxation, propofol reduced plasma epinephrine concentrations in the present study. Therefore, pancuronium infusion may not attenuate methacholine-induced bronchoconstriction.

In the present study, plasma catecholamines were significantly reduced by propofol. This is consistent with a previous paper which showed that propofol produces sympatholysis more potently than vagolysis. As the direct neural supply of the sympathetic system is limited in the lung, sympathetic influence on the airway tone is dependent on the plasma concentration of catecholamines. Therefore, plasma catecholamines, especially epinephrine, may modulate airway smooth muscle tone. Indeed, we observed previously that histamine-induced bronchoconstriction was worsened by phenylephrine and lidocaine, with a reduction in plasma catecholamines, and reversed by droperidol and divalent cations (Mg^{2+} and Zn^{2+}) with an increase in plasma catecholamines. Therefore, the decrease in plasma catecholamines may partially antagonize the relaxant effects of propofol.

In conclusion, the present data suggest that propofol inhibits methacholine-induced bronchoconstriction, but relaxation is not mediated via a central vagal pathway. This result is consistent with clinical reports suggesting potential bronchoprotective effects of propofol. However, it remains to be determined whether the relaxant effects are mediated through the peripheral vagal motor pathway or via direct actions.

References

Propofol inhibits methacholine bronchoconstriction

22 Hirota K, Lambert DG. IV anaesthetic agents inhibit dihydropyridine binding to L-type voltage-sensitive Ca2+ channels in rat cerebrocortical membranes. Br J Anaesth 1996; 77: 248–53